Trends in Microbiology

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REVIEWS

Review

Herpes Simplex Virus-1 in the Brain: The Dark Side of a Sneaky Infection

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Herpes simplex virus-1 (HSV-1) establishes latency preferentially in sensory neurons of peripheral ganglia. A variety of stresses can induce recurrent reactivations of the virus, which spreads and then actively replicates to the site of primary infection (usually the lips or eyes). Viral particles produced following reactivation can also reach the brain, causing a rare but severe form of diffuse acute infection, namely herpes simplex encephalitis. Most of the time, this infection is clinically asymptomatic. However, it was recently correlated with the production and accumulation of neuropathological biomarkers of Alzheimer's disease. In this review we discuss the different cellular and molecular mechanisms underlying the acute and long-term damage caused by HSV-1 infection in the brain.

HSV-1 Infection

HSV-1 is a widely distributed neurotropic human pathogen that is transmitted mainly by intimate contact between infected and susceptible individuals, and it causes labial, ocular, or genital infections [1]. Primary infection usually occurs during childhood: over 60% of individuals under 50 years of age are infected with HSV-1 worldwide [2]. After primary infection of epithelial cells, the virus becomes latent in neurons of the peripheral nervous system (PNS) and can be periodically reactivated resulting in recurrent clinical or subclinical episodes throughout life [1]. Although sympathetic neurons can be also affected, HSV-1 mainly infects sensory neurons close to the site of primary infection [3,4], subsequently traveling retrogradely along the axon to the cell body in the peripheral ganglia. Studies in animal models have shown that the virus can also reach the central nervous system (CNS) [5–14] (Figures 1 and 2A). In humans, viral replication in the brain may result in herpes simplex encephalitis (HSE) (reviewed in [15]) or milder/asymptomatic infections eventually followed by latency [16]. A growing body of evidence indicates that the cumulative effects of repeated 'mild' HSV-1 brain infections may result in neuronal damage similar to that found in neurodegenerative disorders such as Alzheimer's disease (AD), the most common form of dementia in the elderly (Box 1) [17]. Here, we review recent knowledge on the pathogenic mechanisms underlying neuronal damage caused by HSV-1 infection and discuss the effects of severe acute infection and repeated viral reactivations.

HSV-1 Replication and Latency in Neurons

HSV-1 consists of a linear double-stranded DNA genome within an icosapentahedral capsid. The capsid is surrounded by an amorphous proteinaceous coating (the tegument) and the viral envelope, in which multiple glycoprotein spikes are embedded. Virus binding and entry into host cells are mediated by specific association between viral glycoproteins (gB, gC, gD, and the gH/gL complex) and receptors located on target cells, including the herpesvirus entry mediator (HVEM), heparan sulfate moieties, and the cell-adhesion proteins nectin-1 and nectin-2 (reviewed in [18]). After primary replication in epithelial cells, HSV-1 enters peripheral neurons following fusion

Highlights

After primary infection, HSV-1 can reach the central nervous system where, in rare cases, it replicates and triggers an acute and inflammatory response resulting in herpes simplex encephalitis (HSE).

The presence of the HSV-1 genome has been revealed in tissues of the peripheral and central nervous system of individuals with no clinical signs of HSE.

In humans, levels of circulating anti-HSV immunoglobulins, considered as markers of HSV-1 reactivation, have been positively correlated with an increased risk of Alzheimer's disease (AD).

Experimental data show that HSV-1 infection of neurons activates neurotoxic pathways typical of AD, and repeated HSV-1 reactivations in the brain of infected mice produce an AD-like phenotype.

Further studies are required to get greater mechanistic understanding of the causal links between recurrent HSV-1 infections in the brain and AD as well as to validate experimental findings in humans.

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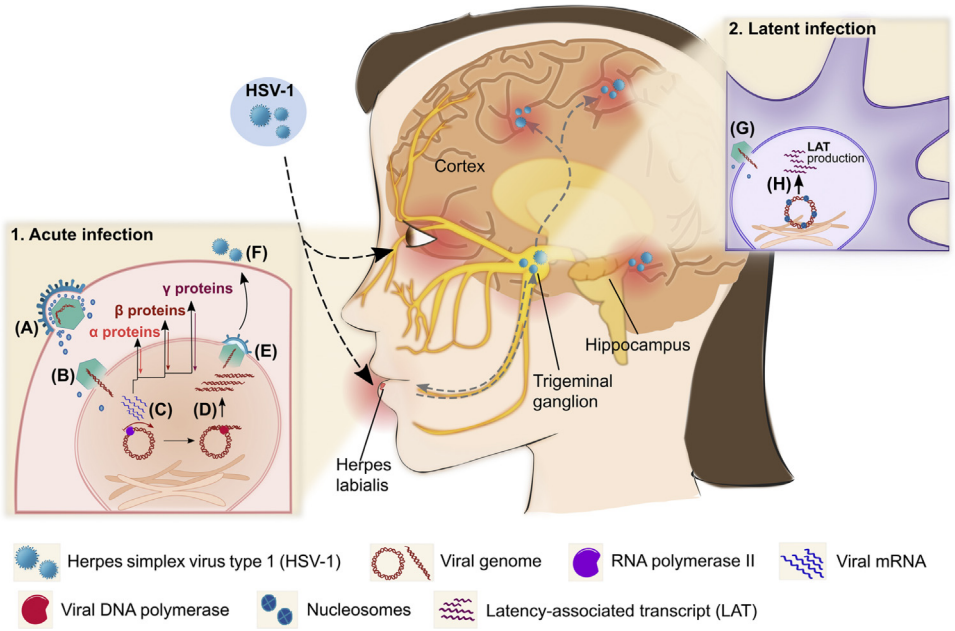
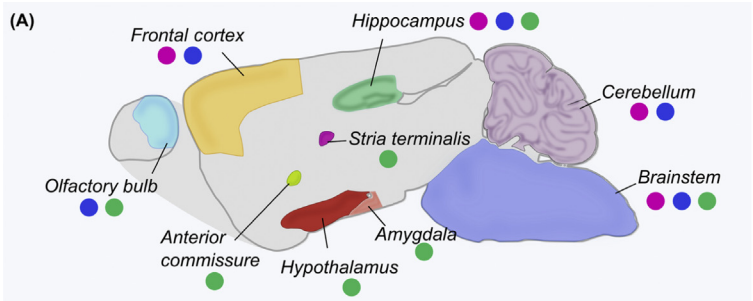


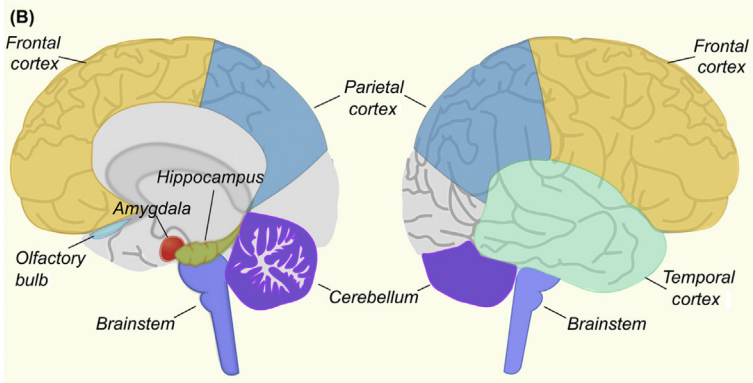
Figure 1. Schematic Representation of Acute and Latent Herpes Simplex Virus-1 (HSV-1) Infection in Humans. Acute infection: (A) HSV-1 virion enters the epithelial cell via envelope fusion with the plasma membrane. Tegument proteins and the capsid containing the linear viral genome are released into the cytoplasm. (B) The capsid reaches the nucleopores by microtubule transport and releases the viral genome into the nucleus, where (C) the genome circularizes and sequential transcription of α, β, and γ genes begins. Arrows indicate nucleocytoplasmic traffic of viral mRNA (black) and related proteins (red). (D) DNA replication begins upon completion of the production of α and β proteins, including viral DNA polymerase. Viral DNA synthesis stimulates the production of γ proteins, which, in turn, participate in capsid assembly and virion formation. (E) The newly synthesized genome is enclosed in the capsid, and the nucleocapsid buds through the nuclear membranes, endoplasmic reticulum, and/or trans-Golgi network vesicles to form an enveloped virion. (F) Newly formed viral particles are released. Latent infection: (G) HSV-1 capsid reaches the neuronal cell body in the sensory ganglia and is transported to the nucleopore by retrograde axonal transport. In the nucleus, viral DNA circularizes and is assembled with the nucleosome, causing the host cell to silence viral genome transcription (H), except for latency-associated transcript (LAT) genes.

of viral and cellular membranes, a process mediated by the interaction between gD and nectin-1 [19]. This usually occurs at the axon termini of peripheral trigeminal ganglia (TG) neurons that innervate the orofacial or corneal epithelial layer. HSV-1 capsids travel to the cell body by retrograde axonal transport, enter the ganglion neurons, and release the viral DNA into the nucleus to establish latency [1]. The latency may be favored by inefficient transport to the neuronal cell body of the tegument protein viral transactivator VP16, required to efficiently activate the viral lytic program, as described below [20] (reviewed in [21]). Latent HSV-1 genomes persist in episomal forms within the nucleus, where they can interact with promyelocytic leukemia (PML) nuclear bodies (NBs) which are involved in the establishment of latency [22–24]. During latency, HSV-1 DNA is chromatinized with heterochromatic histone marks, whereby only a small subset of viral genes is expressed [25,26].

The most abundant products of viral gene expression during latency are latency-associated transcripts (LATs), a primary 8.3/9 kb transcript and two stable introns (2.0 and 1.5 kb) derived from rapid splicing of primary LAT [1,25,26]. In addition to LATs, latent virus produces several **microRNAs** (**miRNA**) (see Glossary) [27,28] that act synergistically with LATs to repress viral replication and may contribute to inhibition of apoptosis and stimulation of viral reactivation [29]. A range of stimuli, including fever, emotional stress, hormone imbalance, UV exposure,



	Infection route	CNS areas	Detection method	Refs
Murine	Lip	BS, CB	SPA	[5]
	Lip	BS	SPA	[8]
	Lip	FC, HP, CB	SPA,PCR, IF	[14]
	Eye	BS, CB	SPA	[6]
	Eye	OB, BS	SPA	[7]
	Eye	OB, FC, HP, BS, CB	SPA, PCR	[10]
	Eye	BS	PCR	[11]
	Eye	BS	SPA, PCR	[13]
	Nose	OB, HT, AM, ST	IHC	[9]
	Nose	AC, HP, BS	PCR	[12]



	Infection route	CNS areas	Detection method	Refs
Human	-	FC, PC, BS, CB	Southern Blot	[36]
	-	FC, TC, HP	PCR	[68]
	-	OB, AM, HP, BS	PCR	[37]
	-	FC, TC	PCR	[115]
	-	FC, TC	PCR	[116]
	-	FC, TC, HP	PCR	[69]
	-	FC, CB	PCR	[117]
	-	FC, TC	PCR	[105]

OB = Olfactory bulb; FC = Frontal Cortex; PC = Parietal cortex; TC = Temporal cortex; HP = Hippocampus; AM = Amygdala; AC = Anterior commissure; ST = Stria terminalis; HT = Hypothalamus; BS = Brainstem; CB = Cerebellum

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Glossary

Avidity and avidity index: avidity is a measure of the strength of antibody binding to the antigen; the avidity index is the percentage of antibodies bound to the antigen after denaturation treatment.
cGAS–STING: cyclic guanosine monophosphate (GMP)–adenosine monophosphate (AMP) synthase (cGAS) is a sensor of cytosolic DNA that produces cGAMP, a secondary messenger, to activate the adaptor protein stimulator of interferon genes (STING) and, in turn, to induce a type I interferon response.
Cytokines: small proteins secreted by different cell types (primarily T and B lymphocytes, monocytes, leukocytes, macrophages, microglia) to enhance and regulate interactions and communication among cells. Chemokines are cytokines with chemotactic activity.
Membrane depolarization: after binding of signaling molecules (e.g., neurotransmitters) to the plasma membrane, the membrane potential (different ionic distribution on both sides of the plasma membrane) shifts from negative to positive charge, modifying membrane permeability to various ions.
microRNAs (miRNAs): small endogenous molecules of noncoding single-stranded RNA that bind complementary messenger RNAs (mRNAs) to inhibit their translation.

Box 1. Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia: an estimated 40–50 million individuals are currently affected worldwide, and this number is expected to double in the next 20 years [63]. AD is characterized by the progressive impairment of cognitive functions, particularly memory. While genetic mutations in amyloid precursor protein (APP) and presenilin proteins account for a small percentage of AD cases [familial AD (FAD)], the majority of AD cases are sporadic (SAD), and are thought to involve as-yet-unidentified environmental factors. There is no effective therapy for AD, and currently available pharmacological treatments are primarily aimed at enhancing cholinergic activity, which is reduced in AD patients, or delaying the formation of amyloid plaques in the brain (see Box 3). Several pathogenic mechanisms, including accumulation of amyloid beta (Aβ) and phospho Tau (pTau) oligomers, oxidative stress, mitochondrial dysfunction, and neuroinflammation have been proposed to contribute to AD onset and progression. Recent epidemiological and experimental evidence strongly support the hypothesis that microbial agents are risk factors for AD. Specifically, herpes simplex virus type-1 (HSV-1) is gaining greater attention because of its ability to cause recurrent, life-long infection.

trauma, and immunosuppression, can reactivate the latent virus within infected ganglia [1]. These factors can directly affect HSV-1-infected neurons or can act at the level of surrounding, non-neuronal cells (e.g., satellite glia and $CD8^+$ T cells), promoting nuclear accumulation of host transcription factors and viral VP16 in neurons [21]. Newly synthesized VP16 may also sustain HSV-1 reactivation in neurons [30]. Reactivation usually results in productive infection although abortive reactivations may also occur [21,31]. In the nucleus, productive infection begins with the sequential expression of three subsets of viral genes [immediate early (IE), early (E), and late (L) genes] mediated by cellular RNA polymerase II. Specifically, viral transactivator VP16 induces the initial transcription of IE genes, the products of which activate E gene expression, in turn promoting transcription of L genes. Structural proteins and new viral genomes are then assembled into capsids, which translocate to the cytoplasm, probably by budding from the inner nuclear membrane (envelopment phase) and fusing with the outer nuclear membrane (de-envelopment phase). Naked capsids acquire tegument and their definitive envelope (re-envelopment phase) in the vesicles of the trans-Golgi network, and the mature virion exits the host cells near the cell body [1] (reviewed in [32]). Alternatively, naked capsids and viral glycoproteins, or complete virions, can travel anterogradely inside separate vesicles anchored to the microtubule scaffolding, reaching the axonal shaft and tip in the periphery, where they are released [32]. Usually, this gives rise to blisters, sores, or ulcers at the site of primary infection, although reactivation can be asymptomatic, despite the shedding of newly produced infectious virus [33]. Because sensory trigeminal neurons are pseudounipolar, new viral particles can also reach the CNS via anterograde transport. Specifically, one of the two branches of TG neurons projects to the trigeminal nuclei in the brainstem, from which projections reach the thalamus and, from there, the sensory cortex. TG neurons have therefore been proposed as a direct route for HSV-1 entry to the CNS (reviewed in [34]). HSV-1 infection of the CNS can cause HSE, a severe inflammatory brain disease (estimated worldwide incidence: 2.5–12 cases/million/year [35]) that causes 70% mortality in untreated patients and up to 30% mortality combined with a high incidence of neurological sequelae in patients treated with antivirals. Primary HSV-1 infection and HSV-1 reactivation account for one-third and two-thirds of all HSE cases, respectively (reviewed in [15]). The HSV-1 genome has been detected in postmortem brain tissue from individuals with no clinical signs of HSE, suggesting that HSV-1 may establish latency in the CNS [36,37]. Postmortem findings in brain tissue must be interpreted with caution, and multiple confounding factors must be taken into account (e.g., fixation/storage time, lateralization, handling, contamination, protein/nucleic acid degradation, impact of antemortem factors such as drug treatments and the duration of

Figure 2. Schematic Representation of Herpes Simplex Virus-1 (HSV-1) Detection in Human and Murine Brain Areas. (A) Sagittal representation of murine brain showing the areas in which HSV-1 is detected after oral, nasal, or ocular inoculation (indicated with colored dots) in *in vivo* models; related studies, detection methods, and primary site of HSV-1 inoculation are listed below. (B) Sagittal (left) and external (right) representation of human brain showing the presence of HSV-1 DNA as detected in postmortem brains; related studies and detection methods are listed below. Colored areas are HSV-1-positive. (See [5–14,36,37,68,69,105,115–117].)

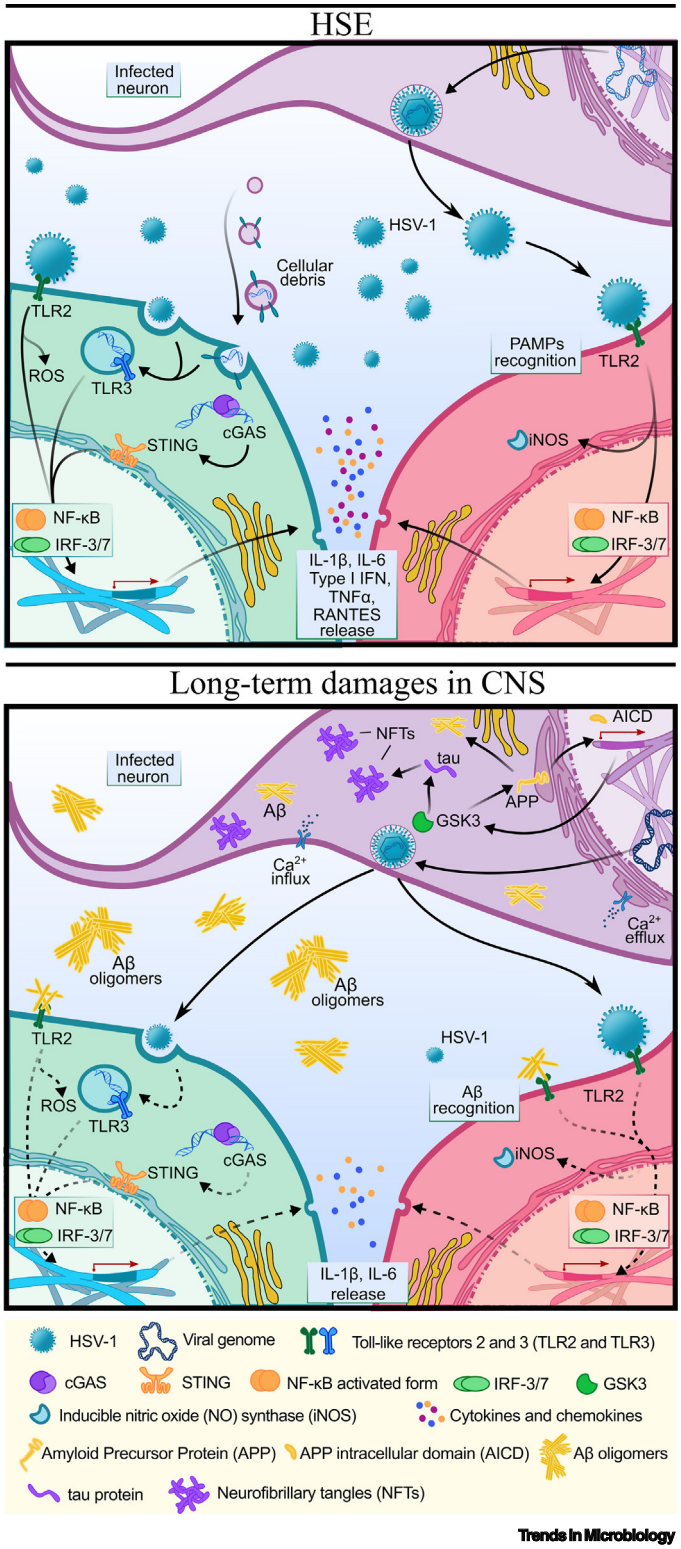
agonal status (discussed in [37,38]). However, these confounding factors are unlikely to have influenced the aforementioned reports of HSV-1 detection, in which very strict positivity criteria were applied. HSV-1 has also been detected both in cerebrospinal fluid (CSF) and brain tissue taken from neurosurgery patients who showed no signs of HSV-1 infection before surgery (HSV-1 detection in both postmortem brains and clinical samples are reviewed in [39]). Recent, albeit controversial, *in vivo* findings suggest that latent HSV-1 may be directly reactivated in the CNS. In latently infected C57BL/6N mice, HSV-1 reactivation occurred in the brainstem independently of reactivation in the TG [11]. In the same mouse model, Doll *et al*. [13] reported larger amounts of infectious virus, as detected by direct plaque assay, and a higher reactivation frequency in the TG than in the brainstem, and suggested that the virus is reactivated in the TG and subsequently transported to the brainstem. We detected infectious virus in several brain regions (i.e., neocortex, hippocampus, and cerebellum) in BALB/c mice 4 days after labial HSV-1 inoculation, and active virus replication in the same cerebral tissues in response to a reactivation stimulus [14]. However, whether viral reactivation in the brain occurred independently of reactivation of a latent viral reservoir in the TG remains to be clarified. In conclusion, although animal models of infection do not perfectly reflect HSV-1 latency and reactivation in humans, several observations in animal models, together with findings in postmortem human brain samples, indicate that the virus can reach the brain (Figure 2). Further studies are required to clarify how and when the virus reaches and replicates in the human brain, and to unravel the role of host-related factors (e.g., sex, genetic background, immune response) in determining the severity and outcome of infection.

Acute HSV-1 Infection of the CNS: Focus on HSE

HSV-1 replication in the brain and the consequent activation of host immune responses may result in HSE [15,40]. As demonstrated *in vivo* in experimental models [41], acute HSV-1 infection of the brain induces an influx of peripheral lymphocytes to help virus clearance. Circulating lymphocytes are recruited by CNS-resident cells (e.g., astrocytes and microglia) (Box 2) that secrete specific **cytokines** and chemokines in response to virus infection [42]. Specific pattern-recognition receptors (PRRs) on microglia and astrocytes (Figure 3, upper panel) recognize invariant HSV-1 structures, such as proteins or nucleic acids, known as pathogen-associated molecular patterns (PAMPs). Toll-like receptors (TLRs) are PRRs that play key roles in extracellular (TLR2, TLR4) and intracellular (TLR3, TLR9) HSV-1 detection [43–45]. Binding of HSV-1 PAMPs to TLRs activates intracellular signaling mediated by nuclear factor κB (NF-κB) or interferon regulatory factors (IRFs) [46]. Activated NF-κB and IRFs, alone or synergistically, increase gene transcription of proinflammatory cytokines and chemokines, including type-1 interferon (IFN), TNF-α, IL-1β, IL-6, IL-12B, CXCL1, and CXCL2 in mice [47], and TNF-α, IL-1, RANTES, and IP-10 in humans [48]. Microglial

Box 2. Microglia

The term 'glia', from the Greek word *glia* meaning glue, refers to a variety of types of brain cell (astrocytes, oligodendrocytes, and microglia) playing a critical role in neuronal function and dysfunction. Microglia are macrophage-like cells that reside within the parenchyma of the nervous system. They differ from other glial cells in that they are not derived from ectodermal tissue, but from yolk-sac progenitors during embryonic hematopoiesis, and therefore belong to the myeloid lineage. Microglia primarily mediate the clearance of neurotoxic molecules, such as cellular debris, dying cells, and misfolded proteins, preserve brain homeostasis, and enhance the innate immune response in the nervous system. Microglia exist in a resting state under physiological conditions, and undergo two forms of activation: (i) the M1 state, characterized by the production and secretion of proinflammatory cytokines (IL-1β, IL-6, IL-12, TNF-α, CCL2) and reactive oxygen and nitrogen species (ROS and RNS, respectively); and (ii) the M2 state, during which microglia produce anti-inflammatory cytokines (IL-10, TGF-β), growth factors (IGF-1, FGF, NGF), and the neurotrophin brain-derived neurotrophic factor (BDNF) [111]. In Alzheimer's disease (AD), amyloid beta (Aβ) binds to pattern-recognition receptors (PRRs) on the microglial surface, activating resting microglia. These microglia, in turn, release cytokines to enhance phagocytosis and Aβ uptake and clearance, inhibit inflammatory processes, and promote tissue repair. Long-term activation of microglia gives rise to neuroinflammation causing synaptic dysfunction and neurotoxicity that significantly contribute to neurodegeneration.



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TLR3, which is located in endosomal compartments, recognizes both cytosolic HSV-1 dsDNA [49] and an HSV-1 dsRNA intermediate [50], and its signaling cascade converges on IRF-3/7 and NF-κB, which subsequently induce the production of type-I IFNs and IL-1β, respectively [51]. Microglial type-I IFN, which is crucial for brain clearance of HSV-1 [52], is also induced in a **cGAS–STING**-dependent manner upon HSV-1 infection, as demonstrated in a study in which primary cells isolated from the brains of cGAS- or STING-deficient mice were infected with HSV-1 [53]. These mice showed increased susceptibility to HSE and concomitant impairment of type-I IFN expression in the CNS.

TLR2, which is expressed on the plasma membrane of microglia and astrocytes, binds lipoprotein components of HSV-1, including glycoproteins gH/gL and gB [54], and induces the transcription of multiple cytokines, including IL-6, and MCP-1, as demonstrated in analyses of HSV-1-infected TLR2 knockout mice [43], which have a lower mortality rate than HSV-1-infected wild-type mice. Thus, while the innate immune response is essential to suppress HSV-1 infection, its redundant and exacerbated activation may be detrimental, contributing to HSE pathogenesis. Furthermore, HSV-1-infected microglial cells produce reactive oxygen species (ROS) via a TLR2-dependent mechanism, and ROS-mediated mechanisms exacerbate disease progression [55]. The strong inflammatory response to HSV-1 infection in the brain is also associated with increased expression of inducible nitric oxide (NO) synthase (iNOS) enzyme, leading to release of cytotoxic NO [56]. Astrocytes upregulate iNOS in response to elevated levels of proinflammatory cytokines during CNS injury [57], but it remains unclear whether this also occurs during HSE. Evidence suggests that microglial cells are the main source of iNOS produced during HSE, and that increased iNOS activity (i.e., increased NO production) enhances the expression of the antioxidant enzyme heme oxygenase-1 (HO-1) in an attempt to restore oxidative balance and attenuate CNS damage [58]. In conclusion, the delicate balance between an effective antiviral/innate response resulting in viral clearance and a strong inflammatory reaction across multiple brain areas distinguishes acute HSE from a mild 'self-limiting' infection. Several factors may influence this balance, including viral load, immune and general health status, and age. Further studies will be needed to better understand how virus–host relationships affect the severity of infection and its outcomes in the brain.

Recurrent HSV-1 Infection in the CNS: Focus on Long-term Damage

Epidemiological and experimental findings from the past 20 years implicate repeated HSV-1 reactivation in the pathogenesis of amnestic mild cognitive impairment (aMCI) and AD (reviewed in [59]). aMCI results in memory deficits that do not impair normal daily activities [60,61], and may be prodromal to AD [62], that is a progressive neurodegenerative disease characterized by irreversible memory loss, cognitive decline, and deterioration of emotional control, daily routines, and social function [63]. AD and aMCI patients show alterations in certain brain areas,

Figure 3. Schematic Representation of the Mechanisms Underlying Acute [Herpes Simplex Encephalitis (HSE)] and Long-term Central Nervous System (CNS) Damage Caused by Herpes Simplex Virus-1 (HSV-1). Upper panel: HSE. Toll-like receptors (TLRs) on astrocytes (pink) and microglia (green) recognize HSV-1 pathogen-associated molecular patterns (PAMPs), to which they bind, inducing the production of specific cytokines and chemokines through activation of intracellular signaling pathways mediated by NF-κB or interferon (IFN) regulatory factors (IRFs). Type I IFN is also induced upon recognition of viral nucleotides by the microglial cGAS–STING axis. PAMP recognition also induces the expression of the enzyme inducible nitric oxide (NO) synthase (iNOS) and production of reactive oxygen species (ROS) in glial cells, resulting in massive inflammation. Lower panel: long-term damage caused by recurrent HSV-1 infection. Neuronal HSV-1 infection triggers intracellular Ca^{2+} signaling and induces glycogen synthase kinase 3 (GSK3) activation and consequent production of Aβ peptides (Aβs) and several amyloid precursor protein (APP) fragments, including APP intracellular domain (AICD). AICD may act as a transcriptional regulator at the promoter of *GSK3*, promoting expression and activation of GSK3, which is also involved in Tau hyperphosphorylation. Aβs accumulate both inside and outside neurons where they form plaques. Recognition of Aβ oligomers by TLR2 on the surface of glial cells induces localized neuroinflammation through the release of proinflammatory cytokines, ROS, and NOS, and the consequent decrease in Aβ phagocytosis.

including the entorhinal cortex and hippocampus [64], which exhibit distinctive histological features (Box 3) including extraneuronal insoluble aggregates (plaques) of amyloid beta (Aβ) peptides and intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated Tau protein (pTau) [65]. Ball [66,67] first proposed a role for cerebral HSV-1 infection in AD pathogenesis, based on the potential capability of HSV-1 to move from TG to the brain regions most affected in AD. Subsequent postmortem studies of small patient cohorts detected latent HSV-I in brain samples from both AD patients and healthy elderly controls [68], and showed a correlation in AD patients between HSV-1 infection and the ε4 allele of apolipoprotein E (ApoE-ε4) [69], a known risk factor for AD [70] that is associated with the incidence of recurrent herpes labialis in humans [69] and with increased HSV-1 neuroinvasiveness in mice [71]. A recent molecular and bioinformatics analysis of postmortem brain samples from four independent cohorts of AD and control patients reported increased expression of human herpesvirus (HHV)-6A and HHV-7, as well as HSV-1, in AD patients versus controls, and a correlation between increased herpesvirus expression and AD-related changes in gene expression [72]. Conversely, RNA and DNA sequencing analyses of AD brain repositories revealed no association between AD and HHV-6, and did not examine associations with HSV-1 [73]. These conflicting findings emphasize the need for parallel multidisciplinary approaches to investigate the potential microbial component of AD pathogenesis. Two studies [74,75] of small sample populations (19 versus 21, and 33 versus 28 AD patients and healthy controls, respectively) reported no association between AD and levels of anti-HSV immunoglobulin G (IgG), which were similar to [74] or higher than those in AD patient samples [75]. In 2008, a study evaluated levels of anti-HSV-1 immunoglobulin M (IgM), considered to be a marker of primary HSV-1 infection and/or viral reactivation, in a cohort of over 500 elderly individuals that were initially dementia-free [76]. After 14 years of follow up, individuals positive for anti-HSV-1 IgM had a significantly higher risk of developing AD, suggesting a strong correlation between HSV-1 reactivation and AD incidence. These findings were later replicated in a larger cohort [77]. Kobayashi and coworkers reported a correlation between repeated HSV-1 reactivation and increased anti-HSV-1 IgG **avidity** [78], and a higher anti-HSV-1 IgG **avidity index** in aMCI patients than in AD patients and healthy subjects. These correlative findings do not demonstrate

Box 3. Molecular Hallmarks of Alzheimer's Disease

The main histopathological lesions observed in the brains of Alzheimer's disease (AD) patients are amyloid plaques and neurofibrillary tangles (NFTs). Amyloid plaques are formed by the accumulation of insoluble aggregates of amyloid beta peptides (Aβs) that are produced by amyloidogenic cleavage of amyloid precursor protein (APP) [65]. Full-length APP is a transmembrane protein that is physiologically processed by multiple secretases. In the non-amyloidogenic pathway, α-secretase cleaves APP within the Aβ peptide region. The C-terminal fragment, which remains associated to the membrane, can be further processed by γ-secretase. In the amyloidogenic pathway, subsequent processing by β- and γ-secretases yields Aβ peptides (mainly Aβ40 and Aβ42, consisting of 40 or 42 amino acids, respectively). Aβ production occurs under physiological conditions and may support synaptic function [112]. However, abnormal increases in Aβ levels caused by imbalanced production/clearance results in the formation of Aβ aggregates, with corresponding neurotoxic effects. These aggregate forms include Aβ oligomers and fibrils, and, ultimately, insoluble senile plaques. Intracellular and extracellular accumulation of Aβ oligomers correlates strongly with synaptic and memory deficits caused by impairment of critical components of the synaptic machinery [112]. Aβ oligomers also activate microglia and astrocytes, stimulating cytokine and ROS production and triggering neuroinflammation and neuronal damage.

NFTs are formed by the aggregation of the cytoskeletal protein Tau. In healthy individuals Tau binds and stabilizes microtubules, mediates axonal transport, and modulates synaptic structure and function. Tau undergoes several post-translational modifications (e.g., phosphorylation, glycosylation, nitration, methylation, proteolytic cleavage). In neurodegenerative diseases, especially AD, Tau hyperphosphorylation leads to conformational changes, impairing the protein's ability to associate with microtubules [113]. Many kinases, including GSK3β, CDK5, MAPK, JNK, and p38, finely regulate this process. Once soluble, Tau undergoes dimerization and then self-associates into oligomers, filaments [better known as paired helical filaments (PHFs)], and ultimately organized structures such as NFTs. Tau alterations are a characteristic of many neurodegenerative diseases, collectively known as tauopathies. A large body of evidence suggests that Aβ and phospho Tau (pTau) oligomers are critical determinants of AD pathophysiology [114], although the relative contribution of each to AD pathogenesis, their interplay, and the complex factors underlying their production and accumulation in the brain are only partly understood.

a causal relationship between HSV-1 infection and aMCI/AD onset, but suggest that repeated HSV-1 reactivation may contribute to the transition between health and aMCI, or between aMCI and AD. Specific anti-HSV-1 immune responses (as determined by anti-HSV-1 IgG titer/avidity index) may play a protective role in early-stage AD [79,80], and protect against progression from aMCI to AD [81]. In addition, two recent studies exploring anti-HSV-1 immunoglobulins in aged individuals provide evidence supporting the synergy between ApoE-ε4 and HSV-1 in increasing the risk of AD [82,83]. However, further studies will be required to replicate these findings in larger patient cohorts and to clarify the role of specific anti-HSV-1 IgG in AD onset.

In the past two decades, numerous research groups, including ours, have worked to unravel the cellular and molecular mechanisms underlying the potential role of HSV-1 infection in AD pathogenesis (reviewed in [59]). Initial evidence supporting this hypothesis came from a study of human neuroblastoma cells, in which HSV-1 infection induced cleavage of amyloid precursor protein (APP) and subsequent production of a C-terminal APP fragment containing the Aβ sequence [84]. Another study attributed the formation and accumulation of Aβ to HSV-1-induced upregulation of secretases, the enzymes implicated in Aβ production via APP cleavage [85]. Piacentini and coworkers reported that HSV-1 binding to the plasma membrane of rat primary cortical neurons induced **membrane depolarization** and consequent intracellular Ca^{2+} signaling [86], mimicking observations in cultured neurons from a triple transgenic (3×Tg-AD) mouse model of AD [87]. Intracellular Ca^{2+} signals, in turn, were shown to elicit APP phosphorylation at Thr668 via glycogen synthase kinase 3 (GSK3) activation and consequent amyloidogenic cleavage of APP [86,88]. Interestingly, *in vitro* studies have shown that GSK3, which is markedly activated in AD brains [89], contributes to Tau hyperphosphorylation during HSV-1 infection [90], and to the Aβ-mediated decrease in expression of the presynaptic proteins synapsin-1 and synaptophysin [88]. Furthermore, HSV-1-induced processing of APP leads to the production of Aβs and of APP intracellular domain (AICD) [91,92], a C-terminal fragment of APP that regulates the transcription of several genes implicated in AD [93]. Specifically, both intracellular and extracellular Aβ monomers and oligomers have been detected in HSV-1-infected human neuroblastoma cells and rat primary cortical neurons [91]. AICD is reported to bind the promoter region of the GSK3 and neprilysin (NEP) genes, the latter of which encodes an enzyme involved in Aβ clearance [94], inducing their transcription during the early phases of HSV-1 infection [92]. Santana and colleagues [95] showed that HSV-1 infection induced Aβ accumulation by impairing the autophagy machinery in human neuroblastoma cells. Together, these findings clearly demonstrate that HSV-1 infection of neurons activates several distinct mechanisms that can cause Aβ accumulation and consequent neurotoxic events. Interestingly, Aβs, to which antimicrobial activity has been ascribed [96,97], have been shown to limit HSV-1 infection both *in vitro* [98,99] and *in vivo* [100]. In their study in 5×FAD mice (a transgenic mouse model of AD that overexpresses human Aβ [101]) subjected to hippocampal HSV-1 inoculation, Eimer and colleagues demonstrated improved survival compared with wild-type littermates, and accelerated Aβ deposition in the brain following viral infection [100]. The authors suggested that the production and aggregation of neurotoxic peptides could counteract viral infection by sequestering the virus. However, all mice succumbed to acute lethal infection after 125 h. A very recent study showed that HSV-1 accelerates Aβ aggregation kinetics *in vitro* and proposed that this occurs via surface-assisted heterogeneous nucleation of the peptide [102]. The authors also observed increased Aβ levels in the brains of 3-month-old 5×FAD mice 48 h after intracranial HSV-1 inoculation compared with uninfected mice, confirming the virus's ability to catalyze Aβ formation and accelerate the pathogenesis of AD. Based on these findings, it is tempting to speculate that the protective and pathogenic actions of Aβ could reflect a Janus behavior of the protein: an initial protective (i.e., antimicrobial) effect may transition to a neurotoxic effect due to overproduction and accumulation of Aβ induced by repeated HSV-1 reactivations in the brain.

Further supporting the neurodegenerative potential of HSV-1, HSV-1 infection in neurons *in vitro* induces Tau hyperphosphorylation [103] and results in DNA damage by impairing DNA repair mechanisms [104]. Some of the aforementioned findings have been validated in *in vivo* studies, although most were performed using animal models of acute HSV-1 infection [105–107]. Based on previous models of *in vivo* HSV-1 infection and reactivation [108], we induced up to seven HSV-1 reactivations at 1-month intervals in mice and showed that recurrent HSV-1 brain infection leads to progressive accumulation of several molecular biomarkers of AD, including Aβ, hyperphosphorylated Tau, and the proinflammatory cytokines IL-6 and IL-1β [14]. Remarkably, accumulation of these markers was associated with impaired adult hippocampal neurogenesis and correlated with an increasing, irreversible cognitive decline in HSV-1-infected mice subjected to multiple virus reactivations [14,109]. Clearly, this model does not perfectly mirror HSV-1 infection in humans, in which viral reactivation varies both in frequency and severity. Moreover, whether each instance of reactivation leads to active viral replication in the brain remains unknown.

A recent retrospective cohort study [110] reported a 2.56-fold increase in the risk of developing dementia, including AD, over a 10-year follow-up period in Taiwanese HSV patients over 50 years of age compared with a matched control group. The study examined the incidence of dementia during the period 2001–2010 in 8362 subjects newly diagnosed with HSV-1 in 2000, and in 25 086 matched subjects who had no history of HSV infection during that same year. Moreover, the incidence of dementia was lower in HSV-infected patients who had been treated with anti-herpes agents ($n = 7215$) than those who had not ($n = 1147$). Although merely correlative, the findings support the view that virus reactivation and replication in the brain may contribute to the incidence of dementia, including AD. To date, no prospective longitudinal cohort study has investigated the link between HSV-1 reactivation (symptomatic and asymptomatic) and the presence of AD biomarkers.

In summary, despite a number of conflicting results, there is a growing body of evidence supporting that repeated, mild HSV-1-infections in the brain may contribute to the onset and/or progression of neurodegeneration (Figure 3, lower panel).

Concluding Remarks

Evidence from epidemiological and experimental studies suggests a causal link between recurrent HSV-1 infection and neurodegenerative processes typical of AD, although further studies are required to validate this correlation in humans. The data reviewed here highlight several key challenges for future research: (i) to identify AD biomarkers in patients with recurring HSV-1 infection; (ii) to understand the virus- and host-related factors (e.g., viral yield, genetic/metabolic features, concurrent diseases or infections) involved in determining the frequency and extent of virus spread to the brain, and their correlation with neurodegenerative damage; and (iii) to identify novel strategies to limit virus reactivation and diffusion to the brain, and evaluate their potential to prevent neurodegenerative damage (see Outstanding Questions).

Outstanding Questions

Is periodic reactivation of HSV-1 in humans accompanied by spreading and replication of the virus in the brain?

To what extent do the different outcomes of HSV-1 brain infection (herpes simplex encephalitis versus mild or asymptomatic infection) depend on viral load, host antiviral response, and/or the host's genetic and metabolic characteristics?

Repeated reactivation of HSV-1 causes long-term damage in the mouse brain. Is this effect dependent on the number or frequency of reactivations, and/or the impairment of host ability to counteract the accumulation of neurotoxic damage?

What is the role of glia in (i) controlling HSV-1 infection, and (ii) exacerbating neurodegenerative damage?

Could antiviral treatments or preventive vaccines effectively block the neurodegenerative processes triggered by repeated HSV-1 reactivation?

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