

Update on Herpes Virus Infections of the Nervous System

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Abstract Herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) are human neurotropic viruses that establish latent infection in dorsal root ganglia (DRG) for the entire life of the host. From the DRG they can reactivate to cause human morbidity and mortality. Although they vary, in part, in the clinical disorders they cause, and in their molecular structure, they share several features that govern the biology of their infection of the human nervous system. HSV-1 is the causative agent of encephalitis, corneal blindness, and several peripheral nervous system disorders; HSV-2 is responsible for meningoencephalitis in neonates and meningitis in adults. The biology of their ability to establish latency, maintain it for the entire life of the host, reactivate, and cause primary and recurrent disease is being studied in animal models and in humans. This review covers recent advances in understanding the biology and pathogenesis of HSV-related disease.

Keywords Herpes virus infections · HSV · Nervous system · HSV-1 · HSV-2 · Herpes simplex encephalitis · HSE

Introduction

“An inefficient virus kills its host. A clever virus stays with it” by the British scientist James Lovelock is a charming description of the one common characteristic feature of all herpes viruses: their ability to become latent and establish a lifelong infection. The *Herpesviridae* family is comprised of about 130 large double-stranded DNA viruses found in mammals,

but also in birds, fish, and others. Eight human herpesviruses (HHVs) are known: herpes simplex virus (HSV) 1 and 2, varicella zoster virus (or HHV-3), Epstein–Barr virus (or HHV-4), cytomegalovirus (or HHV-5), HHV-6, and HHV-7, and HHV-8 [1], also known as Kaposi sarcoma associated herpesvirus. Here, we focus on the two neurotropic herpes simplex viruses HSV-1 and HSV-2, not trying to provide a comprehensive overview, but rather covering newest developments related to pathophysiology, clinical disorders, and treatment.

Structure, Primary Infection, Latency, and Reactivation

Structure

HSV-1 and HSV-2 are closely related, with nearly 70 % genomic homology [2–4]. The genome is a double-stranded DNA molecule located within an icosahedral capsid consisting of 162 capsomers. An amorphous material called the tegument that is enclosed by an envelope consisting of polyamines, lipids, and glycoproteins. Of the 12 or more viral envelope proteins, four glycoproteins (gD, gB, gH, and gL) seem to be of importance for cell entry. After cell surface attachment, binding to gD receptor occurs, activating the membrane fusion machinery. HSV-2 is usually the cause of genital herpes, whereas HSV-1 is typically transmitted during childhood via the orolabial mucocutaneous surfaces and primarily causes herpes labialis [5•].

Primary Infection

Most primary infections occur during the first two decades of life, and a high seroprevalence of HSV-1 (up to 90 %) is typical [6]. HSV infects the human host via mucosal surfaces

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or damaged skin, and most primary infections are asymptomatic [7]. The neurotropic herpesvirus infectious cycle begins with replication in the somatic cells (e.g., epithelial cells) and secondary transmission to dorsal root ganglia and cranial nerve neurons. The transmission between cells and the dual tropism (epithelial and neuronal cells) is still poorly understood and under investigation [8]. Viral effectors are clearly required for neuroinvasion and cell–cell transmission. It seems apparent that the lately discovered pseudorabies virus deubiquitinase activity residing in the amino terminus of a specific tegmentum protein specifically controls viral transmission into the nervous system [9, 10]. After entering the nerve endings, in a fusion-mediated form, the herpesvirus envelope is lost and capsid and tegument are deposited in the cytosol. Retrograde axonal transport delivers capsids to the distant nucleus along microtubules at a rate of approximately 1 $\mu\text{m/s}$, beautifully imaged using time-lapse fluorescent imaging [11–13].

Latency

Once reaching the neuronal soma, HSV-1 capsids are observed docked at the nuclear pore complex. Replication, as occurred in the peripheral epithelial cells, can resume now. However, replication will cause the destruction of the host cell, ruling out the possibility of latent infection. Thus, the suppression of replication by cellular and viral factors is an advantage, favoring virus survival and establishment of latent infection. Some neurons seem to be prone to productive infection, whereas others are more susceptible to viral latency based, in part, on the differential presence of regulatory RNAs or regulatory proteins [14–16]. The latent infection is characterized as maintenance of the viral genome within the neuron in the absence of production of new infectious virus. The genome of the herpes virus localizes in the nucleus of the host neuron where they remain as multi-copy chromatinized episomes, which do not integrate into the host cell genome [17, 18]. In this circular form the HSV genome is maintained during the course of viral latency [19]. During latency in human dorsal root ganglia, restricted HSV-1 gene expression takes place [20]. Throughout the persistent stage, from establishing latency to reactivation, the *latency-associated transcript (LAT)* locus is the only gene to be highly expressed [21]. *LAT* expression has been linked to several aspects of the latency process, including neuron survival, viral genome chromatin status, lytic gene expression, number of latently infected neurons, and efficiency of reactivation in animal [22–25]. In addition, anti-apoptotic properties of the *LAT* help maintain latency by promoting the survival of infected neurons [26]. Noncoding RNAs that participate in gene regulation (microRNA) seem to mediate the function of *LAT* and, until today, protein products have not been identified [27, 28•, 29], but *LAT* has been shown to bind to the polyribosomal fraction in cells [30].

Reactivation

The tegument protein VP16 is crucial for replication. It is a transactivator of immediate early gene expression and enters the nucleus forming complexes that promote productive infection [31, 32]. In mice models it has been shown that the de novo production of VP16 directly triggers HSV exit from the latency state and reactivation [33]. Once reactivation has occurred, viral particles are transported via fast anterograde microtubule-based axonal transport to the distal axon. Evidence is controversial and suggests either naked capsids or fully enveloped viruses are transported from the nucleus to the periphery. Work from several laboratories, including recently published new electron microscopy studies and time-lapse imaging of viral particle composition during active anterograde transport, suggests that fully-assembled enveloped capsids of HSV-1 are anterogradely transported in axons [34–36].

HSV-1

Reactivation of the virus is often asymptomatic, but once HSV has been transported to the periphery, its replication in peripheral epithelial cells can lead to symptomatic disease. Herpes labialis is caused by the formation of vesicular lesions, the result of its replication in epithelial cells. By rupture of these lesions, spread of the virus to neighboring cells or transmission to a new host is guaranteed. In immune-competent hosts, the disease is self-limiting, but in the immunocompromised patient, extensive life-threatening reactivation may occur. Replication in corneal epithelial cells can lead to herpes stromal keratitis with corneal scarring and is one of the leading causes of blindness in the USA [37]. Interestingly, permanent sensory loss in the areas of recurrent HSV reactivations is not observed, suggesting that the reactivation in the ganglion cells is not associated with neuronal cell death [38•].

Herpes Simplex Encephalitis

Of all clinical syndromes associated with the human herpes viruses, herpes simplex encephalitis (HSE) is most feared. It is the most common fatal sporadic encephalitis and the importance of rapid diagnosis cannot be overemphasized as delayed treatment is associated with worse outcomes [39]. Symptoms and signs of encephalitis include fever, headache, lethargy, irritability, confusion, focal deficit, aphasia, and seizures. About 90 % of all HSE cases in adults and children are due to HSV-1. HSE in neonates is mainly caused by HSV-2, where it is a disseminated infection. The neuropathology of HSE typically shows signs of necrotizing encephalitis localized to the orbitofrontal and temporal lobes, usually asymmetrically, but in most cases bilaterally. Progressive temporal lobe edema can lead to uncal herniation, with tachycardia, hyperventilation,

flexor (and later extensor) posturing, and a dilated pupil (usually on the side of the herniated temporal lobe). If untreated, mortality rates are around 70 %, and 97 % of untreated survivors do not return to normal function [40–42]. The neuropathogenesis of HSE has intrigued clinicians and scientists, and debate continues surrounding three major questions: 1) While 90 % of normal individuals are seropositive for the herpes virus, why is the incidence of HSE so extremely low, estimated at only about one case per million a year?; 2) Is the encephalitis due to reactivation or primary infection of the virus? (see [38*, 43, 44]); and 3) What underlies the propensity of the disease process to localize to the fronto-temporal region? Increasingly, evidence suggests that a primary infection independent of the latent highly prevalent infection is responsible for the encephalitic form of the disease. Indeed, recurrent herpes labialis as a reactivation from ganglionic reservoirs almost never leads to HSE [45]. In most instances, the viral strain causing encephalitis in an individual differs from the one responsible for their cold sores [46]. Moreover, only about 25 % of patients with HSE give a prior history of cold sores, an incidence that is no different from that seen in the normal population. Regarding the site specificity of HSE, the pathway of viral spread is probably more important than cell-type viral susceptibility. The unique anatomical localization of the encephalitis is probably the outcome of passage of the virus via the olfactory pathway and along the base of the brain to the temporal lobes. This explains the common finding on magnetic resonance imaging of patients with HSE showing mostly bilateral involvement, even though asymmetric [47, 48]. Immunocytochemical evidence of HSV antigens in the olfactory tract, cortex, and temporal lobes on autopsies support this theory [49]. However, reactivation of herpes labialis occurs in most cases unilaterally, making it less likely to be the source of HSE.

Immunology of HSE

During primary infection, the immune system plays a major role in suppressing viral replication. Later, limiting reactivation once the virus entered its latent phase becomes the major task. Detection by the immune system primarily occurs during the replication phase of the virus in epithelial cells by secretion of interferons (IFNs) and cytokines. Activated natural killer cells induce apoptosis of infected epithelial cells, dendritic cells and B-cells act as antigen presenting cells, and CD4+ and CD8+ T-cells are crucial for elimination of primary infections. Recently, it has been shown that Toll-like receptor 3 (TLR-3) can detect endosomal double-stranded DNA. Its expression can be induced in the state of infection by type-1 IFNs [50]. TLR-3, which has previously been associated with neural plasticity and neurodegeneration [51•, 52], seems to be essential in HSV-1 infections. Children with a genetic defect in the expression of TLR-3 were

shown to be more susceptible to HSE [53–56]. It is therefore possible that cells mutated or lacking TLR-3 induce fewer type-1 IFNs in response to HSV infections [57, 58]. Mouse experimental data are more conflicting and the relation of TLR-3 and HSE is being investigated [59, 60]. The IFN-induced antiviral state is important for limiting the immediate viral replication in susceptible cells at the periphery; this is not HSV-specific, but is also valid for other virus infections. Major histocompatibility complex (MHC) class I molecules presenting viral antigens are the major activator for CD8+ T-cells, which exert their antiviral function by secretion of IFN- γ , tumor necrosis factor- α , perforin, and granzymes. Once activated, CD8+ T-cells and cytotoxic T-lymphocytes (CTL) damage infected cells by forming pores on the cell surface and facilitating the entry of granzymes and granulysin [61]. Once intracellular, these proteins induce apoptosis by cleaving single-stranded DNA and by hydrolyzing histone proteins or by cleaving IL-1 β propeptide into the active IL-1 β , and thus inducing apoptosis in a caspase-dependent manner [62, 63]. In relation to HSV infection of the central nervous system (CNS), the described anti-viral apoptotic mechanism bears specific and crucial difficulty. It has been shown that neurons in culture infected with herpes viruses fail to express MHC antigens avoiding both the immune response and apoptosis—mechanisms to maintain latency [64, 65]. CTLs infiltrating the trigeminal ganglia surrounding infected neurons release preformed granules, but these molecules do not induce apoptosis of latently infected neurons [66]. In relation to HSV and HSE, once infection of temporo-frontal principle neurons has occurred, the immune response leads to apoptosis of infected cells, and allows the virus-specific immune response to efficiently eliminate virus-infected cells from the body and thereby limits the virus capacity to produce new virions. Studies suggest that neuronal apoptosis is an important contributing factor to acute CNS injury and may serve as a novel therapeutic target in these patients. The clearance mechanism has been shown to be an effective response to viruses in peripheral tissues where apoptotic cells can be replaced by mitogenic precursors. However, the neurons that reside in the nervous system are non-mitogenic. This presents a problem for the body wherein clearance of virus-infected neurons cannot be replaced and their elimination might help restrict the infection with the cost of immune cell-induced destruction leading to much greater and widespread brain tissue damage. This mechanism is known well from other viral-induced diseases like hepatitis, where immune responses can cause greater damage than the primary infection itself [67, 68].

Steroids in HSE

The probable increased brain damage due to the anti-viral immune response during HSE fueled the debate of a concomitant use of steroids during treatment with acyclovir. The

mainstay of treatment is still acyclovir to prevent viral replication by inhibiting the viral DNA polymerase in infected cells. Acyclovir is a pro-drug, being activated only in cells that harbor replicating virus [40, 69]. HSE is treated with acyclovir 10 mg/kg intravenously (IV) every 8 h for 14–21 days. Dosage should be adjusted in cases of renal malfunction. Identification of viral DNA by polymerase chain reaction (PCR) on re-examination of the CSF may indicate the need for an additional 1–2 weeks of acyclovir therapy or reconsider the initial diagnosis. Some centers practise cerebrospinal fluid (CSF) PCR at the end of acyclovir treatment and advocate a further course of acyclovir if it remains HSV-positive [70]. Steroids have been shown to improve outcome of bacterial meningitis if used concomitantly with appropriate antibiotic therapy [71, 72] and are, today, a part of standard treatment protocols. The immunosuppressant effect of corticosteroids is the main concern of their use in viral infections. Reactivation of latent HSV in patients receiving radiation and corticosteroids was shown to lead to HSE [73]. In animal experiments, steroid therapy does not correlate with HSV dissemination or viral load [74, 75]. Animal models of HSE and the use of steroids in selected cases in human HSE have shown benefits of their concomitant use with acyclovir [76–78]. No large cohort studies or randomized trials with sufficiently robust evidence for the use of adjunctive steroids in HSV encephalitis have been published until today. The results of the only randomized clinical trial investigating the use of adjunctive dexamethasone to treat HSV encephalitis (GACHE trial) are anxiously awaited [79]. In this trial, 372 patients were supposed to be recruited and randomly assigned to two groups both receiving standard treatment of acyclovir and either dexamethasone or placebo. The trial ended in 2011, but the results have still not been published. Adjunctive dexamethasone might be considered for patients with HSV encephalitis and severe brain edema or vasculitis, but, again, the use of this agent for this purpose is not supported by systematic evidence.

N-methyl-D-aspartate Receptor Antibodies in HSE

The use of immune modulation during HSE is additionally supported by recent evidence of an immune-mediated pathogenesis of relapsing encephalitis mainly in children. HSE usually follows a monophasic course, but 14–27 % of the patients develop a recurrent encephalitic episode after successful treatment of the initial infection [80–84]. As seen in anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis (targeting the NR1 subunit of the NMDAR), IgG NMDAR antibodies were detected in 7 % of patients with HSE [85]. Post-HSE, abnormal movements or relapse of symptoms could be related to anti-NMDAR antibodies. Immunotherapy had a beneficial effect in children with antibodies against the NMDAR following HSE [86].

HSV-2

Similar to the pathophysiology of HSV-1 infection, HSV-2 is associated with neurological abnormalities, an outcome of both primary infection or reactivation. Rather than trigeminal ganglion neurons, the sacral ganglia are the major sites of HSV-2 latency. This is not exclusive and, using PCR, HSV-2 DNA has been detected in ganglia throughout the whole neuro-axis, but with significantly larger copy numbers in the sacral ganglia [87]. The mechanism of latency in HSV-2 is likely to be similar to the one in the type-1 virus, but hasn't been studied as extensively. HSV-2 is principally, but not exclusively, acquired through sexual activity, and transmission from male to female partner is more common than the opposite. It is estimated that 16.2 % of all Americans aged 14–49 years have genital herpes. Interestingly, an association between HSV-2 and HIV was recognized. Infection with HSV-2 increases the risk of HIV by up to fourfold; treatment of the herpes virus infection reduces this increase.

HSV-2 Meningitis

Primary HSV-2 infection is usually asymptomatic and causes genital herpes in a few with recurrences, but of those symptomatic with primary genital herpes 36 % of women and 13 % of men experience meningeal signs and pleocytosis on CSF examination [88]. Once HSV-2 is detected by PCR, treatment with acyclovir can be initiated, but the benefit in aseptic meningitis has not been established. Recurrence of meningitis is common in HSV-2 meningitis and if large endothelial cells termed “Mollaret cells” are present in CSF analysis, the diagnosis of Mollaret's meningitis is established. Even in the recurrent disease, treatment with acyclovir has not been shown to prevent relapses [87].

Neonatal HSV-2 Encephalitis

Infection of newborns by HSV-2 virus during vaginal delivery causes a dissemination of the virus to the CNS in 70 % of babies; it is most commonly heralded by the appearance of focal or generalized seizures, and is devastating. In most cases, the mothers didn't show any clinical signs of infection. If acute infection in the mother is known, cesarean section as the method of delivery is preferred and significantly reduces the incidence of the neonatal disease [89, 90]. Once the diagnosis is established, treatment using either acyclovir or vidarabine has been shown to reduce the morbidity and mortality. Owing to a better safety profile, acyclovir is preferred, being used at 30 mg/kg/day IV in divided doses every 8 h for 14 days in infants with disease localized to skin, eyes, and mouth, and for 21 days when the infection is disseminated or involves the CNS [91].

Vaccine

Strategies to prevent this devastating disease by reducing the incidence of HSV-2 infections have mainly focused on antiviral chemotherapy, the use of condoms, and education programs. In 2002, a vaccine was tested to prevent HSV-1 and HSV-2 infections, and showed efficacy in women who were seronegative for both viruses, but not in men, regardless of their serology status, or women positive for HSV-1 [92]. Unfortunately, a large randomized, double-blind efficacy field trial including 8,323 women negative for HSV-1 and HSV-2 antibodies was only effective in preventing HSV-1 genital disease without preventing HSV-2-related genital infections [93]. Until today, no glycoprotein subunit vaccine has been effective in preventing HSV-2 genital infections. Currently, an Australian study is recruiting 20 women for a phase 1 clinical trial for a new HSV-2 vaccine. Professor Frazer from the University of Queensland, Australia, is conducting the trial, and the results are highly anticipated.

Conclusions

HSV is an important human pathogen responsible for severe morbidity and mortality. The biology of its ability to infect the organism, evade the immune response, reactivate, and cause recurrent peripheral cutaneous disease, as well as neurological disease fueled intensive research. Intensive ongoing research shed light on pathogenesis and opened up the possibility for new treatment approaches, but our understanding of the mechanisms of latency and reactivation as hallmarks of the herpes viruses is still limited. We encourage further exploration and a possible new direction of drug development focusing on the prevention of latency of neuro-invasion instead of only responding to the reactivation of the virus with limited treatment options.

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Compliance with Ethics Guidelines

Conflict of Interest Israel Steiner serves on the editorial board of the *Journal of Neurological Sciences*, *Journal of Neurovirology*, and *Medicine Neurology* (Hebrew). He is a consultant and member of DSMB for Actelion and Genentech/Roche. He has received honoraria from Teva Pharmaceutical Industries Ltd. He has also received travel/accommodations expenses covered or reimbursed from Beilinson Hospital, Petach Tikva, Israel.

Felix Benninger declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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