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An insight into pathogenesis of herpes simplex virus

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Abstract

Herpes has been known for at least 2000 years and it is one of the most common sexually transmitted diseases (STD) worldwide. Herpes belong to the family Herpesviridae, which includes dsDNA viruses causing diseases in animals and humans. There are more than 130 herpes virus known, the common eight herpes known to infect humans include: HSV-1 and 2 that cause oral herpes and genital herpes, the virus that causes chickenpox, otherwise known as zoster virus, the Epstein-Barr-Virus associated with several types of cancer, the roseola virus, the cytomegalovirus and the herpes virus associated with Kaposi sarcoma in AIDS patients. A notable characteristic of HSV is that once it infects a host, it often remains as a persistent latent infection for the lifetime which reactivates from time to time, especially when the host becomes immuno-compromised. Despite the fact that much evolutionary development has taken place in antiviral agents in past two decades, viral infection is still remains the cause of significant mortality worldwide. This review article focuses on the pathogenesis by HSV and the recent development in herpes antiviral targets.

Key words :

INTRODUCTION

Herpes is an infection caused by herpes simplex virus (HSV). They have a unique four-layered structure: a core containing the large, dsDNA genome enclosed by an icosapentahedral capsid which is composed of capsomers. The capsid is surrounded by an amorphous protein coat called the tegument. Envelope contains 12 glycoproteins, each of which is prefixed 'g', for example gB, gC and gD. The capsid is constructed of 162 capsomeres, 12 of which are pentons and the remainder of which are hexons.HSV-1 and HSV-2 each contain at least 74 genes (ORFs) within its genome [1].

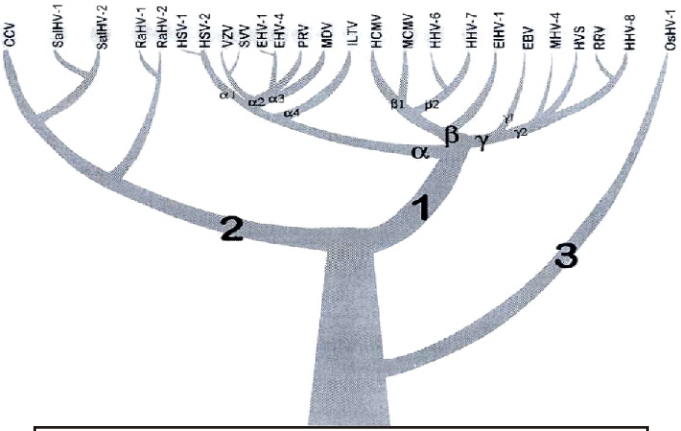
CLASSIFICATIONS AND PATHOGENESIS OF HSV

HSV taxonomy has recently undergone a revision by the ICTV[2]. The family Herpesviridae was raised to the order Herpesvirale and split into three families: a) Herpesviridae, which was divided into three subfamilies Alpha (α), Beta (β) and Gamma(γ) which include mammalian, avian and reptilian viruses. b) Alloherpesvirinae includes fish and amphibian viruses; and c) Malacoherpesvirinae includes one single virus Ostreid herpes virus (OsHV-1).

The eight routinely infecting human herpes are: herpes simplex virus types 1 (HSV1 or HHV1) and 2(HSV2 or HHV2), varicella-zoster virus (VZV or HHV3), Epstein-Barr virus (EBV or HHV4), Cytomegalovirus (HHV5), human herpes virus 6 (variants A and B), human herpesvirus 7 and Kaposi's sarcoma virus (HSV 8) [4]. All herpes viruses establish latent infection with specific tissue characteristic of each virus.

The α-herpes viruses include important human herpes simplex virus HSV1, HSV2 and VZV [5], the causative agents of cold sores, genital ulcerous disease and chickenpox/shingles, respectively. The veterinary α-herpes viruses include bovine herpes virus type I (BHV-1), equine herpes virus type I (EHV-1) and pseudorabies virus (PRV) [6].

In the host, α-HSV infections typically initiate at peripheral sites such as mucosal epithelia. Next, viral particles enter at the termini of sensory neurons of the peripheral nervous system (PNS). These particles are transported long distances along axons in the retrograde direction towards cell bodies, where the genomes are deposited in the nucleus to establish lifelong latency. Following reactivation from latency, new viral particles are



a): Mammals, reptiles, birds b): Fish and aphhibians
c): Mollusk (Oyster)

Fig 1: Herpesvirales evolutionary hypothesis (2000) [3]

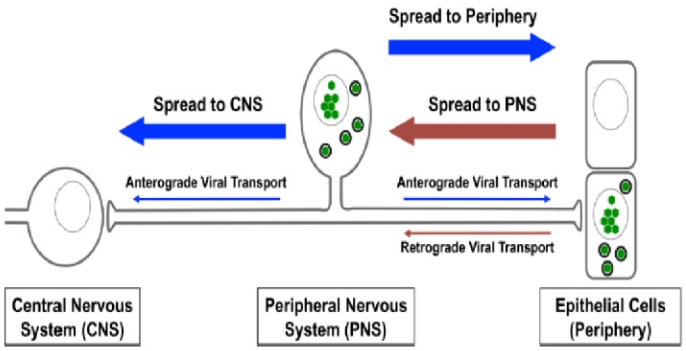


Fig 2: Directional spread of alphaherpes virus infection [7]

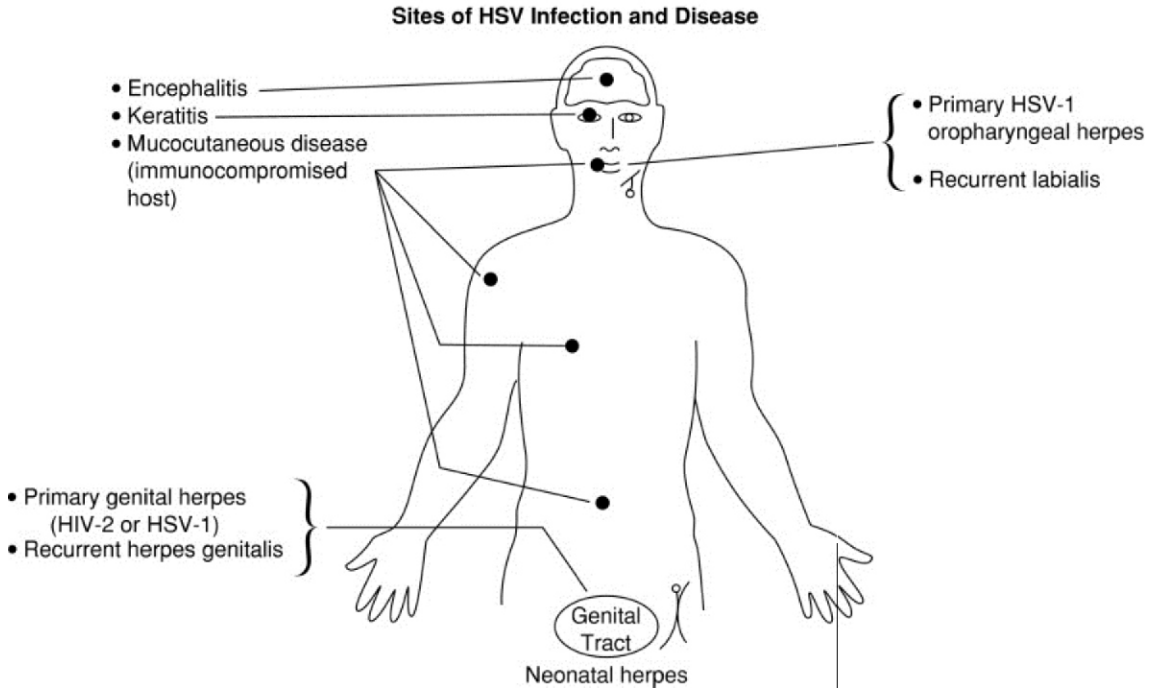


Fig 3: Sites of HSV infection and diseases [13]

assembled and transported towards sites of egress. Typically, infections spreads in the anterograde direction, back out towards the periphery. This is essential for spread between hosts. Infection may also spread *trans*-neuronally,from the PNS to the central nervous system (CNS) [7].

The β- herpes viruses includes cytomegalovirus (HCMV, HSV-5), human herpesviruses 6 (HHV-6A and HHV-6B) and 7 (HHV-7), with a long replicative cycle (days) and restricted host range. HHV-6A is more neurovirulent [8] i.e. it is more frequently found in patients with neuroinflammatory diseases such as multiple sclerosis [8]. HHV-6B and HHV-7 can cause skin conditions in infants known as exanthema subitum or roseolainfantum. The characteristic of these viruses is to form enlarged cells and establish latent infection in leukocytes, secretary glands, cells of the reticuloendothelial system and the kidneys [5].

The γ-herpesvirusesare lymphotopic viruses and includes Epstein-Barr virus (HHV-4) and human herpesvirus 8 (HHV-8, KSHV) and MHV-68, with a very restricted host range. The latent virus has been demonstrated in lymphoid tissue. The EBV, HHV-8 as well as MHV-68 DNA may persist lifelong in an episomal form in host carrier cells (lymphocytes).

Alloherpesviridae includes viruses that infect fish and amphibians[9]. Letaluridherpesvirus 1 (catfish virus), including carp pox herpesvirus, hematopoietic necrosis herpesvirus of goldfish (cyprinid herpesvirus 2) and koi herpesvirus (cyprinid herpesvirus 3) [10].

Malacoherpesviridae causes diseases in molluscs. This family includes two species:Ostreidherpesvirus 1 and Haliotidherpesvirus 1 [11].

1. SITES OF INFECTION

The most common sites of HSV infection include the skin and mucosal surfaces [12]. Mucocutaneous infections are more common with HSV. The recurrent HSV-1 infections of the oropharynx is manifested as herpes simplex labialis (cold sores), and usually appear on the vermillion border of the lip. Genital herpes is caused by herpes simplex virus 2; the primary infection in women usually involves the vulva, vagina, and cervix while in men it is associated with lesions on the glans-penis, prepuce or penile shaft. Herpes simplex keratitis is usually caused by HSV-1 and is accompanied by conjunctivitis. Manifestations of neonatal HSV infection can be divided into three categories: 1) skin, eye and mouth disease (cutaneous lesions); 2) encephalitis (CNS); and 3) disseminated infection that involve multiple organ systems and can produce disseminated intravascular coagulation, hemorrhagic pneumonitis, encephalitis, and cutaneous lesions. Herpes simplex encephalitis is characterized by hemorrhagic necrosis of the inferiomedial portion of the temporal lobe [5].

2. CELLULAR ENTRY AND REPLICATION

Entry of HSV into the host cell involves interaction of several glycoproteins on the surface of the enveloped virus with the receptors on the surface of the host cell.HSV entry occurs via fusion of the viral envelope with either the plasma or endocytic membranes [14] Five glycoproteins are implicated in HSV entry viz., gC, gB, gD and the heterodimer gH/gL[15]. The virus initially attaches to cells through the interaction of gC and gBwith cell surface proteoglycans [16]. The interaction between the receptor-binding protein gD and a specific cell surface receptor is also essential. This triggers the membrane fusion mediated by gB and gH/gL.

Following this, the viral DNA uncoats and transports to the nucleus of the host cell.The transcription, genome replication, and capsid assembly occur in the host cell nucleus. The genes are replicated in a specific order: (1) immediate-early genes (encode regulatory proteins); (2) early genes (enzymes for replicating viral DNA) and (3) late genes(encode structural proteins).

Perkins *et.al* have proposed that ICP27 is an essential

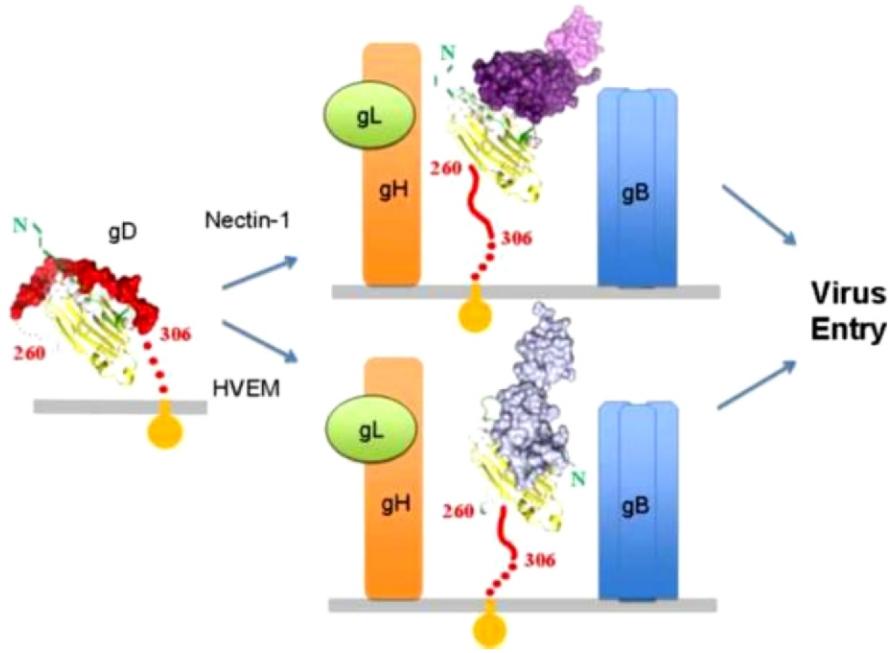


Fig 4: Involvement of the protein in host cell entry [17]

Table 1. Cells that support different types of human HSV infection [20]

Family	Virus	Lytic replication	Site of latency	References
α	HSV-1	Epithelial and Keratinocytes	Neuron	21
	HSV-2	Epithelial and Keratinocytes	Neuron	
	VZV	Epithelial, Keratinocytes, T cell, Sebocyte, monocyte, endothelial, Langerhans and PBMC	Neuron	
β	HCMV	macrophage, dendritic, endothelial, smooth muscle, epithelial and fibroblast	CD34+, HSC, monocyte	22
	HHV-6	T cell	BMP	
	HHV-7	T cell	T cell	
?	EBV	B cell and epithelial	B cell	23-24
	KSHV	Lyphocyte	B cell	

immediate early protein in HSV-1 that stimulates viral mRNA expression. The late gene, glycoprotein C (gC) expression is highly dependent on ICP27 [18]. Assembly of the viral core and capsid takes place within the nucleus. This is followed by envelopment at the nuclear membrane and transport out of the nucleus through the ER and the Golgi apparatus, wherein glycosylation of the viral membrane occurs. Mature virions are transported to the outer membrane of the host cell. Release of progeny virus is accompanied by cell death (lytic cycle).

Alternatively, in selected cell types, the virus may be maintained in a latent state [5].Latent virus may be reactivated and enter a replicative cycle at any point of time by receiving the appropriate stimulus, This is known as latency. The resumption and completion of productive, lytic replication after a period of latency is called an activation event [19].

IE genes play an important role in thelytic cycle and these genes might be suppressed in latency. HSV-1 IE gene expression is activated by a complex, consisting of the viral tegument protein VP16, which is delivered to the cell upon entry, and two cellular proteins, host cell factor 1 (HCF) [25] and the POU homeodomain protein Oct-1[26]. Tegument-deliveredVP16 encounters HCF in the cytoplasm, and this association is absolutely essential for VP16 translocation to the nucleus. Tegument-delivered VP16 remains in the cytoplasm of infected cells if binding to HCF is disrupted by mutation, or if the nuclear localization sequence (NLS) of HCF is deleted [27]. Under these circumstances, viral IE gene expression is inhibited. Once in the nucleus, the VP16/HCF pair interacts with Oct-1associated with TAATGARAT motifs (where R is a purine) found in HSV-1 IE promoters [28]. Now tethered to viral genomes, the VP16/HCF/Oct-1 complex activates viral gene expression by recruiting cellular RNA Polymerase II and by modulating both histone occupancy and chromatin structure of the viral genome [29].VP16 contains a prototypical acidic activation domain [30]that interacts with RNA Polymerase II as well as several cellular components of the basal transcriptional machinery, including transcription factor IIB (TFIIB)and IIH (TFIIH), TATA-binding protein (TBP) and other transcription associated factors (TAFs) [16,31].The viral latency associated transcript (LAT) encodes microRNAs (miRNA) that

suppress the expression of viral IE gene ICPO. ICP0 can activate the expression of other viral genes and thus promote the lytic replication cycle [16,32]

The human herpesviruses share four significant biological properties. First, they code for unique enzymes (thymidine kinase and viral DNA polymerase)[33] involved in the biosynthesis of viral nucleic acids. These enzymes are structurally diverse and provide unique sites for inhibition by antiviral agents eg; Acyclovir, Valacylovir and Famciclovir[33]. Second, the synthesis and assembly of viral DNA is initiated in the nucleus. Assembly of the capsid is also initiated in the nucleus. Third, release of progeny virus from the infected cell is accompanied by cell death. Finally, all herpes viruses establish latent infection within tissues that are characteristic for each virus, reflecting the unique tissue trophism of each member of this family [5].

3. MECHANISMS OF COMPLICATIONS CAUSED BY HSV

When HSV get active, they cause cold sores, blindness, encephalitis or cancer. The complications of HSV-2 includes painful genital sores, psychological distress and if transmitted from mother to foetus resulting in fatal infections in newborns.

Cold sores: For reactivation, HSV makes more messenger RNA than microRNA (LAT RNA that blocks viral replication proteins) that expresses the replication proteins. This new supply of viruses then travels back down the trigeminal nerve, to the site of the initial infection at the mouth. A cold sore always erupts in the same place and is the source of viruses that might infect another person either from direct or indirect contact [34].

Blindness : HSV-1 infection may be more serious than HSV-2, because it can travel from the mouth and affect the eye, in a condition known as "eye herpes" and "ocular herpes simplex". It affects cornea, the infection is known as keratitis. Other parts of the eye are sometimes affected. A minor and temporary inflammation of the conjunctiva (conjunctivitis) or eyelids (blepharitis) may occur with active infection, often at the same time as the cornea is infected. Deeper structures such as the retina or iris may also sometimes be affected [35].

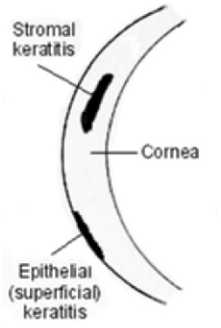


Fig 5: Infection by HSV in the eye

Encephalitis: It is the inflammation of brain.It can develop shortly after an initial HSV infection, or it can develop when a virus reactivates. There are two ways that viruses can infect brain cells and cause encephalitis:

- ***Primaryencephalitis*** occurs when the virus directly affects the brain or spinal cord. The resulting inflammation can occur in one area (focal) or can occur throughout the brain (diffuse).
- ***Secondaryencephalitis***, also called post-infectious encephalitis, occurs when the virus first attacks another part of the body and the infection then spreads to the brain [36].

Cancer: HSV inserts its DNA into the host genome and results into uncontrolled cell growth. By inserting itself into the cells' DNA, the virus "hides" from the body's immune system, so the body's defenses can't eliminate it [37].

Genital herpes:HSV-2 infections occur in the genital region. However, because of increasing oral-genital contact, either HSV type may be found in either location. The vaginal mucosa is commonly inflamed and edematous. The cervix is involved in 70-90% of patients [38].

Mother to foetus transmission: HSV can be vertically transmitted to the infant before, during or after delivery. Maternal age of less than 21 years is a risk factor for vertical transmission. HSV has the potential for hematogenous spread to the placenta and to the fetus (antenatal transmission), the infection to the foetus through the infected birth canal (intra partum transmission) and maternal viral shedding during delivery (post natal transmission) [39]

6. HSV ANTIVIRAL TARGETS

Rosenthal (2010) reported the anti-herpes nanoviricides dose-dependent maximal inhibition of herpes virus infectivity in a culture (H129 strain of HSV-1) model. The anti-HSV nanoviricide is drug for the treatment of cold sores or genital herpes simplex virus infection. It mimics a natural host cell receptor by which the virus binds and infects cells [40]. Raltegravir, a drug approved in 2007 for the treatment of AIDS inhibits the function of an essential protein for the replication of one kind of herpes virus. Terminase, an enzyme required during replicative cycle is formed by three protein subunits. The terminase cuts the new DNA into small fragments (single viral genome) and introduces into the capsid. These new viruses leave the cell to continue infection. This terminase resembles the integrase of AIDS virus.Raltegravir acts on the subunit UL89of the terminase and cancels its scissor function, and thereby stops viral replication [40]

Researchers at Tufts University and University of Pennsylvania (2010), studiedstructure and function of the cell-entry protein fusion events carried out by HSV-2by x-ray crystallography and cell microscopy technique. The herpes virus requires fusogen along with a complex of two other proteins (gB and heterodimer gH-gL),in order to invade the cell i.e. the protein complex is not a fusogen itself but, it regulates the fusogen. The scientists proposed that, gH-gL activates gB fusion. The formation of gB-gH-gL complex is critical for fusion and is inhibited by a neutralizing antibody [41].

Tansy, *Tanacetumvulgare* has antiviral properties, especially for the treatment of herpes (2011). 3,5-dicaffeoylquinic acid (3, 5-DCQA) and axillarin are the important antiviral agents present in Tansy . A research group at the Lund and Carnegie Mellon University measured the internal pressure, that enables the virus to infect cells by ejecting their genes at high force and speed. These results suggested new treatment avenue, that could reduce the pressure within the virus shell. This medication would work on mutated viruses as well since mutation does not change the internal pressure [42]. The immunization with GEN-003 reduces the viral shedding associated with activation of T cell immunity.

GEN-003 is a T cell vaccine designed to induce B cell (antibody) and T cell immune response and includes the fragment of ICP4 and gD2 antigens, as wellas adjuvant Matrix-M. This adjuvant is a novel saponin-derived product [43].

7. RECENT DEVELOPMENTS IN HSV VACCINES

The HSV replication cycle is central to understanding immunity to HSV and vaccine design. Interactions between HSVencoded glycoproteins and cellular glycosoaminoglycans, such as heparansulfate, are involved in virion binding to the cell surface. The HSV envelope glycoproteins gB, gD, and gH-gL are required for HSV binding and entry into cells. The neutralizing activity of antibodies directed against these proteins was one of the major rationales for the use of HSV glycoproteins as immunogens for HSV subunit vaccines. Peptide Vaccines

It was proposed that immunization with a singleimmuno-dominant CD8 CTL epitope [44] or neutralizing epitope[45] can be protective. An immunization with HLA A*0201-restricted epitope in gB2 and a heat shock protein adjuvant been designed, to determine if the peptide "concept" can elicit high levels ofCD8-T cells to an HSV antigen.

Subunit Vaccines

Two similar subunit vaccines have recently completed phase III clinical trials. The Chiron vaccine contains gB2, truncated at amino acid 696 and gD2, truncated at amino acid 302, expressed in transfected CHO cells. The truncations of cytoplasmic tail and transmembrane domains were made to help the proteins exit the producing cells, allowing the manufacturing process to yield large quantities of protein. Phase I studies with gD2 and alum adjuvant established that this vaccine could both induce ELISA and neutralizing antibodies in seronegative subjects and boost pre-existing responses in HSV-1- and HSV-2-infected subjects [46]

Killed-Virus Vaccines

This type of vaccine is used to prevent genital herpes (Skinner *et al.),* where cells infected with a mixture of clinical HSV-2 isolates were sonicated and clarified. The resulting supernatant, which was likely to contain virions and viral protein, was treated with formaldehyde to make the vaccine.[46]

Fractionated-Virus Vaccines

Several groups have prepared HSV vaccines by subjecting infected cells to procedures for inactivatingthe virus and to partially purify subsets of viral proteins. HSV-2-infected cells were treated with detergent and DNase to inactivate virus and glycoproteins were enriched by binding to a plant lectin. The vaccine contained gD2, gB2, and other HSV-2 glycoproteins. The bound fraction was further inactivated with formalin and compounded with alum [44,45].

CONCLUSION

HSV are divided into three families, Herpesviridae, Alloherpesviridae, Malacoherpesviridae, and are responsible for various diseases. All herpes viruses establish latent infection, the most common sites of infection being skin and mucosal surfaces. When HSV get active, they can cause cold sores, blindness, encephalitis or cancer. Once herpes infection starts into the host cell, viral DNA is uncoated and transported to the nucleus of the host cell. The transcription of immediate-early genes encodes the regulatory proteins. Expression of immediate-early gene products is followed by the expression of proteins encoded by early and then late genes. The transmission of HSV infection is dependent upon intimate and personal contact of a susceptible seronegative individual with someone excreting HSV. There are various diagnostic tests available to diagnose herpes infection like Antigen detection test, PCR etc. Also various new vaccines are available to fight against HSV infection beforehand.

This review article sheds light on classification, modes of transmission, mechanism of infection, available diagnostics and current vaccines available diagnostics and current vaccines available for HSV.

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