STATE-OF-THE-ART CLINICAL ARTICLE

Herpes Simplex Viruses

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Herpes simplex virus (HSV) infections of humans were first documented in ancient Greece. Greek scholars, particularly Hippocrates, used the word ''herpes,'' meaning to creep or crawl, to describe spreading lesions. The classification now in use came into being in the late eighteenth century, and although the vesicular nature of lesions associated with herpetic infections was previously well characterized, it was not until 1893 that Vidal specifically recognized person-to-person transmission of HSV infections [1].

Following primary infection, neutralizing antibodies to HSV develop in the serum. Subsequently, some seropositive individuals develop clinically mild recurrent labial or genital lesions, typifying the unique biological property of HSV, namely an ability to recur in the presence of humoral immunity or reactivation of latent infection. The spectrum of disease caused by HSV includes primary and recurrent infections of mucous membranes (e.g., gingivostomatitis, herpes labialis, and genital HSV infections), keratoconjunctivitis, neonatal HSV infection, visceral HSV infections in immunocompromised hosts, HSV encephalitis, Kaposi's varicella-like eruption, and an association with erythema multiforme. An important advance in our knowledge of HSV infections has been the ability to distinguish between HSV-1 and HSV-2. HSV-1 is more frequently associated with nongenital infection, while HSV-2 is associated with genital disease.

HSV Structure and Replication

HSV is a member of a family of viruses whose genomes consist of a single large double-stranded DNA molecule [1a]. The herpes simplex virion consists of four components: (1) an electron-dense core containing viral DNA; (2) an icosadeltahedral capsid; (3) an amorphous, at times eccentric layer of proteins, designated tegument, which surrounds the capsid; and (4) an envelope. The capsid consists of 162 capsomeres and is surrounded by the tightly adhering tegument. The envelope surrounds the capsid-tegument structure and consists of at least 10 glycosylated and several nonglycosylated viral proteins, lipids, and polyamines. Viral DNA contains at least 152 kbp. The variability in size is due chiefly to the variation in the number of reiterations of specific terminal and internal sequences. HSV-1 and HSV-2 DNAs consist of two covalently linked components, designated as L (long) and S (short). Each component is composed of unique sequences (U_L or U_S, respectively) flanked by relatively large inverted repeats. The inverted repeat sequences flanking U_L are *ab* and $b'a'$, whereas those flanking U_S are $a'c'$ and *ca*. The two components can invert relative to one another to yield four populations of DNA molecules differing solely in the relative orientation of these DNA sequences [2].

Current studies indicate that HSV-1 and, by extension, HSV-2 encode at least 84 different polypeptides (figure 1) [3]. Of this number, 14 map in the U_S, four map in each of the sequences flanking U_L, one maps in each of the sequences flanking U_S, and the remainder map in U_L. The genes mapping in the repeats are present in two copies per genome.

To initiate infection, HSV must attach to cell-surface receptors, fuse its envelope to the plasma membrane, and allow the deenveloped capsid to be transported to the nuclear pores. The DNA is released into the nucleus at the core. The key events in viral replication that occur in the nucleus include transcription, DNA synthesis, capsid assembly, DNA packaging, and envelopment (figure 2). Viral surface glycoproteins mediate attachment and penetration of the virus into cells. They also elicit host immune responses to the virus. Currently, at least 10 viral glycoproteins (designated gB, gC, gD, gE, gG, gH, gI, gK, gL, and gM) are known, and an eleventh (gJ) is predicted. gC and gB are required for attachment to the cell surface, whereas gD is required for entry of the virus into cells. gE and gI form a rather potent Fc receptor; gE is also required for basolateral transmission of virus in polarized cells and for efficient expression of late genes.

The synthesis of viral gene products—both RNA and proteins—takes place in three sequential periods. The first set of

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Ab	U_L	B'a'c' U_S ca
Genome domains	Open reading frames	No.
L component		
Ab, b'a'	α0, $\lambda_1$34.5, ORF-P, O	8
U_L	U_L1-56, 8.5, 9.5, 10.5 12.5, 20.5, 26.5, 27.5 43.5, 49.5	65
S component		
A'c', ca	α4	2
U_S	U_S1-12, 1.5, 8.5	14
	Total	89
	Total single copy	84

Figure 1. Gene distribution in the herpes simplex virus genome.

viral gene products consists of six proteins known as α or immediate early proteins. Of these, five regulate the reproductive cycle of the virus, and one blocks the presentation of antigenic peptides on the infected cell surfaces. α Proteins are essential for the synthesis of the second set of viral gene products, known as β or early proteins. Most of the β proteins are responsible for viral nucleic acid metabolism and are the main target of antiviral chemotherapy (i.e., viral thymidine kinase and viral DNA polymerase). The third class of proteins is for the most part the structural components of the virion. They assemble to form the capsid and tegument, and they incorporate into nuclear membranes for eventual envelopment of virions.

In the process of viral assembly, a procapsid is formed from scaffolding and capsid proteins. The scaffolding proteins consist of a protease and its substrate, with the protease cleaving the substrate during capsid assembly. The newly synthesized DNA is cleaved into unit-length molecules and packaged into the virions. Generally, envelopment favors capsids containing DNA. Capsids containing DNA attach to the nuclear surface of the inner nuclear membrane and are rapidly enveloped and released into the space between the inner and outer nuclear membranes. Here, the virions become encased in transport vesicles and are transported through vesicles formed by fragmented and dispersed Golgi stacks to the extracellular space. This process takes ~18 hours.

Several aspects of the viral replication cycle and of the viral proteins are relevant to the pathogenesis of human diseases and to present and future developments in antiviral chemotherapy.

1. At least five viral proteins play a key role in insuring a robust expression of viral genes and the mobilization of cellular proteins necessary for efficient synthesis of viral DNA and proteins. The first of these, αTIF or VP16, is a protein packaged in the tegument that augments the basal level of expression of α genes. Like many other viral proteins, αTIF is both a transactivator and an essential structural protein of the virion.

   The second, an α protein known as the infected cell protein 4 (ICP4), binds directly to both high- and low-affinity sites on viral DNA. ICP4 acts both as a transactivator at low affinity sites and as a potent repressor at high affinity sites located at the transcription initiation sites of its own gene and those of a few other genes. Another α protein, ICP0, acts as a promiscuous transactivator of genes introduced by infection or transfection. ICP0 does not bind DNA. Recent studies indicate that it binds an array of cellular proteins. The functions of the interactive proteins suggest that ICP0 has at least two roles: to stabilize important cell cycle regulatory proteins (e.g., cyclin D3), forcing the cell to the brink of the S phase (but not allowing cell division), and to maintain a vigorous protein synthesis for its own use.

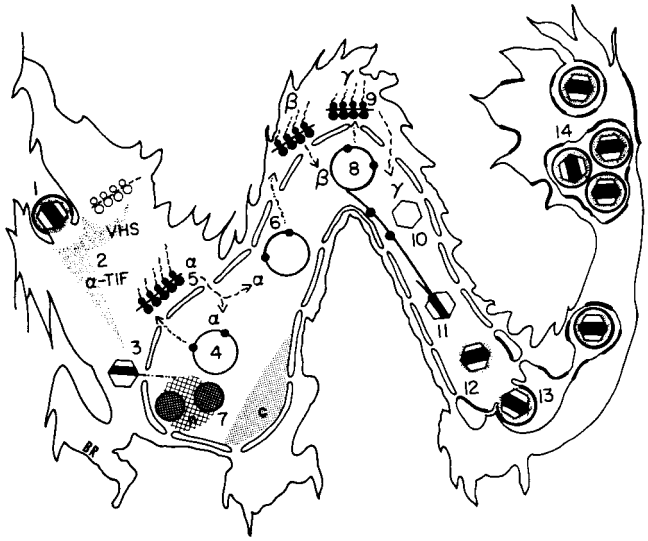

Figure 2. Schematic representation of the replication of herpes simplex viruses in susceptible cells. 1: The virus initiates infection by the fusion of the viral envelope with the plasma membrane following attachment to the cell surface. 2: Fusion of the membranes releases two proteins from the virion. VHS (virion host shutoff) shuts off protein synthesis (broken RNA in open polyribosomes). α-TIF (the α gene transinducing factor) is transported to the nucleus. 3: The capsid is transported to the nuclear pore, where viral DNA is released into the nucleus and immediately circularizes. 4: The transcription of a gene by cellular enzymes is induced by α-TIF. 5: The five amRNAs are transported into the cytoplasm and translated (filled polyribosome); the proteins are transported into the nucleus. 6: A new round of transcription results in the synthesis of β proteins. 7: At this stage in the infection, the chromatin (c) is degraded and displaced toward the nuclear membrane, whereas the nucleoli (round hatched structures) become disaggregated. 8: Viral DNA is replicated by a rolling circle mechanism, which yields head-to-tail concatemers of unit length viral DNA. 9: A new round of transcription/translation yields the g proteins, consisting primarily of structural proteins of the virus. 10: The capsid proteins form empty capsids. 11: Unit length viral DNA is cleaved from concatemers and packaged into the preformed capsids. 12: Capsids containing viral DNA acquire a new protein. 13: Viral glycoproteins and tegument proteins accumulate and form patches in cellular membranes. The capsids containing DNA and the additional protein attach to the underside of the membrane patches containing viral proteins and are enveloped. 14: The enveloped proteins accumulate in the endoplasmic reticulum and are transported into the extracellular space. Reprinted with permission from the *New England Journal of Medicine* [17].

ICP27, another α protein, also has several functions, the most remarkable of which is control of posttranslational processing of RNA. Unlike cellular genes, the majority of viral genes lack introns. ICp27 blocks the splicing of mRNA. While it reduces the amounts of spliced viral mRNAs, the net effect is on host mRNAs and results in diminished cellular protein synthesis. In addition, ICP27 acts as a transporter, or late RNA, from the nucleus to the cytoplasm and, as a consequence, regulates the expression of late proteins. ICP22 is another multifunctional α protein. Few of this protein's functions have been elucidated, but it appears to regulate the longevity of ICP0 mRNA. It is also involved in the use of specific splice acceptor sites of ICP0 mRNA and in the expression of a subset of late genes.

2. The tegument proteins function to enhance the capacity of HSV to replicate efficiently. In addition to the αTIF described above, two viral proteins play an important role in viral infection. One of these proteins, *vhs*, the product of the gene U_L41, induces a RNA activity, which degrades all mRNA. Because viral transcription occurs at a very high rate, viral protein synthesis is less affected than cellular protein synthesis. The tegument also includes a protein kinase encoded by U_L13. Although dispensable in cell culture, this kinase is essential for viral replication in experimental animal systems, and its substrate is both viral and cellular proteins.
3. Viral proteins interact with cellular proteins, either to stabilize them and redirect their function or to block their function. For example, as noted above, ICP0 binds to and prolongs the half-life of cyclin D3. The two other types of interactions of viral and cellular proteins are involved in blocking host cell response to infection. Small peptides derived from degradation of cellular DNA are normally transported through the endoplasmic reticulum to the cell surface. In the first of the two interactions that interfere with function, HSV blocks the presentation of antigenic peptides by encoding an α protein, ICP47, which binds to TAP1/TAP2, the protein that binds to and translocate peptides into the endoplasmic reticulum.

A second antiviral host defense mechanism is based on the activation of dsRNA-dependent protein kinase (PKR). Activated PKR phosphorylates the α subunit of translation initiation factor (eIF-2α) and shifts off protein synthesis. Nearly all viruses that make double-stranded RNA, either as part of their replicative cycle or because both strands of the nucleic acid are transcribed, activate PKR. Furthermore, all of these viruses have functions that have evolved to block the shutoff of protein synthesis. A totally different technique has evolved in HSV: the protein encoded by the viral $\gamma_1$34.5 gene binds protein phosphatase 1 and redirects its activity from common substrate to dephosphorylate eIF-2α. The rate of dephosphorylation of eIF-2α in cells infected with wild-type virus increases 3,000-fold over that in uninfected cells. Expression of several viral functions, including the stabilization and prolonged function of cyclin D3, is likely to induce apoptosis. HSV has evolved at least one gene, the protein kinase expressed by the U_S3 gene, to block apoptosis induced by the virus as well as that induced by other agents.

4. Viral replication is highly deleterious to the integrity of the infected cell. Cellular chromosomes are marginated to nuclear membrane and degraded, the nucleolus falls apart, the Golgi complex is fragmented and dispersed, and the microtubules are rearranged. All of these events are designed to create the environment for viral DNA synthesis, to enhance the glycosylation and exocytosis of virions, and to prevent a host response to infection.
5. Of the 84 genes known to be encoded by HSV-1 and HSV-2, at least 45 genes are dispensable for viral growth in cells. The 45 genes include those whose products enable the virus to attach and enter both apical and basolateral aspects of polarized cells, to fine-tune expression of individual genes, to provide efficient cell-to-cell spread, to sort proteins and viral exocytosis, to increase the precursor pool and repair viral DNA, and to block cellular and immune response to viral infection. It should be stressed that these genes are not truly dispensable: viruses lacking these genes have not been isolated from humans, and viruses from which these genes have been deleted by genetic engineering frequently exhibit reduced capacity to multiply and spread in experimental animals. On the other hand, the observation that so many HSV genes are dispensable and can be replaced by non-HSV genes sustains the expectation that a live attenuated vaccine, capable of acting as a vector for foreign gene expression, is feasible.

Neurovirulence

HSV has two unique biological properties that influence human disease. These properties are the capacity to invade and replicate in the CNS and the capacity to establish a latent infection. Neurovirulence encompasses both neuroinvasiveness from peripheral sites and replication in neuronal cells, and this property appears to be the function of numerous genes; in fact, deletion of virtually any of the genes dispensable for viral replication in cell cultures reduces the capacity of the virus to invade and replicate in the CNS. Mutations affecting neuroinvasiveness have also been mapped in genes encoding glycoproteins. These findings are not unexpected: access to neuronal cells from usual portals of entry into the body requires postsynaptic transmission of virus and, therefore, a particularly vigorous capacity to multiply and to sort the virions to appropriate membranes. In addition, since neuronal cells do not make cellular DNA, they lack the precursors for viral DNA synthesis that are also encoded by the viral genes dispensable for replication in cell cultures. A gene cited earlier in the test, $\gamma_1$34.5, is of particular interest in that mutants deleted in this gene are among the most avirulent known, even though they multiply well in a variety of cells in culture [4]. The molecular basis for the failure of $\gamma_1$34.5-mutants to multiply in the CNS is

unclear and is probably different from the function of the protein in directing the dephosphorylation of eIF-2α.

Latent Infections

The latency of HSV has been recognized biologically since the beginning of the century. Following entry and infection of nerve endings, both HSV-1 and HSV-2 are transported by retrograde movement to the nuclei of sensory ganglia [5]. The available evidence indicates that the virus multiplies in a small number of sensory neurons. In the majority of the infected neurons, the viral genome remains in an episomal state for the entire life of the individual. Reactivations occur following a variety of local or systemic stimuli such as physical or emotional stress, fever, exposure to ultraviolet light, tissue damage, and immunosuppression.

Patients treated for trigeminal neuralgia by the sectioning of a branch of the trigeminal nerve have developed herpetic lesions along the innervated areas of the sectioned branch. Reactivation of latent virus appears dependent on an intact anterior nerve route and peripheral nerve pathways. Recurrences occur in the presence of both cell-mediated and humoral immunity. Recurrent herpes labialis is three times more frequent in febrile patients than in nonfebrile controls. Latent virus can be retrieved either unilaterally or bilaterally from the trigeminal, sacral, and vagal ganglia of humans [6]. The recovery of virus by in vitro cultivation of trigeminal ganglia helps explain the observation that vesicles recur at the same site in humans, usually the vermilion border of the lip.

Little is known regarding the mechanisms by which the virus establishes and maintains a latent state or is reactivated. In fact, disagreements exist on the fate of neurons in which latent virus has become reactivated [7]. The relevant issues may be summarized as follows:

1. Sensory neurons harboring virus contain nuclear transcripts that arise from the sequences *ab* and *b′a′* flanking U_L. To date, two sets of transcripts, designated Latency Associated transcripts (LAT), have been detected. The most abundant transcripts arise from 2.0 domain mapping 3′ to the gene encoding ICP0. This domain encodes two RNAs, a 2.0-kb and a 1.5-kb RNA. Both RNAs are nuclear, and both have characteristics of stable introns. They do not appear to encode proteins. The other RNA detected to date is a 8.5-kb species of much lower abundance. This species appears to include the 2.0-kb sequence contained in the stable intron. The issue then is if the 2.0-kb RNA and the 1.5-kb RNA are introns, where is the product of the splicing event? Furthermore, deletion of the domain of the 2.0-kb RNA or of its promoter decreases the number of neurons harboring latent virus and the frequency of reactivation, but it does not abolish either process.

   Recent studies on the 8.5-kbp DNA domain transcribed during latency led to the discovery of two open reading frames (ORFs) totally repressed by ICP4 during productive infection. These ORFs, designated ORF P and ORF O, are antisense to the $\gamma_1$34.5 gene. ORF P protein has been shown to bind p32, a protein involved in splicing. In cells in which ORF P is derepressed, ICP0 and ICP22, two α proteins made from spliced mRNAs, are grossly underproduced early in infection. Late in infection, the two proteins accumulate concomitant with extensive posttranslational processing of ORF P protein. The ORF O protein binds to ICP4 and blocks it from binding its cognate site on DNA in vitro.

   The mechanism whereby HSV establishes latency remains obscure, but probably will not be for long. Likely, establishment of latency is based on several events occurring simultaneously. These could well be expression of viral genes that block α proteins from being made, absence of factors that enable high level expression of α genes, and cell factors that do not favor or actually participate in the repression of viral gene expression.
2. As noted above, replication of HSV-1 and HSV-2 results in the destruction of the infected cell. It has been suggested that reactivation of latent virus in sensory neurons does not destroy neurons harboring the virus. This suggestion is based on the observation that patients do not have anesthesia at the site of frequent, multiple recurrences. Alternatively, nerve endings from adjacent tissues innervated by other neurons could extend into the site of the healed lesion. Finally, it has also been suggested that small amounts of virus could remain latent in peripheral tissues. The answer to this puzzle has not been forthcoming.

Epidemiology

Although HSV-1 and HSV-2 are usually transmitted by different routes and involve different areas of the body, there is an overlap in the epidemiology and clinical manifestations. These viruses are distributed worldwide, and infection occurs in both developed and developing countries. Animal vectors of human HSV infections have not been described, and humans remain the sole reservoir for transmission to other humans. Virus is transmitted from infected to susceptible individuals during close personal contact. There is no seasonal variation in the incidence of infection. Because HSV infection is rarely fatal, and HSV establishes latency, more than one-third of the world's population has recurrent HSV infections and, therefore, the capability of transmitting HSV during episodes of productive infection.

Geographic location, socioeconomic status, and age influence the frequency of HSV-1 infection. In developing countries, seroconversion occurs early in life. In lower socioeconomic populations, approximately one-third of children have serological evidence of HSV infection by 5 years of age; this frequency increases to 70%–80% by early adolescence. Predictably, middle-class individuals acquire antibodies later in life, such that seroconversion over the first 5 years occurs in

20% of children, followed by no significant increase until the second and third decades of life, at which time the prevalence of antibodies increases to 40%–60%. The annualized rate of infection among university students averages 5%–10%.

The seroprevalence of HSV-1 and HSV-2 infections has been redefined by using type-specific serological assays and sera obtained from the randomized National Health and Nutrition Examination Survey. By the age of 5 years, >35% of African American children are infected by HSV-1, as compared with 18% of Caucasian children. Through adolescence, the prevalence of antibodies to HSV-1 is approximately twofold higher among African Americans than among Caucasians, and the prevalence of antibodies is slightly higher among females than among males. By the age of 40 years, the prevalence of antibodies to HSV-1 is similar among both African Americans and Caucasians. Similarly, a high prevalence of antibodies to HSV-1 exists worldwide, but there is a high rate of country-to-country variation.

Infections with HSV-2 are usually acquired through sexual contact and, therefore, antibodies to this virus are rarely found before the age of onset of sexual activity. Although most genital HSV infections are caused by HSV-2, an ever-increasing proportion is attributable to HSV-1. The distinction in virus type is not insignificant, since genital HSV-1 infections are usually both less severe clinically and less prone to recur. The number of new cases of genital HSV infections has been conservatively estimated to be ~500,000 annually, and approximately 40 million to 60 million Americans are infected latently with HSV-2 [8, 9].

The seroprevalence of HSV-2 increases from ~10% at 15–29 years of age to 35% by the age of 60 [10]. When the populations are analyzed according to race, the seroprevalence among African Americans is three- to fourfold higher than among Caucasians. Factors found to influence acquisition of HSV-2 include gender (more women than men), race (more African Americans than Caucasians), marital status (more divorced persons than single or married persons), and place of residence (more city residents than suburb residents). The highest prevalence of antibodies to HSV-2 in the United States is among female prostitutes (75%) and male homosexuals (83%) [10].

The number of sexual partners correlates directly with acquisition of HSV-2. For heterosexual women living with one partner in the United States, the probability of acquisition of HSV-2 is <10%. The probability increases to 40%, 62%, and >80% as the number of lifetime sexual partners increases to 2–10, 11–50, or >50, respectively. For heterosexual men, similar data are zero probability for one lifetime sexual partner, and 20%, 35%, and 70% for each of the subsequent three risk groups. In contrast, for homosexual men, seroprevalence increases from >60% to 90% for those with 11–50 partners and >50 partners. Thus, multiple sexual partners, irrespective of sexual preference, correlates directly with acquisition of HSV-2 infection.

Women have higher rates of infection than men: the estimated risk of a susceptible female contracting HSV from infected males is 80% following a single contact. Among college students, the rate of acquisition is ~2%, compared with an annual rate of 4% for homosexual men. The incidence of HSV-2 infection during pregnancy is ~2.5% per gestation. Transmission of HSV-2 infection between monogamous sexual partners with discordant infection statuses is 10%–15% yearly. It is important to note that because HSV-2 infection is an ulcerative disease, it is associated with acquisition of HIV, as indicated by increased relative risks of 1.5–2.0 [11, 12].

HSV-2, like HSV-1 infection of the mouth, can be excreted asymptomatically at the time of primary, initial, or recurrent infection. The frequency of clinical recurrences varies somewhat between males and females, with calculations of 2.7 and 1.9 recurrences per 100 days, respectively. Overall, several studies have implied that the frequency of clinical recurrences is as high as 60% [13]. Among women evaluated prospectively after the first episode of genital herpes, asymptomatic shedding is detected in ~12%, 18%, and 23% of women with primary HSV-1, primary HSV-2, and nonprimary HSV-2 infection, respectively. For women with established genital HSV-2 infection, asymptomatic shedding is detected on 1%–5% of days when cultures are performed. When PCR is used to evaluate serial genital swabs from women with genital infection, a significant increase in the frequency of HSV DNA shedding is observed, suggesting chronic infection rather than intermittent infection.

Genital HSV infection in pregnant women must be considered separately from that in nonpregnant populations because of the risk to the fetus or newborn. Transmission of infection to the fetus is related to shedding of virus at the time of delivery. The prevalence of excretion at delivery varies from ~0.5% to 1.0% for all women, irrespective of history.

Pathology and Pathogenesis

Operative definitions of the nature of HSV infection are of pathogenic relevance. Susceptible individuals (namely, those without preexisting antibodies to HSV) develop *primary infection* after their first exposure to either HSV-1 or HSV-2. A recurrence of HSV is known as *recurrent infection. Initial infection* occurs when an individual with preexisting antibodies to one type of HSV experiences a first infection with the opposite virus type. Reinfection with a different strain of HSV can occur, albeit extremely uncommonly in healthy hosts, and is called *exogenous reinfection.*

The histopathologic characteristics of a primary or recurrent HSV infection reflect virus-mediated cellular death and associated inflammatory response. Viral infection induces ballooning of cells, with condensed chromatin within the nuclei of the cells, followed by degeneration of the cellular nuclei, generally within the parabasal and intermediate cells of the epithelium. Infected cells lose intact plasma membranes and form multinucleated giant cells. When cell lysis occurs, a clear (vesicular) fluid containing large quantities of virus appears between the

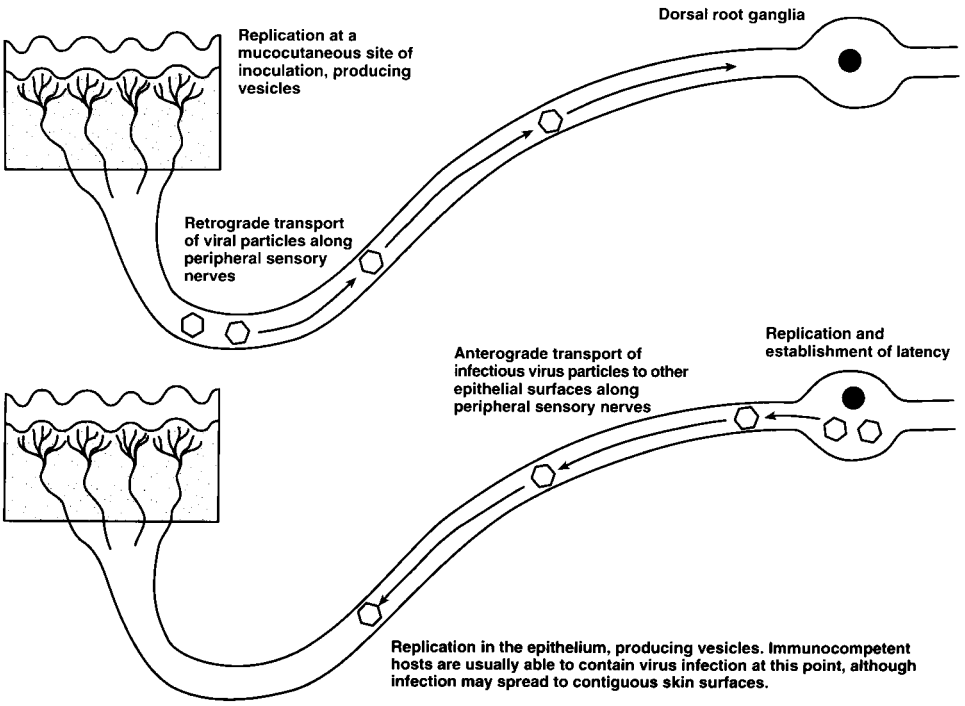

Figure 3. Schematic diagram of primary herpes simplex virus infection.

epidermal and dermal layers. The vesicular fluid contains cellular debris, inflammatory cells, and, often, multinucleated giant cells. In dermal substructures there is an intense inflammatory response, usually in the corium of the skin and more intense with primary infection than with recurrent infection. When healing occurs, the vesicular fluid becomes pustular, with the recruitment of inflammatory cells and subsequent formation of scabs. Scarring is uncommon. When mucous membranes are involved, the vesicles are replaced by shallow ulcers.

The pathogenesis of human HSV disease depends on intimate, personal contact between a susceptible individual (namely, one who is seronegative) and an individual who is excreting HSV. Virus must come in contact with mucosal surfaces or abraded skin for infection to be initiated. With viral replication at the site of infection, either an intact virion or, more simply, the capsid is transported retrograde by neurons to the dorsal root ganglia where, after another round of viral replication, latency is established. Figure 3 depicts the events of primary infection. Although replication sometimes leads to disease and infrequently results in life-threatening infection (e.g., encephalitis), the predominant host-virus interaction leads to the establishment of latency. After latency is established, a stimulus that can produce viral reactivation can cause the virus to again appear as skin vesicles or mucosal ulcers. A model of reactivation appears in figure 4. Infection with HSV-1 generally occurs in the oropharyngeal mucosa. The trigeminal ganglion becomes colonized and harbors latent virus. Acquisition of HSV-2 results in infection at genital, perigenital, or anal skin sites, with seeding of the sacral ganglia.

The natural history of HSV infections is influenced by both specific and nonspecific host defense mechanisms. With the appearance of nonspecific inflammatory changes that parallel a peak in viral replication, specific host responses can be quantitated but vary between animal systems. In the mouse, delayed-type hypersensitivity responses are identified within 4–6 days after the onset of disease and are followed by a cytotoxic T lymphocyte response and by the appearance of both IgM- and IgG-specific antibodies. Host responses in humans are delayed, developing approximately 7–10 days later. Immunodepletion studies have identified the importance of cytotoxic T lymphocytes (CTLs) in resolving cutaneous disease [14]. Adoptive transfer of $CD8^+$ or HSV-immune $CD4^+$ T lymphocytes also reduce viral replication or provide protection from challenge. Neutralizing and antibody-dependent cellular cytotoxic antibodies generally appear 2–6 weeks after infection and persist for the lifetime of the host. Immunoblot and immunoprecipitation antibody responses have defined host response to infected cell polypeptides and have been correlated with the development of neutralizing antibodies. After the onset of infection, antibodies that are directed against gD, gB, ICP-4, gE, gG-1 or gG-2, and gC appear.

Reactivity of lymphocyte blastogenesis develops within 4–6 weeks after the onset of infection, and sometimes as early as 2 weeks after infection. With recurrences, boosts in blastogenic responses can be defined; however, these responses decrease in intensity with time. Nonspecific blastogenic responses do not correlate with a history of recurrences.

Humoral immunity does not prevent either recurrences or exogenous reinfection. Thus, it is not surprising that antibodies

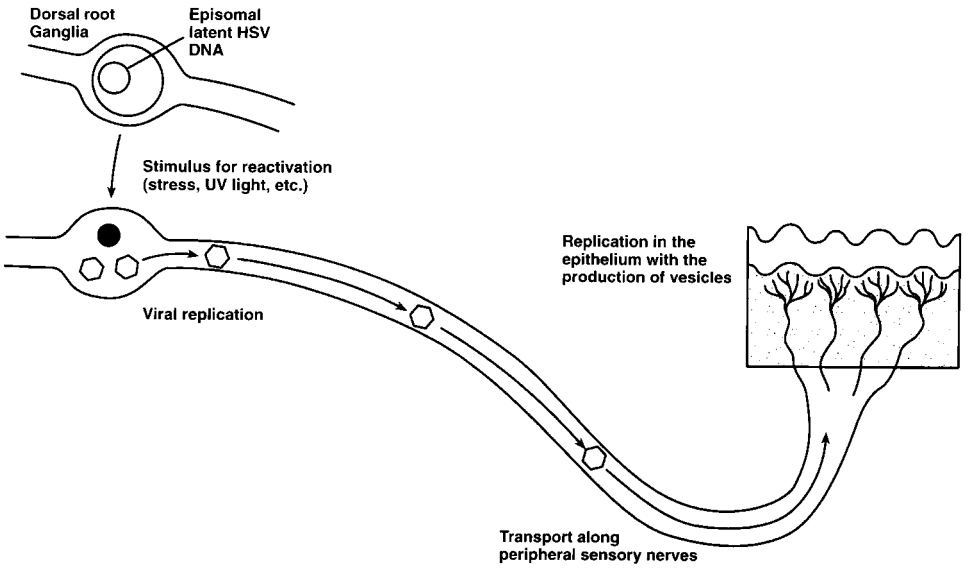

Figure 4. Schematic diagram of latency and reactivation of herpes simplex virus (HSV). UV = ultraviolet.

acquired transplacentally from mothers are not totally protective against infection in newborns. Transplacentally acquired neutralizing antibodies may either prevent infection or ameliorate disease in exposed newborns, as do antibody-dependent cell-mediated cytotoxic antibodies.

Diagnosis

Virus isolation in culture remains the definitive diagnostic method. If skin lesions are present, a scraping of skin vesicles should be obtained and transferred in appropriate virus transport media to a diagnostic virology laboratory. Clinical specimens should be shipped on ice for inoculation onto cell cultures (e.g., human foreskin fibroblasts or Vero cells) that are susceptible to cytopathic effects characteristic of HSV replication. Cytopathic effect usually develops within 24–48 hours after inoculation of specimens containing infectious virus. In addition to skin vesicles, other sites from which virus may be isolated include the CSF (in newborns), stool, urine, throat, nasopharynx, and conjunctivae. Cytological examination of cells from the maternal cervix or from an infant's skin, mouth, conjunctivae, or corneal lesions is of low sensitivity (~60%–70%).

Serological diagnosis of HSV infection is of little clinical value. Therapeutic decisions cannot be postponed until the results of serological studies are obtained. Serological assays that allow distinction between antibodies to HSV types 1 and 2 can be performed only in research laboratories. The use of ELISA for detecting antibodies allows only definition of past infection or seroconversion but cannot distinguish infection due to HSV-1 from that due to HSV-2.

PCR assay for evaluation of CSF for HSV DNA is the diagnostic method of choice for HSV infections of the CNS. Primers from an HSV DNA sequence that is common to both HSV 1 and 2 (either the glycoprotein B domain or HSV DNA polymerase) identify HSV DNA in the CSF. The evaluation of CSF specimens obtained from patients with biopsy-proven herpes simplex encephalitis (HSE) and those with other proven diseases indicates a sensitivity of >95% at the time of clinical presentation and a specificity that approaches 100% [15]. False-negative assessments occur when there is contamination of hemoglobin in the CSF or inhibitors, such as heparin, are present. PCR has also been used to detect HSV DNA in skin lesions but is not a cost-efficient diagnostic method.

Clinical Manifestations of Disease

Mucocutaneous Infection

Great variability exists in the clinical symptomatology of primary HSV-1 infections; asymptomatic infection is the rule rather than the exception [16]. The incubation period ranges from 2 days to 12 days, with a mean of ~4 days. Primary HSV-1 infection results in oral shedding of virus in the mouth for as long as 23 days (mean, 7–10 days). Neutralizing antibodies appear between days 4 and 7 after the onset of disease, and levels of these antibodies peak in ~3 weeks. Symptomatic disease in children is characterized by involvement of the buccal and gingival mucosa. The duration of illness is 2–3 weeks; temperatures range from 101°F to 104°F. Children with symptomatic primary infection are often unable to swallow liquids because of the associated pain. Lesions within the mouth evolve from vesicles to shallow ulcerations on an erythematous base before healing. Submandibular lymphadenopathy is common in patients with primary gingivostomatitis but rare in those with recurrent infections. Other findings include sore throat and mouth, malaise, tender cervical lymphadenopathy, and an inability to eat.

A clinical distinction should be drawn between intraoral gingival lesions, indicative of presumed primary infection, and lip lesions indicative of recurrent infections. Pharyngitis is common, along with a mononucleosis-like syndrome, among patients with primary HSV infections that develop later in life. The differential diagnosis of HSV gingivostomatitis includes herpangina (usually caused by the coxsackieviruses), candidal infections of the mouth, Epstein-Barr virus mononucleosis, lesions induced by chemotherapy or radiation therapy, and Stevens-Johnson syndrome.

The onset of recurrent orolabial lesions is heralded by a prodrome of pain, burning, tingling, or itching, which generally lasts for 6 hours and is followed by the appearance of vesicles [17]. Vesicles appear most commonly at the vermilion border of the lip and persist on average for only 48 hours. The vesicles generally number three to five. Lesions progress to the pustular or ulcerative and crusting stage within 72–96 hours, and healing is complete within 8–10 days. Pain is most severe at the outset and resolves quickly over 96–120 hours. The frequency of recurrences varies among individuals.

Other cutaneous HSV-1 lesions occur. Skin infections caused by HSV generally manifest as eczema herpeticum in patients with underlying atopic dermatitis. Lesions can be either localized, resembling herpes zoster, or disseminated, as occurs with Kaposi's varicella-like eruption. Infections of the digits, known as herpetic whitlow, are particularly common among medical and dental personnel. The estimated incidence of herpetic whitlow is 2.4 cases per 100,000 population per year, and it is caused by HSV-1 or HSV-2. Disseminated cutaneous HSV infections have been reported among wrestlers (herpes gladitorium). Other skin disorders associated with extensive cutaneous lesions include Darier's disease and Sézary syndrome. HSV infections of either type can trigger erythema multiforme.

Primary genital herpes manifests as macules and papules, followed by vesicles, pustules, and ulcers [18, 19]. Lesions persist ~3 weeks, and viral shedding can occur throughout this time period. Systemic complications are relatively uncommon in males; however, aseptic meningitis can develop. Paresthesias and dysesthesias that involve the lower extremities and perineum can result from genital herpetic infection. Primary infections are usually associated with fever, dysuria, localized inguinal adenopathy, and malaise in both men and women. Primary infections are more severe and more often associated with complications in women than in men.

Systemic complaints are common in both sexes, approaching 70% of patients. In women with primary infection, lesions appear on the vulva and are usually bilateral; the cervix is invariably involved. The actual frequency of primary cervical infection in the absence of vulvar infection is unknown. The lesions usually are excruciatingly painful, associated with inguinal adenopathy and dysuria, and may involve the vulva, perineum, buttocks, cervix, and vagina. A urinary retention syndrome occurs in 10%–15% of female patients, and as many as 25% of women will develop aseptic meningitis. In males, primary genital HSV infections are most often associated with vesicular lesions superimposed on an erythematous base, usually appearing on the glans penis or the penile shaft. Extragenital lesions of the thigh, buttocks, and perineum can occur.

Other complications following primary genital herpetic infection in either sex include sacral radioculomyelitis, neuralgias, and meningoencephalitis. Primary perianal HSV-2 infections and proctitis are more common in male homosexuals. Nonprimary initial genital infection is less severe symptomatically and heals more quickly. The duration of infection is usually 2 weeks. The number of lesions, severity of pain, and likelihood of complications are significantly decreased. The presence of antibodies to HSV-1 renders disease due to HSV-2 less severe.

With recurrent genital herpetic infection, a limited number of vesicles (three to five) appear on the shaft of the penis in males or as simply vulvar irritation in females. The duration of disease parallels that of recurrent HSV labialis, i.e., ~8–10 days. Neurological or systemic complications are uncommon with recurrent disease; however, paresthesias and dysesthesias occur. Virus is shed for an average of 2–5 days. Recurrent genital herpetic infection in both men and women is characterized by a prodrome and by localized irritation. The frequency of recurrences varies among individuals. The severity of primary infection appears to correlate with the frequency of recurrences; i.e., the more severe the primary infection, the more likely and frequent are the recurrences. One-third of patients are estimated to have recurrences in excess of eight or nine per year, one-third will have two to three recurrences per year, and the remaining one-third will have between four and seven recurrences per year. Patients with symptomatic or asymptomatic recurrences can transmit infection to sexual partners [20]. Recent studies have suggested a high frequency of HSV DNA in genital secretions, as detected by PCR, between clinical recurrences [21].

Genital HSV infection can rarely become disseminated during pregnancy, involving multiple visceral sites and leading to necrotizing hepatitis with or without thrombocytopenia, disseminated intravascular coagulopathy, and encephalitis. The associated mortality among pregnant women is reported to be >50%. Fetal deaths have also occurred in >50% of cases.

Primary or initial maternal genital HSV infection poses the major risk to the fetus. Thus, identification of women at risk for primary infection (i.e., those seronegative for HSV-2) is of paramount importance. The rate of serological discordance, where the mother is HSV-2 seronegative and her partner is HSV-2 seropositive, averages 15%–20%. The risk for transmission from the father is 10%–15% [22].

Neonatal Disease

The estimated incidence of neonatal HSV infection is approximately one case in 2,000 deliveries per year to one case in 5,000 deliveries per year. At least four factors influence

transmission of infection from mother to fetus: (1) The rate of transmission is 30%–50% with maternal primary or initial infection, as compared with ⩽3% with recurrent infection [23]; (2) paralleling the type of maternal infection, the mother's antibody status before delivery influences both the severity of infection and the likelihood of transmission; (3) prolonged rupture of membranes (greater than 6 hours) increases the risk of acquisition of infection as a consequence of ascending infection from the cervix; and (4) when fetal scalp monitors are used, they can be a site of inoculation of virus.

Infection of a newborn can be acquired via three different routes, and the mother is the most common source of infection in all cases. The first route, in utero infection, is rare and requires stringent diagnostic criteria (namely, identification of infected neonates within the first 48 hours of life by means of viral cultures). The second route of infection is that of intrapartum contact of the fetus with infected maternal genital secretions. It is likely that ~75%–80% of neonates acquire HSV infection by this route. The third route of transmission is postnatal acquisition. Relatives and hospital personnel with orolabial herpes are reservoirs for HSV infection in newborns.

HSV infection in neonates is almost invariably symptomatic and frequently lethal. As stated above, neonates with congenital infection should be identified within 48 hours after birth. Such congenital disease is characterized by the triad of skin vesicles or scarring, eye disease, and microcephaly or hydranencephaly. HSV infections in neonates who become infected during or after birth can be divided into the following three categories [24].

1. Disease localized to the skin, eye, and mouth occurs in 40% of neonates and is characterized by the presence of discrete vesicles and keratoconjunctivitis. Disease localized to the skin, eye, or mouth generally presents at ~10–11 days of life. Neonates with skin lesions will frequently have recurrences over the first 6 months (and longer) of life, regardless of whether therapy was administered. In the era before antiviral therapy was available, ~30% of children eventually developed evidence of neurological impairment.
2. Encephalitis, with or without skin involvement, occurs in 35% of neonates. Infection of the CNS alone or in combination with disseminated disease presents as findings indicative of encephalitis. Clinical manifestations of encephalitis (alone or in association with disseminated disease) include seizures (both focal and generalized), lethargy, irritability, tremors, poor feeding, temperature instability, bulging fontanelle, and pyramidal tract signs. Virus can be cultured from CSF in 25%–40% of all cases. Anticipated findings on CSF examination include pleocytosis and proteinosis (protein level, ⩽500–1,000 mg/dL), although a few neonates with CNS infection have no CSF abnormalities. Fifty percent of newborns with CNS disease who are not treated will die, and death is usually related to brain-stem involvement. The long-term prognosis of encephalitis is particularly poor. As many as 50% of surviving children have some degree of psychomotor retardation, often in association with microcephaly, hydranencephaly, porencephalic cysts, spasticity, blindness, retinitis, or learning disabilities. Skin vesicles, the classic sign of disease, may not be present in ⩽40% of neonates with disease localized to the CNS. Thus, for neonates with cells and protein in the CSF at 2–3 weeks of life, other diagnostic clues, such as skin vesicles, may not be present. For the neonate with CSF findings indicative of infection, HSV must be considered along with bacterial pathogens (e.g., group B streptococcus and *Escherichia coli*).
3. Disseminated infection, which occurs in 25% of neonates, involves multiple organs, including the CNS, lungs, liver, adrenal glands, skin, eye, and/or mouth and is associated with the poorest prognosis. Infants with disseminated infection present for therapy between 9 and 11 days of age. Constitutional signs and symptoms include irritability, seizures, respiratory distress, jaundice, bleeding diatheses, shock, and, frequently, the characteristic vesicular exanthem that is often considered pathognomonic for HSV infection. Encephalitis is a common component of disseminated infection, occurring in ~60%–75% of these infants. The vesicular rash does not occur in >20% of these children. Mortality in the absence of therapy exceeds 80%; all but a few survivors are impaired.

HSV Keratoconjunctivitis

Viral infections of the eye that occur after the neonatal period are usually caused by HSV-1. Approximately 300,000 cases of HSV infections of the eye are diagnosed annually in the United States. HSV keratoconjunctivitis is associated with either unilateral or bilateral conjunctivitis, which can be follicular in nature and is followed soon thereafter by preauricular adenopathy. Herpetic keratoconjunctivitis is also associated with photophobia, tearing, eyelid edema, and chemosis, as well as the pathognomonic findings of branching dendritic lesions. Geographic ulcers of the cornea develop in patients with advanced disease. The rate of recurrence parallels that described for herpes labialis. Most frequently, recurrences are unilateral but in a small percentage of cases involve both eyes.

Infections of the Immunocompromised Host

Immunocompromised patients, especially organ transplant recipients, are at increased risk for severe HSV infections. These patients may develop progressive disease involving the respiratory tract, esophagus, or gastrointestinal tract. The severe nature of progressive disease in these patients appears to be directly related to the amount and duration of immunosuppressive therapy. Esophagitis occurs commonly in immunocompromised patients and can be caused by HSV, cytomegalovirus, or *Candida albicans.* Ayclovir-resistant HSV disease can occur in immunocompromised patients and can be progressive, especially in those with AIDS.

Infections of the CNS

Encephalitis is one of the most devastating of all HSV infections; HSV is considered the most common cause of sporadic, fatal encephalitis. The manifestations of HSV encephalitis in older children and adults are indicative of the areas of the brain affected. These manifestations include primarily focal encephalitis that is associated with fever, altered consciousness, bizarre behavior, disordered mentation, and localized neurological findings. Clinical signs and symptoms reflect localized temporal lobe disease. No signs are pathognomonic for HSV encephalitis; however, a progressively deteriorating level of consciousness in association with fever, an abnormal CSF profile, and focal neurological findings in the absence of other causes should make this disease highly suspect. Diagnostic evaluations should be initiated immediately, since other treatable diseases mimic HSV encephalitis. The mortality among untreated patients exceeds 70%, and only 2.5% of patients who survive regain normal neurological function [25].

Standard neurodiagnostic procedures are used in the evaluation of patients with suspected HSV encephalitis and include CSF examination, electroencephalography, and scanning procedures such as CT or MRI. Characteristic abnormalities of the CSF include elevated levels of WBCs (usually mononuclear), RBCs, and protein. Spike and slow wave activity are generally localized to the temporal lobe on electroencephalograms. A burst suppression pattern is characteristic of HSV encephalitis. Imaging will allow for localization of disease to the temporal lobe. Early after the onset of disease, only evidence of edema is detectable, if at all. This finding is followed by evidence of hemorrhage and midline shift in the cortical structures.

In addition to the brain, HSV can involve virtually all anatomic areas of the nervous system, causing manifestations such as meningitis, myelitis, and radiculitis.

Other Forms of Infection

HSV has been isolated from the respiratory tracts of adults with adult respiratory distress syndrome and acute-onset bronchospasm. Both conditions are associated with increased mortality and morbidity.

Prevention of HSV Infections

Because of the increased awareness of the increasing incidence of genital herpes and neonatal herpes and the association between HSV infection and an increased risk of acquiring HIV, every effort should be made to prevent HSV-2 infections. Until a vaccine is proved effective, educational efforts must be developed for adolescents and adults at greatest risk. The use of condoms should be promoted. Individuals known to be infected should be educated about the risks of transmission of infection, particularly to seronegative female sexual partners who are pregnant. In addition, the incidence of neonatal HSV infection can be decreased by performing a cesarean section if lesions are present in the mother at delivery. Vaccination remains the ideal method for prevention of viral infection, but the use of vaccination to prevent HSV infections introduces unique problems because of recurrences in the presence of humoral and cell-mediated immunity [26].

The vaccines that have been studied to the greatest extent are subunit glycoprotein constructs. Biocine/Chiron (Emeryville, CA) and SmithKline Beecham (Philadelphia) subunit vaccines have been or are being assessed in clinical trials. Both neutralizing and ELISA antibodies can be detected after administration of these vaccines. Three controlled trials of the Biocine/Chiron gD-2 or gB/gD-2 vaccine have been performed. For individuals with recurrent genital HSV infection, a phase 2 trial demonstrated a significant decrease in the number of culture-positive episodes and in the total number of episodes (by approximately one-third) [27]. However, these findings were not reproduced in a definitive phase 3 trial. Indeed, in a phase 3 trial in seronegative sexual partners of persons with known HSV-2 infection, no degree of protection was conferred. Thus, the Biocine/Chiron HSV vaccine trials have been abandoned.

Alternative approaches are to genetically engineer HSV to create an attenuated construct or to administer naked DNA. Naked HSV DNA vaccines are now in phase 1 trials in humans. On the other hand, knowledge of the $\gamma_1$34.5 gene of HSV-1 as a mediator of CNS replication has just now been taken into consideration in the engineering of HSV. Furthermore, deletions of $\gamma_1$34.5 significantly decrease the ability of the engineered viruses to establish latency. Such constructs in HSV-2 are being developed. Other approaches to the manipulation of HSV, including mutations in ICP8 and gH, are under way for vaccine development.

Treatment

Acyclovir (9-[2-hydroxyethoxymethyl] guanine), a synthetic acyclic purine nucleoside analogue, has become the standard of therapy for HSV infections [16]. It is the most widely prescribed and clinically effective antiviral drug available to date. The prodrug valacyclovir (converted to acyclovir) and famciclovir (converted to penciclovir) have recently been licensed and have greater oral bioavailability than acyclovir and penciclovir.

Genital Herpes

Initial genital HSV infection can be treated with topical, oral, or intravenous acyclovir. While topical application of acyclovir reduces the duration of viral shedding and the length of time before all lesions become crusted, this treatment is less effective than that with oral or intravenous acyclovir. Intravenous acyclovir is the most effective treatment for a first episode of genital herpes and results in a significant reduction

Table 1. Indications for acyclovir therapy among patients with herpes simplex virus infection.

Type of infection	Route and dosage*	Comments
Genital HSV		
Initial episode	200 mg po 5 times per d for 10 d	Preferred route in healthy host
	5 mg/kg iv q8h for 5 d	Reserved for severe cases
	5% ointment topically q6h for 7 d	Less effective than oral therapy
Recurrent episode	200 mg po 5 times/day for 5 days	Limited clinical benefit
Suppression	400 mg po twice daily	Titrate dose as required
Mucocutaneous HSV in an immunocompromised patient	200–400 mg po 5 times per d for 10 d	
	5 mg/kg iv q8h for 7–10 d	
	5% ointment topically q6h for 7 d	For minor lesions only
HSV encephalitis	10 mg/kg iv q8h for 10–14 d	Alternative therapy: vidarabine, valacyclovir, or famciclovir
Neonatal HSV†	10 mg/kg iv q8h for 10–14 d	Alternative therapy: vidarabine, valacyclovir, or famciclovir

NOTE. HSV = herpes simplex virus.
* The dose are for adults with normal renal function unless otherwise noted.
† Not currently approved by the U.S. Food and Drug Administration.

in the median duration of viral shedding, pain, and length of time to complete healing (8 days vs. 14 days). Since intravenous acyclovir therapy usually requires hospitalization, it should be reserved for patients with severe local disease or systemic complications. Oral therapy (200 mg five times daily) is nearly as effective as intravenous therapy for initial episodes of genital herpes and has become the standard treatment (table 1). Both valacyclovir and famciclovir offer similar degrees of therapeutic benefit as acyclovir. Neither intravenous nor oral treatment with acyclovir for acute HSV infection reduces the frequency of recurrences.

Recurrent genital herpes is less severe and resolves more rapidly than primary infection; thus, there is less time to successfully introduce antiviral chemotherapy. Oral acyclovir therapy shortens both the duration of viral shedding and the length of time to healing (6 days vs. 7 days) when initiated early (within 24 hours of onset), but the duration of symptoms and length of time to recurrence are not affected. Valacyclovir and famciclovir likely provide little added benefit.

Long-term oral administration of acyclovir, valacyclovir, or famciclovir effectively suppresses genital herpes in patients who have frequent recurrences. Daily administration of acyclovir reduces the frequency of recurrences by ⩽80%, and 25%–30% of patients have no further recurrences while taking acyclovir. Successful suppression for as long as 3 years has been reported, with no evidence of significant adverse effects. Titration of the dose of acyclovir (400 mg twice daily or 200 mg two-to-five times daily) may be required to establish the minimal dose that is most effective and economical. Treatment should be interrupted every 12 months to reassess the need for continued suppression. The emergence of acyclovir-resistant strains of HSV appears to be infrequent in immunologically normal individuals. It is important to note that asymptomatic shedding of virus can continue despite clinically effective suppression with acyclovir; thus, the possibility of person-to-person transmission persists.

Herpes Labialis

Topical therapy with penciclovir (Deavir; SmithKline Beecham, Philadelphia) will accelerate clinical healing by ~1 day. Oral administration of acyclovir (at a dose of 200 mg five times daily for 5 days) reduces the length of time to the loss of crusts by ~1 day (7 days vs. 8 days) but does not alter the duration of pain or the length of time to complete healing. Oral administration of acyclovir can alter the severity of sun-induced reactivation of labial HSV infections. The administration of 200 mg five times daily to skiers did not decrease the frequency of recurrent labial infections as compared with placebo, but significantly fewer lesions formed on days 5–7 among acyclovir recipients [28]. Short-term prophylactic therapy with acyclovir may benefit some patients with recurrent herpes labialis who anticipate engaging in a high-risk activity (e.g., intense exposure to sunlight). The intermittent administration of acyclovir does not alter the frequency of subsequent recurrences. No data support long-term treatment with any of these drugs for the prevention of herpes labialis.

Mucocutaneous HSV Infections in Immunocompromised Patients

Intravenous acyclovir therapy for HSV disease in the immunocompromised host is very beneficial. Immunocompromised patients receiving acyclovir have a shorter duration of viral shedding and more rapid healing of lesions than do patients receiving placebo. Oral acyclovir therapy is also very effective in immunocompromised patients. Acyclovir prophylaxis for HSV infections is of clinical value in immunocompromised patients, especially those undergoing induction chemotherapy or transplantation. Intravenous or oral administration of acyclovir reduces the incidence of symptomatic HSV infection from ~70% to 5%–20%. Similar data exist for famciclovir and valacyclovir. A sequential regimen of intravenous acyclovir,

followed by oral acyclovir for 3–6 months, can virtually eliminate symptomatic HSV infections in organ transplant recipients.

A variety of oral dosing regimens, ranging from 200 mg three times daily to 800 mg twice daily, have been used successfully. Acyclovir-resistant HSV isolates have been identified more frequently after therapeutic acyclovir administration than during prophylaxis among bone marrow recipients and patients with AIDS. Acyclovir-resistant HSV isolates usually are cross-resistant to famciclovir/penciclovir. Acyclovir has become the therapeutic mainstay for the treatment and suppression of HSV infections in immunocompromised patients.

HSE

HSE is associated with substantial morbidity and mortality despite the use of antiviral therapy. The administration of acyclovir in a dose of 10 mg/kg every 8 hours for 10–14 days reduces mortality at 3 months to 19%, as compared with ~50% among patients treated with vidarabine. Furthermore, 38% of the patients treated with acyclovir regain normal neurological function. The outcome is poor for patients with a Glasgow coma score of <6, for those >30 years of age, and for those who have had encephalitis longer than 4 days. For the most favorable outcome, therapy must be instituted before semicoma or coma develops.

Neonatal HSV Infections

Newborns with HSV infections can be classified as having disease that is localized to the skin, eye, and mouth; affects the CNS; or is disseminated. In a comparative study [29], acyclovir was as effective as (but not superior to) vidarabine in neonates with HSV infections. No neonate with disease localized to the skin, eye, or mouth died, whereas 18% of neonates with CNS infection and 55% of those with disseminated infection died. Among neonates with HSV infections of the skin, eye, and mouth, 90% of those treated with vidarabine and 98% of those treated with acyclovir were developing normally 2 years after infection. The comparable values were 50% and 43%, respectively, among neonates who survived encephalitis and 62% and 57%, respectively, among those who survived disseminated infection [30].

Thus, unlike the results of therapy in older patients with herpes simplex encephalitis, there were no significant differences in either morbidity or mortality among neonates treated with acyclovir or vidarabine. Clearance of virus was slower in neonates who received acyclovir than in immunocompromised adults, implying a requirement for host defense as well. The safety and ease of administration of acyclovir make it the treatment of choice for neonatal HSV infections. The currently recommended intravenous dose is 10 mg/kg every 8 hours. Long-term oral suppressive therapy may be of value and warrants further study.

Table 2. Investigational uses of acyclovir.

- Herpes labialis (prophylaxis)
- HSV keratitis
- Suppression of HSV infections in immunocompromised patients
- Disseminated or visceral HSV infections (e.g., hepatitis)
- Suppression of cytomegalovirus infections in organ transplant recipients
- Oral hairy leukoplakia
- Monkey B virus infections of humans

NOTE. HSV = herpes simplex virus.

Other HSV Infections

Case reports have described the successful use of acyclovir in the treatment of other HSV infections such as hepatitis, pulmonary infections, herpetic esophagitis, proctitis, eczema herpeticum, erythema multiforme, and herpetic whitlow (table 2). Topical therapy with acyclovir for HSV ocular infections is effective but probably not superior to that with trifluridine.

Viral Resistance

HSV can develop resistance to acyclovir through mutations in the viral gene encoding thymidine kinase (TK), either through the generation of TK-deficient mutants or through the selection of mutants possessing TK that is unable to phosphorylate acyclovir. Clinical isolates resistant to acyclovir are almost uniformly deficient in TK, although isolates with altered DNA polymerase have been recovered from HSV-infected patients. Drug resistance was considered rare, and resistant isolates were believed to be less pathogenic until a series of acyclovir-resistant HSV isolates from patients with AIDS were characterized [31]. These resistant mutants were deficient in TK. Although acyclovir-resistant HSV is susceptible to vidarabine and foscarnet in vitro, only foscarnet has been shown to be effective in the treatment of infection due to acyclovir-resistant HSV. Acyclovir-resistant HSV isolates have been identified as the cause of pneumonia, encephalitis, esophagitis, and mucocutaneous infections in immunocompromised patients.

Toxicity

Treatment with acyclovir, valacyclovir, and famciclovir is associated with few adverse effects. Renal dysfunction has been reported, especially in patients given large doses of acyclovir by rapid intravenous infusion, but appears to be uncommon and is usually reversible. The risk of nephrotoxicity can be minimized by administering acyclovir by slow infusion and by ensuring adequate hydration. Oral acyclovir therapy, even at doses of 800 mg five times daily, has not been associated with renal dysfunction. A few reports have linked intravenous administration of acyclovir with disturbances of the CNS, in-

cluding agitation, hallucinations, disorientation, tremors, and myoclonus.

The Acyclovir in Pregnancy Registry has gathered data on prenatal exposure to acyclovir [32]. No increase in the risk to mothers or fetuses has been documented, but the total number of monitored pregnancies is too small to detect any low-frequency events. Since acyclovir crosses the placenta and is concentrated in amniotic fluid, there is concern about the potential for fetal nephrotoxicity, although none has been observed.

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CME Sponsor

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Certificates of CME credit will be awarded on a per-volume (biannual) basis. Each answer card must be submitted within 3 months of the date of the issue.

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1. As an investigator, you are interested in the phenotypic property of neurovirulence of herpes simplex virus (HSV). In your studies of the virus, you perform a series of genetic deletions to determine which gene confers the property of neurovirulence to HSV. The resulting viruses are then inoculated directly into the central nervous system of mice. In order to determine pFU/LD_{50} ratios, the gene that is believed to control neurovirulence is
   A. Glycoprotein B.
   B. Glycoprotein C.
   C. $\gamma_1$34.5.
   D. LAT.
   E. Ribonucleotide reductase.
2. A 16-day-old infant presents to the emergency department with fever (temperature, to 39°C), irritability, and fussiness. The CSF has a protein level of 150 mg/dL, 100 WBCs equally divided among polymorphonuclear cells and lymphocytes, and a normal glucose concentration. The gram stain is negative, and antigen studies performed on the CSF are also negative. Bacterial cultures are negative at 24 hours. The most likely cause of infection is
   A. enterovirus.
   B. cytomegalovirus.
   C. Epstein-Barr virus.
   D. HSV.
   E. varicella-zoster virus.
3. A 67-year-old male presents to the emergency department with fever (temperature, to 38.5°C), right-sided focal seizures, and disorientation. His CSF contains 35 WBCs, all of which are mononuclear cells; the protein level is 70 mg/dL, and the glucose concentration is normal. A MRI of the CNS reveals an area of hemorrhage and edema in the left temporal lobe. The diagnostic method of choice is
   A. brain biopsy.
   B. viral culture of CSF.
   C. fluorescent antibody staining of CSF.
   D. antibody determination in CSF.
   E. PCR assessment of CSF.
4. The seroprevalence of genital herpes is increasing in the United States. Which of the following factors appears to correlate most directly with this increased prevalence of infection?
   A. City of residence
   B. HLA type
   C. Number of sexual partners
   D. Years of education
   E. Immunocompromised host
5. A 24-year-old male presents to your office with recurrent genital lesions that are vesicular in nature and cropped. You elect to treat the individual with acyclovir. Which of the following statements is true regarding acyclovir?
   A. It has a terminal 3′-hydroxyl group.
   B. It is selectively activated by viral thymidine kinase.
   C. It is selectively activated by host cell thymidine kinase.
   D. It exists in a nucleotide form.
   E. It is a competitive inhibitor of viral DNA polymerase but not a chain terminator.
6. You provide care for an immunocompromised host with HIV/AIDS. The individual has recurrent fever blisters. You prescribed acyclovir in the past; however, over the past 2 months, the patient's lesions have been unresponsive to acyclovir at doses even as high as 800 mg five times daily. The most likely cause for the failure of the patient to respond to therapy is

A. a ribonucleotide reductase mutation.
B. a viral thymidine kinase mutation.
C. inadequate plasma levels of acyclovir.
D. a viral phosphotransferase deficiency.
E. a viral protease deficiency.

7. A child is born to a woman with primary genital herpes at term. On examination in the newborn nursery, there is no evidence of an abnormality. On day 7 of life, the child develops fever and irritability. The most likely organ providing evidence of herpes simplex infection is
A. the brain.
B. the lungs.
C. the adrenal glands.
D. the liver.
E. the kidneys.

8. You are the director of a vaccine company. You elect to develop a vaccine that includes the major immunodominant glycoprotein from the envelope of herpes simplex virus. This glycoprotein is
A. gC.
B. gD.
C. gE.
D. gI.
E. gN.

9. In considering the use of valacyclovir for suppressive therapy for frequently recurrent genital herpes, you recognize that the drug is cleared by which route?
A. The kidneys
B. The liver
C. The gastrointestinal tract
D. The lungs
E. The saliva

10. You are interested in the pathogenesis of latency. From necropsy specimens, you probe trigeminal ganglia for gene expression. Which of the following is found?
A. LAT
B. Thymidine kinase
C. ORF R
D. $\gamma_1$34.5
E. Protein kinase