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Herpes Simplex Viruses

Edited by

**Marie Studahl, Paola Cinque,
Tomas Bergström**



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Herpes Simplex Viruses

INFECTIOUS DISEASE AND THERAPY

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Herpes Simplex Viruses

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CRC Press

Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

First published 2006 by CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Reissued 2018 by CRC Press

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A Library of Congress record exists under LC control number: 2005052899

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ISBN 13: 978-1-138-10594-2 (hbk)
ISBN 13: 978-0-203-71182-8 (ebk)

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CRC Press Web site at <http://www.crcpress.com>

Preface

Ubiquitous viruses among humans, herpes simplex viruses (HSV) are believed to be several million years old. Infection by these viruses may cause various conditions ranging from mucocutaneous disease to more severe afflictions of the central nervous system (CNS) or life-threatening infections in immunocompromised patients. Physicians from almost all medical specialties will encounter patients with HSV infections that may require treatment. Recently acquired knowledge of pathogenesis and diagnostics in already described clinical diseases caused by HSV, as well as identification of new syndromes or diseases where herpes simplex may or may not play a causal role, may be unknown to a large proportion of medical personnel.

This book on HSV not only summarizes the clinical aspects of HSV infections, but also throws light on basic molecular virology to facilitate a better understanding of different disease manifestations. The purpose of this volume is to compile diagnostics and management of patients with HSV infections with basic virology relevant to an understanding of pathogenetic mechanisms. This book will be a good aid to clinicians treating patients with HSV infections, as well as diagnostic microbiologists who will find suitable and updated information for their respective professions.

The first chapter of Section I deals with the evolution of the viruses followed by an update on the natural course of primary and recurrent infections as well as epidemiology. The next chapter concerns pathogenesis in different experimental and clinical manifestations of HSV infections. The following chapter on diagnostics demonstrates the improvement in this field that has occurred over the last decennium, mainly due to the introduction of genome detection by PCR, and to the development of new and type-discriminating serological methods. The second, more clinical, part of the book begins with a chapter on treatment of HSV infections utilizing established as well as new drugs where clinical trials are ongoing. The subsequent

chapters document the different disease manifestations of HSV infections, including their prognosis and treatment.

In separate chapters, well-known diseases such as gingivostomatitis, recurrent labial infection, HSV infections of the skin, acute and recurrent genital infection, and ocular infection are described. CNS diseases such as herpes simplex encephalitis and meningo/radiculo/myelitis, as well as diseases recently discovered to be caused by herpes simplex such as paresis of the facial nerve are covered. HSV infections in immunocompromised patients and in pregnant women including congenital and neonatal infections are specially addressed in later parts of the book. Lastly, recent advances in prevention of infection and disease by vaccination and future outlooks in HSV research are discussed.

**Marie Studahl
Paola Cinque
Tomas Bergström**

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Evolution of Herpes Simplex Viruses

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INTRODUCTION

The evolution of the two herpes simplex virus (HSV) species, HSV-1 and HSV-2, falls naturally into two main components. The first of these concerns the ancient evolutionary history of the origins and development of HSV-1 and HSV-2 within the contexts of the family Herpesviridae, the sub-family Alphaherpesvirinae, and the *Simplexvirus* genus to which the HSV species are assigned. As we shall explain, we believe that these processes took place over a timeframe of several hundred million years (MY), with the most recent-dated event being the divergence of the HSV-1 and HSV-2 lineages some 9 MY ago. The second part of our treatment concerns the evolutionary processes that have generated and are active in contemporary populations of HSV-1 and HSV-2, which are composed of lineages that we estimate have arisen well within the last million years.

Investigation of both these evolutionary phases depends on availability of herpesviral genomic sequences. For the first phase of ancient development, the primary analytical route comprises comparison of encoded amino acid sequences for equivalent genes from across the herpesvirus family, with prominent use of methods for construction of phylogenetic trees. The second phase, of population biology of each HSV species, was initiated two decades ago using restriction nuclease profiles for the DNAs of HSV isolates to measure diversity, while its modern practice employs DNA sequences from selected genomic regions of isolates. Availability of sufficient comparative

sequence data is only now approaching a level adequate for such analyses, so our appreciation and understanding of recent HSV evolution is still at an early stage.

ORIGINS OF THE FAMILY HERPESVIRIDAE

Common Descent of Mammalian Avian Herpesviruses

DNA sequence analyses of the genomes of mammalian and avian herpesviruses have shown that these viruses are all related by descent from a common ancestral virus. The evidence derives from a large subset (around 40) of the viral genes that have been found to have counterparts in every completely sequenced herpesvirus genome, and representatives of which have been seen in partly sequenced genomes (1,2). The linear arrangements in genomes of these “core” genes are partially preserved even among highly diverged herpesviruses. The identified set of core genes ranges from the most conserved, whose encoded amino acid sequences are unambiguously related across the family, to the least similar, where assignment is based on scant local similarities only. Membership thus fades into uncertainty at this lower end rather than being definitively bounded. In addition, there are three genes not in the core set whose distribution and genomic locations argue that they were present in the common ancestral virus but were subsequently lost in some lineages. We thus identify a “minimal ancestral set” of 43 genes (1,3). The core genes mostly encode components of the icosahedral capsid, proteins concerned with DNA replication and packaging of replicated DNA into nascent capsids, and virion surface glycoproteins involved in entry into and egress from cells. On the other hand, most genes specifying proteins with control functions and most genes encoding proteins of the virion tegument and surface are specific to sublineages of the viruses.

Phylogenetic relationships among mammalian and avian herpesviruses have been investigated using gene sequence data, and detailed phylogenetic trees have been derived (4). In addition, the limited sequence data available for reptilian (turtle) herpesviruses indicate that they are also part of this virus group (5,6). The summary tree shown in Figure 1 depicts major lineages and sublineages, and their relationships. The three deepest branches of the tree correspond to the three taxonomic subfamilies, the Alpha-, Beta-, and Gammaherpesvirinae, and the eight terminal branches to genera in the subfamilies (7). In this chapter, we are concerned with HSV-1 and HSV-2, which belong to the *Simplexvirus* genus or $\alpha 1$ group of the Alphaherpesvirinae.

Robust phylogenetic trees derived from alignments of amino acid sequences of up to eight genes from the core set revealed within several of the sublineages patterns of relationships among virus species that mirrored the branching patterns of corresponding lineages of mammalian host species during divergent evolution of the host lines (4,8,9). This observation was

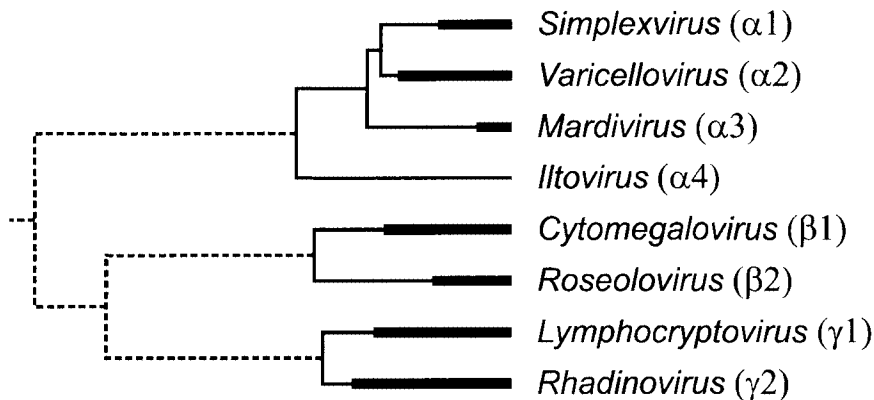


Figure 1 Major lineages for mammalian and avian herpesviruses. This summary phylogenetic tree was derived from alignment of encoded amino acid sequences for sets of conserved gene, using a maximum likelihood method with a molecular clock imposed. Major lineages equivalent to genera are shown, and with recent taxonomic additions (*Mardivirus* and *Illtovirus*). Lineages belonging to the Alpha-, Beta-, and Gammaherpesvirinae are also labeled with the appropriate Greek letter plus a numeral. The least certain portions of the tree adjacent to the root are shown as dashed lines. In terminal branches, heavy lines indicate the region in each lineage with multiple branches. *Source:* From Refs. 4, 7.

consistent with cospeciation of herpesviruses with their hosts being a prominent mechanism in the genesis of herpesvirus lineages. While this idea was not new, the sequence-based trees available in the last few years have provided both good evidence in its support and also a semiquantitative foundation that has then allowed estimation of a timescale for herpesvirus evolution by transfer of speciation dates from mammalian paleontology. It has to be registered that there are complexities and limitations, as with any estimate of evolutionary time based on differences among nucleic acid or protein sequences. The herpesvirus phylogeny also shows instances of lineages whose origins were interpreted as having been by transfer of a founding virus between host species. Overall, we consider that the cospeciation-based timescale for herpesviruses is a powerful tool in interpretation of the family's evolution.

The least certain portions of the tree in Figure 1 are the deep branches nearest the root (shown as dashed lines), primarily because these are most distant from the input sequences (equivalent to the branch tips) and inferences concerning them are accordingly most dependent on the modelling algorithm used. Early work (Ref. 9, using the neighbor-joining algorithm) gave an estimate for the root locus of around 200 million years before the present, while later more extensive and sophisticated analyses gave a figure of 400 million years (based on Ref. 4, with more input data, modelling by

maximum-likelihood methods with gamma distribution of substitution rates and an updated paleontological timescale). For our present purposes, three points regarding the root of the tree are relevant. First, the root corresponds to a very remote period in the history of life on our planet. Second, the most recent common ancestral species (at the root) was already a recognizable herpesvirus, with a complement of at least 43 genes and with a capsid structure and DNA replication systems comparable to the modern versions. Third, this ancestor must therefore have had a substantial previous evolutionary development.

Early Evolution of Herpesviruses

Considering first the early evolution of herpesviruses that gave rise to the most recent common ancestor of present day lineages, we can conclude little of detail or certainty. In addition to the group of herpesviruses so far discussed, of mammals, birds and reptiles, there are two other sets of viruses currently assigned to the family Herpesviridae: a group of fish and amphibian viruses, and a single invertebrate (oyster) virus (2). Both display the characteristic virion architecture of herpesviruses, and indeed this is the basis of their assignment to the family. However, very little of their gene complements are detectably related to those of the mammalian/avian/reptile group. Like the latter, the fish/amphibian group comprises a set of lineages that are clearly related by gene content (although with wide overall divergence), while the oyster virus is distinct in its gene contents from both of the vertebrate virus groups. Our interpretation of this situation is that the three groups are probably genuinely related in having a common origin of their capsid genes, but that most of the remainder of the gene complements of the present day species is in each case acquired after divergence of these three ancient lineages. This comparison thus sketches an early stage of evolutionary development, of what might be termed a “pre-herpesvirus.” A final point regarding the origins of the herpesviruses is that certain aspects of their mechanisms for packaging DNA are similar to those of DNA bacteriophages, suggesting some very distant connection (2).

RELATIONSHIPS WITHIN THE SUBFAMILY ALPHAHERPESVIRINAE

Proposed Cospeciations of Viruses and Hosts

Four major lineages are recognized in the Alphaherpesvirinae. Two of these are populated sparsely and exclusively by avian viruses, namely *Mardivirus* ($\alpha 3$) and *Iltovirus* ($\alpha 4$). It has been speculated that these two genera arose by way of interspecies transfers of early alphaherpesviruses, most likely from mammals to birds. The mammalian viruses in the subfamily lie in a clade with the avian virus lineages as outgroups, and with two genera,

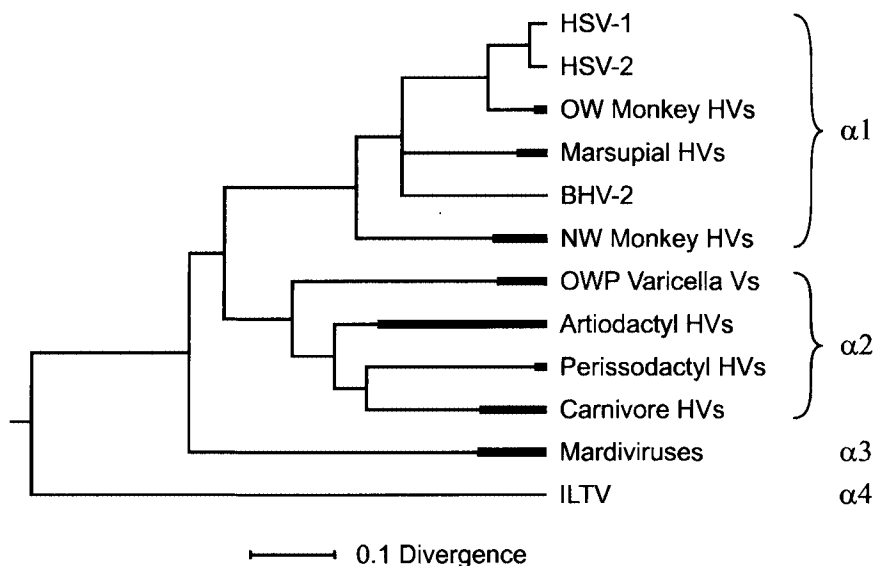


Figure 2 Lineages in the subfamily Alphaherpesvirinae. This tree expands the alphaherpesvirus portion of the phylogenetic tree in Figure 1, with emphasis on the HSV-proximal branches. As in Figure 1, the heavy lines indicate in terminal branches the locations of multiple branches. *Abbreviations:* OW, old world; HSV, herpesvirus; BHV-2, bovine herpesvirus 2; NW, new world; OWP, old world primate; V, virus; ILTV, infectious laryngotracheitis virus.

Simplexvirus ($\alpha 1$) and *Varicellovirus* ($\alpha 2$). The main lineages within the Alphaherpesvirinae are expanded in Figure 2.

In the $\alpha 2$ group, the branching pattern for the primate, artiodactyl, perissodactyl, and carnivore viruses is congruent with the pattern for their host lineages, at least for the level of detail shown in Figure 2; the comparison is effectively at the level of order in mammalian taxonomy. The structure of the $\alpha 1$ portion of the tree is a little more complicated: this group comprises primate viruses, except for the occurrence of one bovine herpesvirus (BHV-2) and two marsupial herpesviruses (MaHV-1 and MaHV-2). Setting aside these anomalies, the branching pattern of old world primate (OWP) and new world primate viruses follows the pattern for the host lineages. Thus, the $\alpha 2$ clade displays characteristics of cospeciation for four orders of placental mammals, while the $\alpha 1$ clade evinces this characteristic for primates only. The fact that primate virus lineages occur in both these groups in a manner consistent with cospeciation leaves unresolved the nature of the original divergence event between the $\alpha 1$ and $\alpha 2$ lineages. We cannot reliably distinguish with available data the branching orders for the lineages of BHV-2, the marsupial viruses, and the OWP viruses. We suppose that BHV-2 and the marsupial viruses arose from two separate transfers

from OWP viruses at some distant time following separation of the OWP and new world primate host lineages. The nearest relatives of the HSV species are in the clade labelled as OW monkey viruses in Figure 2.

Three such viruses are represented in the tree, namely simian B virus (SBV), simian agent 8 (SA8), and herpesvirus papio 2; these have the formal taxonomic names of Cercopithecine herpesvirus 1, 2, and 16, respectively. These OW monkey viruses were placed in the tree shown on the basis of only one gene, that for virion glycoprotein B. However, their relationship to the HSV species and other species in the immediate $\alpha 1$ locality has been confirmed by data for several other genes (*US3*, *US4*, and *US6*; not shown) and is regarded as secure.

Relationships of the HSV Species and Other OWP Viruses

Establishing the origins of the HSV species in the context of their development within the OWP virus clade turns out to be an uncertain undertaking, for several distinct reasons. A major consideration is the paucity of data available for $\alpha 1$ viruses of nonhuman OWPs. As shown in Figure 2, there is a well-defined clade of OW monkey viruses, and the HSV species lie in a sister clade that is unambiguously separated from the OW monkey virus clade by an extended branch. However, the HSV species are the only viruses in this sister clade: there are *no* viruses of other hominoids represented (i.e., species of chimpanzee, gorilla, orangutan, and gibbon), which we might expect to be associated with the HSV clade. Overall, there is surprisingly little information available on $\alpha 1$ herpesviruses of apes. Papers from the 1980s and earlier described HSV-like infections in captive apes, with isolation of viruses that by the criteria of the day were very similar to HSV (10,11). Surveys of sera from captive and wild apes detected antibodies against HSV-like viruses (11,12). However, we have found no recent account of isolation or characterization of such viruses, let alone DNA sequences. This lack of description for a class that we believe must include the closest relatives of the HSV species is a seriously limiting factor for evaluation of the immediate antecedents of HSV-1 and HSV-2.

The next complexity concerns the relationship between the HSV species. HSV-1 and HSV-2 are each other's closest known relative, but their genomes are actually quite diverged. Based on recent phylogenetic analysis (4), our current best estimate is that the two lineages diverged 9 million years ago. Allowing for the uncertainties of such a calculation, it remains clear that the separation is of considerable antiquity. For comparison, the spread of modern humans across the planet took place over the last 0.1 million years, while divergence of the human lineage from those of other hominoids took place between 5.5 (chimpanzee) and 14.6 (gibbon) million years before the present (13). Thus, we estimate that splitting of the two HSV lineages occurred long before the emergence of modern humans, and within

approximately the same timeframe as speciation of the hominoids. We therefore arrive at the evaluation that key to understanding the evolutionary context of the divergence of HSV-1 and HSV-2 would be a phylogenetic tree incorporating related viruses of hominoids—which certainly exist but for which we have no data at all. We also wish to enter reservations regarding use of any argument for cospeciation with host in considering the emergence of the HSVs. The instances outlined above for which postulating occurrence of cospeciation apparently worked well concerned high-level taxa (mammalian orders) and dates involving several tens of millions of years, but it is easy to imagine that the situation might become more complicated with closely related sets of species and more recent divergence dates.

The occurrence in a single host species of more than one herpesvirus of a given genus and with quite closely related genomes, as with HSV-1 and HSV-2, is not uncommon. Other cases are known, for example in the $\alpha 2$ group with artiodactyl and perissodactyl viruses and in the $\gamma 2$ group with primate viruses (14–16). We imagine that such cases could arise by several routes, such as an episode of geographic isolation of two host populations followed by re-establishment of contact, or divergence of virus lines in two related host species followed by transfer between these hosts, or by evolution within one host species into a novel tissue niche. The present day differential propensities of HSV-1 and HSV-2 are compatible with the last of these, although it is to be noted that there is no absolute difference in their capability for growth in different tissues. Overall, we do not perceive any cogent reason for supporting any particular speciation scenario. Gentry et al. (17) proposed that changes in mating habits of human ancestors provided conditions for divergence of HSV-1 and HSV-2, but we regard this hypothesis as over-specific, given the various possibilities.

Our general vision of the lineage leading to HSV, in the timeframe of the last 10 or 20 million years, is of existence in hominoid species, with separation into separate HSV-1 and HSV-2 lineages perhaps midway in this period, and probably with many related virus species in the evolving range of hominoids. The fact that HSV-1 and HSV-2 appear to us as closest relatives may be misleading, given the lack of appropriate comparisons. Viewing the HSV species separately, we regard the following as plausible scenarios. First and most simply, each HSV might be the human-specific member in one or the other of two distinct groups of hominoid viruses each conforming to cospeciation relationships. Next, they might instead have a closer relationship with viruses of nonhuman hominoids: the HSV species might be very similar to ape viruses as a result of interspecies transfer. Such multiple transfers could generate mixing of lineages for human and nonhuman viruses; examples of this last mode are provided by lineages of primate immunodeficiency viruses (on a much shorter timescale) (18) and primate hepadnaviruses (hepatitis B viruses) (19). Lastly, there is a third class of possible descent of the HSV lineage: the relationship of HSV to other primate viruses might be

neither cospeciation nor especially close to other hominoid viruses; for instance, transfer from some nonhominoid host might have occurred.

EVOLUTION OF ALPHAHERPESVIRUS GENOME STRUCTURES AND GENE SETS

Evolution of Genomic Unique and Repeat Elements

All alphaherpesvirus genomes contain long and short unique regions (U_L and U_S) and a pair of large repeat elements that flank the U_S sequence in opposing orientations (R_S). Some, including HSV-1 and HSV-2, also possess a distinct pair of large repeat elements that flank U_L (R_L). These large-scale features are illustrated for HSV in Figure 3, with conventions of genome segment naming. All of the ancestral genes, common to the three subfamilies, lie in the U_L component. An equivalent arrangement of long and short unique sequences with flanking repeats is also found in the *Cytomegalovirus* genus of the Betaherpesvirinae, but the U_S , R_S , and R_L components of these betaherpesvirus genomes are considered to be unrelated to the alphaherpesvirus sequences. It thus appears that the alphaherpesvirus S region (U_S plus its flanking R_S copies) emerged after divergence from the Beta- and Gammaherpesvirinae and before the appearance of the $\alpha 1$ to $\alpha 4$ lineages.

For the purposes of this analysis, we regard R_L elements as comprising some thousands of base pairs (and containing protein coding genes) and exclude the much smaller similarly placed sequences (of several tens of base pairs) that are found in $\alpha 2$ genomes. By this definition, R_L elements occur only in the $\alpha 1$ and $\alpha 3$ groups. However, on the basis of their sequences and gene contents, $\alpha 1$ R_L and $\alpha 3$ R_L are unrelated (20). We therefore regard as likely that the common ancestral alphaherpesvirus genome was of form

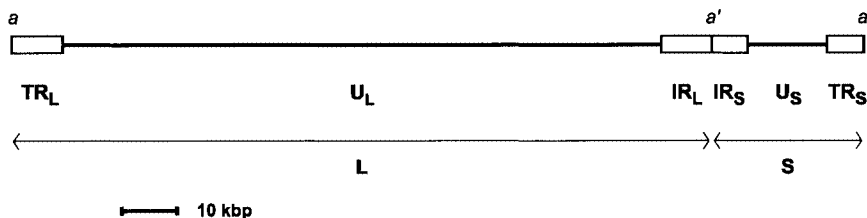


Figure 3 Gross genome structure of HSV. The linear form of the HSV genome as found in virions is depicted. Regions of unique sequence (U_L and U_S) are shown as heavy lines, and major flanking repeat elements as open boxes. The terminal copy of R_L (designated TR_L) is oppositely oriented to the internal copy IR_L , and similarly for TR_S and IR_S . The unit consisting of TR_L – U_L – IR_L is termed the long or L region, and IR_S – U_S – TR_S the short or S region. The locations are indicated by short direct repetitions at the genome termini (a sequences) and of an oppositely oriented copy at the junction between IR_L and IR_S (a'). A scale bar is shown at the foot.

U_L - IR_S - U_S - TR_S , and that R_L elements subsequently developed independently in the $\alpha 1$ and $\alpha 3$ lineages. Inasmuch as HSV R_L contains the ICP0 gene while in $\alpha 2$ genomes the homologous gene is present as a single copy in a near-terminal location within U_L , the $\alpha 1$ R_L elements must have originated by duplication of a region that previously constituted one extremity of U_L . In the $\alpha 1$ group, HSV-1, HSV-2, the OW monkey viruses SBV and SA8 (21,22) and BHV-2 (23) all possess R_L elements, as do MaHV-2 (24) and HVS-1 (25), so we can conclude that R_L developed at an early point in the $\alpha 1$ lineage. However, it has been reported that MaHV-1 (26) lacks R_L regions, and the branching pattern for the $\alpha 1$ sublineages in Figure 2 would then suggest that R_L repeat pairs were present in the genomes of ancestors of MaHV-1 but that one R_L copy has been lost in MaHV-1.

Genes Specific to the *Simplexvirus* Genus

We consider that HSV-1 and HSV-2 possess equivalent sets of genes, which encode 74 distinct proteins (27,28). This estimate excludes some proposals for additional genes that we regard as unproven and leaves aside as unresolved any protein coding function of the latency-associated transcript locus. Forty-three of the HSV genes belong to the ancestral set. In principle, the other 31 HSV genes might have arisen early in the development of the Herpesviridae and then been lost in other lineages, or have first appeared after the early Alphaherpesvirinae lineage split from that leading to the Beta- and Gammaherpesvirinae. We regard as more plausible that new acquisition within the Alphaherpesvirinae was the predominant route and for the present discussion we treat this as the *only* mode. Twenty-two of these 31 genes are also represented in the complete sequences of avian *Mardivirus* genomes (20), present before the $\alpha 3$ lineage diverged from those leading to $\alpha 1$ and $\alpha 2$. Three of the remaining nine HSV genes have counterparts in the $\alpha 1$ lineage [and one of the three also has an $\alpha 4$ homologue (29)], so these were also acquired at an early stage in the development of the subfamily. This leaves six HSV genes that are considered to have arisen within the $\alpha 1$ lineage, namely *UL56*, *RL1*, *US5*, *US8A*, *US11*, and *US12*. The only complete genome sequences presently available for viruses of the $\alpha 1$ group are of HSV-1 and HSV-2 (27,28) so that our view of when the six $\alpha 1$ -specific HSV genes entered the lineage is incomplete. We know from limited sequence data that the OW monkey viruses SA8 and SBV have homologues of five of these HSV genes, namely *UL56*, *US5*, *US8A*, *US11*, and *US12* (30,31) (A. Dolan and D. McGeoch, unpublished data), but there is no counterpart of the sixth gene, *RL1*, at the corresponding locus in the genomes in these OW monkey viruses (A. Dolan and D. McGeoch, unpublished data). However, there is a homologue of HSV *RL1* in the marsupial virus MaHV-1, in an apparently equivalent genomic location (32). Thus, given the lines of descent shown in Figure 2, we have to propose either that the *RL1* gene was gained before divergence of

the marsupial virus and OWP virus lineages and then lost in the OW monkey virus clade or—perceived as less likely—that the gene was independently gained in the marsupial virus and HSV lineages. Our interim conclusion is thus that none of the HSV complement of genes is unique to HSV-1 and HSV-2, and all may have been present in the $\alpha 1$ lineage before the divergence of the HSV and OW monkey virus clades.

Evolution of Genomic S Regions

A number of attributes of the alphaherpesvirus S region indicate that it has experienced a pronounced level of evolutionary activity. Five of the six $\alpha 1$ -specific HSV genes are in S. Among these, *US12* and also *US8A* may have evolved de novo (28). HSV U_S also contains five genes for virion surface glycoproteins, three of which have apparently evolved by way of duplication and subsequent divergence, namely *US4*, *US6*, and *US7*, encoding glycoproteins G, D, and I, respectively (33). Gene *US5* encodes a very small type 1 glycoprotein, which plausibly might be a member of the same gene family that has undergone extensive deletion. Comparisons of the locations of the boundaries between U_S and R_S across the range of alphaherpesvirus sequences show that the boundaries are dynamic—in some genomes a given gene may be in U_S while in others it is located across the border in R_S (34). This phenomenon is considered to have its basis in partly illegitimate recombination between genomes, with one homologous crossover between copies of R_S and one heterologous crossover involving two loci in U_S copies; an equivalent mechanism is presumed to have been involved in genesis of $\alpha 1$ R_L elements. R_S elements typically have a higher G+C content than the unique portions of the same genome; in the HSV species, R_S exhibits the highest G+C content of the genome, at 80% G+C over its 6.7 kbp. This feature too may be a result of recombination processes, with a biased gene conversion mechanism acting to drive shift in overall base composition (35,36). The R_L element of HSV has similar characteristics to R_S , with a high G+C content (72% in HSV-1, 75% in HSV-2). The R_S and R_L elements are involved in generating genome orientation isomers by recombination during normal virus replication so that with HSV four isomers in total are produced, with each genomic region (S and L) occurring in either orientation with respect to the other.

Comparison of Genes of HSV-1 and HSV-2

While neither HSV-1 nor HSV-2 possesses any genes absent in the other, their coding sequences are moderately diverged. Alignment of the two sets of coding sequences (excluding *US4*, which is grossly truncated in HSV-1) requires introduction of gapping characters equivalent to 2% of the alignment length. These aligned coding regions have an average of 0.14 non-synonymous substitutions per nonsynonymous site, while the corresponding figure for synonymous substitution is 0.47 (28). There are only two

genuinely striking instances of differences between HSV-1 and HSV-2 in the organization of equivalent genes, which concern genes *US4* and *RL1*.

HSV gene *US4* encodes the virion surface glycoprotein G (gG), and has homologues in other $\alpha 1$ viruses, in most $\alpha 2$ viruses and in ILTV ($\alpha 4$). The HSV-2 *US4* coding region comprises 699 codons and specifies a type 1 transmembrane protein, with an N-proximal “head” domain containing Cys–Cys disulfide bonds and a heavily *O*-glycosylated extended “stalk” between the head and the transmembrane anchor (33,37). The Cys-bonded domain is homologous to N-proximal regions in glycoproteins D and I, encoded by genes *US6* and *US7*. In contrast, the HSV-1 *US4* gene contains only 238 codons, and has evidently suffered a large deletion near its 5' end so that it encodes a “headless” variant of the glycoprotein. Neither the HSV-1 nor the HSV-2 protein is essential for virus growth in vitro (38,39). Their roles remain obscure; recently, a chemokine-binding activity was detected in some $\alpha 2$ gG species (but not, as yet, in HSV) (40). The continued existence of the HSV-1 version argues that the truncated protein does have a significant function, which is reported to be in allowing virus entry to cell apical surfaces (38). In the absence of a common defined role, we can speculate that this divergence between the two HSV types might represent a significant difference in function.

As discussed above, gene *RL1* is specific to the $\alpha 1$ lineage. This gene is an important determinant of virulence (41–43). The encoded protein ICP34.5 has a 63 amino acid C-proximal domain that is strongly similar to a domain of a host cell protein; evidently the gene has been captured, at least in part, from a host genome (44). The *RL1* gene provides the only example of a difference in exon/intron organization between HSV-1 and HSV-2 genes: in HSV-2 it has two coding exons separated by an intron, while the HSV-1 version is intronless (44,45). With availability of the human genome sequence, we have ascertained that in the homologous human gene [*GADD34* (46) or GenBank *PPP1R15A* in chromosome 19] there is an intron whose acceptor site is located exactly equivalently to the acceptor site of the HSV-2 gene's intron, at the upstream end of coding sequence for the conserved domain. This could be taken as support for the intron-containing HSV-2 version of the *RL1* gene being the earlier form in evolutionary development in $\alpha 1$ herpesviruses, with the gene's capture from a host cell having been by way of an unspliced nucleic acid species, whether unprocessed RNA transcript or genomic DNA.

CLASSES AND MECHANISMS OF HSV GENOMIC VARIATION

Nucleotide Substitutions

We now turn to more recent events and processes in HSV evolution. Although the most recent common ancestors of contemporary HSV-1 and

HSV-2 lineages appeared recently enough that large-scale evolutionary divergences have not occurred within each species, ample time has passed for substantial variability to accumulate within populations and for populations to diverge significantly from one another. This variability and divergence are potentially informative about aspects of virus demography and population history, with relevance to epidemiology and the coexistence of host and virus populations.

Molecular variation provides a record of genetic changes, which can be used to estimate rates and timescales of evolution, while deviation from the patterns of variability expected under a standard, neutral model (47) can be used to examine whether interesting processes such as selection, changes in demography, genetic structuring, and migration may have occurred in the history of one or more populations. The existence of genomic variation within each of the HSV species has long been recognized (48–52). However, its molecular details and the molecular and population processes that produce detectable patterns of HSV variability are still not fully characterized.

Nucleotide variability in HSV takes several forms. Of these, nucleotide substitutions are the type of genetic change most relevant to estimating evolutionary rates in phylogenetic and population genetic approaches, whose models of sequence change typically and do not accommodate insertion–deletion events or other types of sequence rearrangement. Substitution polymorphisms in HSV have been assayed both directly by sequencing specific regions of the genome and indirectly from patterns of restriction endonuclease cleavage, which yields a picture of variability patterns across the genome. With yearly rates of substitution as low as 10^{-8} to 10^{-7} per nucleotide, it would be difficult to estimate directly the rate of misincorporation by the HSV DNA polymerase complex in each round of replication, for example by sequencing progeny DNA. Instead, assays have been used which measure the appearance of spontaneous loss-of-function mutations in the thymidine kinase gene in the absence of drug selection (53). By this approach, HSV-2 has a higher spontaneous mutation rate than HSV-1 (54,55) but, because it is biased towards frameshift mutations and nonsynonymous substitutions at functionally critical sites and because mutations accumulate over multiple cycles of DNA synthesis, such an assay is more suited to providing comparisons than absolute estimates of the misincorporation rate. Two activities of the DNA polymerase have been identified that affect replication fidelity: exonuclease-deficient polymerases have enhanced substitution rates, while some polymerases conferring phosphonoacetic acid resistance possess increased nucleotide selectivity, reducing the rate of substitution (56–58). As more sequence data become available, it will be possible to characterize further the nucleotide substitution process in terms of the mutation spectrum in synonymous or noncoding sequences, and to compare such data with patterns of substitution inferred from phylogenetic analyses.

Variable Tandem Repeats

Low complexity or repetitive DNA sequences have long been known as a feature of HSV genomes [(27,35,59–61), reviewed in Ref. 62]. Their high variability may contribute to HSV evolution by providing a mechanism for rapid changes in genome and protein structure. Homopolymeric runs are the simplest form of reiteration and the proliferation of C_n and G_n motifs in HSV sequences, apparent on even a casual inspection, can be ascribed to the high G+C content of HSV genomes. Nucleotide insertions or deletions at such sequences are common and are responsible for frameshifting in the thymidine kinase gene associated with the bulk of cases of acyclovir resistance during natural infection or in culture (63,64). Similar homopolymer instability in the *US4* gene of HSV-2 also leads to gG protein truncation in a small proportion of natural isolates (65), indicating short-term dispensability for infection or perhaps the trade-off between a so far unknown function and immune escape. In the above cases, mutant viruses probably do not persist in virus populations, but instead result from new mutation events. Variable length homopolymers are also common in intergenic sequences (R. Bowden, unpublished data), where instability increases with tract length. Template slippage during DNA synthesis is a likely explanation for this mutability; however, recombination may also play a role. There are lower frequencies of C_n and G_n tracts in coding sequences than in noncoding sequences in both the HSV-1 and HSV-2 genomes and this effect becomes marked for n of 6 or more, with no runs of more than 10 identical bases in coding sequences, while noncoding sequences may have up to 19 successive identical nucleotides (R. Bowden, unpublished work). There is evidently selection against longer runs in HSV coding sequences, presumably due to their enhanced frameshifting rate, an explanation that is substantiated by the finding that the DNA sequences encoding repeated proline or glycine motifs contain lower proportions of homopolymeric (CCC or GGG) codons than do those encoding single and tandem prolines and glycines.

HSV DNA also contains many reiterated sequences with longer repeat units, of which the best known occurs within the *a* sequences (66,67), but which also include three imperfect repeats of a 54 nt sequence in the first intron of HSV-1 *RL2* (68) and various other reiterations catalogued in the HSV-1 and HSV-2 complete genome sequences (27,28,35,60,61). Some of these are known to be highly variable between strains. The mutational mechanism responsible for variation of longer tandem repeats is not well characterized; for *a* sequence repeats at least, recombination of mispaired repeat arrays is implicated (69). The impression given by the composition and variability of reiterations in coding sequences is that they probably contribute to protein function through general biophysical properties of the encoded amino acids rather than as critical structural units. Consistent with

this view are variable repetitive acidic or basic motifs: (Glu)₇₋₉ encoded within HSV-1 *US4* (70) and (Arg)_n in HSV-1 *RL1* (71). Other variable repetitive motifs are proline rich, and therefore might be expected to form unstructured polypeptide domains of variable size. An extreme example is the approximately 10–100 reiterations of (Pro–Gln) encoded by repeats of CCCC^a/_G near the 3' end of HSV-1 *UL36* (27,72), with variation detectable between isolates and also at smaller scales between clones of the same isolate. The equivalent locus in HSV-2 contains a 15 nt repeat encoding (Pro–Gln–Pro–Pro–Leu), which is also variable, indicating a similarity of amino acid content but a measure of evolutionary divergence between the HSV-1 and HSV-2 proteins.

A selection of variable tandem repeat loci in *U_S* and the *R_L–R_S* segment of the HSV-1 genome (73) has been termed “common type variation” (CTV) (74) and characterized as a potential tool to distinguish closely related strains more effectively than even a large panel of restriction endonucleases, since their mutation rate far exceeds that of single nucleotides. CTV is detected as hypervariability in the lengths of specific restriction fragments overlapping the CTV loci, and is often manifested as ladders of fragments, differing in length by one repeat unit, for a single isolate. Most recently, CTV has been used to demonstrate the diversification of the latent population of HSV genomes during natural persistent infection (75), suggesting either reinfection of single neurons or that a reactivation episode may involve several latently infected neurons seeded by successive recurrences.

Recombination in HSV DNA Replication and Biology

Homologous recombination, mediated by the viral DNA replication machinery (76), has long been recognized as the mechanism underlying HSV genome isomerization (77). However, there has been a continuing lack of information about the molecular details of both replication and recombination in HSV. Current models of HSV recombination take account of the close analogy that has become apparent between HSV replication and recombination-dependent DNA replication in phage, bacteria, and yeast (78–81). Theoretical arguments about the necessity for genetic exchange in maintaining a population's fitness (82) notwithstanding, an inescapable conclusion of observations concerning the putative mechanisms and origins of recombination in HSV is that, as has been asserted for other organisms (83), its primary significance is in ensuring successful replication.

Apart from genome isomerization, the detectable consequences of recombination during HSV replication include inversions, insertions and deletions of repeated sequences, the exchange of homologous DNA fragments in doubly infected cells, and perhaps, as mentioned earlier in this chapter, an effect on the G+C content of HSV DNA through a biased

mechanism for resolving heteroduplex replicative intermediates. Recombination occurs at high rates between heterologous strains *in vitro*, with cross-over rates estimated in the range 0.2–0.7% per 1000 bp (84–88), and in coinfections of experimental animals (89–91).

Evidence from patterns of nucleotide variability in populations has recently demonstrated that recombination is also readily detectable in natural HSV-1 infection (92). In contiguous sequences, recombination is manifested as homoplasies, and estimated rates of recombination are comparable to the rate of new substitutions (92). In data from widely separated loci, recombination is apparent as reassortment of markers so that different sequence fragments show different patterns of similarity between strains (92). In retrospect, reassortment consistent with recombination is evident in thymidine kinase and gB gene variability for both HSV-1 and HSV-2 (93), although it went unnoticed in the original analyses.

The conceptual and practical effects of finding high levels of recombination in HSV-1 natural infection on our understanding of the genetic patterns in virus populations are substantial: (i) genetic exchange increases the probability that individual deleterious mutations are lost from the population by selection (82), though the importance of this effect in HSV is difficult to quantify; (ii) similarly, the reassortment of uniquely occurring mutations has dramatic implications for the selection of advantageous combinations of variants, so the rate of virus evolution in response to environmental stimuli is potentially increased (82); (iii) instead of a clonal evolutionary process in which all segments of DNA along the genome are assumed to share the same history, virus genealogies must now be thought of as complex, non-tree-like structures. Therefore, phylogenetic (tree-based) approaches become inapplicable because trees are an inadequate representation of the relationships between strains in a population and even networks capable of representing limited recombination are useful only for short contiguous sequences (92). Adding recombination to models of sequence evolution increases the complexity of computations, for example in estimating the age of a particular mutation in the context of population history. On the other hand, the presence of recombination, with the incorporation of many partially independent genealogies into a single data set, means that there is more information available to infer the underlying population history than if the whole genome were evolving as one locus (94,95). We note that in interspecies studies the working assumption of congruent phylogenies for each gene remains, to the best of our present understanding, unaffected by intraspecies recombination.

In view of HSV's propensity for recombination when the opportunity is presented, it is clear that infection with two or more strains at the host and cellular level is the key stage in the formation of recombinants, and the immunological, epidemiological, and biological factors influencing coinfection will be important in understanding the interactions of strains in

populations, where previously such competitive or genetic interactions were treated simplistically or overlooked.

STUDIES OF HSV VARIABILITY USING RESTRICTION SITES AND DNA SEQUENCES

Sources of Information About HSV Variability

Patterns of population variability are the key to characterizing contemporary molecular evolution in HSV. Comparisons made between HSV types, among genes and among populations are each relevant to different aspects of our developing understanding of HSV evolution. Diversity can be estimated directly from sequence alignments for specific genes, or indirectly from single nucleotide polymorphisms assayed using variation at restriction sites for genome-wide estimates. Throughout this section, we focus on mean pairwise diversity [π , the average number of substitutional differences between pairs of sequences in a sample, divided by the length of sequences compared (96,97)] as a means of comparison. Sequence data come from public databases and published studies for *UL44* (gC) (98–100), *US4* (gG) (70,101), *UL23* (thymidine kinase) (102–104) for HSV-1 and HSV-2 and for several genes in an extended study of HSV-1 variability (92).

Genotyping of HSV isolates using panels of restriction endonucleases has proved useful in epidemiological and evolutionary contexts. In early studies, it was established that isolates of HSV-1 or HSV-2 that are epidemiologically unrelated are generally distinguishable by their restriction patterns (48,49,51,52,105), and conversely that successive isolates from the same individual are usually identical or almost so (106–108). A qualitative biological interpretation of these findings is that carriers of HSV-1 or HSV-2 are usually infected on a single occasion with one homogeneous virus strain and that subsequent infections, if they occur, do not lead as efficiently to reactivatable latent infection. Furthermore, comparisons of siblings' HSV-1 patterns suggest that the virus is regularly transmitted within families (109). Restriction profiling therefore provides a means to distinguish reliably the majority of isolates in population samples that may also be used to characterize strain variation between populations (52) and provide an unbiased estimate of molecular diversity (π) across the genome (96,110). The bulk of restriction profiling studies of HSV population evolution are by Sakaoka and co-workers, focusing on East Asia, Sweden, the United States, and Kenya, and investigating hypotheses about the relationships between human and HSV population histories (108,110–116).

Comparisons of HSV-1 and HSV-2 Variability

Although there is relatively little information that allows a direct comparison of diversity levels between HSV-1 and HSV-2, a consistent pattern of

lower diversity in HSV-2 than in HSV-1 is evident. From comparisons of sequence data for *UL44*, *US4*, and *UL23* genes, we can conclude that HSV-1 is around 3–5 times more diverse than HSV-2. However, no available single-gene sequence data set is ideal: either populations are not matched (*UL44*), or a sample has had to be collated from multiple studies (*UL23*), or a functional divergence between orthologous sequences is suspected (*US4*). Relatively few variable restriction sites have been assayed for HSV-2, but restriction patterns tend to confirm that this pattern is not a chance observation at specific genes (108,110,117) and comparisons of HSV-1 and HSV-2 variability by RNaseA mismatch cleavage examining *UL23* and *UL27* also indicate higher diversity for HSV-1 than HSV-2 (93).

Under the standard neutral population genetic model, the expected molecular diversity is proportional to the product of the molecular mutation rate per generation (i.e., per transmission from one host to another) and the effective population size (118), and does not depend on, for example, mean generation time. Accordingly, there are three broad explanations for the lower diversity found in HSV-2 than in HSV-1. First, HSV-1 may have a higher mutation rate per host infection (virus generation) than HSV-2. However, this seems unlikely, since HSV-2 DNA polymerase is if anything the more mutagenic (54,55) in each round of productive cell infection and there is little reason to suppose that HSV-1 undergoes many more generations of cell–cell transmission per host infection than does HSV-2. Second, there may be real differences in the time (number of virus generations), since each virus became established in the sampled human populations. This would imply the existence of an unsampled (human or animal) population in which HSV-2 has existed throughout human history, a possibility that requires investigation. A third class of possibilities involves epidemiological factors: either the higher diversity in HSV-1 may simply reflect its higher prevalence (i.e., absolute population size) over a long period, or differences in the way HSV-1 and HSV-2 are transmitted may affect the relationship between prevalence and effective population size for each virus. If HSV-1 is transmitted predominantly within families and HSV-2 occurs in clusters of epidemiologically related cases, then it follows that the number of new infections arising from each HSV-2 infection will be more variable than that from each HSV-1 infection, so genetic drift in HSV-2 populations will be faster and HSV-2 should then sustain fewer distinct lineages and lower molecular diversity. In our view, a combination of the second and third factors is likely to be important in explaining the differences in variability between HSV-1 and HSV-2.

Molecular Diversity Across the Genome

We know of no studies whose primary aim was to compare patterns of variability between many different genes of HSV. For HSV-2, the sequences

of three glycoprotein genes were determined in five strains and revealed a higher density of variable sites in *UL27* (gB) than in the genes for gC and gD ($\pi = 0.0035$ vs. 0.0012 , 0.0015 nt⁻¹) (100). However, such samples are a little small for statistical reliability. The 3' part of HSV-2 *US4* ($\pi = 0.0030$ nt⁻¹) (101) is comparable to *UL27* in diversity.

For HSV-1, there is more information about levels of diversity for different genes. Data are available for genes *UL23*, *US4* (70), and *UL44* (98) and from a survey of molecular sequence diversity in global HSV-1 populations (92,119), covering at least substantial fragments of the following genes: *UL4*, *UL5*, *UL40*, *UL41*, *US4*, and *US5*. The most variable HSV protein-encoding sequence so far reported is HSV-1 *US4*, with approximately one difference per 100 nt ($\pi = 0.010$ nt⁻¹) between randomly chosen pairs of isolates from Europe (70,92). *UL23* diversity is on a similar scale to *US4* in both HSV-1 and HSV-2, but ad hoc comparisons such as these are hampered by the small size of most samples or differences in the populations studied.

Heterogeneity in the constraint on amino acid replacements is evident in the widely variable ratio of nonsynonymous to synonymous substitutions (K_a/K_s) amongst HSV-1 genes. Data from genes belonging to different functional classes fit with broad expectations: K_a/K_s ranges from 0.05 in *UL40*, encoding the small subunit of ribonucleotide reductase, a protein that is highly conserved on evolutionary timescales, to 0.5–0.6 in the virion glycoproteins *US4* (gG) and *US5* (gJ) (92). Constraint on the gene for gC, a glycoprotein with an essential role in virus entry, is stronger than in *US4* and *US5*. Variation in the rate of nonsynonymous substitution rather than in overall mutation rate therefore appears to be primarily responsible for observed differences in gene diversity in HSV-1. K_a/K_s measures from intraspecific comparisons seem consistent with the genome-wide ratio of nonsynonymous to synonymous divergences of *c.* 0.3 for the comparison of HSV-1 and HSV-2 earlier in the chapter.

The *US4* gene features several times in this chapter, in discussions of HSV evolution both at inter- and intraspecies timescales. *US4* encodes gG, the only protein whose structure is grossly different in HSV-1 and HSV-2, implying divergence between the species in functions that are not yet completely defined but for which there are some clues (38,40). In line with its large-scale divergence between types, in population samples *US4* is the most variable HSV gene yet described, implying at least the possibility that positive selection on a subset of amino acid positions may drive diversification. In addition, gG is a prominent target of the humoral immune response, used in type-specific serological assays that exploit reactivity to epitopes in both conserved and nonconserved domains (see Chap. 4). While techniques to identify sites undergoing conventional positive selection are impaired by the high rates of recombination affecting HSV sequences, the possibility has also been explored that HSV-1 *US4* might be the object of

balancing selection (92), a form of positive selection characterized by the stable existence of two or more lineages along which both synonymous and nonsynonymous substitutions accumulate, creating deeper branches in the gene genealogy than would be expected under a neutral model of evolution. This proposition is supported by the existence of two clusters amongst European HSV-1 *US4* sequences (120) and by the fact that several replacement polymorphisms coincide with known humoral epitopes, providing an attractive potential mechanism for immune-mediated selection. Although tantalizing, we do not consider this evidence to be conclusive proof of balancing selection in the absence of comparative data from other loci. The attention given to the evolution of *US4* serves to emphasize the lack of information about other genes that are likely to be of similar interest because of their important or novel functions, including for example homologues of cellular proteins and proteins that interact with the host cell or immune system, either of which might undergo various kinds of non-neutral evolution.

Population Molecular Diversity

Comparisons of molecular variability between populations of a single HSV species may tell us about differences in virus population history, and about the relationships between virus populations, which in turn may reflect aspects of host population history (92,110). The magnitude of molecular diversity within populations provides a rough guide to long-term effective population size and the timescale of population variability, while the patterns of sharing of variants and lineages may be informative about relationships between virus populations; these patterns are considered in the following section.

For HSV-1, pairwise diversity estimates from restriction profile data (110) and from sequence variability in parallel population samples (92,119) are in general agreement, with π in the range 0.002–0.010 nt⁻¹ in different populations, depending on the gene analyzed. European populations have higher diversity than Asian populations, and limited data indicate that African diversity is higher still, presumably reflecting the long history of human populations in Africa. Calculations based on a median diversity estimate of 0.005 nt⁻¹ show that observed diversity levels are consistent with virus population ages similar to human population ages (*c.* 10⁵ years) (92) for mutation rates at the upper end of published estimates, around 10⁻⁷ nt⁻¹ yr⁻¹ (8). Referring to comments earlier in this chapter, we note that the genomic divergence between HSV-1 and HSV-2 is about two orders of magnitude greater than the diversity of HSV-1 in populations, and so represents a proportionately longer timescale of *c.* 10⁷ years. The similarity of this figure to the estimated time of the split between HSV-1 and HSV-2 divergence dates is of course expected since substitution rates for HSV have

mostly been calculated using alphaherpesvirus interspecies comparisons (8). An alternative approach is to assume that virus and human populations share the same timescale, using population diversity or divergence data to estimate substitution rates. Sakaoka et al. (110) used restriction patterns and an assumed date for the split between African and European populations of 110 thousand years ago to calculate a genome-wide average substitution rate of $3.5 \times 10^{-8} \text{ nt}^{-1} \text{ yr}^{-1}$, which is in especially close agreement with the previously quoted rate once a correction for the proportions of synonymous and nonsynonymous substitutions is included (92).

The limited information about HSV-2 diversity in different populations (108,113) justifies only very general comments: (i) HSV-2's lower diversity within populations suggests that it has accumulated variability over a proportionately shorter timescale than has HSV-1; however, as stated previously, this does not necessarily constitute evidence of a shorter population history. Data on strain divergence between populations will be helpful in establishing whether HSV-2 has existed continuously in all human populations or has spread relatively recently. (ii) Calculations from data in Ref. 108, analyzing restriction site polymorphism in 309 HSV-2 isolates from Japan, Korea, Sweden, and the United States, suggest the highest population diversity in Japan. However, the strong possibility of bias concerning the choice of variable restriction sites typed in a study dominated by East Asian isolates indicates that further investigation is required to reliably assess HSV-2 variability in world populations.

STUDIES OF HSV-1 POPULATION RELATIONSHIPS AND ORIGINS

Restriction Site Data and Japanese Human Origins

Most investigations of the relationships between populations of HSV have used restriction profiling. Early on it became clear that countries with close anthropological relationships had similar allele frequencies at many variant sites and shared many restriction patterns, while populations without such ties or from different continents tended to have distinct sets of variants and profiles. An analysis of the distributions of HSV-1 strains in six countries (Japan, Korea, China, Kenya, Sweden, and the United States) (110) summarized and extended earlier studies (111,112,114), providing estimates of within- and between-population diversity and a qualitative description of patterns of profile sharing. The picture of host population-dependent HSV-1 evolution that has built up from timescale estimates is supported by qualitative observations: European and Asian HSV-1 populations are less divergent from each other than either is from an African population, while all non-African populations studied are similarly diverged from an African population, as expected if continental HSV-1 populations were founded at

the same time as host populations (110) under prevailing models for the origins of human populations (121). Just as for quantitative estimates of timescales, these findings are consistent with population-level human-virus coexistence rather than with substantial recent virus spread between populations.

If HSV-1 variability does reflect events in the shared history of virus and host, it may be useful in investigating hypotheses about particular human populations. The “dual structure model” for Japanese origins was proposed by Hanihara (122) and is supported, at least in part, by archaeology, physical anthropology, and human genetics (123–125). In brief, it contends that modern Japanese human populations comprise descendants of two main waves of human colonizers: the Paleolithic Jomon hunter-gatherers from Southeast Asia who arrived by *c.* 10,000 years before the present, when Japan became isolated from mainland Asia by rising sea levels following the last glacial maximum; and the Neolithic Yayoi Period settlers who arrived from *c.* 2300 years ago from the Korean peninsula and partially supplanted the previous inhabitants. Under the hypothesis, physical and genetic distinctions between the people in different parts of Japan are manifestations of these two waves. Umene and Sakaoka (115,116) identified two dominant restriction profiles in one Korean and several Japanese HSV-1 populations (see map, Fig. 4). The most common profile in most of Japan, known as F1, reaches frequencies of almost 50% in Tottori in the south of the main Japanese island Honshu and Kagawa on nearby Shikoku, and is also the most common profile in Korea, comprising *c.* 30% of isolates there. Moving out through Japan, the frequency of F1 decreases and it is partially replaced by another profile, F35. In Hokkaido, the northernmost large island of Japan and home of the Ainu, putative descendants of Jomon era settlers, F35 occurs in 14% of isolates to F1’s 8%. A parsimonious explanation of these frequency differences involving variable contributions of two distinct ancestral virus populations supports the “dual structure model,” the idea that human and virus populations are related at a range of timescales and the proposition that HSV-1 populations may retain geographic strain patterns for thousands of years at a time.

A potential objection to the above analysis is that it draws on only two profiles from many in East Asian samples and so does not use the data from many isolates. Accordingly, principal components analysis was used in an objective assessment of HSV-1 restriction polymorphism in Korea and Japan (119). In principal components analysis, a data set is transformed onto new axes (PC1, PC2, etc.) that successively account for the largest possible proportions of the total variation in the data. It was found that 90% of the variance in allele frequencies between populations is explained by a factor (PC1) that is consistent with the “dual structure model,” in that it places populations with the most distinct putative origins and most contrasting F1 and F35 frequencies at different extremes of the diagram (Fig. 4).

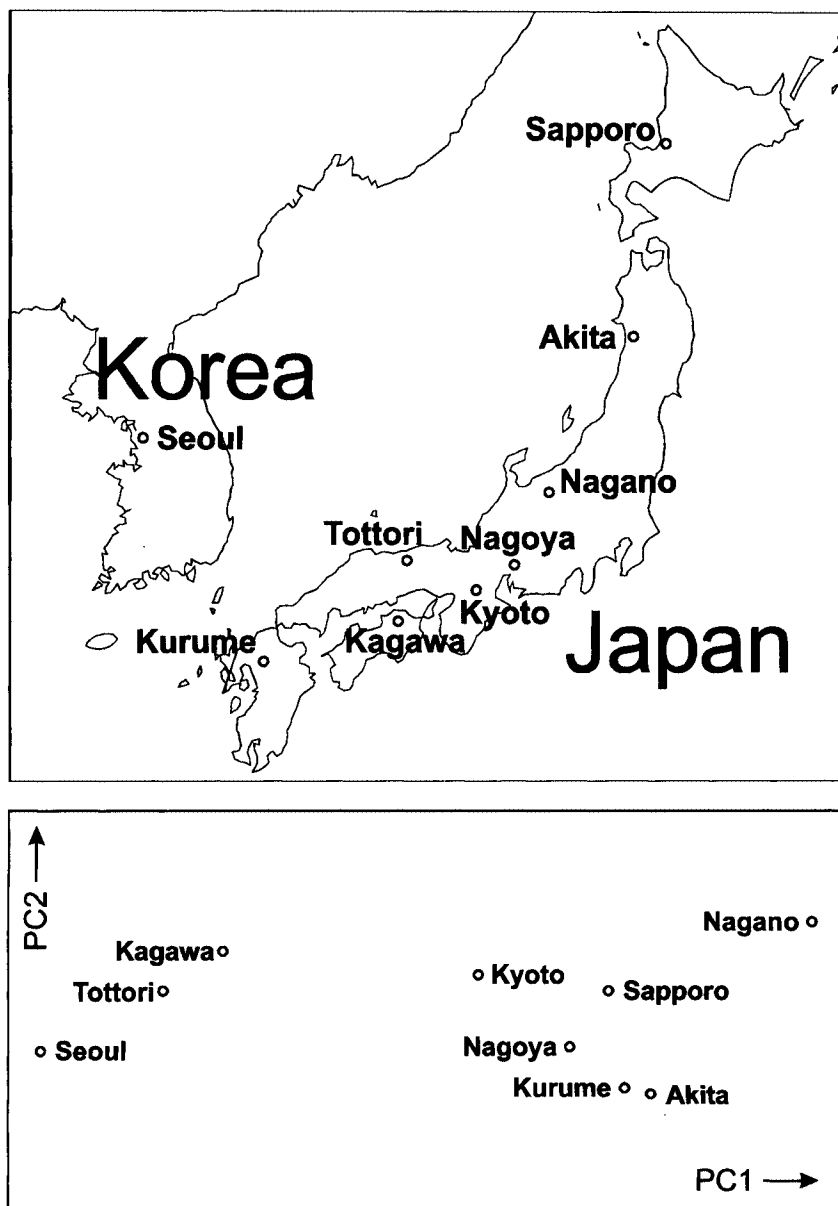


Figure 4 (Caption on facing page)

Sequence Data and Population Relationships

The availability of a sequence data set paralleling the restriction profiling studies above, with virus population samples from China, Japan (separate samples from Kagawa, Nagano, and Sapporo), Kenya, Korea, the United Kingdom, and Sweden, has allowed a more detailed investigation of the relationships between populations (119). Using statistical phylogeographic methods, the primary level at which populations could be distinguished was by continents: All populations from different continents were substantially differentiated from one another, while populations from the same continent had variable levels of differentiation (119). In particular, United Kingdom and Swedish samples could not be distinguished from one another on the basis of their distributions of variants even though structure was detectable amongst East Asian populations.

Analysis of molecular variance (126) provides a way to partition genetic variability at successive levels of geographic organization. As an example, a popularly quoted figure concerning global human variation is the *c.* 85% of total genetic variance that occurs within populations, leaving only a relatively small proportion for between-population, between-continent, or between-race comparisons (127–129). By contrast, 37% of HSV-1 genetic variance is between continents and only 57% occurs within populations, for the samples studied (119). The high level of continental differentiation in HSV-1 is likely to be explained by high rates of mutation and genetic drift, compared with human marker systems, so that an individual's HSV-1 genotype may actually be more informative about their origins than a moderate amount of information from standard genetic markers.

While it is clear that significant geographic structure exists amongst HSV-1 populations in East Asia, widespread recombination between HSV-1 strains means that the lineages underlying that structure are not related by a tree-like genealogy, so a phylogenetic approach cannot be used to classify the sampled isolates. Principal components analysis was therefore used to analyze data from individual isolates to identify clusters equivalent to population-specific lineages (119). Several clusters were identified, including one with clear links to European sequences and two major clusters for

Figure 4 (*Facing page*) Principal components analysis of East Asian HSV variants. The “dual structure model” for modern Japanese origins was investigated using principal components analysis of HSV-1 restriction data from eight locations in Japan and from Seoul in South Korea, indicated on the map (map modified from image at <http://www.lib.utexas.edu/maps>). In the graph, the first principal component (PCI) accounted for 90% of the variance in allele frequencies between samples, and clearly separated the Korean sample and some Japanese subpopulations from other Japanese samples that included those from areas with the highest numbers of Jomon era archaeological sites and putative indigenous Jomon descendants. *Source:* From Ref. 119.

Japan and Korea that varied in their frequencies in different samples (Fig. 5). Cluster “K” is shared between the Korean sample and Kagawa and Sapporo in Japan and cluster “N” is most evident in Japanese samples, comprising 20 of 21 isolates from Nagano. From this, we presume that “K” is related to genotype “F1” of Refs. 115, 116, and “N” is related to their “F35” genotype. Crucially, the distinctions between Japanese populations rely on different contributions of these two lineage groups rather than on viruses that can be linked to either China or Europe. This approach provides a basis to estimate the contributions of different ancestral lineages and populations to modern HSV-1 populations as well as to simplify population

Sequence clusters identified by PCA in 5 East Asian HSV-1 samples

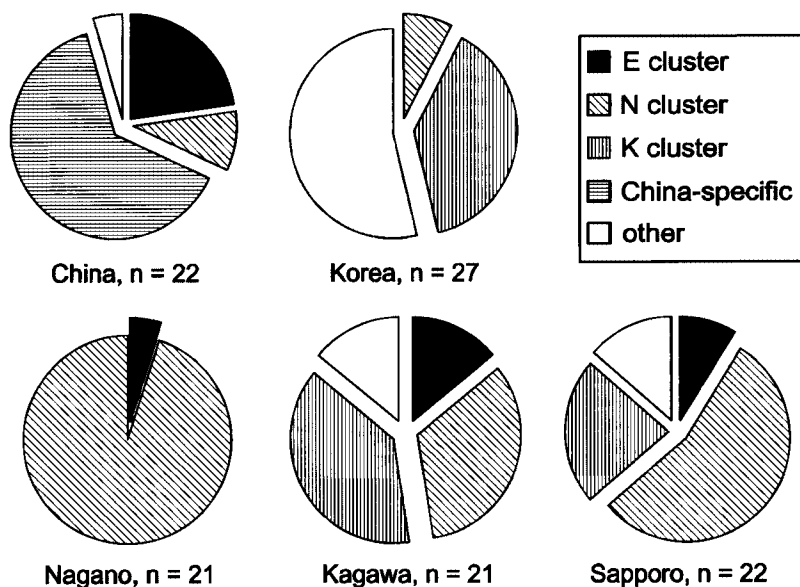


Figure 5 Distribution of sequence clusters in East Asian HSV-1 samples. This figure is a summary of the clustering patterns of DNA sequences from three segments of the HSV-1 genome, determined using principal components analysis on concatenated sequences from individual isolates. Sequences were identified that were similar to European HSV-1 sequences (E cluster), that were specific to particular populations (China-specific), or that did not fall into clusters (other). Of particular interest, Japanese isolates fell into two major clusters (N and K) in proportions that varied substantially between samples: 20 of 21 Nagano isolates are from the N cluster, while Kagawa and Sapporo have isolates in both N and K clusters. The Korean sample had N and K isolates as well as some “others” that were similar to European isolates for one or two of the genomic segments. *Source:* From Refs. 92, 119.

genetic models by focusing on single lineages. It therefore may provide insight into virus and, by extension, host population history.

In terms of the more general patterns of spread of HSV-1 strains within and between human populations, a simple conclusion from East Asian data is that distinct patterns of HSV-1 variability can be maintained, even within a small geographic area, for at least the *c.* 2300 years since the two human groups contributing to modern Japan came into contact. On this basis, further structuring in Asia presumably corresponds to patterns in human colonization and migration that could potentially be analyzed. What does this imply for the rest of the world? Considering that HSV-1 can maintain detectable structure for thousands of years, the relative homogeneity in two populations from Northwest Europe suggests a common single origin, and that mutation and genetic drift have not established significant population-specific variation, rather than the converse, that migration and human contacts have erased the differences between HSV-1 populations with distinct origins. Genetic evidence from mitochondrial DNA and Y chromosome haplotypes suggests that human populations in Western Europe probably had a largely homogeneous origin, expanding from southern refugia after the last glacial maximum, *c.* 13,000 years ago (130,131), and observed patterns are consistent with HSV-1 populations sharing that same history. HSV-1 population sequence data show signs of a modest population expansion that might have occurred since such a bottleneck (92). That patterns are maintained over even modest distances for many human generations may be significant in our understanding of HSV-1 epidemiology in that it suggests that transmission may be dominated by within-family contacts. Epidemiologically and genetically, HSV-1 appears to be amongst the most stable of human viruses.

PROSPECTS

We conclude by pointing to a number of areas in which further research is likely to lead to the most significant immediate advances in the understanding of HSV evolution.

First, as previously discussed, a major source of uncertainty concerning the history of the *Simplexvirus* genus is the absence of any characterized counterparts of the human viruses amongst apes. The identification and genomic analysis of related viruses in species such as chimpanzees has the potential to improve the precision and accuracy of estimates of the divergence date between HSV-1 and HSV-2.

Second, while population variability in HSV-1 has now been extensively investigated, HSV-2 diversity and interpopulation relationships remain poorly characterized. Data for a similar set of populations and genes, as have been analyzed in HSV-1, may allow the construction of epidemiologically based models to explain molecular variability of HSV-1 and HSV-2 in terms of demographic and transmission parameters.

Third, while it is clear from very limited sampling that African HSV-1 populations are far more diverse than other continental populations, their variability remains largely uncharacterized. Just as it has been for human populations, African data will be critical in our developing understanding of the history, timescale, and population relationships of both HSV-1 and HSV-2.

In addition to these key areas, gains in understanding are likely to come from more thorough analyses of the genomes of available viruses in studies at both long- and short-timescales, using more genes to estimate rates of evolution, and from developments in the methods for analysis of interspecies and intraspecies data.

ACKNOWLEDGMENT

We thank Andrew Davison for critical comments on the manuscript.

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Herpes Simplex Virus Vaccines and the Viral Strategies Used to Evade Host Immunity

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VACCINES FOR PREVENTION OR TREATMENT OF HERPES SIMPLEX VIRUS (HSV)

Goals of an HSV Vaccine

Most subjects infected with HSV-1 or HSV-2 remain asymptomatic and are unaware that they have been infected or that they may be transmitting the virus. The prevalence of HSV-1 and HSV-2 infection increases with age and varies considerably worldwide (1). In the United States, approximately 85% of individuals become infected with HSV-1 and 25% with HSV-2 over their lifetime. If infection were asymptomatic in everyone, we would have no need for a vaccine. However, HSV-1 and HSV-2 can cause life-threatening, sight-threatening, or emotionally debilitating infections, which explains the great efforts that have been made to develop effective vaccines. Ideally, a vaccine would prevent infections caused by both HSV-1 and HSV-2, and reduce the incidence of both symptomatic and asymptomatic infection. The latter is desirable to prevent transmission of the virus to unvaccinated individuals.

How important is it that a vaccine protects against both HSV-1 and HSV-2? HSV-1 is the most common cause of sporadic cases of encephalitis in the United States. Most viral causes of encephalitis are not susceptible to

current antiviral therapies; however, HSV-1 can be treated (2,3). Nevertheless, mortality and morbidity are often substantial despite therapy (4). HSV-1 is the causative agent of herpes simplex keratitis, which is the leading infectious cause of blindness in the United States, and a common indication for corneal transplantation (5,6). HSV infections are often more severe in the immunocompromised host. HSV-1 reactivation infections can present as large intraoral ulcers or extensive necrotic lesions around the mouth (7). HSV-1 esophagitis is rare in immunocompetent individuals, but more common in immunocompromised subjects (8). These serious manifestations of HSV-1 infection support the value of vaccine efforts, particularly if the vaccine formulation has efficacy against both HSV-1 and HSV-2.

Neonatal herpes is the most common serious manifestation of HSV-2 infection. The risk of acquiring neonatal herpes is highest in babies born to women who have a primary HSV infection at the time of labor and delivery, although recurrent infection also poses some risk to the infant (9). Herpes genitalis is the fourth most common cause of sexually transmitted diseases in the United States, after *Chlamydia*, gonorrhea, and human papilloma virus infections. Herpes genitalis infection increases the risk of acquiring HIV after sexual exposure (10,11). In recent years, the prevalence of HSV-1 as the cause of genital herpes has increased (12). In some countries, HSV-1 has surpassed HSV-2 as the most common cause of genital herpes. Recurrence rates are higher when HSV-2 is the causative agent; therefore, HSV-2 remains the primary target for vaccines attempting to prevent or treat genital herpes (13).

Based on the spectrum of diseases caused by HSV-1 and HSV-2, it is somewhat surprising that the focus for vaccine development has been predominantly on HSV-2 infections. Marketing strategies helped guide these decisions; however, the changing epidemiology of genital herpes and the increasing number of impaired hosts related to organ transplantation, HIV infection, and cancer chemotherapy suggest that it may be wise to reconsider this decision. A vaccine that offers protection against both pathogens is an important goal.

Ideally, an HSV vaccine should prevent both clinical and subclinical infection. Clinical infection is measured by lesion formation, while subclinical infection is detected by asymptomatic viral shedding or more commonly, by seropositivity in subjects who never knowingly had herpes lesions. The concept of preventing subclinical infection is referred to as sterile immunity. Can an HSV vaccine induce sterile immunity? The markers of sterile immunity include no lesion formation at the initial site of infection, no establishment of viral latency, and no asymptomatic virus shedding on mucosal or skin surfaces. These are high standards for a vaccine to achieve. An alternate outcome that may be more readily achieved is preventing symptoms, but not infection. However, the immunocompromised host is likely to remain at risk for serious recurrent infections in vaccinated populations unless sterile

immunity is achieved. Below we discuss current strategies and progress on developing effective HSV vaccines.

HSV Vaccine Development: Results of Trials in Animal Models

HSV-1 and HSV-2 infect mice, guinea pigs, rabbits, and nonhuman primates; therefore, studies to assess candidate HSV vaccines can be readily performed in laboratory animals. The guinea pig genital model is often preferred because HSV-1 and HSV-2 produce acute and recurrent infections in this model that mimic the disease in humans (14). Generally, candidate vaccines have provided excellent protection against clinical lesion formation at the challenge site and moderate protection against recurrent lesion formation, but have shown little benefit in preventing latency. No immune correlates of protection have been established; therefore, it remains unclear whether potent neutralizing antibodies, CD4⁺ T-cell responses, CD8⁺ T-cell responses or other immune parameters are critical for long-lasting protection against HSV (15). The general approach to vaccine development has been to determine whether candidate immunogens protect mice and guinea pigs, and then to address safety and efficacy of the best immunogens in humans. Current approaches to develop HSV vaccines include using purified viral glycoproteins, HSV DNA, HSV proteins expressed in recombinant vaccinia virus, or replication defective live HSV vaccines.

Purified Glycoprotein Subunit Vaccines

HSV glycoprotein vaccines have been the most thoroughly studied and are the most advanced candidate vaccines. HSV-1 and HSV-2 express at least 11 glycoproteins on the virion envelope. Four glycoproteins, gB, gD, gH, and gL, are required for virus entry into cells; therefore, these glycoproteins are prime targets for neutralizing antibodies to block entry (16). Experimental models have focused mainly on gB and gD. Success in animal models led to large, controlled phase III human trials, as discussed in the following section. Glycoproteins gH and gL have been less thoroughly studied and remain important potential vaccine candidates (17). Studies in animal models continue in an effort to define the most effective combinations of glycoproteins and adjuvants to elicit sterile immunity (18).

DNA Vaccines

HSV DNA subunit vaccines are easy to prepare and can be coadministered with DNA encoding for cytokines or interleukins that serve as immune adjuvants. Animal models demonstrate that plasmids encoding HSV glycoproteins are immunogenic and that immune adjuvants enhance the protection provided by viral DNA alone (19–21). DNA vaccines can also be used to boost immune responses produced by live virus vector vaccines (22). However, to date no human trials have been reported using HSV

DNA vaccines. Therefore, despite theoretical advantages, efficacy remains uncertain.

Recombinant Vaccinia Virus Vector Vaccines

Vaccinia virus has been used in murine and guinea pig models as a live virus vector to deliver HSV glycoproteins and immediate early gene-regulatory proteins as potential T-cell immunogens (23–25). In comparison with DNA glycoprotein vaccines, a vaccinia recombinant expressing HSV-1 glycoproteins gC or gE provided more potent protection against HSV-1 challenge in a murine infection model than plasmid DNA expressing the same two glycoproteins (19). Similar to approaches described for DNA vaccines, HSV-1 glycoproteins have been coexpressed with interleukins in recombinant vaccinia virus to enhance immune responses to HSV antigens (26). An advantage of live virus vectors is that vaccines can be administered via mucosal routes (intranasal or vaginal), which enhances the protection against HSV challenge (23).

Disabled Infectious Single Cycle Vaccines

Several live, attenuated HSV-2 vaccines have been evaluated in animal models and are now in human trials. Perhaps the most advanced is the disabled infectious single cycle (DISC) HSV-2 vaccine being developed by Xenova Group PLC (27). The vaccine formulation consists of HSV-2 deleted in one of the essential genes for virus entry, gH. The vaccine stock is prepared on mammalian cells genetically engineered to contain the gH gene. Glycoprotein gH-deleted HSV-2 DNA is transfected into the gH-complemented cell line. The virus that emerges lacks gH DNA, but incorporates the gH glycoprotein produced in the complementing cell line into the virion envelope. When used as a vaccine, this live virus is capable of undergoing only one round of replication, since the virus produced lacks gH and cannot enter cells for a second round of replication.

Immunization with HSV-2 DISC virus in a guinea pig vaginal model resulted in complete protection against HSV-2 disease when the vaccine was administered prior to challenge and was partially protective against recurrent disease when administered as a therapeutic vaccine (28). A live HSV-1 DISC vaccine offered similar protection against genital challenge (29). However, in a large human trial of HSV-2 DISC virus, the vaccine showed no benefit when used to treat recurrent genital herpes (15). Additional human studies are planned using this vaccine for prevention, rather than treatment of genital infection.

Replication Defective Live Virus Vaccines

Studies by Knipe and coworkers have examined replication defective HSV-1 and HSV-2 strains that are deleted in ICP27 and ICP8 (30,31). ICP27

encodes an essential, immediate-early protein involved in regulation of viral gene expression, while ICP8 encodes the single-stranded DNA-binding protein required for viral DNA replication. These replication defective viruses express many viral proteins at levels similar to those of wild-type virus, including viral glycoproteins gB and gD involved in virus entry. Studies in animal models have shown excellent protection, and the investigators are interested in pursuing human phase I trials (32).

Studies of HSV-2 Glycoprotein Vaccines in Humans

Regulatory agencies, such as the U.S. Food and Drug Administration, have not approved any vaccine for prevention or treatment of HSV infection. However, a subunit-based vaccine showed promise in preventing genital HSV-2 infection in placebo-control trials and is undergoing further evaluations. Below, we review results of human trials using this and another subunit HSV-2 vaccine.

Glycoprotein Subunit Vaccines for Prevention of Genital Herpes

HSV glycoprotein subunit vaccines have the theoretical advantage of being safer than whole virus vaccines, since exposure to foreign DNA is avoided. Hepatitis B vaccine is an example of a highly effective subunit vaccine; therefore, a precedent has been established for a subunit vaccine approach. Chiron Corporation and GlaxoSmithKline (GSK) each performed large-scale human trials to evaluate the safety and efficacy of HSV-2 glycoprotein subunit vaccines. The Chiron Corporation vaccine formulation included HSV-2 glycoproteins gB and gD in adjuvant MF59, which is an oil-in-water emulsion containing squalene, polysorbate 80, and sorbitan trioleate. The GSK vaccine contained HSV-2 gD alone in alum and 3-O-deacylated-monophosphoryl lipid A (MPL) (33–35). The primary study endpoint for the Chiron Corporation vaccine study was prevention of HSV-2 infection (symptomatic and asymptomatic infection), while the endpoint in the GSK study was prevention of genital herpes disease (symptomatic infection only).

Acquisition rates of HSV-2 in subjects receiving placebo or the Chiron vaccine were 4.6 and 4.2 per 100 person-years ($P=0.58$). No significant effect was detected on the duration of the first genital HSV-2 outbreak or subsequent frequency of reactivation, despite high levels of vaccine-induced HSV-2-specific neutralizing antibodies in vaccine recipients. Outcomes following vaccination with the GSK formulation were also not significantly different comparing vaccine and placebo recipients. However, in a subgroup analysis, the vaccine was efficacious in preventing genital herpes disease in women who were seronegative to both HSV-1 and HSV-2 prior to vaccination (73% protection in one study, $P=0.01$, and 74% in another, $P=0.02$) (Table 1).

Table 1 Comparison of Chiron Corporation and GlaxoSmithKline HSV-2 Subunit Vaccine Trials

Parameters	Chiron Corporation	GlaxoSmithKline
HSV-2 antigen	Glycoproteins gB and gD each at 30 µg	Glycoprotein gD at 20 µg
Adjuvant	MF59	MPL
Participants	HSV-2-seronegative subject of an HSV-2-infected partner	Study 1: HSV-1- and HSV-2-seronegative subjects of a partner with genital herpes Study 2: Subjects of any serologic status whose partner has genital herpes
Primary endpoint	Time to acquisition of HSV-2 defined by seroconversion or virus isolation	Study 1: Occurrence of genital herpes disease defined by having each of the following: (1) genital signs or symptoms (pain, itching, swelling, papules, vesicles, ulcers, or crusts); (2) either a positive HSV culture or positive HSV PCR; and (3) HSV seroconversion Study 2: Genital herpes disease as defined above in HSV-2-seronegative female subjects
Outcomes	Vaccine efficacy of 9%, which was not significantly different comparing vaccine and placebo groups	Vaccine efficacy 38% in study 1 and 42% in study 2 (not significantly different from placebo groups)
Subgroup analyses	Time to infection showed a 50% lower acquisition rate in vaccines during first 5 months	Vaccine efficacious in women who were HSV-1 and HSV-2 seronegative in study 1 (73%, $P = 0.01$) and study 2 (74%, $P = 0.02$)
Comments	Subgroup analysis was not done to examine efficacy in preventing genital herpes disease in HSV-1- and HSV-2-seronegative women	Additional randomized trial is in progress to confirm efficacy against genital herpes disease in HSV-1- and HSV-2-seronegative women

Abbreviations: HSV, herpes simple virus; MPL, monophosphoryl lipid; PCR, polymerase chain reaction.

Source: From Refs. 33, 34.

The GSK vaccine trials were not specifically designed to address protection in women; therefore, additional studies are required to confirm these results. If confirmed, the results may suggest that anatomical differences between genders lead to improved genital tract immunity and protection in women. A study sponsored by GSK has now been initiated to address vaccine efficacy against genital disease (symptomatic infection) in HSV-1- and HSV-2-seronegative women. If successful, a gender-specific vaccine may become available. This outcome is likely to be controversial, since the vaccine does not produce sterile immunity (does not prevent asymptomatic infection); therefore, from a public health perspective, men may be at increased risk of acquiring genital herpes, since females may not know whether they have been infected and are potentially contagious to their partners.

Glycoprotein Subunit Vaccines for Treatment of Recurrent Genital Herpes

Two clinical trials were performed using the Chiron Corporation vaccine to assess the role of therapeutic vaccination in controlling frequency and severity of genital herpes. The first used HSV-2 glycoprotein gD (100 µg) in alum as adjuvant (36). Ninety-eight subjects who reported 4–14 recurrences per year received either gD2 vaccine or placebo. Vaccine recipients had fewer virologically confirmed recurrences per month (0.18 vs. 0.28, $P=0.019$), and fewer mean recurrences per year (4 vs. 6, $P=0.039$). In a follow-up study, gB2 was added to the vaccine formulation and combined with gD2, each used at 10 µg, which is lower than the dose used in the first study (37). The adjuvant used was MF59 adjuvant, which was selected based on improved T-cell responses compared with alum. Two hundred and two subjects with 4–14 recurrences annually were randomized to receive either vaccine or placebo. No significant improvement was noted in monthly rate of recurrences; however, the duration and severity of the first outbreak was significantly reduced in the vaccine group. The authors commented that the lower dose of vaccine antigen used in the second study may have contributed to the less impressive protection. These studies suggest that therapeutic vaccination using gB2 or gD2 has only a modest effect on the course of genital herpes.

NOVEL DIRECTIONS IN HSV VACCINE DESIGN

The strategies discussed above involve designing HSV vaccines that induce potent B-cell and T-cell immune responses. In recent years, many different mechanisms have been described that are used by viruses to escape host immunity. An alternate concept for vaccine development is proposed below, in which vaccines not only induce potent immune responses but also stimulate responses to block virus evasion from host immunity.

Immune Evasion Strategies of HSV-1 and HSV-2

Both viruses and the organisms that they infect have evolved elegant mechanisms to protect themselves. Viruses rely on host cells to replicate, using cellular machinery to survive and proliferate and their hosts to disseminate progeny. To combat infection and subsequent disease, hosts mount formidable defense mechanisms consisting of both innate and adaptive immune responses. Viruses respond, having evolved to interfere with host immunity through diverse mechanisms. HSV interferes with immunity mediated by both antibody and the complement system and blocks cytotoxic T lymphocyte (CTL) activation by preventing antigen presentation by the MHC class I complex.

Evasion from CD8⁺ T Cells

HSV evades cytotoxic T-lymphocyte recognition by interfering with the Major Histocompatibility Complex class I antigen presentation pathway. MHC class I is expressed on the surface of most cell types and displays peptides derived from the cytosolic compartment of infected cells to CD8⁺ T cells. MHC class I molecules are composed of three subunits: the class I heavy and light (β 2 microglobulin) chains are inserted into the endoplasmic reticulum (ER) as part of their normal biosynthesis, while the small antigenic peptide itself must be transported from the cytosol into the ER. These small antigenic peptides, derived from proteasome-dependent processes, are transported into the ER by transporters associated with antigen processing (TAP 1 and TAP 2). Once peptides are loaded, and the MHC class I molecules are assembled, the class I molecules exit the ER and enter the secretory pathway to be expressed at the cell surface. Expression of MHC class I molecules displaying antigenic peptides from HSV on the surface of infected cells targets these cells for destruction by CD8⁺ T cells.

Like many viruses, HSV interferes with CTL recognition of virus-infected cells. Viruses prevent MHC class I presentation by a variety of mechanisms including blocking peptide production, transport into the ER and loading onto MHC class I molecules, and interfering with the processing of MHC class I molecules and their subsequent trafficking (38). HSV encodes one protein that interferes with MHC class I presentation.

The HSV immediate early gene ICP47 encodes an 88 amino acid protein, which interferes with MHC class I presentation by preventing the transport of antigenic peptides into the ER (39). ICP47 accomplishes this by binding to the peptide binding site of TAP 1 and TAP 2, which prevents binding and the subsequent transport of peptides through the transporter (40). By blocking TAP-dependent transport, ICP47 interferes with the assembly of MHC class I molecules, resulting in an accumulation of unstable and improperly folded class I molecules that are retained in the ER compartment (41). HSV-infected cells are thus rendered unrecognizable to CD8⁺ T cells and are protected from destruction.

Evasion from Antibody and Complement

HSV escapes immunity mediated by both antibody and complement, by preventing activation of the complement cascade. The complement system plays an important role in both the innate and acquired immune responses against viral infection (42). Complement can be activated by both antibody-dependent and antibody-independent processes, resulting in the accumulation of complement components on the surface of viruses and virus-infected cells. Activation of the complement cascade can block virus entry or lead to phagocytosis of complement-coated viral particles. The formation of the membrane attack complex (MAC) on the surface of virus-infected cells can lead to their lysis.

Activation of complement occurs by one of three mechanisms: the classical, lectin, or alternative complement pathways (Fig. 1). The classical complement pathway is initiated upon the binding of antibody to the surface of pathogens or in an antibody-independent manner when C1q, the first component of the complement cascade, binds directly to targets. These targets include bacterial lipopolysaccharide, nucleic acids, polyanionic compounds,

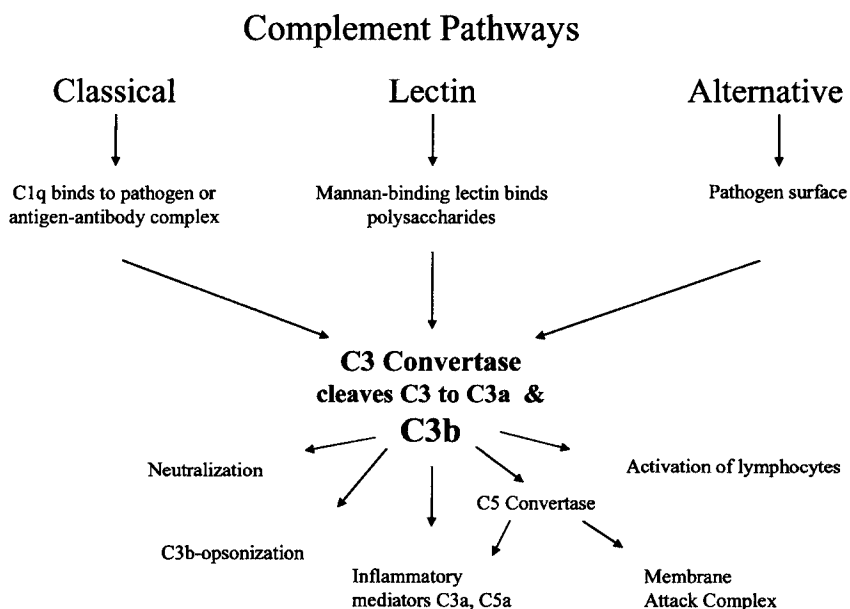


Figure 1 Complement activation pathways. The complement system consists of three pathways, the classical, lectin, and alternative pathways, which are activated in response to numerous microbial stimuli. These pathways converge at the effector arm of the cascade, the C3 convertase, resulting in neutralization, opsonization, and phagocytosis of microbial pathogens, inflammation, and lysis of infected cells.

myelin, and some viruses. The lectin complement pathway recognizes mannose and *N*-acetyl glucosamine residues on bacteria, while the alternative complement pathway recognizes foreign surfaces. Together, these three pathways are able to recognize and activate the complement system against a diverse range of microbial pathogens.

The three pathways converge at the C3 convertase, which converts C3 into two components: C3a, a small inflammatory peptide, and C3b, which binds the surface of pathogens or infected cells. The location of C3b binding is important for its function, as the binding of C3b to adjacent membranes leads to phagocytosis while C3b binding to the C3 convertase leads to the generation of the C5 convertase and subsequent formation of the membrane attack complex (MAC). This terminal pathway of the complement system leads to disruption of cellular membrane and viral envelopes by the formation of pores, resulting in destruction of viruses and infected cells. The MAC can lyse a variety of microorganisms, viruses, red blood cells, and nucleated cells.

Complement plays an important role in host defense against viruses, leading to the destruction of both viruses and infected cells. Viruses have therefore evolved numerous mechanisms to control complement. As C3 plays a key role in each of the three complement activation pathways, it is often the target of viruses. Many viruses, including HSV, interfere with complement activation by expressing complement regulatory proteins. HSV encodes two complement regulatory proteins, glycoprotein C (gC) and glycoprotein E (gE) (Fig. 2).

The Role of gC

The HSV envelope glycoprotein C of both HSV-1 and HSV-2 (gC-1 and gC-2) functions as a receptor for complement component C3, and its enzymatic

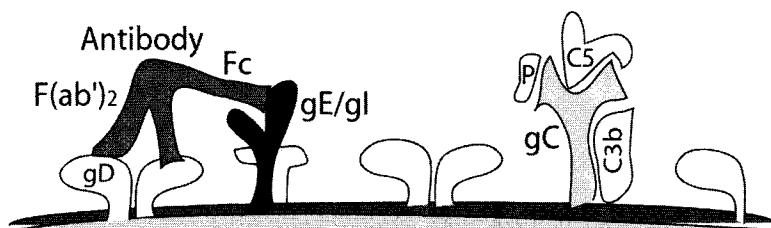


Figure 2 Model of HSV gE and gC immune evasion mechanisms. The left side of the figure shows antibody bridging, where an antibody molecule binds by its F(ab')₂ domain to the target antigen, shown here as gD, and by the Fc domain to the gE/gI complex, preventing the activation of complement and antibody-dependent cellular cytotoxicity. The right side of the figure shows gC-mediated immune evasion. gC binds complement component C3b and blocks the interaction of C5 and properdin (P) with C3b, which inhibits complement activation by multiple mechanisms, including accelerating the decay of the alternative pathway C3 convertase.

cleavage products C3b, iC3b, and C3c (43,44). gC, which is encoded by the UL44 gene of both HSV-1 and -2, is conserved among members of the alpha-herpesvirus family including HSV-1, HSV-2, varicella zoster virus (VZV), pseudorabies virus (PRV), bovine herpesvirus-1, and equine herpesvirus-1 and -4. Sequence analyses revealed that the gC homologues are most similar within the carboxyl-terminal half of the molecule and that each is likely to have similar disulfide bond arrangements (45–47). Moreover, all with the exception of VZV, bind complement component C3b in a species-specific manner.

gC is expressed on both the virion envelope, as well as on the surface of virus-infected cells. gC of both HSV-1 and HSV-2 binds C3b when in a purified form and when expressed on the surface of transfected cells (48,49). Regions of the gC proteins that are involved in C3b binding are well conserved in both proteins. Four regions on gC-1 and three regions on gC-2 are known to be important (50,51). Binding of gC-1 to C3b inhibits activation of the classical pathway on the HSV-1 virion, and may function in a similar manner for gC-2 (49,52). Differences do exist however, as gC-1, but not gC-2, is able to accelerate the decay of the alternative pathway C3 convertase, C3bBb, preventing lysis of HSV-infected cells (49,53). Properdin extends the lifetime of C3bBb three- or four-fold by binding to and stabilizing the C3bBb complex. This extended lifetime should increase the amount of C3b functioning as a component of the alternative C3 convertase, thereby amplifying the complement cascade, as well as the amount of C3b coating the cell surface. gC-1 destabilizes the C3 convertase by inhibiting the binding of properdin to C3b, which limits the effectiveness of the alternative complement pathway in lysing HSV-infected cells (43,50). gC-1 has also been shown to inhibit the interaction of C5 with C3b, leading to the disruption of both the classical and alternative complement activation pathways at the level of the C5 convertase (53). Expression of gC-1 may therefore limit the assembly of the MAC on membranes of HSV virions and infected cells.

The region of gC-1 important in blocking the binding of C5 and properdin to C3b is near the amino-terminus of the protein, which may explain differences in protection observed between gC-1 and gC-2. While gC-1 and gC-2 are well conserved, especially within regions responsible for C3b binding, this similarity does not extend to the amino-terminus of both proteins. Recent studies evaluating the importance of each region in modulating complement activity were performed using a low passage clinical isolate of HSV-1 that was mutated within the C3 binding domain, the C5/P blocking domain, or both to determine the level of protection conferred by gC-1 in neutralization assays (54). Each virus was incubated with HSV-1 and HSV-2 antibody negative human serum as a source of complement for one hour, or complement inactivated serum as a control and virus titers were then determined. Complement alone had little effect on the wild-type HSV-1 virus, reducing the titer by only two-fold. The HSV-1 virus mutated within the C5/P blocking domain was also relatively resistant being

neutralized only three-fold by complement. The virus mutated within the C3 binding domain was more susceptible and was neutralized five-fold, while complement had the greatest effect on the HSV-1 virus mutated in both the C5/P blocking and C3 binding domains, neutralizing it by 38-fold.

The importance of these domains in modulating complement activity was also examined *in vivo* using a murine model of infection (54). The HSV-1 viruses mutated in either the C5/P blocking or C3 binding domains were significantly less virulent than the wild-type HSV-1 virus. Interestingly, the C3 binding domain mutant virus was more attenuated than the C5/P domain mutant virus and as attenuated as the gC double mutant virus, which suggests that the C3 domain contributes more to HSV-1 virulence than the C5/P domain.

The Role of gE

Immunoglobulin G (IgG) Fc receptors (FcγRs) are expressed on many hematopoietic cell types including macrophages, neutrophils, natural killer cells, dendritic cells, platelets, B cells, and some T cells. These receptors provide an important link between the innate and acquired immune responses, as the interaction of the IgG Fc domain with FcγRs results in numerous effector activities including complement activation, phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), cell activation and proliferation, and inflammation.

The HSV envelope glycoproteins E and I (gE and gI) of both HSV-1 and HSV-2 form a complex that functions as a receptor for IgG, interfering with activities mediated by the IgG Fc region (55–58). HSV was originally implicated in displaying this FcγR activity when erythrocytes coated with IgG formed rosettes when added to HSV-infected cells, which indicated that HSV induces the expression of an IgG binding protein (59). This HSV-dependent IgG binding protein was shown to bind the Fc region of IgG. gE and gI, which are encoded by US8 and US7, respectively, were identified by IgG affinity chromatography. Together they form a noncovalent heterodimeric complex that binds the Fc region of both monomeric as well as aggregates of IgG. The regions responsible for IgG Fc binding were mapped to amino acids 235–380 on gE and 128–145 on gI (60,61). When expressed alone, gE functions as a low-affinity Fc receptor, binding IgG aggregates, but not IgG monomers. The binding of monomeric IgG requires the formation of the gE/gI complex, which is responsible for the higher affinity binding of IgG (60). This Fc receptor activity is well conserved among members of the alphaherpesvirus family including HSV, VZV, and PRV (62,63).

The HSV Fc receptor protects cell free virus and virus-infected cells from immunity mediated by both antibody and complement (55,64). While studies initially focused on the protection conferred by the gE/gI complex against nonimmune IgG, further analysis indicated that the HSV Fc receptor is more likely to interfere with the activity of HSV-specific antibodies

through a process called antibody bipolar bridging (Fig. 2) (55). Antibody bipolar bridging by HSV occurs when immune IgG binds to a viral target by its hypervariable region and to the gE/gI complex by its Fc end. By preventing the proper orientation of the Fc domain, antibody bipolar bridging renders immune IgG molecules unable to perform Fc-mediated immune functions, including classical complement activation by C1q binding to the Fc domain, ADCC, and phagocytosis (65,66). In vitro studies demonstrated that the HSV Fc receptor prevented complement-enhanced antibody neutralization, ADCC, and attachment of granulocytes to the Fc domain of antibodies bound to HSV-infected cells, supporting a role for the gE/gI complex in immune evasion.

Recently, the Fc receptor activity of gE was shown to mediate immune evasion of the HSV-1 virus in vivo (64). An HSV-1 mutant virus was generated with a four-amino-acid insert within the gE IgG Fc binding domain. This mutant virus, HSV-1 gE339, is deficient in Fc binding, yet retains other functions associated with gE and gI, including normal cell to cell spread (67). Experiments were performed in the murine flank model and indicated that the HSV-1 Fc receptor plays a significant role in protecting the virus against antibody and complement-mediated immunity.

Further studies were performed to examine the contribution of both gC and gE immune evasion in the pathogenesis of HSV (68). The virulence of HSV-1 viruses defective in either IgG Fc or C3 binding alone or both was compared with the wild-type HSV-1 virus in the murine model of infection. Complement-intact mice were passively immunized with 200 µg/mouse of pooled human IgG containing high antibody titers against HSV. Sixteen hours later, mice were infected by scratch inoculation on the denuded flank. Each single mutant virus was significantly more attenuated than the wild-type virus. The virus deficient in both IgG Fc and C3 immune evasion was more impaired than either mutant alone.

Efforts to Block HSV-1 Immune Evasion to Enhance Host Resistance to Infection

The studies discussed above establish that HSV-1 immune evasion molecules are important virulence factors. The question arises whether methods can be devised to block evasion activities mediated by these molecules. HSV-1 gC is expressed on the virus envelope and infected cell surface; therefore, gC is a potential target for vaccines that induce antibodies that bind to gC and block its immune evasion activities. Studies were performed in mice that were passively immunized with gC monoclonal antibodies prior to infection. Animals were protected against HSV-1 challenge by monoclonal antibodies that bind to the gC domain involved in C3b binding, but not by antibodies that recognize other regions on gC (69). Mice treated 1 or 2 days postinfection with gC monoclonal antibodies that block C3b binding also protected

against virus challenge. Therefore, antibodies that target immune evasion domains can reduce the severity of infection.

Experiments were then performed to determine if immunization with gC protein could produce antibodies that bind to gC and block gC-mediated immune evasion (69). Mice immunized with gC protein produced antibodies that blocked C3b binding to gC and significantly protected mice against HSV-1 challenge. Mice challenged with a mutant HSV-1 strain that lacks the ability to interact with C3b were not protected by the vaccine, which suggests that protection was mediated by antibodies that specifically target the gC immune evasion domain.

HSV-1 gE is also expressed on the virion envelope and infected cell surface. We examined whether immunizing with gE protein could produce antibodies that bind by the F(ab')₂ domain to gE and block the HSV-1 FcγR (70). A gE immunogen that comprised most of the gE ectodomain (amino acids 24–409) was highly effective at producing antibodies that block the FcγR. These results suggest that immunizing with gE fragments has potential for preventing immune evasion by blocking activities mediated by the HSV-1 FcγR.

CONCLUSIONS

Studies were performed to more directly link HSV-1 immune evasion with vaccine performance. IgG and complement from subjects immunized with the experimental GSK gD-2 HSV vaccine were tested for neutralizing activity against a mutant virus defective in gC and gE immune evasion. The vaccine serum was far more effective against the gC/gE mutant virus than wild-type virus (69). This result supports a critical role for immune evasion molecules in reducing vaccine potency. This finding raises an important consideration, which is whether adding gC and gE proteins to a gD vaccine will improve vaccine efficacy. The hypothesis is that gD will induce potent immune responses, while gC and gE will stimulate antibodies that prevent the virus from evading these immune responses. Our findings suggest that it is possible to block certain HSV-1 immune evasion domains. Therefore, one consideration for future vaccine development is to use a combination of immunogens, including some that induce strong neutralizing antibody responses, others that produce potent T-cell responses, and a third group that prevents HSV from evading B- and T-cell responses.

HSV vaccines are showing promise in human clinical trials, protecting some subjects from symptomatic infection, and controlling the frequency and severity of outbreaks in those already infected. Several other vaccines are currently undergoing early phase I trials. However, despite high levels of virus-specific antibodies and immune T cells in vaccinated or previously infected individuals, subjects are not protected from primary or recurrent infection. HSV is well adapted to the human host and is able to evade host

immunity through a variety of mechanisms, warranting consideration of alternative strategies for vaccine development, including efforts to block viral evasion of host immunity.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI 33063 from NIAID, HL 28220 from NHLBI, and DE 14152 from NIDCR.

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The Natural History and Epidemiology of Herpes Simplex Viruses

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Dans ignorance où nous vivons des processus exact de l'herpès, nous voyons chaque génération médicale créer une théorie adaptée aux idées, aux découvertes du moment.

—Du Castel, 1901*

INTRODUCTION

We are interfacing here between chapters 1 and 2 on the evolution and molecular aspects of herpes simplex virus (HSV) and later chapters, by denoting first the epidemiologically relevant aspects within four periods (Table 1). Phase I encompasses ~5 million years of coevolution—when it is estimated that the viruses differentiated into HSV-1 and HSV-2, and around which time proto-humans evolved from African apes. This phase includes the period during which the ancestral *Homo sapiens* evolved from Africa ~100,000 years ago, remaining hunter-gatherers until ~10,000 years ago (with some such tribes still currently existing), and up to the first written recording of likely herpesvirus lesions (“creeping” eruptions) ~2500 years ago. Phase II (from that time to the 1960s A.D.) is most

* In our ignorance of the exact processes of herpes, we see each medical generation create a theory adapted to the ideas and discoveries of the moment.

Table 1 The Different Phases of the Epidemiology of HSV-1 and HSV-2 Throughout Time

Phase	Period
I. The coevolutionary (EVO-EPI) phase ^a	~5 million to ~2500 years ago
II. The infrastructure phase	~2500 years ago to 1960s (A.D.)
III. The modern phase	From 1960s to 2004
IV. The near future phase ^a	From 2004 to 2024

^aPhase I and IV are the more speculative. Phase I is based on evolutionary information, studies in existing hunter-gatherer tribes, and knowledge of α herpesviruses in lower species; Phase IV is based on available information obtained over Phase II, but mostly Phase III.

prominent in providing the infrastructure of human knowledge on the subject—the ability to diagnose herpes by laboratory means, the reporting of most of the various body sites of HSV involvement and diseases produced, as well as the recording of several important epidemiological observations. Phase III (from the 1960s to the present) has been particularly enriched by many laboratory- and epidemiologically related innovations and by basic information on many aspects of the viruses and their interactions with human hosts. Most helpful for epidemiological understanding has been the modern awareness that HSV could be differentiated into two distinct viral types, and the ability to measure HSV-1 and HSV-2 type-specific antibody responses.

Presented briefly first is substantiation of the relevant rationale for this separation into phases, using evolutionary and historical perspectives. We then provide both a general picture and supporting data, in context of major factors that have developed in the world—mostly recognized since the 1960s, which are affecting the natural history and epidemiology of HSV-1 and HSV-2, and are likely to continue to do so for at least the next two decades (Phase IV). Thus, the earlier clinicoepidemiological distinction made four decades ago between the two HSV types is changing in some populations. This paradigm shift is due to several major factors that have occurred in the past half-century including: (i) the improved socioeconomic status (SES) in some, but not other, populations in the world, and (ii) the marked alterations in sexual and other behavioral practices, within developed and developing countries (including minority groups^{*}). Influencing throughout the clinicoepidemiological patterns are the interactions between the viruses and the host immune systems, related to whether HSV-1 is acquired before HSV-2, or whether HSV-2 is acquired before HSV-1. The impact of the human immunodeficiency virus (HIV)[†] on both HSV types—and that of HSV (particularly

^{*} Used here to comprise certain ethnic groups, immigrants, and homosexual men and women in developed countries.

[†] HIV refers only to HIV-1, for which data are primarily available.

HSV-2) on HIV is also becoming a major factor affecting the natural history and epidemiology of these viral infections, as well as their prevention.

Since we have detailed previously the progress of knowledge on the natural history and epidemiology of HSV during the modern phase first in 1973 (2), then in 1976, in the first edition and, in 1997, in the fourth edition of the four volumes on “Viral Infections in Humans” (3,4), we will refer briefly to key observations made in earlier years, mostly for comparative purposes. Focus is placed on the more recent data obtained in the past two decades on the mounting number of reports of viral isolate type studies, and type-specific antibody surveys in different developing and developed countries, denoting their strengths and limitations. Special attention is given to the results of the U.S. national seroepidemiological surveys obtained repeatedly over time [three National Health and Nutrition Examination Survey (NHANES); i.e., studies obtained, since 1976, at different periods within a quarter of a century]—that span a quarter of a century. We conclude by discussing preventive measures primarily at the population level, in context of the research and public health policy challenges facing us in the next two decades (Phase IV).

PHASE I—THE COEVOLUTION (OR EVO-EPI) PHASE

Chapters 1 and 2 have provided current molecular evidence related to the characterization and divergence of herpes simplex viruses into HSV-1 and HSV-2.[†] The bridging of general evolutionary and epidemiological principles, and of specific information on herpesviruses in currently existing animals and hunter-gatherer tribes, allows a reconstruction of the likely natural history and epidemiological patterns over a 5-million-year period. HSV-1 and HSV-2 are α herpesviruses which evolved by coevolution with their lower species hosts—hence into chimpanzees and then the proto-human line, eventually resulting in *H. sapiens* ~100,000 years ago. Japanese investigators have obtained data to infer that the close association of HSV-1 with the human population could actually serve as a marker for the geographical migration of human populations (7).

During the coevolution phases, the viruses continued to evolve to resist the concomitant evolving immune systems of the hominid, including *H. sapiens*, host (8). This conclusion is supported by the fact that, with few exceptions, subclinical or mild-to-moderate diseases are produced by all α herpesviruses in their *natural* hosts. Severe or fatal disease in hominid neonates or older hosts, having no impact on the evolutionary survival of the

[†] This section on HSV fits best with the newer approaches being made to bridge evolution and epidemiology (which we have called EVO-EPI) in the same way as previously done by others to bridge evolution and developmental processes of organisms (EVO-DEVO), and by us to bridge evolution and virology—coined earlier as Evovirology (5,6).

viruses, can then be viewed as stochastic events (e.g., encephalitis or eczema herpeticum), in relation to abnormal immune responses: a hyperactive response in case of stromal keratitis and erythema multiforme, or a hypoactive one, in case of conditions related to cellular and/or immune deficiencies, such as severe malnutrition (Kwashiorkor), that must have plagued hominids throughout their 5-million-year history.

The intermittent reactivation of latent α herpesviruses, including HSV-1 and HSV-2 in the nervous system of their natural host, must have provided a great advantage for viral survival, at a time when hominids lived in small tribes. *H. sapiens* also lived in similar small groups, until the past 10,000 or so years, with the advent of farming and urbanization in several parts of the world, resulting in many technological and socioeconomic changes. Unlike several zoonotic agents most likely acquired from domesticated animals, such as measles and influenza viruses that require large populations to be transmitted (9), the latency property of HSV enables its transmission from an infected index case, not only to contemporary individuals within a tribe, but also possibly many decades later to other contacts, including children or members of other tribes. Supporting this concept in still existing hunter-scavengers among *H. sapiens* are recent data on the HSV prevalence in South American Amazon Indians. These individuals have remained isolated for around 10,000 years as hunter-gatherers, with entry of outsiders occurring in relatively recent years (~20 to 200 years ago, depending on the tribe). Recent collaborative studies conducted by our group indicate that, no matter which of the 22 Amazon Indian tribes surveyed, 80% or more of the children were already HSV-1 antibody positive by the age of 5 years (10,11). The high prevalence of HSV-1 in the Amazon Indians correlates very well with their lower socioeconomic status, as noted in U.S. Navajo Indians (12) and in several recently studied populations of Africa, Asia, and Latin America (13,14). Such a high prevalence in children of antibodies to HSV (mostly untyped, but likely to be HSV-1) was commonly noted in some Western countries in the 1960s and 1970s (15–19).

HSV-2 antibody reactivity ranged from 0 to 79% among the adult members of the 22 different Amazon tribes living in separate villages with an average population of 200 individuals—except for two tribes of >1000 (11). This wide range might be explained by the possibility that HSV-2 has been endogenous in some tribes, while in others the sexually transmitted viruses were introduced more recently by “outsiders.” In either case, HSV-2 would most likely have survived best in tribes with poly-partnered sexual behavior patterns, with transmission occurring perhaps from females conquered in tribal wars (20).

The different transmission patterns assumed by HSV-1 or HSV-2 via saliva, sexual transmission, or from mother to progeny are well exemplified in several lower species naturally infected with their own α herpesviruses. In some host species for example horses, the virus that is spread by an oral

route is differentiable, as to type, from the sexually transmitted one (21). The bovine α herpesviruses can be transmitted by both routes, but are not differentiable into types; α herpesviruses of macaques and baboons can also be transmitted by both routes, but no attempts have yet been made to differentiate the orally and sexually transmitted strains into different types (5,22). It is also worth noting that very similar clinicopathological features, as those found in human babies, have been reported in canine pups after maternal transmission of the canine α herpesviruses that can occasionally be sexually transmitted (5). It would be expected that sexually transmitted α herpesviruses would likely to be found in the most poly-partnered and sexually active of all primates—the bonobo or *P. paniscus* (23). Twelve bonobos have been shown recently (24,25) to possess a genital herpesvirus very similar to the human HSV-2, with their sera reacting with human HSV-2 type-specific glycoprotein gG₂ (26). Transmission of the bonobo virus sexually to two *P. troglodytes* chimpanzees has been noted within a primate center setting, and sera of only one of 10 other troglodyte chimpanzees had antibodies to the gG₂ or HSV-2. It has not been possible as yet to determine whether *Homo sapiens* descended from bonobos, as suggested (27), or that the (pro) bonobos transmitted the HSV-2 to the proto-humans, or that the HSV-2 like virus was acquired by sexual relations with old or more recent hominids, including *H. sapiens*.

PHASE II—THE INFRASTRUCTURE PHASE

The earlier historical aspects have been reviewed elsewhere (4,28,29) and only relevant aspects related to natural history and epidemiology will be noted here briefly. Skin creeping eruptions were described about 2500 years ago, and blisters associated with fever, which occurred at the same site, were noted by the Greek—Herodotus, and later by the Roman—Galen. Skin lesions of herpesviruses were differentiated from those due to other agents, including smallpox, in the late 19th century by Unna, who noted the cytopathological findings of multinucleated giant cells of the herpesviruses. A few decades later, Lipschütz described the herpetic intranuclear inclusions in fixed cells.* It had also been shown, in the 19th century, that herpetic skin lesions could be transmitted from one human to another. However, it was not until around World War I that Grüter and Lowenstein applied a new diagnostic method—the production of characteristic dendritic lesions in the rabbit cornea, which helped to differentiate the filterable agent (virus) causing herpes “febrilis” and herpes “genitalis” from that causing varicella-zoster. Much earlier, in 1736, Astruc in France recorded herpes “genitalis”

* Of still current importance is that, in unfixed—Czank—smears of suspect herpetic lesions, inclusions are absent, making this indirect, but rapid diagnostic methods less reliable than one using cell-fixation (30).

for the first time, and a book “*Les herpes génitaux*” was published, in 1836, by Diday and Doyon. On the basis of the recognition in the early 20th century that herpes “genitalis” occurred primarily among adults and mostly in venereal disease (VD) clinics, unlike herpes “febrilis” that was most commonly observed in children, Lipschütz—from 1921 to 1932, tried to persuade the medical/scientific community that they were due to two different virus types, without success.

During the 1930s and 1940s, it became possible to identify HSV, based on the ability of the virus to produce pocks in egg embryo chorioallantoic membranes, as well as causing disease in infected mice.[†] Assays were developed to measure HSV antibodies using neutralization or complement fixation. Such serological tests provided the first evidence that “fever blisters” recurred in individuals who had been infected earlier, as they already possessed serum HSV antibodies. Later in Phase III, it was shown that the virus remained latent in the local nerve ganglia in mice and humans. The use of cell cultures in the 1950s, together with the earlier methods to identify the virus, expanded the body sites of involvement associated with primary and recurrent HSV infections: eyes, brain, skin, external genitals, as well as infection of internal organs, such as the liver—occurring in neonates and in older individuals severely malnourished or eczematous (Kaposi’s varicelliform eruption). However, the usual maternal genital source of neonatal herpes was not appreciated until Phase III, neither was subclinical genital involvement and particularly the pivotal role of the maternal cervix in the intrapartum transmission to the neonate. Although non-type-specific serological tests provided information on the age-related rise of HSV, from about 50% by age 5 years to 80% or more by young adulthood, there were few other epidemiological data obtained from the 1930s to the early 1960s. These included primarily studies on gingivostomatitis in children (32), and of fever blister rates in adults—varying between 16 and 45% in the different populations studied (4).

PHASE III—THE MODERN PHASE

The noted Australian scientists, F.M. Burnet and Lush (33), made a substantial contribution in the 1930s regarding herpetic recurrences being due to an earlier acquired latent HSV. In 1939, Burnet and Williams (34) reported on a study involving only two HSV isolates from fever blisters (one, an old laboratory strain—“HF” and the other, a new isolate from the same site). They generalized that, since these two strains were antigenically similar, all HSV strains belonged to the same type. This view was

[†] Our first hints of differences between genital and nongenital herpes was noting that the pocks of nongenital isolates were small, whereas those produced by genital isolates were much larger (31). Another early difference was the higher frequency of encephalitis occurring in mice vaginally inoculated with genital isolates, as compared to nongenital ones (28).

apparently accepted until the 1960s, when it was found to be erroneous, based on the findings by Schneweis (35) in Germany, and by one of the authors (A.J.N.), together with Center for Disease Control (CDC) colleagues (28,36). Two distinct HSV types were identified, differentiated initially by the use of several immunological and biological methods, and then with molecular technology [including the currently diagnostically useful polymerase chain reactions—PCR (see chap. 5)]. Clinical isolates could also be differentiated into distinct variants within each type, using restriction enzyme analyses, opening the way to studies on the “molecular epidemiology” of HSV (37). This method has been applied, for instance, in identifying or refuting the source of neonatal HSV-1 or HSV-2 infection in nursery outbreaks, as well as in determining the geographical clustering of HSV-1 in different parts of the world (7,37,38). Unfortunately, unlike several countries that use methods to differentiate the two viral types by culture or PCR for clinicoepidemiological purposes, in the United States such tests have been performed primarily in research laboratories and mostly in populations (STD- and HIV-clinics, and minorities, that are not), that are likely to acquire genital HSV-1. As a consequence, the United States has generally fallen behind, for almost two decades, in appreciating the increase in genital HSV-1 infection observed by many European and Japanese workers* (see below).

The early recognition of the extensive antigenic cross-reactivity between two HSV types[†] (42) rendered type-specific antibody differentiation difficult, despite the large variety of serological assays used in the 1960s and 1970s (18). Type-specific serological determination was only accomplished in the early 1980s, when the newly recognized HSV-1 and HSV-2 type-specific glycoprotein G (gG) proteins were purified with monoclonal antibody-affinity columns (26,43). To save on the volume of the gG₁ and gG₂ antigens that are used in an enzyme-linked immunosorbant assay (ELISA) (50 μ L), we adapted an immunodot assay, which uses only 1 μ L of the antigen per duplicate test. Much effort was placed on ascertaining that this new assay possessed the high sensitivity, specificity, and reproducibility required for epidemiological surveys (44).[‡] Immunoglobulin G antibodies are tested,

* We are among the U.S. workers who failed to appreciate the growing importance of HSV-1 as a cause of genital herpes, despite several early hints: (a) when Dr. T. Kawana from Tokyo joined our laboratory in 1978, we confirmed that about half of his Japanese genital HSV isolates were indeed HSV-1; (b) in studies in a state university in the 1980s (39), we noted a 12% rise in HSV-1 antibodies in students followed for 4 years (40), and observed that, when clinically manifest, about half of genital isolates were HSV-1—but the number of individuals were small and the findings went unreported.

[†] Such cross-reactivity also impacts immunologically, both as regards cross-protective aspects between the two types, and also to help explain why seronegative, and not HSV-1-positive, women benefit from a recombinant gD2 vaccine in preventing HSV-2 infection and disease (41).

[‡] Specificity was ascertained in the larger NHANES II studies by ensuring that blinded sera from children 1–10 years of age were almost always negative (0.1%).

since IgM antibodies are short-lived, and are also often detectable after a recurrent infection. The immunodot assay has been applied, over the past two decades, to three U.S. NHANES surveys (45–50), and to several populations in the United States and 20 other countries (13,51–53). The same assay was also used to demonstrate the likely role of HSV-2 as a facilitator of HIV acquisition and transmission in African, European, and American heterosexual or homosexual cohorts (54–60).

A Western blot assay used to detect the gG₁ and/or gG₂ bands was later developed by the Seattle group and was validated, using specimens we provided (61). More laborious to perform, the Western blot was slightly more sensitive than the immunodot assay in detecting antibodies in early convalescent sera, although both tests were later shown to occasionally fail to detect antibodies up to 2 months after first infection. Such events are expected to be so few, as not to affect large seroepidemiological studies significantly. In recent years, more than five homemade assays and 10 commercial ELISAs have been used for various seroepidemiological surveys (reviewed in Ref. 13, 14, 107, 108). Of importance is that many of these tests have not had the same intense validation as to sensitivity, specificity, and reproducibility, as established with the immunodot and the Western blot methods [e.g., one of the most widely used commercial assays (Gull) was found to yield 11% false results compared to the Western blot]. Few studies have been performed with commercial HSV-1 antibody assays, which appear to have poorer specificity than HSV-2 antibody tests (62). In view of the possible misleading information that can impact on a person's future well-being, whenever results of HSV-2 serological tests are reported to individuals, a second confirmatory serological assay is advisable.

For reasons noted later, HSV-1 antibody testing is important to obtain, in addition to that for HSV-2 antibodies. The results of tests on sera/plasma, which show positive or negative reactivity to gG₁ and gG₂, are interpreted as follows:

1. gG₁+ only reflects
 - a. a *primary* HSV-1 infection in the past; or
 - b. an *initial first* HSV-2 infection, in case HSV-2 can be isolated for the first time in the presence of a negative gG₂ antibody reactivity;
2. gG₂+ only reflects a primary HSV-2 infection in the past;
3. gG₁+ / gG₂+ reflects prior dual infections with HSV-1 and HSV-2;
4. gG₁- / gG₂- reflects total seronegative sera, usually signifying no prior experience with either HSV-1 or HSV-2—unless the serum was obtained during the acute phase of the primary infection, before antibodies can be demonstrated.

The prevalence of HSV-1 antibodies in the population tested is calculated to be a + c, and that of HSV-2 antibodies to be b + c. Worth

emphasizing here is that the presence of HSV-2 antibodies almost always represents sexually acquired infections, in view of the relative infrequency of neonatal or nosocomial HSV-2 infections. Also noteworthy is that, when HSV-2 antibodies are present, “pure” HSV-2 antibodies (with no HSV-1 antibodies) are found more frequently in higher SES populations, while “dual” HSV-2 + HSV-1 antibodies occur more commonly in lower SES populations, who usually experience HSV-1 infections during childhood and HSV-2 infections at a later age. Compared to oral infection—the most common source of HSV-1 antibodies in most populations—only a relatively small proportion of the total HSV-1 antibody prevalence is a result of facial and other skin manifestations, and even less frequently due to ocular or Central Nervous System HSV-1 infections. However, in some groups within the higher SES populations (as discussed later), genital HSV-1 is now representing a more significant proportion of HSV-1 antibody positivity, particularly in adolescent females, in men or women < 25 years of age, and in homosexual individuals. [A formula (13) can be used to estimate the proportion of sexually-transmitted HSV-1 antibodies (Fig. 1), when HSV-2 antibody prevalence is > 10%.]

In case of a primary genital HSV-1 infection, involvement of other sites is not uncommon—either from concomitant genital and oral sex acquisition (63), or occasionally in young children by autoinoculation from an oral herpetic infection to the genitalia (64). It is worth noting that obtaining

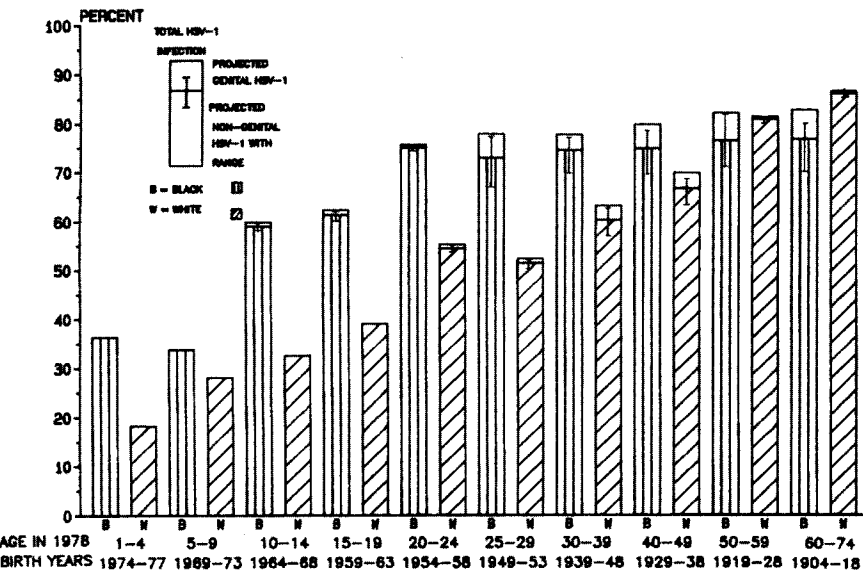


Figure 1 Prevalence of HSV-1 antibodies in Whites and Blacks in the United States (1976-1980)—estimates of proportion of past HSV1 infections, which are sexually acquired. *Source:* From Ref. 13.

HSV-1 or (HSV-2) antibody *titers* are usually of little help, as they are commonly boosted by recurrences. Those individuals with more frequent recurrences will usually have higher antibody titers in their sera than those with low antibody titers. Antibodies appear to play little role in preventing local or contiguous site recurrences, but provide an important factor in the prevention of disseminated neonatal infections (65,66). Visceral dissemination of herpetic infections is rare in HSV antibody-positive AIDS patients, even those with very low T-cell lymphocytes in the peripheral blood. However, the lower cell-mediated immunity associated with HIV and other immunocompromised hosts is associated with the more frequent and longer-lasting herpetic recurrences, and also with the occasional involvement of contiguous sites, such as the esophagus or rectum. As a consequence, HSV transmissibility is increased from such compromised hosts.

A prior HSV-1 infection will generally prevent a genital HSV-1 infection, but only infrequently will it prevent a genital HSV-2 infection (2,4). However, individuals with such *initial first* HSV-2 infections usually demonstrate lesser clinical manifestations than primary ones. In comparison, "true" primary HSV-2 infections, which also may be subclinical, often exhibit an increased occurrence of fever and severe pain, as well as the number and the duration of lesions. The same clinical and subclinical patterns observed with primary HSV-2 also occur with primary HSV-1 infections; however, meningitis is more common with HSV-2 and encephalitis with HSV-1.

Obtaining HSV-1, concurrently with HSV-2, antibody assays was helpful in providing the first suggestion that HSV-2, acquired early in life, generally protects from HSV-1 acquisition (13). It was noted in four separate adult populations that the unexpected low prevalence of HSV-1 antibodies could be made up to expected levels if one added the proportion of HSV-2 antibodies that were "pure"—indicative of a primary type 2 infection. Similar observations were made in two more recent studies (67,68). Thus, in HIV-positive U.S. pregnant women, with a very high prevalence of HSV-2 antibodies (50+%), the proportion of HSV-2 antibodies was found to be greater than that of HSV-1. The proportion of "pure" HSV-2 antibodies was found to fill the virtual gap left by the lower HSV-1 antibodies, to around 100%.* These findings were interpreted as due to the acquisition of HSV-2 at an earlier age than HSV-1, as a result of sexual relations at a young age, including sexual abuse. Such a conclusion has great immunological relevance, as it infers that HSV-2 protects from acquisition of HSV-1, and is particularly important as regards the possibility that an HSV-2 vaccine could provide protection for both types. More direct

* An example might facilitate understanding of these findings: assume the HSV-1 antibody prevalence to be 70%, that of total HSV-2 antibodies to be 75%, and of "pure" HSV-2 antibodies to be 30%. The 30% "pure" HSV-2 rate would raise the 70% HSV-1 antibody rate actually noted to virtually 100%.

prospective studies in two populations have supported this concept (25,65). A recent study in a guinea pig model of genital HSV-1 and HSV-2 infection has also revealed that a vaccine, composed of recombinant gD protein from HSV-2 (41), could partially protect the animals from a genital HSV-1 infection (69). It should be noted that HSV comprises large numbers of protein antigens, so that an HSV-2 vaccine containing more than one HSV protein may prove even more capable of preventing infection and disease due to both viruses. Also needing emphasis is that not only would it be helpful to prevent genital HSV-2 or HSV-1 infections and possible ensuing neonatal infections but also to prevent HSV-1 infections of other important sites, particularly the eyes, the brain, and various body sites involved in immunocompromised hosts. What is not often remembered is that, even though genital HSV-2 infections have received more study in Phase III than HSV-1 infections, evidence from many sources supports the view that HSV-1 infections represent overall a greater cause of *physical* morbidity and mortality than HSV-2 infections.

Reactivation of a primary genital HSV-2 infection has been noted to occur more commonly than that of a primary genital HSV-1 infection (63), also shown in the guinea pig genital model (69). However, preliminary data suggest that recurrent genital HSV-1 infections in humans may be increasing in frequency (71,72). Although primary HSV-2 infections can occur in the oral cavity, reactivations are very uncommon (73). With both HSV types, reinfections with the same viral type is unusual (74,75), as is superinfection of genital HSV-1 with HSV-2 (76). In such instances, the newly acquired genital HSV-2 has tended to recur more frequently than the earlier-acquired HSV-1.

VIEWING THE NATURAL HISTORY AND EPIDEMIOLOGY OF HSV-1 AND HSV-2 IN CONTEXT OF THE MAJOR RECENT CHANGES IN THE WORLD

Herpes simplex virus infections have been regarded—if not defined—by many as being “ubiquitous”—based on the findings that, until the 1970s, the HSV antibody prevalence approached 100% in all populations studied (see above). HSV infections have also frequently been considered as “pandemic,” as well as “emerging” infections. Such terms have too often been applied loosely in relation to the contemporary epidemiological understanding of the particular HSV type in the various (sub) populations within different countries of the world.

“Ubiquitous”—implies infections occurring in (almost) *everybody* in the world, without defining the particular group. Is HSV-1 ubiquitous in those below 20 years of age, or is HSV-2 ubiquitous at any age in populations of developed countries?

“Pandemic”—implies occurring all over the world in *epidemic* proportions and has been attributed heretofore primarily to HSV-2. Although, HSV-2 may be epidemic in some areas, it has been endemic at a relatively constant low or high rate in some populations and at a high rate in others.

“Emerging infection”—HSV-2, as a major cause of genital herpes, emerged when first recognized, mostly in the newly “sexually liberated” individuals of the 1960s and 1970s as a persistent sexually transmitted infection, which was incurable, and could lead to newborn herpes and was associated with cervical neoplasia (2,3). Actually, genital herpes had been recognized for two centuries and was already endemic in developing countries and in some minority groups within developed ones. On the other hand, current data presented below suggest that genital HSV-1 infection might already be considered as an “emerging” ongoing new epidemic among adolescents and young adults within higher SES populations.

The differentiation of HSV into two types in the 1960s (28,35,36) permitted, what appeared to be a general—but *never absolute*, association with site of involvement and with certain forms of herpetic diseases. Thus, a large series of close to 1000 viral isolates from different body sites and disease entities, typed in the 1960s and 1970s (73), revealed that—beyond the neonatal age—the great majority of oro-labial, facial, and ocular infections, as well as of encephalitis and the more severe infections, involving different sites in compromised hosts, were associated with HSV-1. During the same two decades, HSV-2 was noted to be the major type isolated from the genital and ano-rectal sites, and in cerebrospinal fluid (CSF), when associated with the meningeal complications of primary genital/anal infections. Since most maternal genital isolates around the time of delivery were due to HSV-2, isolates from infected neonates from various local skin, eye, and oral sites, as well from the CSF, brain, and various visceral organs were also HSV-2. Beyond the newborn age, infections of the skin below the waist were primarily due to HSV-2, and those of the hands were caused about equally by either type. Isolates of a limited number of latent viruses recovered from trigeminal nerve ganglia yielded HSV-1 and those from the sacral ganglia HSV-2. A large series in the 1990s from Sweden (77) confirmed most of these results, but found a larger proportion of HSV-1 isolates associated with genital/anal sites and of HSV-2 in upper body skin areas below the face.

EPIDEMIOLOGY OF HSV-1 INFECTION

Studies performed in Yugoslavia (32) on close to 20,000 children with primary herpetic oral infections—gingivostomatitis—noted a pattern likely to be still present in those populations with high HSV-1 acquisition in the first

5 years of life, e.g., the Amazon or Navajo Indians (11,12). The Yugoslavian studies (32) revealed no cases of gingivostomatitis at 1–6 months if age, 12% at 6–10 months, 35% at 1–2 years, 23% at 2–3 years, 11% at 4–5 years, and 8% at 5–6 years of age. Outbreaks of oral herpes, many asymptomatic and only detected by frequent examinations of the oral cavity, were detected in orphanages (4). Although a Japanese study recorded a day care nursery outbreak of gingivostomatitis (78), NHANES surveys failed to find an association of HSV-1 antibodies with day care attendance (50). In a more hands-on study, which identified the common spread of cytomegalovirus in day care centers, acquisition of oral HSV-1 was mostly associated with family members with prior oro-labial infection (79). The major significant variables identified in both the United States national surveys (50) and in Navajo Indians (12) were low socioeconomic status, and crowded households with high rates of HSV-1-positive family members. Females and black individuals in the national surveys were noted to be more likely to be HSV-1 antibody positive than males or whites (Fig. 1).

Oral herpes has been associated occasionally with pharyngitis, tonsillitis, and other respiratory infections (4), although reactivated HSV associated with another causal agent, makes one suspicious regarding causality. Reactivated herpes within the oral cavity is common but difficult to diagnose, as it is most often subclinical. Reactivation rates of oral HSV-1 appears to be as common as those of genital HSV-2, varying among individuals, being more frequent in the immunocompromised (126). Manifest orolabial HSV-1 occurs in 15–45% of HIV-1 antibody positive persons (4).

Seroepidemiological studies conducted in the 1980s of the HSV-1 prevalence in different adult populations in the United States and 20 countries (13) noted a marked correlation between HSV-1 prevalence and SES of a country or minority ethnic group. Adults, ranging mostly from 20 to 40 years of age, within limited groups of different types of populations from several cities in developing countries of Asia, Central America, and Africa, showed prevalence rates of 85% to close to 100%. Populations from more developed countries—France, Japan, Holland, Scandinavia, and whites from the United States—had rates between 50% and 70%. More recent surveys conducted mostly in the 1990s (14), with similar limitations as to the types of populations studied—but with more concerns regarding serological specificity (62)—showed essentially similar trends, in relation to (SES), e.g., Syria with >85% HSV-1 prevalence by age 20.

Studies performed in the last 10 to 15 years in individuals <30 years of age in Sweden, Japan, Switzerland, and the United Kingdom, as well as in U.S. university students, noted an HSV-1 antibody prevalence of <40% (19,39,80–82). Repeated surveys of children <15 years of age in the United Kingdom revealed a decrease over the years of HSV (untyped), or of HSV-1 antibody rates from 85% in 1953, 34% in 1987, and down to 24% in 1995 (83). Figure 1 shows that, in the U.S. National Health and Examination

Survey (NHANES) II (1976–1980), HSV-1 antibody prevalence rates in <15-year-old individuals were about twice as high in U.S. blacks (60%), as compared to whites (34%). The rates in the age group <20 years of age were 64% for blacks and 40% for whites. The NHANES III (1986–1994) survey (50), which also included Mexican Americans, found that the rates in those <20 years of age were 40% in white, 58% in black, and 72% in Mexican American females. The prevalence in males <20 years of age was overall slightly lower.

It must be emphasized that the bar graphs, shown in Figure 1, represent prevalence—and not incidence—rates, i.e., they do not necessarily reflect new acquisition in the same individuals with increasing age. Thus, while it is most likely that in the younger age groups (<30 years of age), there may well have been an actual increase in viral acquisition, the difference in prevalence rates between age groups >30 years is almost all due to a *cohort effect*. Thus, it can be noted that there is a rise in the HSV-1 antibody rates among African Americans up to around 25 years of age, after which they plateau. Among white Americans, the prevalence rate after age 30 increases for each age group. It is well appreciated that the overall socioeconomic improvement in much of the U.S. white population was mostly manifest in children born after the Second World War, while socioeconomic improvement in the minority population did not improve for some time thereafter. This is partly substantiated by comparisons between the NHANES II and III surveys, obtained about 12 years apart. Black males and females, who were below 30 years of age, showed a decrease of about 10% in HSV-1 antibody prevalence rates over this period. The decrease in similar aged white males is less marked, while white females <30 showed no difference in prevalence rates. These findings also suggest that the improvement in SES among whites was counterbalanced by an increased acquisition of primary genital HSV-1 infections, more common in females than males—as is now being confirmed from studies in young, mostly white U.S. populations (84–86,126).

In comparison to NHANES II, the prevalence of HSV-1 in the NHANES III survey increased in U.S. whites about 10% per age group, until the 70 years of age group, in contrast to the plateauing in the rates in African Americans (and Mexicans) after 30 years of age—again suggestive of a cohort effect. If this were not the case, during the period of two decades comprised within the two NHANES surveys, one would have expected a marked increase in primary HSV-1 infections in older majority white adults, which has not been recorded, to our knowledge, in the literature.

The decline in the prevalence of HSV-1 antibodies in the younger age groups among higher SES populations in various countries represents one of the major factors responsible for the increasing trends of genital HSV-1 infections. In such young individuals, 60–80% are seronegative for both HSV-1 and HSV-2 antibodies, and are thereby more likely to acquire HSV infection of either type. As noted later for HSV-2 in the U.S.

NHANES studies (46–49), the pool of HSV-2 in young white male or female individuals, particularly < 20 years of age, is still very low, affecting 0.5–2%. The pool of HSV-1, mostly related to oral herpes, is 20–40%. Assuming that the reactivation of both oral HSV-1 and genital HSV-2 is overall approximately the same, it would appear that heterosexual females are ~20 times more likely to contract genital herpes from a male partner with recurrent oral HSV-1—via oral sex—than one with a recurrent genital HSV-2 infection. Assuming also that the frequency of oral–genital and genital–genital sexual acts are about the same, and with the appreciation of the greater vulnerability of the female genital tract to infection with either HSV type, it would not be unexpected to find that primary genital HSV-2 is being replaced by genital HSV-1, particularly in adolescent females and young women. A coincidence of several events has apparently increased the more general oral-sex practices—both fellatio and cunnilingus—in adolescent and young adults during Phase III (reviewed in 87, 126): (a) the overall “sexual liberation” with several surveys indicating that 50–80% of teenagers practice oral sex, often as part of a casual relationship; (b) the impact of HIV with the belief that oral sex is “safe sex”; (c) the concept that oral sex is not “real sex,” fulfilling virginity desires and abstinence pledges.

The transmission of oral HSV-1 from hand masturbation by a partner, who uses HSV-1-contaminated saliva, provides another possible, though difficult to validate, viral transmission method. Limited data are available in lesbians, regarding genital HSV-1 acquisition (88), whereas some homosexual men (MSM) are likely to have a high rate of genital/anal HSV-1 infection (84). It is not known whether the greater prevalence observed in the 1980s, of HSV-2 antibodies than HSV-1 antibodies still pertains (55).

Of the many reports of genital HSV-1 infection in several Western European countries and Japan, and more recently, Israel and the United States (71,72,81,84–86,89–95,126), a few deserve special note. The data from Glasgow, Scotland (94), from the mid-1980s over 15 years, indicate a general increase in the proportion of genital HSV-1 isolates (Fig. 2). By the year 2000, the increase was found to be greatest in females below 25 years (~70%), lower in males <25 year old (~60%) and in females >25 years (~55%), and lowest in males >25 years (~35%). The earliest appreciation of the growing proportion of genital isolates being HSV-1 appears to have occurred also in Edinburgh (89), and in other places in the United Kingdom (England and Northern Ireland) (71,90). Japan has also recorded a high proportion of genital herpes since the mid-1970s (81).

Most of the studies are the result of viral isolate-type surveillance without concurrent HSV-type antibody studies. This combined approach has been applied in a Swedish sexually-transmitted disease (STD) clinic (91). The findings indicate that 84% of primary genital infections were due to HSV-1 in females and 54% in male patients, with 90% giving a history of oral sex, and 50% of contact with a partner with oro-labial herpes. A Seattle

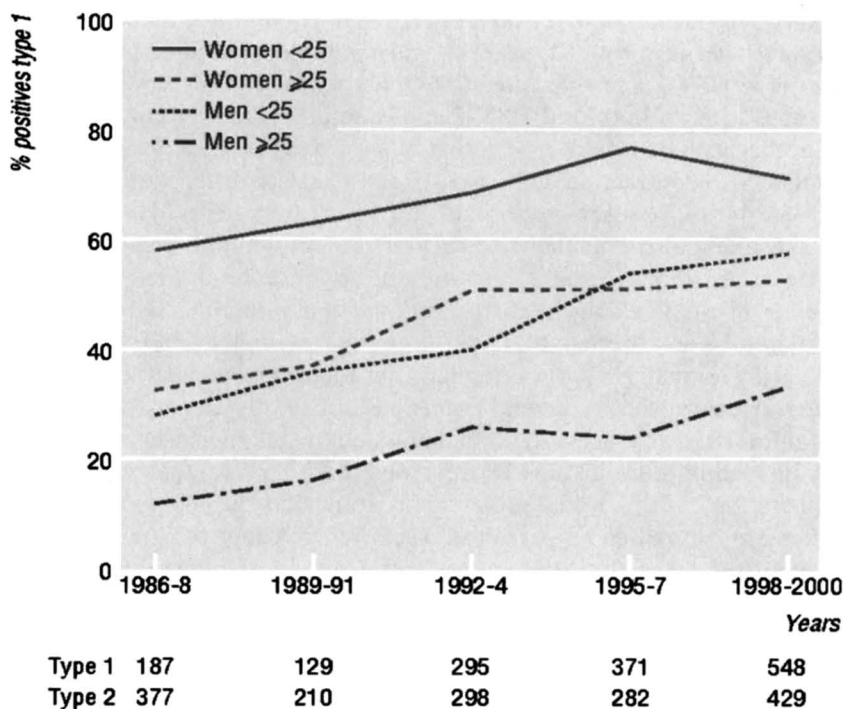


Figure 2 HSV-1 prevalence in western Scotland over 15 years (1986–2000). *Source:* From Ref. 94.

report also indicated the correlation of genital/anal HSV-1 with oral sex, and the higher prevalence of genital HSV-1 in young females than males, and in whites than blacks (84).

Of particular interest is a report from Northern Ireland, based on a retrospective study of HSV isolates from clinically manifest genital herpes (71). The authors found that genital herpetic recurrences in their patients were more commonly due to HSV-1 than HSV-2 in females, while infrequent in males. An increasing prevalence in recurrent genital HSV-1, from 4 to 15%, has also been noted in Norway (72). As schematized in Figure 3, in case higher reactivation rates of genital HSV-1 infections are confirmed by appropriate longitudinal studies, it might be possible to identify HSV-1 subtypes with increasing abilities to reactivate. It is well appreciated that the main epidemiological advantage of HSV-2 in the genital tract is its more common reactivation rate than HSV-1 (96), noted also in a guinea pig model (69). Such new strains of HSV-1 would enhance the frequency of genital-genital/anal

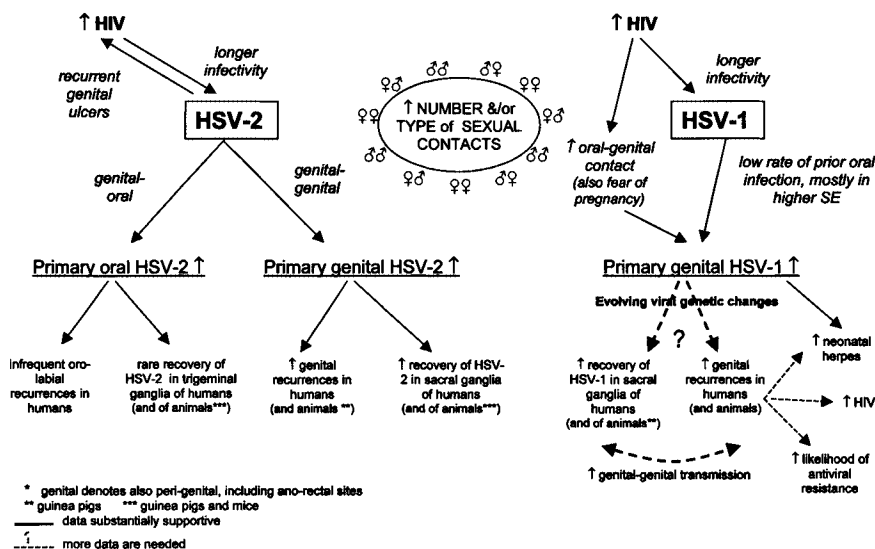


Figure 3 Increasing emergence of genital HSV infection associated with changes in socioeconomic status (SE), sexual behavioral patterns, and interactions with the human immunodeficiency virus (HIV-1)—possible effects on the ongoing viral evolution of HSV-1 affecting reactivation of latent virus in sacral ganglia, recurrence rates, genital-genital/anal transmissibility, antiviral resistance and neonatal herpes.

HSV-1 infections. In turn, this might provide greater facilitation of HIV acquisition and transmission. Furthermore, any increase in genital HSV-1 in pregnant women, particularly primary infections, is likely to cause an increase in neonatal herpes due to HSV-1. Such increases have already been reported in the United States (97) and in Europe (98).

Beyond what has been discussed regarding oral, genital, and newborn herpes, there has been relatively little new information on the epidemiology of other important HSV-1-related infections (4). In a recent French review of 104 articles from 1995 to 2001 on HSV-1 infections (other than genital or neonatal), Martin emphasizes how badly needed are current studies on the epidemiology of oral, ocular, and skin HSV infections, particularly of primary cases in adult populations (99). Estimates of the incidence of HSV encephalitis, almost always caused by HSV-1 beyond the newborn age, vary widely from 1 in 100,000 to 1 in a million per year (100,101). These Finnish and U.S. studies indicate that HSV, primarily HSV-1, accounts for about 10–15% of cases of encephalitis. Infection of the brain can be a result of primary or recurrent infection, or of reinfection with the same strain of HSV-1, as determined by restriction enzyme analyses (75).

The natural history of the various forms of ocular involvement, which occur in about 20% of cases of newborn herpes, has been reviewed by our

group earlier (102). The virus can cause external lesions—conjunctivitis and keratitis, internal lesions, e.g., chorioretinitis, as well as visual cortical involvement. Outside the newborn age, it is estimated that about half a million Americans have experienced ocular HSV disease and that approximately 50,000 new and recurrent cases occur every year in the United States (103). The many forms of ocular involvement in adults have been reviewed recently by Liesegang (104). The epidemiological data used by that author were derived from a U.S. multi-institutional (HEDNA) study that demonstrated the effectiveness of acyclovir for the prevention of recurrent HSV eye disease.

EPIDEMIOLOGY OF HERPES SIMPLEX VIRUS TYPE 2

As noted earlier (Phase II), genital herpes and its sexual transmission were well appreciated, particularly in France and Germany, from the 18th century up to around the 1930s. Thereafter, for several decades, the English literature suggested a more common mode of transmission to be autoinoculation from oral herpes. To our knowledge, that mode of genital transmission has only been reported in the past few decades in children (e.g., Ref. 65). In 1946, Slavin and Gavett (105) were the first to obtain HSV genital isolates from two patients. These workers found that one of the genital isolates was antigenically similar to a laboratory strain (“HF” from a fever blister), now known to be HSV-1. The second isolate was noted to be antigenically different (the report presents an incidental comment that the partner of the case with that isolate had genital herpes). If both genital isolates had been different from the HF (HSV-1) control, it is likely that these workers would have pursued their findings. Instead, it took almost two decades before it was shown that all eight genital isolates tested by our group (36) were HSV-2, in contrast to dozens of isolates from nongenital sites. HSV strains—now known to be HSV-2—had been isolated from the urine and the thigh by Schneeweis in Germany; the “MS” strain (the prototype in many laboratories of HSV-2 for many years) was isolated by Gudnadottir in Iceland from the brain of a patient with multiple sclerosis (28,35). For over a decade, HSV-type identification proved much easier to perform (73) than HSV type-specific antibody differentiation (18). When the latter became possible (26,43), we showed that sera collected in the 1960s from patients in our Atlanta hospital, serving a predominantly black population, were already 50–60% HSV-2 positive (13). We also found that HSV-2 was already common in Zaire in 1959, with an HSV-2 antibody prevalence of 21% in Kinshasa; by 1985, the rates had climbed to 60%.

In Zaire, as in many other developing countries during the Phase III period, many socioeconomic and cultural changes occurred after World War II with the end of colonization and ensuing civil wars, associated with a great movement of populations and urbanization. These changes most likely contributed to the growing AIDS epidemic, already present from

the 1950s or earlier (106). Sexual cultural patterns apart, all this turmoil has worsened SE conditions, and caused many adolescent girls to have early sexual relations with older men. For example, whereas HSV-2 antibody prevalence is less than 2% in U.S. adolescent whites, and less than 10% in U.S. adolescents blacks (46,49), it may be as much as 50% or more in some sub-Saharan adolescents (e.g., Ref. 108).

The earliest natural history and epidemiological studies of HSV-2 in the 1960s and 1970s were performed mostly in minority low SES hospital or STD populations in Atlanta (13). Further subpopulations were studied thereafter in many parts of the world (reviewed in Refs. 13, 14, 107 and 108- the latter two reviews adapted the data from Ref. 14). Of note is that the main U.S. hospital populations studied by our Atlanta and the Seattle team (63,65,93,96,97,109–115) varies widely in demographic characteristics, particularly as regards SES (Atlanta low, Seattle high), and the proportion of ethnic minorities (Atlanta—mostly black, Seattle—mostly white). In many respects, the principal Atlanta hospital population studied has more of the characteristics of developing countries and of ethnic minorities in developed countries. The Seattle population is more representative of the majority in developed countries. Thus, the high HSV-2 prevalence of 50–60% in pregnant women in the Atlanta hospital, studied over two decades, is about double than in Seattle. Yet, the rate of neonatal infections, still mostly due to HSV-2 in Atlanta, is less than half that of Seattle, which has a greater proportion of both genital and neonatal HSV-1 (66,97,116). Surveys in private practice, Health Maintenance Organizations and Sexually Transmitted Diseases or HIV clinics, as well as in homosexual men are more comparable between Atlanta and Seattle.

The more general availability of viral typing and antibody-type differentiation has provided a wider perspective of HSV-1 and HSV-2 infections in several areas of the world. While there are some controversial points, noted later, regarding seroepidemiological results (13,14,107,108), the overall picture of the natural history of HSV-2 infection, denotes the following:

1. HSV-2 infections are almost always associated with *sexual transmission* and thereby have the major attributes of a “true” *sexually transmitted* infection, whereas HSV-1 is still mainly a sexually transmissible infection, as it has another major route of transmission (oral) for evolutionary survival (5). HSV-2 can be acquired nosocomially, e.g., the hand of a nurse or in nursery outbreaks (3).
2. The majority of HSV-2 infections—whether primary, initial first, or recurrent, are subclinical, with the cervix in women being an unrecognized site most important to maternal HSV transmission intrapartum to a neonate. The maternal infection can be transmitted from a pregnant woman to her infant—less frequently with recurrent HSV-2 (1–5%), than with primary HSV-2 (or HSV-1)

- infections (~50%); the virus may infrequently also be acquired in utero or postnatally (66,117).
3. HSV-2 is more common in those individuals: (a) whose sexual debut occurs early, including sexually abused boys or girls; (b) who have large numbers of partners, with whom they have genital-genital/anal sexual contacts; (c) who have experienced earlier, or concurrently, other sexually transmitted infections, e.g., HIV or syphilis (see below).
 4. HSV-2 is found most commonly in commercial sex workers, and more frequently in homosexual men than in heterosexual men or women (13), and appears to be less common in lesbians (88).
 5. Significant risk factors for HSV-2, found in some, but not all, populations are: (a) lower SES; (b) lesser education; (c) use of drugs, alcohol, smoking, and douching (the effect of circumcision is controversial).
 6. Genital recurrences are 2–3 times more commonly detected by PCR than by viral cultures, but it is not clear whether viral DNA detected close to delivery represents potentially transmissible virus to the neonate (113); HSV-2 recurrences in the genital tract have been (so far) recognized as more common than those caused by HSV-1; HSV-2, acquired orally by fellatio can cause stomatitis, and is less likely to recur in the mouth or lips, than is orally acquired HSV-1.
 7. The rate of acquisition of genital HSV infection has been evaluated in a number of studies on heterosexual mono-partnered relationships of immunocompetent individuals, in which only one partner is positive for genital HSV-2 (112,115,118). In many cases, the studies have differed as to the original study purpose (e.g., serving as placebo controls of some preventive measures), and to the number of subjects and their demographics and adherence to abstinence when lesions were present, as well as to whether the susceptible partner was initially seronegative or HSV-1 antibody positive. Overall, the majority of acquisitions were from an asymptomatic positive partner, with the rate being greater (2 or more times) in females than males, and with an increased number of sexual acts per month. The rate of acquisition was lower in case of longer duration of the disease in the partner and of the relationship on entry into the studies.

Prior to the development of type-specific antibody surveys, conflicting epidemiological findings, based on different methods, were observed. We employed the characteristic cytological changes of HSV found on Papanicolaou smears to detect genital herpes in our Atlanta hospital, serving mostly low SES black women (13). From 1964 to 1979, a relatively uniform annual

rate of 1–2% annually was noted in over 250,000 Papanicolaou smears, in which the typical herpetic cytological markers (30) were observed by the same examiner. In contrast, in the 1970s, clinical surveys in the United Kingdom and United States appeared to demonstrate very large increases in clinically manifest genital herpes (13). In the United States, the surveys involved different private practice doctors over time, during a period when genital herpes was receiving a great deal of medical and media “notoriety.” U.S. whites were more likely to develop primary infections, often clinically manifest, compared to our cytologically studied Atlanta black population, who had acquired a generally clinically protective immunity due to HSV-1 infection earlier in life. When seroepidemiological studies became available in the 1980s, it was noted that only 1–10% of HSV-2 antibody-positive individuals in the Atlanta community hospital black population, and in many other such lower SES groups, gave a *history* of genital herpes. In comparison, higher SES individuals were more likely to give such a history (from 20% to 50%).

In order to interpret the large number of seroepidemiological surveys obtained since the early 1980s, it is important to differentiate two available reviews of large numbers of surveys: (a) those performed mostly in the 1980s, in varied groups in the United States and 20 countries, using the *same* immunodot assay (13), and (b) those performed mostly in the 1990s, in various groups over 40 different countries, using over a dozen different serological tests (14,107,108). Whereas we always tested for HSV-1 concomitantly, only about 1/3 of those tested for HSV-2 antibody in the 1990 surveys [reviewed by Smith and Robinson (14)] included HSV-1 antibody testing. We generally concur with the latter reviewers (14) that “comparisons of the prevalence of HSV-2 and HSV-1 infections by geographic area or country are hampered by differences among populations surveyed, and the use of different serological assays. Similar caution was expressed in Malkin’s review (107). Two other issues are of concern:

- a. *Time factor*—The results obtained most often from a single survey performed, e.g., in 1985 or 1995, is not representative of the prevalence of either HSV-2 or HSV-1 in that geographic area or group studied 8–18 or more years later—in view of the changing dynamics of several influential factors emphasized earlier. Thus, it is not clear whether incidence rates based on studies we conducted in the 1980s (13) still hold: (i) in university students followed for 4 years—2% per year for HSV-2; (ii) in a health maintenance organization (HMO), 2% per year for individuals followed over 3 years; (iii) in an STD clinic, 5% for patients followed over 6 months; (iv) in homosexual men followed over 7 years, 5%; and (v) for white women during pregnancy, 2%.

Comparisons of prevalence rates, as noted earlier in the Zaire urban area, have also provided trends of increases over the 16-year

period, even if not performed in the same individuals. Increased prevalence rates over time have been noted in other populations followed over some years, e.g., in Sweden—with prevalence rates showing an increase over the 1970s, and plateauing thereafter (119).

- b. *Extrapolation of limited data*—In estimating infection or disease incidence or prevalence, many epidemiologists have had to use data obtained in a relatively small, often convenient sample, not truly representative of the larger population. These limited prevalence rates are sometimes extrapolated to the larger area (city, state, province, or country) and—together with similar limited data obtained in some other parts of the country—may then even be extrapolated to a whole continent, and even to the whole world. There are many exceptions in the generalizations made in the reviews of disparate groups within a few cities of a continent, e.g., that Western and Southern Europe have lower rates of HSV-2 than Northern Europe and North America. Differences can, in fact, be found within the same country, or even the same city, e.g., we noted that HSV-2 antibody rates were 2–3 times higher in a large Swedish city (Stockholm) than in a small city (Örebro), and the same rate differences were found between a large (Atlanta) and a small (Birmingham, Alabama) U.S. city (13). Even within one city—Atlanta—the rates were two times greater in lower SES than in higher SE groups. A French report has also noted differences according to geographical area (120). Other examples of over-generalization are the low rates noted for the whole continent of Asia, based in the review of the 1990s surveys (14), with only ~300 individuals tested in China (including Hong Kong) and none at all from India. We had noted earlier (13) a very large difference, in the 1980s, of HSV-2 antibody prevalence rates in one region of China between women > 50 years of age (55% HSV-2 positive) and those < 30 years of age (2% HSV-2 positive). We attribute this large difference to the Chinese Revolution with its stringent regulation of sexually transmitted diseases affecting the younger age group. HSV-2 is likely to have been endemic pre-revolution as indicated by the high rates in older women; however, the current increase in HIV in some parts of China suggests that the HSV-2 prevalence is likely to be on the rise again.

The most consistent trends, found in all reviews (13,14,107,108), was the greater prevalence of HSV-2 antibodies in individuals with high-risk sexual behaviors, e.g., commercial sex workers, who demonstrate extremely high HSV-2 prevalence rates of 60–100%. As regard interpretation of the age-specific rates, we concur with the general increases by age, but the more

recent reviews (14,107,108) do not mention the likely *cohort effects* noted earlier in some of the older age groups.

Most, but not all of the weaknesses of the reviewed seroepidemiological national surveys can be remedied by using NHANES-like approaches. The U.S. NHANES surveys comprise numbers of target individuals (tens of thousands), representative of the whole U.S. population. Weighted and multivariate statistical analyses are used to derive the national prevalence data. The repeated (now continuous) surveys permit following trends over the years, according to age, gender, ethnic grouping, and region of the country. In addition, information from questionnaires has enabled various socioeconomic, educational, and possible risk factors to be correlated, including an HSV history, and a limited sexual history that does not include questions related to same-gender relations or oral sex.

NHANES II—1976–1980 (45) demonstrated a marked difference in HSV-2 prevalence rates between men and women, and between whites and blacks, which was observed again in the following two surveys (46,48). Suggestive of a cohort effect is that the prevalence rates plateaued among whites by 30–40 years of age, but increased with age in the minority groups, indicating that the HSV-2 pool had been higher one or more decades earlier. In all ethnic groups, the rates noted below 30–40 years of

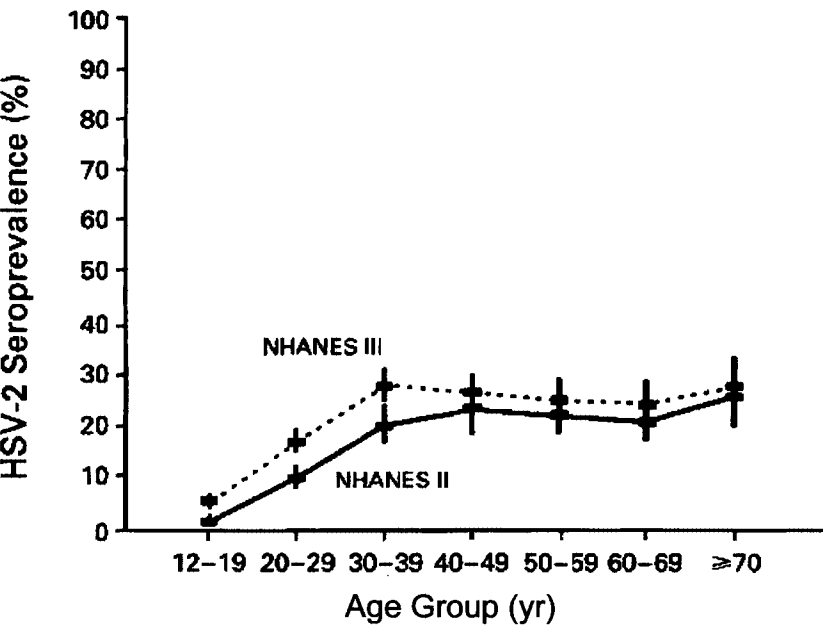


Figure 4 HSV-2 seroprevalence according to age in NHANES II (1976–1980) and NHANES III (1988–1994). *Source:* From Ref. 46.

age represent most likely increasing incidence. This is substantiated by the mathematical modeling report (47), which compared the HSV-2 antibody prevalence between NHANES II and III, pointing to a significant increase in 20–40-year olds, almost exclusively in whites (Fig. 4). Over a 12-year period, the incidence was estimated to increase from 4.6 to 8.4 per 1000, or 83%. Estimates were also made that, by 1992, 45 million Americans had already been HSV-2 infected and that, annually 700,000 men and 900,000 women were likely to become infected anew. These estimates did not include the contribution of genital HSV-1. It is also important to appreciate that, even though the overall prevalence rates in blacks did not differ between the two surveys, the *total number* of blacks with genital herpes has increased, in view of the rise in the black population over the 12 years between the two surveys.

The epidemiological aspects of neonatal herpes, reviewed in 1970 (121) and again recently (66,116,117), have also changed over the decades. Most important is the increasing role of primary maternal HSV-1 infection acquired close to the time of delivery (97), most likely by oral–genital contact. However, the highest morbidity, despite antiviral therapy (122), is in neonates who develop local central nervous system (CNS) disease; this entity is more commonly a result of maternal HSV-2 reactivation, which has a much lower risk of maternal–neonatal transmission (1–5%) than a primary HSV infection (~50%). Discussed later is the fact that, despite their great advantages, the NHANES-like surveys do not provide detailed information on particularly important groups such as pregnant women.

HSV INTERACTIONS WITH OTHER SEXUALLY TRANSMITTED INFECTIONS, PARTICULARLY HIV

Bacterial vaginosis has been reported to increase seroconversion of HSV-2 (123). HSV-2 antibody prevalence has been associated with increased Human T-cell Lymphotropic Virus-1 infection in the Caribbean, but not in Japan (59,124). The disparity between the Caribbean and Japanese results is that HTLV-1 is mostly acquired sexually in the Caribbeans. On the other hand, in the Japanese area, where HTLV-1 has been prevalent, the retrovirus has been transmitted most commonly by breast milk of mothers of lower SES populations, in whom the antibody prevalence of HSV-1 is higher than that in Tokyo (53). In the U.S. NHANES II studies, it was noted that for every four cases of HSV-2-positive antibodies, there was one of positive syphilis antibodies. Adding these two markers of sexual activity can help estimate the proportion of the sexual, versus nonsexual, transmission of other infectious agents, e.g., hepatitis B.

With the onset of the AIDS epidemic, it became evident that AIDS patients were prone to develop more frequent HSV-2 (and HSV-1) recurrent infections, which tended to last longer and involve larger skin or genital areas, with contiguous involvement of the esophagus and rectum (125–127).

Indeed, such herpetic manifestations were considered to be evidence of HIV clinical progression towards AIDS. Once the HIV antibody assay was developed, we initiated studies with our new HSV type-specific antibody assays (26,44) to ascertain the possible role of genital HSV-2 in facilitating acquisition and transmission of HIV-1.

Our earliest study in Africa, reported in 1987, revealed that HIV-positive individuals were three times more likely to be HSV-2 antibody positive than HIV-negative individuals (54). Although no data on sexual behavior patterns were available in the African study, such information was gathered in other later studies, in which multivariate analyses were used. The results of studies, performed with collaborators, in homosexual men in San Francisco and Amsterdam, and in heterosexual men and women in Baltimore, and Haiti were supportive of an HSV-2–HIV causal relationship, and we suggested the potential use of HSV-suppressive acyclovir therapy to prevent HIV acquisition and/or transmission (54–59).

A role of genital ulcer disease in HIV acquisition had been noted in large numbers of studies (70). HSV-2 represents the most common cause of genital ulcers in most countries of the world, including many where HIV is prominent (129–134). Besides being of greater prevalence than syphilis, chancroid or other causes of genital ulcers, HSV genital lesions are much more common, by the fact that they recur frequently. Such first or reactivated HSV ulcers could provide a portal of entry for HIV in HIV-negative individuals. In case of HIV-positive/HSV-2-positive individuals, the HIV commonly found within genital herpetic ulcers (135) could provide another source of HIV inoculum, other than HIV-contaminated genital secretion. It has also been shown that genital herpes reactivation increases the viral load of HIV (126,136). In addition, *in vitro* studies have demonstrated that HSV coinfection of cells with HIV will increase the latter's viral replication (137).

A recent report has provided a meta-analysis of a large number of seroepidemiological studies performed in different populations by various workers (114). This approach revealed that, with the exception of two studies, the odds ratios for HIV prevalence were significantly higher ($1.5\times$ – $10\times$) in HSV-2-positive than in HSV-2-negative individuals. Since this January 2002 report (114), there have been several other linkage studies performed in Ethiopia, Tanzania, Zimbabwe, South Africa, India, and the United States (138–147). The reports are generally consistent with a significant HSV-2/HIV association, with several authors suggesting the need to pursue trials using suppressive HSV therapy to prevent HIV acquisition and/or transmission. A particularly interesting study performed by a United Nations Acquired Immune-Deficiency Syndrome (UNAIDS) group (147) compared a variety of risk factors to possibly explain the low rates of HIV in two African cities (<5%), in contrast to the high HIV rates (>20%) in two other cities. These workers found that the two major HIV risk factors were HSV-2 infection, rather than other sexually transmitted infections, as well as the lack of circumcision.

The possibility of decreasing the spread of HIV, using not only acyclovir but other drugs and methods to reduce HSV (see next section), is an important challenge for research and public health policies. Positive results of such HIV preventive studies, to be conducted during Phase IV, could have a great impact on both genital HSV and HIV in many respects.

CHALLENGES FOR RESEARCH AND PUBLIC HEALTH POLICIES DURING PHASE IV*

The main message of this chapter has been to emphasize that we are dealing, and will likely continue to deal, with a generally moving target, due to the earlier described major factors that affect the natural history and global epidemiology of HSV-1 and HSV-2. It is apparent that, however great is our desire to define common and applicable general public health policies, these have to be guided primarily by results of research with *reproducible* evidence, geared to any one country's particular changing variables, including not only population make-up, sexual behavior patterns, and HIV prevalence but also related priority and budgetary constraints. International organizations such as the World Health Organization (WHO), UNAIDS, and the Gates Foundation have recently begun to raise the priority of HSV as a global challenge, primarily because of the likely role of genital herpetic ulcers as facilitators of HIV acquisition and transmission (4,70,147,149). The impact of the interactions between genital herpes and HIV is bound to affect greatly Phase IV, both regarding the global epidemiology of HSV-1 and HSV-2, as well as the availability of greater resources to improve support for the research and applications needed to reduce herpetic infections.

We need to expand our knowledge on mechanisms of HSV-1 and HSV-2 latency (see chap. 4) in order to understand factors involved in reactivation that could be prevented. For instance, the recent research noting that the induction of a cellular gene (COX-2)—involved in the prostaglandin system—may cause viral recurrences is leading to studies using COX-2 inhibitors that might affect viral reactivation (103). Other approaches to reduce HSV recurrences, which also require further investigation, include local immunopotentiators (150) and acyclovir suppression (151,153). The recent report of a relatively short-term study on the partial effectiveness of acyclovir-induced HSV suppression to prevent acquisition of genital HSV-2 infection in discordant couples (115) provides difficult challenges for physicians and particularly public health officials. Should a vigorous search be made for every discordant couple? Only if one is symptomatic and only if both are long-term mono-partners? Should suppression be continuous—if so, for

* "The writer neither focuses nor conjectures; he projects." Jean-Paul Sartre, in "Why write?" (148).

how long? or can it be used intermittently with every sexual act—oral, genital, or anal? There is a particular lack of data on the intermittent approach of using the drug by one, the other, or both partners with each sexual act.

Use of suppressive acyclovir therapy in pregnant women identified with genital herpes was suggested as a means to prevent cesarean sections and possibly neonatal herpes (152). Despite several studies [reviewed by Watts et al. (161)], the only consistent finding was that the procedure decreases the rate of genital virus isolation, albeit less so in pregnant than nonpregnant women. The report noted that the widespread clinical use of acyclovir by community physicians required their trial to be prematurely terminated before a determination could be made on whether the drug could reduce cesarean delivery, let alone maternal–neonatal transmission. Recommendations without better evidence will bedevil physicians and public health advisors, as it may be too late to develop the needed data. It is worth emphasizing that the recent report by Brown et al. (97), of a study conducted over 17 years, finally provided evidence for the effectiveness of C-sections to prevent neonatal herpes—over 30 years after this approach was proposed, largely on indirect grounds (121).

Oral acyclovir suppressive therapy is currently recommended in several countries for genital herpes during pregnancy, in discordant couples, for organ transplantation prophylaxis and for some forms of ocular herpes (70,104,152). Although in immunocompetent individuals less than 1% of isolates are acyclovir resistant, about 5% of the isolates in immunocompromised hosts, e.g., HIV, are already acyclovir resistant (154,155). In the event of even more widespread use of acyclovir prophylaxis in areas where HIV is prevalent, acyclovir resistance is likely to increase. It therefore appears necessary to stimulate the development and clinical evaluations of oral anti-HSV drugs, which affect other viral molecular targets than thymidine kinase (153,156) with minimal side-effects.

Various types of HSV vaccines have been under study for several decades (41,157)—see also chapter 2. The use of condoms in preventing transmission or acquisition of a genital herpetic infection is another consideration (158). Recent evaluation groups believe that the data on condoms are not very supportive (159,160); however, partial effectiveness has been noted in protecting women (13,161). Microbicides are currently being explored (162), but there is particular concern about development of resistance to the agent applied after frequent use. The general counseling regarding sex abstinence is causing a paradoxical increase in the prevalence of genital HSV-1, as noted earlier. More counseling is needed regarding oral sexual practices and other measures to prevent salivary HSV-1 transmission. Effective means to protect the genital/anal tract from salivary HSV transmission are particularly problematic. Despite the reluctance of some public officials to pose relevant questions about the various types of sexual practices, more information is needed to obtain trends in their varied use and of possible

effects of different educational efforts and other methods to control these possible routes of transmission. Surveillance is particularly important to guide many of the public health policies of the future.

Alexander Langmuir's Epidemiology Program at the CDC in Atlanta pioneered in the 1950s the application of national surveillance of a variety of diseases and infection-related entities. These have proven their value over the past half-century and similar approaches are needed in other countries.* Yet, surveillance approaches may prove fallible if not based on good diagnostic criteria and solid scientific research, as exemplified below when applied specifically to HSV.†

1. Without substantial and continuous national surveillance, mathematical modeling will yield false predictions. A recent report (163) projected a huge increase in genital HSV-2 infection and related higher costs by the year 2025, based on the increased prevalence reported between NHANES II and III over a 12-year period (47). However, analyses of the now continuous US NHANES demonstrate a significant decrease in HSV-2 prevalence, primarily in the younger age groups, compared to the previous NHANES III about a decade earlier (46).
2. The 50% or so decline in the prevalence, in developed countries, of HSV-1 infections over the past half-century could well have been attributed to an HSV-1 vaccine (assuming one had been found to be effective in a Phase III clinical trial, and had been administered widely to infants in the 1950s); however, most of the decrease can really be attributed to the improved socioeconomic conditions over the past 50 years.
3. An attempt at developing national surveillance data in France was based on testing for HSV-2 antibodies in available sera (obtained for other purposes) in individuals 35 years or older (120). Not only is there a likely cohort effect but also a failure to characterize the age group in whom acquisition of HSV-2 is most common. Indeed, it may be cost effective, for most purposes, to test randomized individuals aged 18–35 years, over repeated periods of time, in order to establish the HSV-2 patterns in seroepidemiological surveys in a particular locality. Results can then be analyzed

* A European Union CDC-like institution, to be placed soon in Stockholm, Sweden, is likely to contribute to increased overall surveillance in countries of that continent.

† It should be emphasized that, besides obtaining information relevant to HSV-1 and HSV-2, application of HSV type-specific antibody assays can prove useful for the surveillance of other entities, nationally or in particular groups of interest. For instance, *objective* measurements of sexual behavior patterns or of sex education evaluations can be obtained by following HSV-2 antibody changes over time. Similarly, the prevalence of HSV-1 antibodies in children 5 or 10 years of age can provide one of the best *objective* measurements of socioeconomic changes over time.

according to gender, age, SES, minority considerations, and changes in risk factors and prevalence over time. One might miss possible effects of Viagra, or other such drugs, in older individuals, but that age group could be part of a more specialized survey. For many of the reasons noted earlier, our bias is to test concomitantly for HSV-1 antibodies and to use well-validated serological assays (with a confirmatory test performed on positive sera if the results are to be reported to the participating individuals).

4. Neonatal herpes represents the best example of the common failure of attempts at national surveillance, primarily due to difficulties in diagnosis. Australia and several European countries, mostly in the 1980s and early 1990s, have reported rates of 2–8 per 100,000 births (reviewed in Ref. 66). Similar low rates of 4/100,000 were obtained in a U.S. national survey in the 1980s, conducted over an 18-month period in hundreds of hospitals (164). All of the above rates are 5–10-fold lower than those experienced in an Atlanta hospital for low SES pregnant women, in three Seattle hospitals for mostly higher SES women than those in Atlanta, as well as in a children's hospital in Helsinki (66,97,165). The Seattle experience over a 17-year period represents the most accurate data available, as it combined serological and clinical observations in pregnant women, with identification of the genital virus at delivery. Also evaluated was the transmission rate of the maternal genital virus to the progeny, with or without cesarean section interventions. Major problems in diagnosis in the mother are the commonly asymptomatic nature of her genital infection, mostly of the cervix at delivery. In the baby, lower reported rates can be due to the fact that the more visible signs of the baby's skin lesions are often absent or actually misdiagnosed. In our recent review (66), we pointed to the need for better training of health professionals in the diagnosis of maternal genital herpes and neonatal herpes with the best tools possible—including paying more attention to infection of the cervix in the mother and of ocular infections in the baby (102). Use of type-antibody serological testing of pregnant women and their male partners is controversial (166), as HSV-1 is becoming more commonly involved in genital infections, and as late primary infections would yield seronegative results (167). It has been suggested that vaginal sex and cunnilingus be avoided in the last 1–2 months of pregnancy to avoid primary or initial first maternal genital infections (168).

An accurate diagnosis of neonatal herpes is also needed, in order to isolate the baby, since nosocomial outbreaks, particularly in intensive care units, have been reported (4). The data available do not support advocating removal

of nursery or other personnel with fever blisters—only advising no kissing and vigorous hand washing. However, it is recommended to remove from baby care any personnel with a herpetic infection of the finger (paronychia), or other parts of the hands or uncovered lower arms. Medical and public health practitioners should also be aware of the potential of outbreaks of herpes gladiatorum, usually due to HSV-1 in wrestlers, and take effective measures, e.g., suppressive therapy to prevent further cases (169,170).

Virological surveillance has been mostly performed, using retrospective analyses of the relative proportion of HIV-1 or HSV-2 identified in clinically suspicious lesions by viral culture and/or PCR. As noted earlier, recently in many European countries, Japan, Australia and Israel, the emergence of HSV-1 as the major cause of genital herpes, particularly in young women <25 years of age, was recognized 10–20 years before being appreciated recently in parts of the United States (84–86). Other than results of viral identification, including typing of clinical lesions, it is important to obtain clinical data and serological assays, as well as asymptomatic viral shedding in a subset. The combined efforts applied in appropriate longitudinal studies, particularly in young individuals, such as university students, are needed to provide data on the proportion of primary and non-primary first genital HSV-1 and HSV-2 infections and their clinical and subclinical recurrence rates. Basic studies are needed to identify possible subtypes of HSV-1, which may reactivate more commonly and their potentially important consequences (Fig. 3).

Besides national surveys, and noting the same concerns regarding SES and minority aspects, specific population groups of particular public health importance and with accessibility, include:

- a. *Pregnant women*, with an age span close to that noted above; family planning clinics also provide women within a young age group.
- b. *STD clinics*—with the proviso that, in some developed countries, the public STD clinics are representative of the general population, whereas, in other countries—including developed ones like the United States—public STD clinics are not truly representative.
- c. *University students*—As noted earlier, this group, at least in the United States, provides the best means to ascertain the progress of the “emerging” genital HSV-1 epidemic. Such studies could also be performed in homosexual men or women, who appear more likely to acquire genital/anal HSV-1 infections.
- d. *Military personnel*—Usually representative of a country’s population, they comprise a sizeable accessible group of young age that can be surveyed at repeated intervals.

- e. *Groups with HSV-1 disease other than of the genitals, or in newborns*—Consideration should also be given to obtain improved epidemiological information on some herpetic diseases, most often due to HSV-1, because of their role in inducing a higher physical morbidity and occasional mortality. These include HSV-1 ocular infection and encephalitis, and HSV-1 (and HSV-2) infections in immunocompromised hosts. Such data are particularly important to obtain, in view of the increased seronegativity rate in young adults in developed countries, rendering them potentially more vulnerable to acquisition of a primary HSV-1 infection and possible severe diseases. Current data are limited on these disease entities, but they all share common problems as related to the importance of accurate diagnostic criteria, often requiring medical expertise and special technology, e.g., indirect ophthalmoscopy and magnetic resonance imaging.
- f. *HIV clinics* (and groups with a high incidence of HIV infection)—The correlation of HSV-2 infection with HIV has by now been well established; still needed are studies in various localities among different groups with a high incidence of HIV infection to complement studies underway, so as to ascertain the relative effectiveness of HSV antiviral suppressive therapy within different settings. Two approaches are possible: (1) to suppress genital HSV reactivation in HSV-2-positive individuals who are still HIV negative, and have a high risk of acquiring HIV (e.g., in discordant couples or migrant workers); (2) to suppress, in both HIV- and HSV-2-positive individuals, reactivated genital ulcers, which might contain HIV from being transmitted to HIV-negative sexual contacts; one group very likely to be positive for both HIV and HSV-2 is of commercial sex workers.

CONCLUSIONS

As HSV-1 antibody prevalence declines in younger age populations in the developed (or developing) world, there will be a continued increase in genital HIV-1 infections. In the event that some HSV-1 subtypes have developed to reactivate more commonly (Fig. 3), there would be an accelerated rate of increase in genital HSV-1 infections, with their related consequences—particularly neonatal herpes, which anyway requires improved diagnosis. It also appears that a vigorous search is needed to identify possible increases of nongenital forms of primary HSV-1 diseases in the growing population of seronegative adolescents and adults in higher SES populations.

HSV-2 infections are likely to stabilize in older age groups in many of the higher SES countries, but will probably continue to increase in adolescents and young adults in low SES populations and certain minority groups

within developed countries. Research on vaccines and other potential preventive means, such as suppressive antiviral therapy, should be aimed at both HSV-1 and HSV-2, and not only at genital HSV-2 infections. More studies are needed to validate the relative effectiveness of antiviral suppression for various forms of HSV infection, as well as to prevent acquisition and/or transmission of HIV. Concern for an increasing rate of acyclovir resistance should stimulate greater efforts to evaluate other potentially effective oral drugs.

Currently, resources that are needed to support the basic and applied research on unresolved issues regarding herpes simplex viruses, and on application of evidence-based public health preventive approaches, are likely to be relatively slim in the near future. However, more resources would likely become available, if HSV-suppressive antivirals are shown to reduce significantly HIV acquisition and/or transmission.* Such additional support would have an important impact on the natural history and epidemiology of herpes simplex virus infection during Phase IV—and be of assistance, for future generations, to deal with the formidable challenges of HSV *eradication* in Phase V by the end of this century or millennium.

ACKNOWLEDGMENTS

Dedicated to AJN's mentors: Dr. Alexander Langmuir in epidemiology, Dr. Sidney Kibrick in virology and Dr. Darryl Reanney in evolution of viruses (evovirology).

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* Already over \$35 million have become available for performing HSV antiviral suppression studies in a variety of populations in several countries.

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Pathogenesis

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INTRODUCTION

Herpes simplex virus-1 (HSV-1) is one of the most thoroughly investigated human viruses, but the number of unanswered questions regarding its pathogenesis and natural course of infection tend to increase with understanding. This chapter reviews some aspects of the pathogenesis of HSV-1 and HSV-2 infections, with emphasis on data derived from humans as hosts and from their viral isolates. An important limitation in the field of HSV pathogenesis is the difficulty in studying asymptomatic infection, which therefore remains enigmatic. As transmission seems to occur commonly during asymptomatic phases, clinical manifestations including mucocutaneous lesions may be regarded as extrapolations of events occurring during a silent, natural infection. In fact, disease events may be unnecessary for the further existence of HSV in the human population, yet they still exist. However, the complications encountered during HSV-1 and HSV-2 infections, thoroughly reviewed in this volume, strongly indicate a need for further defining determinants of pathogenicity, such as viral virulence factors and host susceptibility traits, to improve treatment and prophylaxis.

HSV STRUCTURE AND REPLICATION

HSV Virion

HSV-1 is a typical herpes virus consisting of a double-stranded DNA constituting of an electron-dense core within the icosahedral nucleocapsid built up by 162 capsomers. The nucleocapsid is surrounded by the adherent tegument, which in turn is tightly connected to the envelope. The structure of the enveloped particle was recently resolved by cryo-electron tomography (Fig. 1), where the tegument appeared to be asymmetric due to eccentric positioning of the nucleocapsid and displayed a partly filamentous, actin-like structure (1). Moreover, protruding envelope glycoproteins are of several morphological types, and these spikes tended to be nonrandomly clustered, which could be of possible functional importance during viral entry. Although HSV-2 virions seem to be more fragile and therefore less studied, the similarity of viral genes and their organization and expression between the two subtypes (see chap. 1) argues for a similarity in structure also.

The HSV genomics and proteomics during the infectious cell cycle has been thoroughly studied, and because it is beyond the scope of this chapter to summarize recent findings within that vast field in detail, the reader is referred to existing comprehensive reviews (2).

To initiate infection, free virus particles enter the cells through direct fusion of the viral and cellular membranes, and the naked nucleocapsids are transported to the nucleopores by the microtubular pathways of the

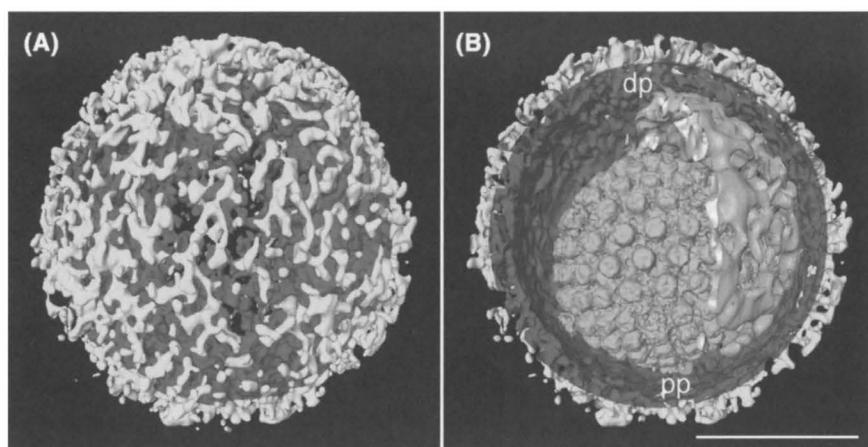


Figure 1 Structure of an HSV virus as determined by cryo-electron tomography. (A) Outer surface showing the distribution of glycoprotein spikes (■) protruding from the membrane (■). (B) Cutaway view of the virion interior showing the capsid (■) and the tegument “cap” (■) inside the envelope (■ and ■). Scale bar, 100. *Abbreviations:* pp, proximal pole; dp, distal pole. *Source:* From Ref. 1.

cytoskeleton (3). The nucleocapsids release their linear DNA content into the nucleus and transcription occurs after circularization of the DNA. After synthesis and assembly in the nucleus, nucleocapsids are temporarily enveloped by the inner nuclear membrane into the perinuclear space through budding (Fig. 2A). This enveloped particle is deenveloped while passing through the outer nuclear membrane. In the trans-Golgi network, secondary envelopment by the permanent membrane containing surface projections

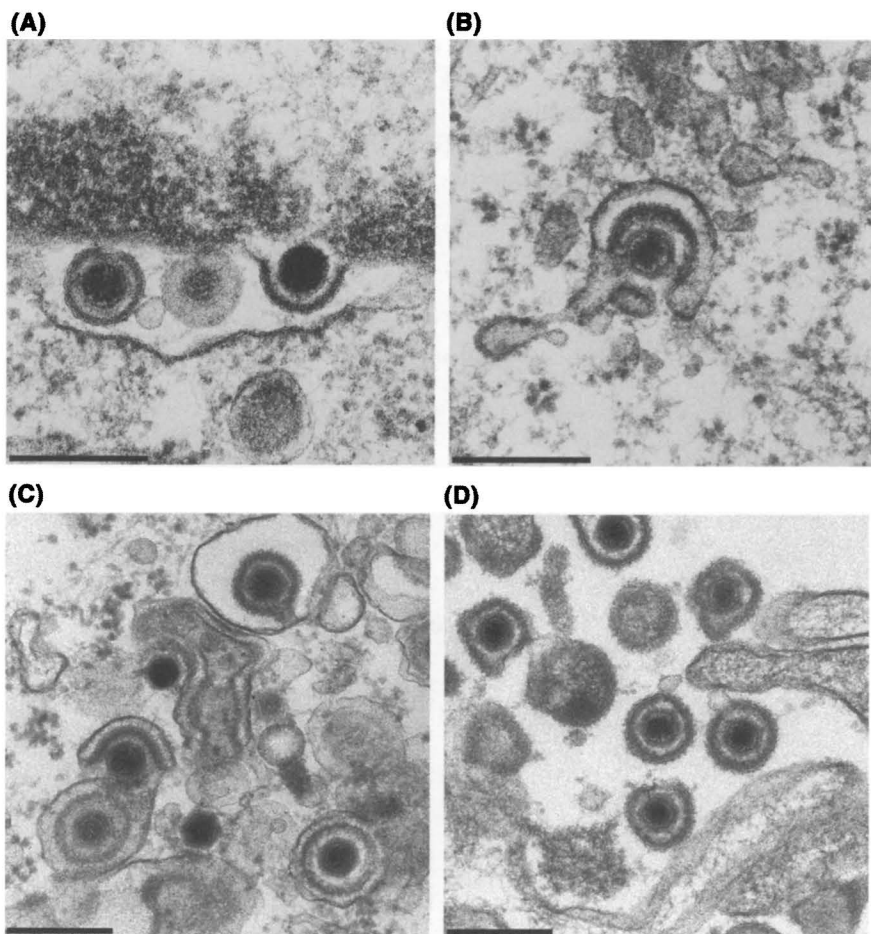


Figure 2. Processing of the HSV-1 particles. (A) Primary envelopment at the inner nuclear membrane into the perinuclear space. (B) Secondary envelopment at the membranes of the trans-Golgi network. (C) Different stages of secondary envelopment at membranes of the trans-Golgi network. (D) Mature extracellular HSV-1 particles. Scale bars, 250. *Source:* From Ref. 4 and Mettenleiter TC. Herpesvirus assembly and egress. *J Virol.* 2002; 76:1537–1547.

occurs (Fig. 2B), and the mature virions are transported in Golgi-derived vesicles (Fig. 2C) to the cell surface where mature extracellular viruses (Fig. 2D) are formed (4).

HSV Envelope Glycoproteins and Their Functions

HSV envelope glycoproteins play a major role during viral entry and egress, and are dominant targets for human B- and T-cell mediated immune responses as well as mediators of immune evasion such as binding of complement factors and the Fc part of IgG. This has motivated a role for several of the glycoproteins as important constituents of subunit vaccine candidates (see Chap. 2). Of the 11 genes encoding for HSV glycoproteins, six (gB, gC, gH, gK, gL, and gM) are encoded by the U_L region and 5 (gD, gE, gG, gI, and gJ) by the U_S region (Table 1). Table 1 presents the current understanding of their respective functions during the infectious cell cycle.

HSV entry is a cascade reaction initiated by interaction of gC with cell surface glycosaminoglycans such as heparan sulfate (HS) and chondroitin sulfate (5,6). The initial binding is followed by a stable attachment in which gD

Table 1 Functions of HSV Glycoproteins

Gene	Protein	Necessary for replication in vitro	Function ^a
UL 1	gL	Yes	Participates in fusion
UL 10	gM	No	Regulates membrane protein trafficking Downregulates cell-cell fusion
UL 22	gH	Yes	Participates in fusion
UL 27	gB	Yes	Participates in fusion Accessory binding of GAG molecules
UL 44	gC	No	Initial attachment to GAG molecules Binds complement factors
UL 53	gK	No	Promotes egress Downregulates fusion
US 4	gG	No	Promotes apical entry of polarized cells
US 5	gJ	No	Blocks apoptosis
US 6	gD	Yes	Promotes stable attachment by binding to HVEM, nectin 1, nectin 2, and 3-O HS, participates in fusion
US 7	gI	No	Promotes cell-to-cell spread in complex with gE
US 8	gE	No	Promotes cell-to-cell spread in complex with gI Binds Fc-part of IgG antibody

^aSee text for references.

may utilize several cell surface proteins (such as HVEM, nectin 1, and nectin 2; for review, see Ref. 7) or 3-*O*-sulfated HS (8) as receptors. The crystal structure of gD has been resolved (9) and its functionally important regions defined and found to overlap with neutralizing epitopes (10). Interaction of gD with cell surface receptors is a necessary preparatory step for fusion of the viral and cellular membranes. The fusion process is rapid and difficult to study, but seems to also involve at least gB and the complex of gH and gL, with gK providing a downregulating function (11,12). In addition, an accessory role for gG-1 during apical entry of polarized cells has been demonstrated (13).

Cell-to-cell spread is a crucial facet of HSV pathogenesis, both during formation of vesicular lesions and spread within the nervous system. A complex of gE/gI is necessary for this process by sorting the nascent virions to cell-cell junctions, while gM may participate as a downregulator by controlling membrane protein trafficking (14–16). It is interesting to note that gE-negative strains of pseudorabies virus or swine herpes have been successfully utilized as vaccines for this animal, underscoring the importance of this viral protein for virulence further (17). During viral egress, many of the processes during entry have to be counteracted, and one example is that the downregulator of fusion, gK, was reported to promote egress (18). Whether HSV uses similar or different receptors for entry, cell-to-cell spread, and egress is a crucial question that remains to be fully elucidated.

NATURAL INFECTION

Asymptomatic Infection

The most common course of HSV infection is an asymptomatic one, since anamnestic reports of clinical manifestations in form of oral or genital herpes indicate an incidence of substantially less than 50% of subjects seropositive to HSV-1 and HSV-2. HSV-1 is transmitted by body secretions such as saliva and tears, fluids that by repeated sampling may be Polymerase chain reaction (PCR)-positive in almost all asymptomatic subjects including some of those that are seronegative (19). Recent serological studies on Swedish children indicate that infection mainly occurs early during life (< 3 years) or during puberty and later (20). The incidence of asymptomatic oral-genital transmission of HSV-1, a common cause of primary episodes of genital herpes (21), is unknown. HSV-2 is almost exclusively spread by the genital route, a reason why seroprevalence of this virus is low before puberty, and asymptomatic shedding may also probably account for the major part of transmission (22).

Primary Infection and Viraemia

When symptoms develop, primary HSV-1 infection often manifests itself in form of gingivostomatitis (see chap. 7), after an incubation period of two to three weeks. A prevailing view is that the oral cavity is the site of primary viral

replication, from where the virus spreads to trigeminal ganglia (see subsequent paragraphs). The first round of replication may be extensive in the oral mucosa, thus explaining the signs and symptoms of disseminated gingivostomatitis. After this, the infection may be confined to the interplay between sensory neurons and keratinocytes. A haematogenous phase (as is evident in Varicella Zoster Virus) would thus be absent for HSV after the neonatal phase.

However, a recent study of immunocompetent children with herpetic gingivostomatitis revealed that more than one-third was PCR-positive for HSV-1 DNA in peripheral blood at the time of stomatitis (23). This is in line with earlier successful attempts to isolate HSV-1 from the blood during various manifestations of primary infection (24), which is why it is likely that viraemia is also part of the natural infection of HSV-1. This would explain why some adults develop long-term fever during primary infection. Regarding HSV-2, this virus was isolated early from white blood cells during primary episodes of meningitis in adults (25). Moreover, viraemia appears to be common during neonatal herpes (26). A haematogenous spread of HSV may also be relevant for the pathogenesis of the skin disease erythema multiforme (27), and may explain the occurrence of HSV-induced hepatitis (28). These findings complement rather than disprove the prevailing concept that HSV mainly resides and spreads neuronally, and that a substantial part of the disease is related to viral interaction with the nervous system.

Axonal Transport

Two discoveries in the early 20th century linked oral herpetic lesions to infection of the sensory cranial nerves and their ganglia: (i) herpes labialis occurring during pneumonia was related to trigeminal ganglionitis (29), and (ii) surgical section of the proximal trigeminal root induced facial herpetic lesions in the anesthetic area (30). In a series of experiments on rabbits, Goodpasture and Teague were able to show that after peripheral infection, the agent of herpes febrilis traveled along the axis cylinders to induce ganglionitis and encephalitis (31). Later studies demonstrated that HSV could utilize both the retrograde (to reach the sensory nuclei) and antegrade (to exit nerve endings for entering into keratinocytes) axonal transport machineries (32–34). Nucleocapsids, together with some tegument components such as US11 (35), probably exploit cellular-encoded microtubular motor molecules like kinesin and dynein for the transport (for review, see Ref. 36). The envelope glycoproteins may utilize other mechanisms, because they are separately carried nearer the axonal surface during the outwards transportation and before final assembly in the periphery (37).

Persistency/Latency

The finding that HSV could be postmortally isolated from trigeminal ganglia even in asymptomatic human subjects indicated that the virus was

harbored within the nervous system in a persistent/latent state (38). Successive studies showed a broader range of ganglionic infections including cranial, cervical, thoracic, and vagal ganglia by HSV-1 and lumbosacral ganglia by HSV-2 (39,40). The question how the virus maintains ganglionic latency during its lifelong habitat, to avoid extensive neuronal death, has attracted substantial scientific interest. Much of the work has been centered on the HSV latency-associated transcript (LAT) expressed in neurons containing latent genomes. The complex functions of this protein include protection against neuronal cell death by apoptosis (see subsequently) as well as establishment of latency and enhancement of reactivation (for a recent review, see Ref. 41).

In contrast to the findings in ganglia, HSV has only been isolated from brain tissue during active infection such as herpes simplex encephalitis (HSE), brain stem encephalitis, or during inflammatory disease (42–44). Hence, the central nervous system (CNS) seems to be free from infectious virus particles during normal conditions. However, since sensory neurons are pseudounipolar, i.e., the same axonal process extend both to the periphery (skin) and the Central Nervous System (brain stem, spinal cord), HSV could easily be transported centrally to cause lytic infections. Why is then Herpes Simplex Encephalitis such a rare event? To resolve this contradiction, a sorting mechanism that preferentially targets the virus to ganglia and periphery has been postulated (36).

Despite such possible control mechanisms, viral DNA has been detected, both by hybridization and PCR, in brains from patients without signs and symptoms of infection even in areas such as the hippocampus and temporal lobe that are commonly involved in HSE (45,46). One explanation could be that latent virions are not only harbored within sensory ganglia, but are distributed in a more disseminated fashion. Thus, also brain infection could be part of the subclinical infectious cycle of HSV, including projection areas for sensory neurons innervating the oral region. Most likely, this would require a strong downregulation of HSV replication within the brain, which might explain why such HSV genomes do not produce infectious particles. Whether HSV DNA exists in complete genomes in the brain is still unknown.

Recent studies suggest that ganglionic persistence/latency may be a more complex and active process than previously thought, and that neuronal HSV infection may be permanently controlled by the cell-mediated immune response. Using eye infection of mice, Feldman et al. (47) discovered that, in addition to numerous neurons in the trigeminal ganglia that express latency associated transcript RNA, rare neurons show evidence of productive infection including antigen expression. Moreover, these neurons were surrounded by inflammatory infiltrates. Such neurons may represent abortive reactivations, or alternatively foci of persistent viral replication, a hypothesis that broadens our current concept of ganglionic latency. In a study on human

trigeminal ganglia by Theil et al. (48), an inflammatory response dominated by CD8⁺ T-cells and cytokines such as IFN- γ and TNF- α was demonstrated in HSV-1-infected but not in uninfected trigeminal ganglia. Since HSV-1 antigens were not detected by immunohistochemistry, the authors speculated that the inflammatory cells could be left from previous reactivations. With the reservation that age-matched uninfected controls were not included in the study, their findings are compatible with a constant immunosurveillance controlling ganglionic infection with HSV-1. This is in line with a previous work showing that IFN- γ can prevent reactivation in sensory neurons (49).

Reactivation

The biological property of reactivation is central to the medical scope of HSV infection. Common triggering events of HSV induced peripheral lesions caused by viral reactivation include nerve trauma, septicæmia and other infections, Ultraviolet-irradiation, dental procedures, and possibly also persistent mental stress (50–53). This plethora of reactivating stimuli indicates that both central and peripheral factors are involved. Interestingly, in a study of controlled UV-induced oral herpetic lesions, a bimodal distribution (immediate and late) of onset of lesions occurred (54) suggesting that UV-radiation stimulated replication of the virus already present in the epidermis, as well as of neuronally harbored virus.

The example of radiation therapy against a brain tumor initiating HSE suggests that other trigger factors also should be studied (55). Another unusual form of traumatic triggering of HSV reactivation may be neurosurgery. After a delay of approximately one week, destructive encephalitis may develop with fever and seizures, and with typical viral inclusion bodies demonstrated by histopathology (56). With the reservation that these patients most often received steroid therapy postoperatively, the report suggests that surgical trauma and concomitant inflammatory activity may reactivate latent HSV-1 genomes already present in the brain. Whether a common pathway exists for pathogenetic processes induced by these disparate reactivating factors remains to be determined. Studies of the cross talk between HSV and local immunological factors may be a step ahead to elucidate the largely unknown molecular mechanisms responsible for symptomatic viral reactivation in man (57).

Fate of the Infected Cell

HSV disease in man is associated with various cellular damage such as formation of inclusion bodies, oedema, and cell lysis. The finding of frequent asymptomatic HSV DNA shedding in saliva, sometimes in high quantities (19), raises the question of natural defense mechanisms on the cellular level. Even in patients with frequent bouts of oral herpes, sensory loss in areas of herpetic lesions are rarely encountered, arguing that infected neurons may

escape lytic or destructive infection during reactivation. The process is a delicate act of balance between replicative forces and cellular defenses. As an example, upregulation of viral replication during reactivation is stimulated by the early protein ICP0 that among other functions, by selective ubiquitination induces proteasome degradation of key factors within the nuclear bodies harboring viral genomes (58). Since the nuclear bodies themselves become disrupted, this process may lead to further cell damage that has to be counteracted.

Apoptosis, which is a common host innate response to virus infection, may be blocked efficiently by HSV both in nonneuronal and neuronal cells (59,60) in a cell-type specific way. The anti-apoptotic function, which in addition to LAT and other genes also includes genes coding for gD and gJ (61), is initiated within hours by replicating HSV, but not by UV-inactivated virus that instead somewhat surprisingly induced apoptosis. It is currently unknown if similar biological processes are active during natural HSV infection in humans, but an anti-apoptotic viral activity offers an attractive explanation to the nonharmful character of the lifelong ganglionic viral infection. Furthermore, during normal circumstances, excretion of infectious virions also seems to be harmless in the periphery until homeostasis is broken by the different triggering factors. In contrast, apoptosis seems to be a component of the pathogenetic processes during HSE in humans (62).

GENETIC SUSCEPTIBILITY OF THE HOST

Considering the ubiquitous prevalence of HSV-1 in many of the serosurveyed populations, a natural resistance toward infection of this virus in larger groups of humans seems unlikely. However, subjects carrying functional mutations in host genes necessary for crucial stages of the infectious life cycle of the virus may be well protected. Although such a natural resistance linked to genetic alterations in the host is a common finding in other infections such as HIV, this possibility is still an open question as regards HSV, despite sequencing of host genes coding for viral entry mediators or cytokines (63,64). The lack of seroprevalence to HSV-2 described in some populations has rather been linked to behavioral factors such as sexual abstention or geographic isolation (see chaps. 2 and 9). However, the emerging data on single nucleotide polymorphisms within the human genome will provide new tools for investigations of an eventual natural resistance to HSV.

Several studies have been searching for alleles of the human leukocyte antigen (HLA) and other genovariants that may confer protection against symptomatic HSV disease or could be linked to severe manifestations, but the results are still inconclusive. It has been reported that HLA-B27 and -Cw2 may be associated with asymptomatic infection by HSV-2, but the assumption was based on a small number of subjects (65). Given that HSV manifestations vary with time in subjects, a rigid study design would be

required to address this question. In another approach, the allele four of the human apolipoprotein E gene was suggested to be more common in patients with HSE (66), but another study found no such correlation (67).

HSV VIRULENCE

Viral Load

In several viral diseases, such as infections caused by hepatitis B, HIV, and cytomegalovirus, a link between viral load and severity of disease has been proposed, which is supported by data derived from quantification of viral RNA/DNA (68). Such polymerase chain reaction (PCR) technology has been recently applied in clinical HSV research. When real-time PCR was utilized to quantify latent HSV-1 DNA in cranial ganglia from diseased persons, a much greater viral load was unexpectedly discovered in vestibular than in trigeminal and other sensory ganglia (69). In cerebrospinal fluid (CSF), DNA quantities differed between investigated herpesviruses so that EBV and HHV-6 were detected at lower amounts as compared to the alphaherpesviruses and CMV (70). In neonatal patients with encephalitis, CSF concentrations of HSV DNA were significantly higher than in adult patients with HSE, and the authors suggested that the viral load reflects differences in pathogenesis, with more widespread and vigorous viral replication taking place in the neonates (71). Ongoing studies are aiming to thoroughly relate viral loads to outcome in CNS infections such as HSE. A comparison between CSF quantities of HSV-1 DNA in HSE and HSV-2 DNA in meningitis revealed much greater amounts of the former virus (Bergström, unpublished). Such intertypic comparisons are preferably made by targeting primers to type-common sequences and using probes with minor nt differences (72).

HSV Virulence Traits

Almost any deletion mutants constructed *in vitro* may show a reduced virulence in animal models (2), but strains with silenced genes are rarely isolated from patients; an exception to this is Tk-negative strains that are resistant to aciclovir. Although such viruses often are less virulent in animal models, they may induce disease in immunocompromised hosts (73). Furthermore, HSV-2 phenotypes, negative for the envelope glycoprotein G, have been isolated from ordinary recurrent genital lesions suggesting functional redundancy for this gene. Interestingly, both these forms of defect viruses were reported to be mainly due to frame-shift mutations within homopolymer runs of nucleotides (74,75). One implication of these and earlier studies is that a host strain may contain several genetic variants (76), but the extent and functional importance of this viral heterogeneity remains unclear.

Since asymptomatic infection may be regarded as the rule, the question arises whether HSV strains isolated from patients with substantial

disease manifestations represent virulent phenotypes. Previous studies have suggested that virus recovered from patients with frequent recurrences displayed this biological property in experimental infection of rodents also (77). Moreover, our studies on HSV-1 strains isolated from brains of patients with HSE, collected during the era when biopsy and isolation was the diagnostic method of choice, indicated a virulence trait in form of replication to higher titres in neuronal cells in vitro, as well as enhanced neuroinvasiveness in rodents (i.e., enhanced neurovirulence after peripheral infection), as compared to strains isolated from oral lesions (78). DNA sequencing of the genes encoding for gI/gE and gG of these brain isolates has hitherto not revealed any genetic markers that discriminate them from other strains (79). In another approach, direct DNA sequencing of PCR amplicons derived from CSF samples from HSE patients failed to detect viral mutations linked to HSE within the genes encoding for gB or gD (80,81). Some of these genes were previously associated with virulence based on mapping experiments of virulent strains arising after in vivo recombination of avirulent viruses (82,83).

Virulence traits of HSV-2 are much less investigated, despite the severe outcome in neonatal infection. When isolates derived from adults with meningitis were compared with genital isolates, both groups showed similar growth rates in vitro, but the meningitis strains were significantly more neurovirulent after intracranial infection in mice (78). In contrast to the brain-derived HSV-1 isolates, the HSV-2 meningitis strains did not display an enhanced neuroinvasiveness, suggesting that different viral virulence traits are of importance in these two clinical conditions.

The gene with a most profound implication for neurovirulence is the diploid gene ICP 34.5, situated within the terminal repeats of U_L. Deletion of this gene produced a drastic reduction in neuronal infection in mice despite ample replication in various cultured cells (84). One of the functions of this gene, apparently, is to protect the infected cell from shut off of host cell protein synthesis by interacting with a host cell phosphatase (85). Through thorough studies of distinctive strains isolated from different organs of a newborn with fatal neonatal HSV-1, a small-plaque variant from the brain showed enhanced neuroinvasiveness after peripheral infection in mice as compared to a large variant (86). The neuroinvasive variant contained a large number of repeats of nucleotides coding for the amino acids proton-coupled amino acid transporter (PAT) in the middle part of ICP 34.5, and the number of these repeats correlated to neuroinvasiveness (87). Whether the repeats of the PAT motif is a pathogenetic factor of importance for the development of neonatal herpes or merely an effect of local selection is unclear because only one patient was studied and HSV-2 seems to lack a similar sequence. Nonetheless, the studies confirm earlier reports that infection at multiple sites may harbor different viral variants (88) and show that this feature may be present even during primary infection. Furthermore, the authors demonstrate for the

first time a genetic marker detected in clinical samples that could be possibly related to neurovirulence.

Differences in Pathogenicity Between HSV-1 and HSV-2

The existence of two genetically related viruses with similar biology and partly overlapping target tissues focuses the attention on their respective differences in pathogenicity and immunogenicity, and the molecular background of these differences. Recent changes in epidemiological patterns, at least in Australia and parts of Europe, in form of HSV-1 being increasingly detected in primary genital herpes (89–91) may question the concept of type-selective viral oral/genital tropism. However, the following conditions strongly argue for differences in HSV biology and pathogenesis in human infection: (i) uncommon isolation of HSV-2 from the classical oro-labial region and of HSV-1 from cervix; (ii) uncommon reactivation of genital HSV-1 and facial HSV-2; (iii) differences in CNS complications in form of HSV-1 causing encephalitis and HSV-2 meningitis; and (iv) profound differences (HSV-2 > HSV-1) in virulence during neonatal CNS infections (89,92–95).

The ability of HSV-2 to infect HSV-1 seropositive hosts at similar efficiency as HSV-seronegative subjects (96) and its capacity to offer protection against secondary HSV-1 infection (97) indicate that fundamental differences between the two viruses exist regarding the nature of their respective evoked immune responses. In this context, based on the complete discrimination of antibody epitopes between gG-1 and gG-2 (98–100) and their unique low degree of genetic homology, it may be speculated that these two proteins differ in their biological functions. Furthermore, several steps during viral entry are mediated type-selectively that may have bearings on differences in tropism and pathogenesis. Interaction of gC-1 and gC-2 with HS differs significantly in that gC-2 promotes a high-affinity binding (101). 3-*O*-sulfated HS can function as receptor for gD-1 but not for gD-2 (8). Conversely, wild type HSV-2, but not HSV-1 strains, can utilize nectin-2 that is expressed on several relevant cell types, such as neuronal cells and keratinocytes and the region determining this type-selectivity as regards to receptor usage is located to the N-terminal part of gD (102).

The consequences of these HSV-1/HSV-2 differences in immunogenicity and receptor usage for type-differences in the pathogenesis of HSV disease are still unclear. However, envelope glycoprotein and other molecular differences between the two viruses may be exploited by future research aiming to increase understanding of the diseases they cause and the possibilities of prophylaxis.

CONCLUSIONS

Recent advances in molecular diagnostics have linked HSV-1 and HSV-2 to an increasing number of diverse clinical manifestations, indicating a broader

tropism of the two viruses than conceived previously. The efforts of many research groups through the decades have discovered numerous facets of the intricate interplay between these viruses and their hosts, both during asymptomatic infection and severe disease. In addition, these studies have contributed immensely to the general understanding of viral and mammalian biology. Still, viral and host factors that are crucial determinants of HSV pathogenicity in man remain largely undefined.

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Understanding and Diagnosing Herpes Simplex Virus

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INTRODUCTION

A revolution has taken place in laboratory techniques for herpes simplex virus (HSV) diagnosis in recent years (1–3), and it is now possible to produce accurate results within a day, sometimes within hours (4,5). This development is not only driven by the increase in the number of immunosuppressed patients, in whom HSV infections are responsible for significant morbidity and mortality (6–10), but also by the introduction of safe antiviral drugs for the treatment of both acute and chronic HSV infections (11–14). These factors have contributed to a change in the clinician's expectations of diagnostic virology and have created a welcome opportunity for the medical virologist to develop rapid, accurate, and relevant test modalities. Examples of these are nucleic acid (NA) amplification techniques, including HSV DNA quantitative assays (11,15–17), and the introduction of sophisticated laboratory services such as antiviral susceptibility testing (18,19). These improved laboratory services are rapidly redefining the natural history and epidemiological picture of HSV infection (20–24). As clinicians become increasingly reliant on these techniques, the clinical need for robust molecular techniques, such as fully automated real-time polymerase chain reaction (PCR) assays (25) will become evident.

Because of the multitude of available diagnostic tests for HSV-infections (1,3,6,26–32), it is important for the caregiver to understand which test to order. This decision is not only dependent on tests available, but also on the difference in clinical urgency between patients with life-threatening HSV disease, such as infected neonates, and patients with suspected HSV encephalitis (HSE), as opposed to patients with simple recurrent skin lesions. The former group requires a rapid and comprehensive laboratory investigation, whereas the latter may demand a lesser degree of test acuity. For example, the exquisite sensitivity of HSV Polymerase chain reactions is crucial in the diagnosis of HSE, whereas a less sensitive but highly specific direct immunofluorescence assay (DFA) (33–35) may be appropriate to use when analyzing a scraping from a vesicular lesion from skin or a recurrent genital eruption.

The laboratory location and its structure are also crucial for delivery of services, and an “on-site” virology laboratory is ideal for a general hospital, where rapid and accurate HSV diagnosis will not only aid in tailoring treatment but also may shorten or avoid hospital stay, eliminate unnecessary antibiotic treatment and expensive diagnostic tests, as well as help with infection control precautions. In contrast, for the office-based physician, reference laboratory testing with somewhat longer turnaround time (TAT) may suffice in many instances, particularly if combined with point-of-care (POC) assays, such as those recently marketed for the detection of HSV-2 antibodies (36). Future generation real-time PCR assays, based on portable field equipment will further change the way the general practitioner will be able to manage the patients.

The appropriate test battery chosen for each single case is subject to the specific questions asked by the attending physician (Table 1), and the

Table 1 Clinically Relevant Issues (Broad Terms) in the Management of Suspected HSV Disease

Purpose of HSV test

Antiviral treatment

Antiviral prophylaxis

Antiviral suppressive therapy

Suspected disseminated disease

Suspected congenital infection

Suspected neonatal infection

Suspected CNS disease

Suspected eye disease

Primary infection

Recurrent infection

Distinction between HSV-1 and HSV-2 diseases

Infection control

STD counseling

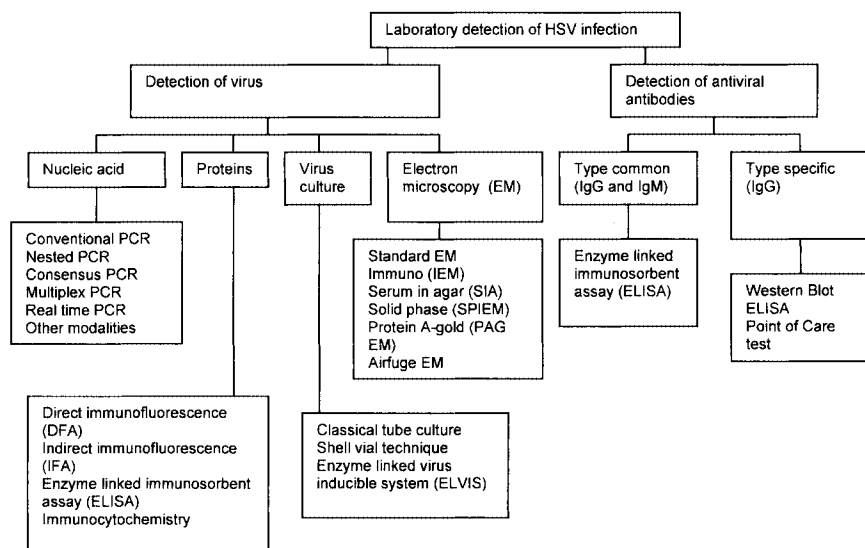


Figure 1 A summary of available HSV diagnostic techniques.

quality of the answer is dependent on specimen collection, transport, TAT, and result interpretation (30,37–39). If the clinician is not completely comfortable in choosing tests, the virologist should be consulted. If this is not possible, relevant clinical information, clearly indicated on the requisition, will help the virologist to determine appropriate tests once the specimen has reached the laboratory. This information will aid in the interpretation of laboratory findings. Such information includes clinical symptoms and signs, date of onset of disease, and immune status of the patient. If HSV serology is requested, the laboratory needs to know whether blood or blood products have been administered to the patient recently.

Two distinctly different approaches exist in HSV laboratory diagnosis: first, the detection of the virus; second, the detection of antiviral antibodies (Fig. 1). Viral detection techniques include molecular assays, virus culture, electron microscopy (EM), and immunoassays. A specific antiviral antibody response may be detected by type-common and type-specific HSV serology.

DETECTION OF VIRAL GENOMES, WHOLE VIRUS, AND VIRAL ANTIGENS

Molecular Detection Techniques

HSV genome can be detected by molecular techniques in a variety of specimens, including serum, plasma, cerebrospinal fluid (CSF), white blood cells, urine, stool, skin, mucosal scrapings, and tissue biopsies. The first such techniques available were based on NA hybridization using radiolabeled

DNA probes (40). Although extensively used in the research setting, these techniques never gained a foothold in the routine diagnostic laboratory as they used radioactivity, were labor intense, were expensive, and no more sensitive than immunologically based assays.

It was not until the principle of NA amplification was introduced that molecular detection assays became a realistic proposal for routine diagnostic virology (41). The specificity of NA amplification assays is derived from careful selection of target molecules, which are amplified using specifically designed primers (25,42,43). The sensitivity of these assays, when optimized, is usually exquisite, allowing the detection of 1 to 10 genome equivalents (GE) in clinical specimens (44,45).

HSV exhibits a complex pathogenesis, including the establishment and maintenance of neuronal latent infection as well as asymptomatic or symptomatic reactivation of a latent genome (46), and a positive HSV NA amplification result must therefore be interpreted carefully and within clinical context. For example, the presence of a low HSV DNA copy number in CSF from an immune competent host should always be considered clinically significant (47), whereas the detection of very low copy number of HSV genome in oral or genital secretions could mean a primary infection, but could also reflect a reactivated infection. In the latter case, the patient may be shedding HSV without symptoms.

The quality of the initial sample as well as appropriate transportation and storage conditions are clearly pivotal for the reliability of NA amplification results. DNA is, in general, more stable than RNA, and HSV DNA has been reported stable when stored for more than 16 months, whether purified or unextracted in a whole specimen (48). HSV DNA has also been recovered from CSF specimens stored at room temperature for up to 30 days (49). Based on these observations and the fact that NA amplification assays do not require "live" virus, transportation time is less of an issue than if HSV virus culture is requested.

Clinical laboratories that use NA amplification for diagnostic purposes, must regularly participate in internal and external quality assurance programs (50,51), as NA amplification assays may generate both false positive and false negative results when not performed under stringent laboratory conditions (43).

False positive results may occur by carry-over contamination from previously generated amplicons. Several methods have been used to diminish this risk, such as the use of uracil *N*-glycosylase (UNG) to degrade products from previous amplifications (52) and the use of negative water controls. Other approaches include the use of designated pre- and post-amplification areas and routine duplicate testing of specimens.

False negative results may be caused by inhibitors present in the clinical samples, such as hemoglobin, proteins, and polysaccharides (43,53), and the efficacy of the DNA purification step (54,55) is therefore an essential part of a reliable NA amplification test.

The choice between manual and automated NA preparation systems is dependent on specimen numbers, volume, and patient population. Manual methods may be suitable for laboratories with few specimens and for pediatric medicine, where the volume of a single specimen is invariably low. For larger laboratories or reference centers, automated methods that allow standardization and high volume throughput are more suitable. However, automated systems are costly, and so far no single instrument has shown superior performance.

Other ways to avoid false negative results include the use of "internal standard" molecules, which are amplified at the same time as the target (56); however, this may decrease the amplification efficacy, and therefore the assay sensitivity. The clinical sample may also be "spiked" with known target NA, then run in parallel with the clinical sample, a practice that will require more reagents and time, but helps monitor for specimen inhibition. Also the use of both strong and weak positive controls in each run will aid in assay standardization.

A number of NA amplification techniques, such as PCR, ligase chain reaction, strand displacement, transcription mediated amplification, NA sequence based amplification (NASBA), branched DNA technique, and hybrid capture assays, have been described (42).

PCR is one of the most widely used NA principles in the clinical diagnostic setting for the detection of HSV genome, and many variants of the first-generation conventional PCR have been reported, each with their own purpose and advantage (Table 2).

Conventional PCR is a two-step procedure including amplification and detection, the latter requiring either gel electrophoresis, using ethidium-bromide staining (57) or radioisotope (44), or an ELISA using dioxigenin, biotin, or enzymes (58,59). The electrophoresis format provides added specificity by displaying the molecular weight of the amplified product (57). Most conventional HSV PCR assays are designed to be HSV-type specific (60,61). The sensitivity and the specificity of conventional HSV PCR are increased in the nested PCR procedure (44), which has two rounds of amplification where the amplicon from the first round contains hybridization sites for a second primer pair. The disadvantage of the nested procedure is prolonged testing time (one to two days) and the risk of carry-over contamination (62). Consensus PCR of herpes group viruses allows single tube screening of clinical specimens by amplifying conserved sequences in the herpes virus (63,64). If a product is detected, it can be identified using specific hybridization. This approach allows for rapid and more economical initial screening of specimens, and several reports of consensus PCR for the herpes group viruses applied to the analysis of CSF specimens exist (64–66). Another variant of PCR multiplexing uses multiple primers to allow amplification of multiple templates within a single reaction (67). Advantages of this approach include rapid screening, decreased cost, and

Table 2 Five Modifications of the Conventional PCR Technique: Principles, Advantages, and Disadvantages

PCR variant	Principle	Advantages	Disadvantages
Nested PCR	Two amplification steps. The amplicons from the first step contain hybridization sites for a second primer pair.	Increases sensitivity. Increases specificity as the second primer set verifies the first-round product.	High likelihood of contamination during transfer of the first-round amplicon to a second reaction tube.
RT PCR	RNA target is first converted to cDNA by RT, which is then amplified by PCR.	Detects RNA and mRNA.	Longer TAT. Technically challenging.
Consensus PCR	Amplifies conserved sequences in viruses belonging to the same family. Product identified using specific hybridization, restriction enzyme analysis, or DNA sequencing.	Allows detection of a broad range of viruses in one reaction. Cost effective and rapid.	Requires second detection step for identification of which virus is in the herpes group.
Multiplex PCR	Two or more sets of primer pairs, specific for different targets, are introduced into the same reaction tube.	Multiple targets can be detected simultaneously. Decreases TAT.	Requires extensive development work. May have lower sensitivity than single reaction PCR because of competition.
Real-time PCR	Amplification and detection simultaneously.	Decreases TAT. Decreases contamination. Robust Commercial assays are being developed. Easy to standardize and automate standard curve for quantification.	Somewhat lower sensitivity. Expensive analyzer required.

Abbreviations: cDNA, complementary deoxyribonucleic acid; mRNA, messenger ribonucleic acid; PCR, polymerase chain reaction; RT, reverse transcriptase; TAT, turnaround time.

the detection of coinfections. A conventional multiplex PCR assay for the detection of herpes viruses in CSF in HIV-infected patients was recently reported (68). Although conceptually attractive, multiplexing has some drawbacks, including lower sensitivity and specificity (68) and significant developmental costs. Reverse transcriptase (RT) PCR allows the detection of HSV mRNA and has been used successfully in experimental settings for studies of HSV pathogenesis (46).

In contrast to conventional PCR assays, real-time PCR allows amplification of a target and detection of the amplicon simultaneously, using fluorophore labels and sensitive methods to detect their emissions in "real-time," thus eliminating post-PCR processing (25). The technique can be used for a variety of sample types including serum, plasma, CSF, tissue, buffy coat, urine, tear samples, nasopharyngeal washings (NPW), vesicle scrapings, cord blood, and amniotic fluid. Instrumentation platforms vary and may use single tubes, glass capillaries, or a 96-well format. The data generated from a real-time PCR reaction are plotted as fluorescent intensity against cycle number and reveals the kinetics of the PCR reaction. Some automated equipment will display the reaction kinetics as it happens, whereas others will display the kinetic curve only at the end of the detection cycle. The entire testing time, compared to conventional PCR, is two hours or less because of reduced cycle times and the elimination of a separate detection step. Ongoing advances in the development of fluorophores and instrument platforms will add to the utility of real-time PCR in diagnostic virology, and the methodology is incrementally gaining acceptance owing to its short TAT, reproducibility, reduced risk of carry-over contamination, and the possibility of automation. Some allow the simultaneous processing of entirely different real-time assays and the use of broad-based probe chemistry, including DNA-binding fluorophores, linear oligoprobes, and 5' nuclease oligoprobes (25). The 5' endonuclease probes are now widely used in diagnostic virology, and a recent report describes a high-throughput quantitative assay for detection of HSV DNA (69). To date there are only a few truly multiplexed HSV real-time assays reported in the literature, most of them require interruption of procedure to transfer the template, their fluorescence to be detected by end-point analysis or the assays are not performed in the same tube (25). One recently reported multiplex assay uses a nonspecific label, SYBR green, to detect HSV, varicella zoster virus (VZV), or cytomegalovirus (CMV) in separate tubes (70). Another assay detects HSV-1, HSV-2, VZV, and enteroviruses within a single capillary by applying fluorescent melting curve analysis (71). Real-time HSV PCR permits easy distinction of HSV subtypes, and a commercial real-time kit for genotypic subtyping was recently evaluated (72).

There is a growing clinical need for quantitative herpes virus PCR results or the monitoring of "viral load," particularly for the detection of the lymphotropic herpes viruses Epstein-Barr virus (EBV), CMV, and

HHV6 in transplant patients, but the determination of HSV viral load in clinical specimens, such as CSF (73) and genital secretions (74), has also been reported to be of clinical use, as it allows monitoring of both disease progression and antiviral treatment. For patients with herpes simplex encephalitis (HSE) a high viral HSV load in CSF is associated with poorer outcome. It has therefore been suggested that quantification of HSV DNA in CSF could be a useful tool for predicting outcome and the extent of neurological sequel (31). Many of the conventional PCR assays used for quantitation have been “semi-quantitative” (25) as opposed to those using competitive co-amplification. In this technique an internal control NA of known concentration and a wild-type target of unknown concentration are both amplified using equal efficiency assay design (25). This principle has been used in the detection of subclinical genital shedding of HSV, which allowed the monitoring of suppressive therapy (74). While conventional competitive PCR may be relatively inexpensive, real-time PCR is far more advantageous and convenient for HSV DNA quantification (31). First, real-time quantitative PCR has a significantly greater dynamic range, and, second, very low inter- and intra-assay variability. Commonly, quantitative real-time PCR employs an external standard curve, where a known HSV standard is titrated and analyzed within the same experimental run as the unknown sample. Some may argue that this assay is also semiquantitative, as the standard curve is “external” to the samples tested.

Although the most common use of quantitative real-time PCR is to measure viral load in clinical specimens, it has also been used to determine HSV antiviral drug susceptibility (18). This technique involves short-term culture of the virus, followed by real-time PCR measurement of HSV-1 replication kinetics in the presence of acyclovir (18). Real-time PCR has also been used in the rapid detection and genotypic subtyping of HSV (75,76). Real-time PCR is also suited to rapid field-testing, and once automation is perfected this test format will allow efficient batch testing.

In summary, the real-time PCR format is becoming increasingly useful in various fields and is likely to become the new generation of HSV qualitative and quantitative detection reference tests.

Virus Culture

For a sensitive and accurate assay, virus culture has remained as the HSV detection gold standard for a long time period (77–89); however, this has been challenged with the introduction of NA amplification, which is now regarded as a specific, sensitive, and diagnostic alternative to HSV culture (24,29,31).

As the role of diagnostic virus culture is being redefined, it is clear that this method provides some unique advantages over NA amplification, particularly because whole active virus is isolated (90). This is necessary

for phenotypic antiviral susceptibility testing (91) and molecular fingerprinting of isolates (18,24,92,93) and for epidemiological and infection control investigations. For example, a recently reported real-time PCR assay was employed for antiviral susceptibility, but required initial viral replication through virus culture (18).

The sensitivity of HSV culture is dependent on rapid specimen transport and the use of appropriate transport medium (39,94–96), as the virus is fastidious and only remains active for two to four hours on inanimate surfaces (97,98).

A multitude of specimens such as vesicular lesions, eye, throat and genital swabs, CSF, blood, buffy coat cells, tissue, urine, NPW, and amniotic fluid (94) can all be cultured for HSV, using a variety of continuous cell lines such as human epidermoid carcinoma lines (HEp-2 and A549), human fibroblasts, rhabdomyosarcoma cells, and mink lung (ML) cells (82,94). Although relatively straightforward for the proficient virus laboratory, conventional culture is labor intense and expensive by requiring frequent inspection for cytopathic effect (CPE) of the inoculated cells using an inverted microscope. The isolation time needed is dependent on the initial inoculum, but the average HSV culture time is between two and seven days. A definitive culture confirmation of HSV CPE requires the use of type-specific monoclonal antibodies and an immunoassay such as DFA or an enzyme-linked immunosorbent assay (ELISA). A variant of classical tube culture, the shell vial technique (77,78), involves centrifugation of the specimen onto a cell monolayer, which is grown on a glass cover slip that is placed at the bottom of a glass vial or for higher throughput a 24-well plate. The cover slip is immunostained after 24 to 48 hours (99,100). The cell line used affects the sensitivity, and human embryonic kidney cells (HEK) or ML cells are reported to have greater sensitivity than human lung fibroblasts (101). This method may decrease the TAT significantly, but is not as sensitive as the conventional tube culture method, where the virus is allowed to incubate with the cells for a longer period of time. A clinical virologist must weigh the advantages and disadvantages before deciding which method should be used in the laboratory. With the introduction of rapid and robust NA amplification methods in the clinical virology laboratory, the virologist may choose a NA amplification method for rapid detection and a classical cell culture method for the recovery of small amounts of live virus for further use in antiviral susceptibility testing or subtyping.

The enzyme-linked virus inducible system (ELVIS) test, another innovative virus culture variant, uses a genetically altered Baby Hamster Kidney (BHK) cell line, where a reporter gene for beta-galactosidase is driven by the promoter from the HSV-1 *UL39* gene. When infected with HSV, these cells will express the beta-galactosidase gene, resulting in a color change that can be visualized by light microscopy (102). This system is commercially available and has sensitivity that is comparable to conventional culture.

In summary, virus culture is for the specialized laboratory, but a useful technique when live virus is required for further analysis.

Antigen Detection

The diagnostic virus laboratory should make every effort to provide a prompt and accurate answer to the clinician, using fiscally responsible techniques. Rapid immunoassays, although less sensitive than culture or NA amplification (103), fit nicely into this category. They are suitable for both body fluids, such as genital, vesicular, and oral secretions, as well as tissues, such as vesicle scrapings, biopsies, and postmortem tissues. They allow same day diagnosis and do not require active virus replication, thus permitting prolonged transportation times (94).

All immunoassays are based on using labeled specific anti-HSV antibodies, which detect HSV specific proteins (104,105), but several different technical formats exist such as peroxidase-based immunocytochemistry, DFA, ELISA, and cytospin DFA (106). Initially, polyclonal antibodies were used in immunoassays, but these did not provide sufficient specificity and sensitivity, and it was not until monoclonal antibodies became available (107,108) that immunoassays became highly specific and sensitive, allowing the accurate distinction between HSV-1 and HSV-2 infections (109,110).

Direct detection of HSV antigen in vesicle scrapings is usually performed using DFA, a rapid and specific technique, where the intracellular location of the fluorescence is discernible, providing an “inbuilt” specificity control as opposed to the ELISA format, where a color change determines whether the test is positive or not. The drawback of the DFA technique is that it requires special expertise, access to an immunofluorescence (IF) microscope, and a skilled technologist (94). In our pediatric virus laboratory we use the DFA technique on a regular basis to distinguish between HSV and VZV infections in both staff and patients from critical areas such as the oncology and neonatal unit, where the distinction between HSV and chickenpox exposures has significant infection control and therapeutic implication. The cytospin DFA is a modified DFA, where the specimen is spun down onto a glass slide prior to DFA staining. This increases the sensitivity of the assay, because the number of cells increases. The sensitivity and specificity varies 30% to equal to classical culture.

A method more suitable for a general pathology laboratory, the ELISA format, works for genital and oral secretions and permits automation. However, the sensitivity is only 47% to 89% of that of culture.

Immunocytochemistry is a technique suited to the study of HSV replication in human biopsy and postmortem tissues. It allows the co-localization of specific cellular and HSV antigens, such as HSV infection in keratinocytes in Paget's disease (111), HSV and papilloma infection in organotypic culture tissue (112), HSE in AIDS (113), Bell's palsy (114), and fatal dissemination

of HSV in pregnancy (liver and brain) (115). Although excellent for morphological studies, immunohistochemistry is not very sensitive as shown in a recent study of HSV esophagitis in the immunocompetent individuals where immunohistochemistry examination alone missed the diagnosis, whereas the addition of viral culture improved the sensitivity (116). Molecular techniques can be combined with immunocytochemistry, and in situ hybridization has been used to co-localize cellular antigens and HSV NA or messenger ribonucleic acid transcripts both in experimental models (117) and in humans (118). Emerging in situ PCR techniques for the detection of HSV DNA should help improve the sensitivity and allow the pathologist to further delineate the relationship between the localization of HSV NA and various cell types (119).

Monoclonal antibodies, which specifically recognize lytic cycle HSV replicative proteins (immediate-early, early, and late), have been used extensively in experimental models for HSV replication (46). Immunohistochemistry has also been used to map HSV-1 and HSV-2 pathogenetic differences in mouse brain tropism and transportation pathways in the CNS (120) as well as murine retinal infection (121).

Although the traditional method of choice for the definitive diagnosis of HSE immunohistochemistry of brain biopsy, this invasive technique has now been supplanted by the establishment of reliable NA amplification techniques.

In conclusion, immunoassays allow the rapid detection of HSV antigen and the study of HSV antigen localization in tissue, but may need to be followed up with NA amplification and/or culture to ensure excellent sensitivity.

Cytological Smear

A presumptive diagnosis of HSV infection can be made by examining cellular material from a vesicular skin or mucosal scraping or tissue using Papanicolau (122), Giemsa, or Wright stain, which may show intranuclear inclusions. A Tzank smear also uses material from vesicular scrapings and may reveal multinucleated giant cells (123). Although these tests may have to be used in small, peripheral hospitals without access to a diagnostic virus laboratory, they are neither sensitive (124) nor specific (125), as they do not distinguish between the viruses in the herpes group.

Electron Microscopy

Specimens that can be processed for HSV EM examination include body fluids and tissue. Secretions, vesicle fluid, exudates, and CSF need little preparation prior to EM examination, but a minimum of 10⁶ virus particles/ml are required for detection (126–128), making this a poor choice for rapid diagnosis of most specimens, in an era when NA amplification permits detection of 1 to 10 GE equivalents. Vesicle fluid for herpetic lesions

may have a sufficient number of virus particles as will specimens that have been amplified for a few days in virus culture. Prior to the introduction of amplified culture systems and NA methods many modifications of classical EM were made to increase the test sensitivity. These include variations of immunoelectron microscopy (IEM): direct IEM (DIEM), serum-in-agar (SIA) IEM, solid-phase IEM (SPIEM), and protein A-gold IEM (PAG IEM) (Doane FW. *Electronmicroscopy in Diagnostic Virology*. Cambridge University Press. 1987. Eds. Doane F.W. and N. Anderson). These techniques will allow detection down to 10^4 virus particles/ml (129). Another approach for the analysis of body fluids is ultracentrifugation directly onto an EM grid using an airfuge. This has been reported to enhance classical EM sensitivity by 25% (130).

Thus, with the introduction of new sensitive techniques such as immunoassays and NA amplification for the detection of HSV, the diagnostic niche for EM has changed significantly. However, EM still has an important role in the detection of HSV in tissue biopsies and postmortem tissue specimens where an ultrastructural search through thin section of fixed and embedded cells may provide important diagnostic and pathogenetic clues. Many experimental studies in vitro and animal models have utilized thin sections of fixed and embedded cells and tissue for the study of pathogenesis, cellular tropism, and HSV cellular processing such as entry, replication, and egress. The clinical utility of EM has been most pronounced in HSE, and recently a group used a combination of immunohistochemistry, in situ DNA hybridization, and EM to confirm a case of relapsing HSE (118).

EM has also been used for structural studies of single HSV particles, and negative staining of enveloped viruses, such as HSV, allows the nucleocapsid to be clearly outlined. A recent review describes the combination of X-ray crystallography and electron cryomicroscopy in the structural study of the herpes virus (131). The authors suggest that this combination of techniques may permit the study of nonicosahedral components in the HSV virion at high resolution and the collection and analysis of structural data has the potential to become a routine tool that complements other molecular tools in the virology laboratory.

In summary, EM is no longer useful as a routine rapid HSV detection technique, because it does not allow batch testing and requires sophisticated skills and the access to an EM; although it may be useful in the detection of HSV infection in tissue biopsies or postmortem specimens its main utility is now in the research setting, where it is a powerful tool for the study of HSV pathogenesis, virus cell interactions, and particle structure.

DETECTION OF ANTIVIRAL ANTIBODIES

The utility of HSV serology has improved gradually during the last decades. The first-generation serological technologies, such as complement fixation

and neutralization tests, were type-common and did not allow distinction between IgM and IgG responses, and therefore acute and convalescent sera were required to diagnose primary HSV infection. The introduction of ELISA and immunofluorescence assays (IFA) enabled the detection of type-common anti-HSV IgG and IgM antibodies, which is useful in the diagnosis of primary infection, where HSV-specific IgM will be positive in a single serum specimen (132–135). IgM rises within a week of primary infection and wanes after two to three months, but it is important to note that type-common HSV IgM will occasionally rise slightly during recurrent HSV infection, which may confound the interpretation of a low titer HSV IgM response. The presence of HSV IgG only determines previous exposure or immunity, and IgG determination is valuable in the management of immunocompromised patients, where the presence of HSV IgG antibodies identifies patients at risk for recurrent HSV infection (136).

A valuable adjunct to virus detection, HSV type-specific antibody testing can be performed both in serum and CSF and will help detect patients with only HSV-1 or HSV-2 antibodies as well as patients with reactivity to both subtypes. This information may be important in a variety of clinical situations such as suspected neonatal herpes infection, following vaginal delivery in culture negative women with no history of genital herpes. The development of reliable HSV type-specific serology has been complex, because HSV-1 and HSV-2 share many epitopes, which results in crossreactivity. This problem is demonstrated in type-specific ELISAs that use crude antigen preparations and display show specificities as low as 70% for HSV-1 and 51% to 85 % for HSV-2 (36).

Ashley et al. (137) circumvented the issue of crossreactivity in their type-specific HSV antibody assay by using the western blot (WB) methodology, in which patient serum is reacted with HSV proteins that have been separated electrophoretically according to molecular weight. The pattern of antibody binding bands is type-specific and the WB therefore allows help to determine whether a patient has been infected with either HSV-1 or HSV-2 or both subtypes. This WB assay has been used in extensive patient studies to define the spectrum of clinical manifestations of genital herpes and the natural history of unrecognized HSV-2 disease (138).

Despite significant crossreactivity between HSV-1 and HSV-2, one major HSV glycoprotein G (gG) is clearly type specific, so that HSV-1 infection will evoke an anti-gG-1 response whereas HSV-2 infection evokes an anti-gG-2 response. This has been explored by several groups, who have created type-specific HSV antibody assays using monoclonal antibody blocking assays, immunodot enzyme assays, gG capture assays, and a HSV-2 ELISA where lectin purified gG-2 is used as an antigen (139). Sera that do not give a clear type-specific pattern in the gG region can be preabsorbed against HSV-1 and HSV-2 proteins and then retested, which will further improve the specificity of the type-specific assays.

Although the WB assay remains the reference standard for subtype-specific HSV serology, moderate to low complexity laboratories can now choose from more user-friendly test formats than WB for HSV type-specific serology, based on type-specific gG response as described earlier. Several FDAs approved ELISA tests, which can be semiautomated, exist. A novel strip immunoblot assay that can be used for low-volume testing has also been introduced to the market. The sensitivity and the specificity of commercially available type-specific assays vary, but in general the specificity has been better for the detection of HSV-2 antibodies. A membrane-based immunoassay, using a native glycoprotein of gG-2 as antigen, is the basis for a newly approved POC HSV-2 antibody assay, which can produce immediate results in the physician's office. This assay has a specificity of 94% to 100% and a sensitivity of 92% to 97.7% when compared to the WB assay (36,140,141). The test takes approximately 6 to 10 minutes to perform and providing that quality assurance procedures are in place, they may become very useful for a practitioner in a SP Sexually Transmitted Disease clinic or in general practice.

In conclusion, several commercially available test kits exist for the determination of type-specific antibody response but only tests based on gG should be used, as ELISA tests based on crude antigen extracts have very poor specificity. One limitation of type-specific assays includes a prolonged time of approximately 13 days to seroconversion to gG-2. Performance data, interpretation, and confirmatory follow-up testing strategies of gG-based assays have recently been reviewed (36).

The utility of HSV type-common and type-specific serology is improved when a qualified clinical virologist is consulted for an interpretation of complex clinical scenarios such as CNS, neonatal, and HSV diseases in the immunocompromised patient. Although the diagnosis of central nervous system (CNS) infections by type-common HSV serology of Cerebral Spinal Fluid is now mainly a thing of the past, it may still occasionally provide useful information. It does require a concomitant serum specimen and a determination of IgG ratios between serum and CSF (142–144). HSV IgM can rarely be found in CSF (145,146). A recent report of a retrospective study of 624 CSF samples from patients suspected to have HSE, detected intrathecal HSV IgM and/or IgG in 3.8% of cases, all more than 14 days after disease onset, and HSV-1 PCR positivity in 1.3% and HSV-2 PCR positivity in 1.1% within 12 days after onset of disease (1,147). Interestingly, no intrathecal antibodies were detected in PCR-positive specimens, and no antibody-positive specimens were PCR positive. Although exceptions exist (148), the authors draw the conclusion that intrathecal antibodies can only be detected when the virus is cleared from the CSF and that PCR is the method of choice for early diagnosis of HSE, whereas intrathecal antibody studies would be of value in later stage disease. Another caveat regarding CSF antibody determination is that the antibody response may be delayed or absent when antiviral treatment is started early (149).

The determination of type-specific HSV antibody response in CSF has been used to investigate the pathogenesis of neo- and postnatal HSV (150,151). The interpretation of the HSV serological response in suspected neonatal or congenital herpes may be obscured by the presence of passively transferred maternal HSV IgG antibodies in the baby during the first six months of life. A type-specific serologic response to glycoprotein gG-1 for HSV-1 and to glycoprotein gG-2 for HSV-2 will help the clinician to determine the etiology of genital herpes and to investigate the route of infection in neonatal herpes. Type-specific HSV serology will also help to identify and to counsel patients and partners with unrecognized genital herpes (36).

The interpretation of HSV serology may be complex in transplant and cancer patients, who often receive blood or blood products and who may not be able to mount an adequate immune response depending on the level of immunosuppression.

LABORATORY DIAGNOSIS OF SPECIFIC HSV INFECTIONS

The interpretation of positive HSV laboratory results must always be done in conjunction with the clinical picture and general laboratory tests such as routine hematology, CSF analysis, and liver function tests. If required, the clinician may consult the clinical virologist, who is qualified to address the specifics when interpreting positive and negative HSV test results.

This section will describe the utility and interpretational quandaries of laboratory testing for the diagnosis of specific HSV infections. A summary of specimens suitable for various clinical conditions is provided in Table 3.

CNS Infection

HSE, predominantly caused by HSV-1, is the most common form of sporadic encephalitis in North America (21,152) with an incidence of 2/1,000,000 per year. Untreated, the disease is often fatal and is difficult to diagnose with standard laboratory techniques. The virus cannot be cultured from CSF, and serology is not diagnostic until late in the illness, when the neurological damage is significant. Traditionally, the only definitive diagnostic test was antigen analysis of brain biopsies using IFA (24,153). With the introduction of safe antiherpetic drugs, this diagnostic dilemma led to the recommendation of empiric treatment of clinically and/or radiologically suspected HSE (153). However, the introduction of type-specific and sensitive NA amplification assay in the early 1990s (44) dramatically improved the service to clinicians, who manage patients with HSE (91,152,154–156) in replacing the need for brain biopsy (152).

PCR is now considered the method of choice for the diagnosis of HSV-1 and HSV-2 CNS infections. Sensitivities and specificities of various

Table 3 Specimens that May Be Collected for Laboratory Analysis for Various Clinical HSV Conditions

Clinical condition	Nucleic acid amplification	Culture	Serology	Antigen detection	Immunocytochemistry
Encephalitis	Cerebrospinal fluid	Cerebrospinal fluid Whole blood Serum	Cerebrospinal fluid Serum	n/a unless skin lesions are part of condition	Brain biopsy Postmortem material
Meningitis	Cerebrospinal fluid	Cerebrospinal fluid Whole blood Serum	Cerebrospinal fluid Serum	n/a unless skin lesions are part of condition	n/a
Neonatal HSV	Cerebrospinal fluid Whole blood Serum Urine Eye secretions Vesicular lesions	Cerebrospinal fluid Whole blood Serum Urine Eye secretions Vesicular lesions	Cerebrospinal fluid Serum	Vesicular scraping	Skin biopsy Brain biopsy Postmortem tissue
Genital HSV	Genital secretions Vesicular exudates Vesicular scraping	Genital secretions Vesicular exudates Vesicular scraping	Serum	Vesicular scraping	Skin biopsy
Skin disease	Vesicular exudates Vesicular scraping Skin biopsy	Vesicular exudates Vesicular scraping Skin biopsy	Serum	Vesicular scraping	Skin biopsy
Eye disease	Eye secretions Tear film Dendritic ulcers Corneal transplant material	Eye secretions Tear film Dendritic ulcers Corneal transplant material	Serum	Vesicular scraping	Corneal scraping Corneal transplant material

Abbreviation: HSV, herpes simplex virus.

HSV PCR-based detection methods in patients with suspected HSE are commonly greater than 95% (24), and in a review of the literature Tebas et al. estimated a PCR sensitivity of 96% and specificity of 99% in patients with HSE proven by brain biopsy or intrathecal antibody production (157). A positive PCR result in an immunocompetent patient is diagnostic, because HSV DNA is not found in CSF in HSV-seropositive immunocompetent individuals who are neurologically normal (158), whereas the picture is less clear in immunosuppressed patients. PCR has been shown to be positive for more than two days after onset of HSE symptoms and for up to four weeks after onset (159), therefore a false-negative HSV PCR result may be generated if the CSF specimen is taken too early following onset of disease. In patients with low clinical suspicion of HSE, a properly performed negative test virtually excludes the disease, whereas if the clinical suspicion is high, there is a greater possibility that a negative result is false (154). In the partially treated HSE patient, the PCR may remain positive for some time, as this assay is not dependent on actively replicating the virus. Sequential HSV PCR in CSF can therefore be used to monitor the progression of the disease during treatment. CSF viral load measurements by quantitative PCR in sequential specimens may provide additional insight into predicting the outcome in HSE, as it has been suggested that a high viral load may reflect more serious disease (47).

HSV-2 most commonly causes meningitis, a less aggressive form of HSV CNS disease. This may occur even in the absence of genital lesions. As opposed to the situation in HSV-1 HSE, CSF may be culture positive in HSV-2 meningitis (160). The use of type-specific HSV-2 PCR analysis of CSF has further increased positive diagnosis (161) and unequivocally demonstrated that this form of HSV CNS disease is most commonly caused by HSV-2 (103,162). The use of diagnostic PCR has also led to the identification of an expanded spectrum of HSV infections of the nervous system (163), including Mollaret's meningitis (156) and myelitis with diverse clinical manifestations (164,165). In preschool-age children, beyond the neonatal period, HSE may present either with multifocal or diffuse involvement of the brain detected most efficiently by magnetic resonance imaging (MRI). In this setting diagnostic PCR is invaluable as an adjunct (166). PCR is also a helpful tool in the monitoring and management of HSV CNS disease in patients who develop a relapse of encephalitic illness after an initial episode of HSE (163), some of which may represent true residual disease and some immunologically mediated disease (167). A recent report of a pediatric cohort of 25 children with HSE showed that relapse occurred more readily in those who had received a lower dose of acyclovir (167). HSV PCR may also be useful in entirely excluding HSV CNS disease, because other treatable diseases may mimic HSE (152). Future PCR investigations of unusual neurological disorders, with a wide variety of symptomatology, may lead to further expansion of the role of HSV in CNS disease. Fodor et al. suggested

that 17% of HSE cases may not have the classical temporal lobe presentation, but present as brainstem encephalitis, myelitis and multifocal, or diffuse encephalitis (7).

It is important to note that many clinicians now express interest in using a negative HSV PCR result in CSF as a means to discontinue acyclovir treatment in a patient with suspected HSE. However, the decision to discontinue acyclovir must be based on the clinical picture (1,168) in combination with PCR test results, as the test sensitivity is not 100%. Some laboratories that culture virus have now modified their routine CSF PCR analysis for encephalitis and meningitis to a screening test, which includes the detection of several herpes viruses simultaneously, using consensus PCR (64,65), multiplex PCR assays (67), or multiplex real-time PCR (70,71).

Neonatal HSV

A life-threatening condition, neonatal HSV infection affects approximately 1500 to 2200 infants per year in the United States. It can occur prenatally, during parturition, or postnatally (169). Although 75% to 80% of neonatal HSV disease occurs during parturition, the other two scenarios need to be considered. Intrauterine and intrapartum HSV infections are transmitted from the mother, whereas postpartum infection may also be spread from other individuals (151). Restriction enzyme (RE) HSV fingerprinting (170,171), typing of an isolated virus strain and determination of HSV type-specific IgG by western blotting (150) are all useful tools in determining by which route an infant was infected.

Although rare, intrauterine HSV infection can result from either primary or recurrent maternal infection and may have severe consequences for the fetus (14,172). HSV IgM determination in maternal blood combined with type-specific serology is a useful tool in defining the infection type. Maternal virus culture, PCR, or immunoassays of genital lesions will allow typing of the virus, but has no bearing on the distinction between maternal primary or recurrent disease. Intrauterine blood samples obtained by cordocentesis are useful specimens for the determination of IgM antibodies in fetal blood and for the detection of viral DNA by PCR (173). Amniotic fluid can be analyzed by PCR and virus culture (173,174).

The outcome of perinatal HSV is dependent on whether the mother has a primary or recurrent infection (14,175,176). If the mother reactivates a latent HSV infection during pregnancy, passively transferred maternal HSV IgG antibodies will modify the fetal disease and the maternal antibodies present that are specific to HSV-2 but not HSV-1, this appears to reduce the neonatal transmission of HSV-2 (176).

A primary maternal HSV infection induces a typical antibody response with HSV-specific IgM and IgG, but a recurrent infection may or may not show an increase in HSV-specific IgG and/or IgM. HSV IgM

antibodies in recurrent infection have been shown to display a less complex reaction with viral polypeptides (134), an observation that could be utilized in the diagnosis of recurrent infection in the specialized laboratory with experience in WB assays.

Infants with neonatal HSV can be divided into three groups: (a) skin, eye, or mouth (SEM) involvement only, (b) encephalitis with or without SEM, (c) disseminated infection (177). The clinical picture and outcome varies significantly between the three groups, as well as the efficacy of antiviral treatment and long-term prognosis. The laboratory diagnostic techniques useful for neonatal HSV infection are dependent on disease presentation. For example, if the baby has skin lesions a presumptive diagnosis can be obtained within an hour, using type-specific IFA of a blister base scraping. For laboratories that provide real-time PCR service, vesicle fluid or scrapings can be analyzed with same day results. However, as approximately 2/3 of babies with neonatal herpes present without skin lesions (178), other test modalities are necessary. If the baby presents with CNS symptomatology, neuroimaging and HSV PCR on CSF will provide a rapid diagnosis. If the baby presents with sepsis and without a history of genital herpes in mother, a comprehensive workup is warranted, including a combination of neuroimaging, PCR on blood, CSF, urine, stool, and conjunctival and nasopharyngeal secretions. Virus culture should also be performed on these specimens but could require anywhere from two to seven days to produce positive results. HSV IgM determination and type-specific serology in mother and in baby are also appropriate in this circumstance (179). It is important to note that HSV subtyping is important in the diagnosis of neonatal HSV, because it helps with both epidemiology and prognostication (180).

In newborns without clinical symptoms but with risk factors for neonatal HSV, such as premature rupture of membranes or the presence of genital lesions during vaginal delivery, viral serology will help to prove whether baby has protective maternally transferred HSV IgG. Screening cultures and/or HSV PCR of throat, urine, stool, nasopharynx, and conjunctiva can be used to monitor such babies.

Genital HSV

Genital herpes is most commonly caused by HSV-2, but may also be caused by HSV-1 (181). Genital herpes is a public health problem for several reasons, including psychosocial suffering, morbidity associated with each recurrence, and transmission of virus from mother to baby, and genital HSV is an identified risk factor for the transmission of HIV (182). The prevalence of genital HSV in the general population has likely been underestimated because of the lack of reliable diagnostic tests. Recent large seroepidemiological studies have illustrated that genital herpes is largely a subclinical disease and that spread can occur inadvertently through asymptomatic shedding

(138). Recent debate has suggested that validated type-specific serology could help in curbing this outbreak and access to the new rapid, type-specific serological assays may further bring this issue to public attention (36). Increased awareness may help in both efficient management of patients and infection control. Now a gold standard in the diagnosis of neurological HSV disease, PCR may soon play an important role in the management of genital HSV disease, in being able to identify subclinical shedding and breakthrough infection. The use of a multiplex PCR assay to screen for several venereal diseases simultaneously, may alter the way these patients are managed and will alleviate the need for expensive, less sensitive, and slower culture techniques, and ultimately reduce cost (3).

Skin Infections

HSV is responsible for a variety of clinical skin manifestations including eczema herpeticum, erythema multiforme, herpes gladiatorum, herpetic whitlow, Kaposi's varicelliform eruption, face, neck, ear and HSV folliculitis (183). HSV infection may also complicate cosmetic procedures of the orofacial region such as laser resurfacing, dermabrasions, dental interventions, and burns. A rapid type-specific IFA test on a skin scraping usually gives the diagnosis, the sensitivity of IFA depends on the quality of the submitted sample, but is between 80% and 90% (94,103). A culture of vesicle fluid, and subsequent collection of cells from the blister base, obtained by vigorous swabbing or preferentially by the use of a scalpel blade, can be sent to the laboratory for rapid antigen detection (103) and a result should be available within a few hours of submission. In critical cases, a stat request should yield an answer within the hour. It should be noted that these tests are usually highly specific but only 80% to 90% sensitive. This means that a positive result is helpful, but a negative result does not exclude the presence of the virus.

The analytical sensitivity of standard IFA in HSV skin infections can be increased by 30% by PCR (184) and this methodology may be useful in suspected cases where antigen detection IFA is negative (67,185). A multiplex nested PCR for VZV, HSV-1/HSV-2 (186), and real-time automated multiplex assay (55) has been reported and may become useful in atypical dermatological presentations of HSV, because it is sometimes clinically useful to differentiate between HSV and early herpes zoster.

Eye Infection

HSV infection of the eye is, second to trauma, the most important cause of blindness in the United States (21), and PCR-based technology is improving the understanding of ocular HSV disease (20). Primary HSV infection of the eye can result in keratoconjunctivitis, which can be diagnosed by virus

culture or PCR of eye secretions. Tear film has been shown to be an adequate sample that is easier to collect and causes the patient less discomfort than a corneal scraping when PCR is used for HSV detection (187).

A reactivated form of ocular HSV infection, dendritic ulcers, and geographic ulcers of the cornea as well as uveitis requires rapid and accurate diagnosis, as safe antiviral treatment is available. Culture and/or PCR of lesions are the diagnostic methods of choice. HSV has also been associated with acute retinal necrosis (188), where PCR is a useful diagnostic technique. Multiplex PCR may allow rapid screening of infectious posterior uveitis (189). PCR-based assays of vitreous specimens are useful in the diagnostic evaluation of patients with infectious retinitis (190).

HSV PCR has recently been shown to be useful in the laboratory investigation of herpetic stromal keratitis (HSK) (191). In this report tear samples appeared sufficient to obtain a diagnosis, which is particularly useful in HSK where corneal scrapings are contraindicated. PCR has also been used during allograft failure after corneal transplantations where a proven diagnosis of previous HSK by PCR will help the clinician to start antiviral treatment immediately, thereby possibly decreasing the number of graft failures (192).

Immunocompromised Patients

Medical progress in the management of transplant and cancer patients has increased the immunocompromised patient pool, as has the increase of HIV-infected individuals. HSV infection in these patient groups is often severe, including chronic, persistent, and active infection as well as severe life threatening disease (193). These patients often receive chronic suppressive antiviral therapy and the development of resistance to such drugs is adding to the complexity of management of these patients (194).

Clinical diagnosis of HSV skin infection in the immunocompromised host can be difficult, because a recurrent ulcer may resemble herpes zoster or appear in multiple sites simultaneously. AIDS patients may have severe facial or anogenital lesions, which often are seen as chronic ulcers. The virus laboratory plays an important role in these patients and culture, IFA or PCR is crucial to determine treatment. Virus culture from lesions in immunocompromised patients receiving long-term suppressive therapy with acyclovir may be particularly relevant if phenotypic antiviral resistance assays are required (91,149).

Virus serology occasionally plays a role in these patients, particularly in cancer patients prior to immunosuppression, because positive serology would identify patients at risk for recurrent HSV infection (136).

Disseminated HSV disease in the immunocompromised host requires a comprehensive laboratory work up, including serology, PCR, and virus culture. Multiplex or consensus PCR and quantitative real-time PCR will likely become routine in the work-up of febrile episodes in this patient group.

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SECTION II. DISEASE MANIFESTATIONS OF HSV AND THEIR TREATMENT

6

Antiviral Treatment

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HISTORICAL ASPECTS OF ANTIVIRAL THERAPY

Though the first vaccine was developed for a viral disease (1) more than 200 years ago (Edward Jenner, small pox, 1798), antibiotics were known for 30 years (Alexander Fleming, penicillin, 1929) before the first antiviral chemotherapeutics (Herbert Kaufman, idoxuridine, 1962) were published (2). Today, the therapeutic standard to treat bacterial infection is well established compared to the emerging field of virology. There are obvious reasons for the delayed development of antiviral agents. For instance, bacteria can easily be detected by light microscopy, retained by a filter (exceptions are chlamydia and mycoplasma) and grown in standard media, while the resolution of an electron microscope is necessary to visualize a virus (Latin for poison) and cell culture techniques had to be established to propagate the obligate intracellular viruses, which pass through a bacterial filter (Chamberland, porcelain tube, 1882; Adolf Mayer, TMV disease, 1886; Dimitri Iwanowski, TMV passes Chamberland filter, 1892; Martinus Beijerinck, filterable virus "TMV alive," 1898). Since viruses exploit many cellular functions to multiply, their genomes are smaller (the genome size of bacteria is in the low Mbp range vs. Kbp for viruses). Hence, less essential proteins/enzymatic functions are provided by the multiplying pathogens that differ enough from the host enzymes and can be exploited as targets for chemotherapy to interfere with growth and survival of the pathogen. Today, the nucleosides acyclovir [Zovirax® (3–7), 1981],

valacyclovir [Valtrex® (8–10), 1995], famciclovir [Famvir® (11–13), 1994], and topical formulations of penciclovir [Vectavir®, Denavir® (13), 1996] represent the standard therapy of herpes simplex disease (Fig. 1 and Tables 1, 2). In contrast to the first antiviral compounds such as idoxuridine [IDU (2,16), topical treatment for herpetic keratitis, 1962], vidarabine [Ara-A (1,50), adenine arabinoside, first systemic (iv) antiviral therapy for biopsy-proven herpes encephalitis, 1977], and trifluridine [Viroptic® (14,16), successor of IDU, 1964], the standard nucleosidic chemotherapeutics [acyclovir (ACV), valacyclovir (VACV), and famciclovir (FCV)] possess a reasonable efficacy and safety with systemic administration. Valacyclovir and famciclovir are prodrugs of acyclovir and a close congener penciclovir (PCV), respectively, and were launched in the mid-1990s at the time when the patent of acyclovir expired. The generics improved the cost/benefit ratio, making it more difficult to market the prodrugs, which offer the only advantage of more convenient dosing schedules based on improved oral bioavailability (VACV 54%, FCV 77% vs. 10–20% for ACV and <5% for PCV, respectively) and pharmacokinetics.

TREATMENT OF HERPES SIMPLEX VIRUS (HSV) INFECTIONS

HSV infections are the cause of diverse disease manifestations (Table 1) ranging from totally asymptomatic infections to common herpetic disease such as herpes labialis (cold sore, Chaps. 7 and 8) and herpes genitalis (genital herpes, Chaps. 8 and 9). In a minority of cases, HSV infections can progress to sight-impairing (HSV infections of the eye, Chap. 10) or life-threatening (encephalitis, Chaps. 11 and 12) disease especially in the immunocompromised patients population and neonates (Chaps. 14–15).

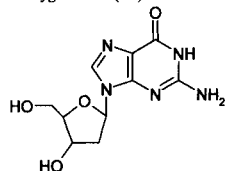
A detailed description of disease manifestations is documented in subsequent (Chaps. 7–15). Once herpes simplex disease has been diagnosed (according to the preceding chapter), treatment can be initiated with standard therapeutics (www.pdr.net, www.rote-liste.de, etc.).

Currently, the antiviral drugs acyclovir [Zovirax (3–7)], valacyclovir [Valtrex (8–10)], famciclovir [Famvir (11–13)], and topical formulations of penciclovir [Vectavir, Denavir (13)] represent the standard therapy of herpes simplex infections (Table 1). The nucleoside mimetics (Figs. 1–2) have successfully been used to treat HSV disease listed in Table 2. The pharmacological profiles of drugs for systemic application are compared in Table 3.

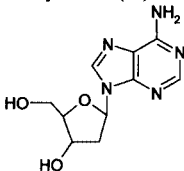
It is of crucial importance to initiate therapy as early (within 24 hours of onset) as possible following diagnosis or onset of signs and symptoms (26–28) of HSV infection or disease. This general rule to treat infections caused by human pathogens applies in particular to HSV infections, due to the fact that the moderate potency of nucleosidic drugs diminishes with increasing viral load, a situation observed late during the disease course. In many clinical trials, it was proved difficult to demonstrate a significant therapeutic effect with respect to a defined parameter such as time to

Structural Formulas

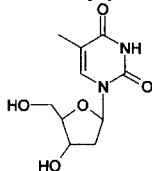
Deoxyguanosine (dG)



Deoxyadenosine (dA)



Deoxythymidine (dT)

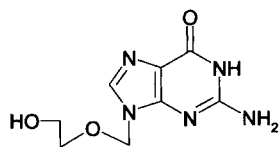


Launched / Marketed Drugs

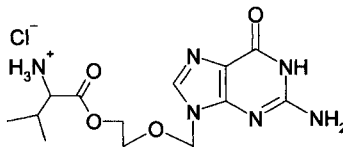
First line or standard therapy

Acyclovir (ACV), Zovirax,

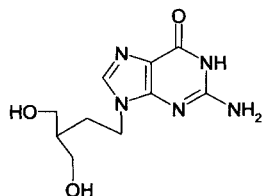
Wellcome 248 U, 9-(2-hydroxyethoxymethyl)guanine
 2-Amino-6-hydroxy-9-[(2-hydroxyethoxy)methyl]-9H-purine
 $C_8H_{11}N_5O_3$ MW 225.21

**Valacyclovir (VACV), Valtrex,**

2-[(2-Amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl-L-(S)-2-amino-3-methylbutyrate
 L-Valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl ester
 $C_{13}H_{20}N_6O_4 \cdot HCl$ MW 360.8

**Penciclovir (PCV), Vectavir, Denavir,**

BRL 39123, 9-[4-hydroxy-3-(hydroxymethyl)-1-butyl]guanine
 $C_{10}H_{15}N_5O_3$ MW 253.26

**Famciclovir (FCV), Famvir,**

BRL 42810, 2-[2-(2-amino-9H-purin-9-yl)ethyl]-1,3-propanediol diacetate
 $C_{14}H_{19}N_5O_4$ MW 321.34

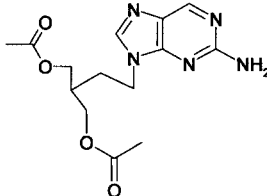


Figure 1 Structural formulas. Acyclovir is a guanosine analog with an acyclic side chain at the ninth position of the base and a broken sugar ring. ACV and PCV mimic dG, whereas IDU, TFT, BVDU, and SVD are dT mimetics. Ara-A is a dA analogue. (Continued on pp. 156–157.)

healing, if therapy is initiated later than 72 hours after onset of disease. Furthermore, the nucleosidic drugs are virostatic and not virucidal; thus, the dose regimen has to be followed to ensure continuous exposure of the patient with drug levels above the IC_{50} , which as indicated above correlates negatively with increasing viral load. In summary, nucleosidic drugs show only moderate efficacy when mucocutaneous HSV infections are treated. For instance, they may shorten duration of viral shedding and healing time

Second line therapy

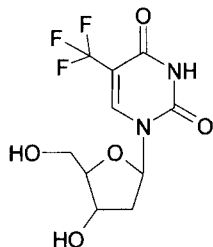
Trifluorothymidine (TFT), Trifluridine, Viroptic Phosphonoformic acid (PFA), Foscarnet, Foscavir

2'-Deoxy-5-trifluoromethyluridine,

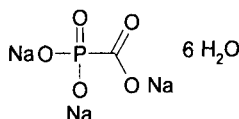
5-trifluoromethyl-2'-deoxy-uridine

1-(2-Desoxy-β-D-ribofuranosyl)-5-(trifluoromethyl)uracil

$C_{10}H_{11}F_3N_2O_5$ MW 296.2



$Na_2CO_3P \cdot 6H_2O$ MW 300.1



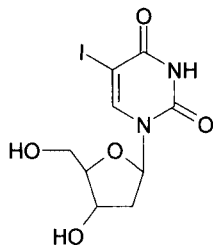
Early days of treatment

Idoxuridine (IDU), Herplex

5-Iodo-2'-deoxyuridine or 2'-Deoxy-5-iodouridine

1-(2-Deoxy-β-D-ribofuranosyl)-5-iodouracil

$C_9H_{11}IN_2O_5$ MW 354.1

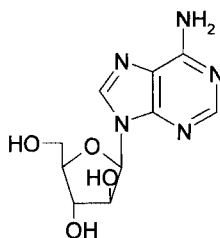


Vidarabine (Ara-A), Arasena A

Adenine 9-β-D-arabinofuranoside, adenine arabinoside

9-β-D-Arabinofuranosyladenine

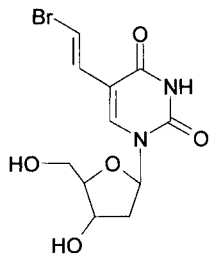
$C_{10}H_{13}N_5O_4$ MW 267.25



Brivudin (BVDU), Helpin, Zostex

(E)-5-(2-bromovinyl)-2'-deoxyuridine

$C_{11}H_{13}BrN_2O_5$ MW 333.14



Isoprinosine, Inosiplex, $C_{24}H_{34}N_6O_8$ MW 847.02

Inosin[(1-dimethylamino-2-propanol)-4-acetamidobenzoat]

$C_{10}H_{12}N_4O_5$ MW 268.23 $C_9H_8NO_3$ MW 178.17 $C_5H_{14}NO$ MW 104.17

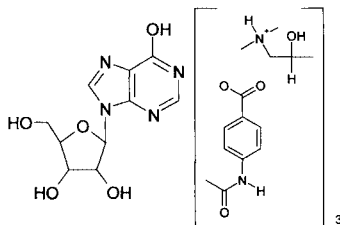
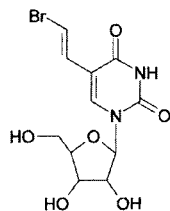


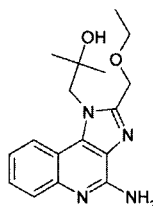
Figure 1 (Continued on p. 157)

Investigational Drugs

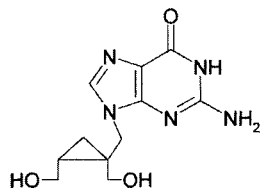
Sorivudine (SVD, BVAU), Bv (bromovinyl) araU,
Brovavir, Usevir $C_{11}H_{13}BrN_2O_6$ MW 349.14



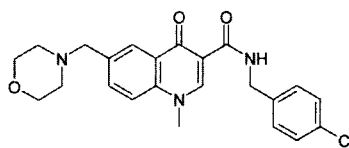
Resiquimod, R-848, S-28463
 $C_{17}H_{23}N_4O_7$ MW 314.39



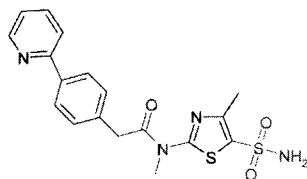
A-5021 (AV-038 prodrug of A-5021)
HSV Pol inhibitor
 $C_{11}H_{13}N_5O_7$ MW 265.27



4-oxo-dihydro quinoline, PNU-183792
HSV Pol inhibitor
 $C_{23}H_{24}ClN_4O_3$ MW 425.92



Thiazolylamide,
HSV Helicase-Primase Inhibitor
BAY 57-1293 $C_{18}H_{18}N_4O_5S_2$ MW 402.5



Aminothiazolyl-phenyl-derivatives,
HSV Helicase-Primase Inhibitors
BILS 45 BS (R=H), BILS 179 BS (R=Me) $C_{23}H_{23}N_5O_2S$ MW 457.56

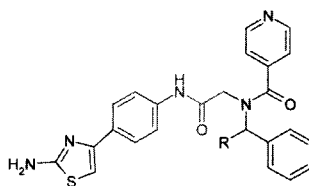


Figure 1 (Continued)

by 1 or 2 days (e.g., 6–7 days treated vs. 7–8 days untreated). Also, neither intravenous nor oral acyclovir treatment of acute HSV infections reduces frequency of recurrences after discontinuing of therapy, a situation that may change with the development of new drugs (18–22). However, oral acyclovir, valacyclovir, and famciclovir suppress genital herpes in patients with frequent recurrences. Suppression therapy can reduce the frequency of recurrences up to 80% and prevents recurrent disease in 25–30% of the patients (29–34). Prophylactic treatment in immunocompromised patients, especially those having induction chemotherapy or transplantation, with the standard drugs ACV, VACV, and FCV reduces the rate of symptomatic

Table 1 Disease Manifestations of Herpes Simplex Virus Infections

Virus	Disease
HSV-1	Encephalitis, Meningoencephalitis herpetica
HSV-2	Aseptic Meningitis
HSV-1 >	Keratitis, Herpes corneae
HSV-2	Keratoconjunctivitis herpetica
	Conjunctivitis
	Yearly 500 000 cases of HSV eye infections or ocular herpes diagnosed in the US
HSV-1 >>	Oropharyngeal / Orofacial Herpes
HSV-2	Gingivostomatitis herpetica (primary/initial)
	Herpes labialis (cold sore, ubiquitous recurrent disease),
	Tonsillitis, Pharyngitis, Esophagitis, Tracheobronchitis
HSV	Mucocutaneous disease (immunocompromised patient)
HSV	Herpes whitlow, Eczema herpeticum
	Herpes gladiatorum
HSV	Hepatitis, Visceral HSV infections
	Disseminated disease, herpes sepsis (Immunocompromised host, Neonates)
	Fetal infections (~ 1 in 300 000 births)
	Neonatal Herpes (< 1 in 1500 births)
HSV-2 >>	Primary/Recurrent Herpes genitalis (Genital herpes)
HSV-1	(~1.6 million new infections per year in the US ~ 6 in 1000)
	Vulvovaginitis herpetica, Perianal herpes

The disease is predominantly caused by the HSV serotype highlighted in bold and additional information such as primary or recurrent disease is indicative but not an absolute statement. Prevalence of HSV-1 and HSV-2 is 30–90% and 6–60% of the population, respectively, and correlates with age and number of sexual partners, besides other factors such as race, marital status, and location of the host but shows no seasonal variation.

HSV infection from about 70% to 5–20%. Thus, prophylaxis is an option to therapeutic ACV, VACV, or FCV, especially because acyclovir-resistant HSV isolates occur more frequently after therapeutic treatment than during prophylaxis (35,36).

First-line treatment or recommended chemotherapy for the particular indication is listed in Table 2 and the prescribing information regarding standard therapy is attached (see appendix). Topical therapy is regarded to be

Table 2 Therapy of Herpes Simplex Virus Infection (www.pdr.net, www.rote-liste.de)

Type of infection	Route and dose ^a	Comments
<i>First-line therapy</i>		
Genital HSV		
Initial episode		
Acyclovir (ACV)	200 mg by mouth 5 times/day for 7–10 days	Preferred route in normal host Severe cases only
	5 mg/kg i.v. over 1 hr every 8 hr for 5–7 days	
	400 mg by mouth 3 times/day	
Valacyclovir (VACV)	1 g by mouth twice daily for 10 days	
Famciclovir (FCV)	250 mg by mouth 3 times, i.e., 3 times/day for 5–10 days	
Recurrent episode		
Acyclovir	400 mg by mouth twice daily for 5 days	Limited clinical benefit
Acyclovir	5% ointment, cover lesions every 3 hr, 6 times per day for 7 days	Topical treatment regarded less efficacious
Valacyclovir	500 mg by mouth twice daily for 3 days	
Famciclovir	125 mg by mouth twice daily for 5 days	
Suppression		
Valacyclovir	500–1000 mg by mouth once daily up to 1 yr	Reduces rate of transmission
Famciclovir	250 mg by mouth twice daily up to 1 yr	
Acyclovir	400 mg by mouth twice daily up to 12 months	Titrate dose as required (200 mg 3–5 times daily)
Intermittent therapy		
Acyclovir	200 mg by mouth every 4 hr 5 times/day for 5 days	Therapy should be initiated at the earliest sign or symptom (prodrome) of recurrence
Primary gingivostomatitis		
Acyclovir	200 mg by mouth 5 times/day for 7–10 days	Preferred route in normal host

(Continued)

Table 2 Therapy of Herpes Simplex Virus Infection (www.pdr.net, www.rote-liste.de) (*Continued*)

Type of infection	Route and dose ^a	Comments
Valacyclovir	500 mg–1 g by mouth twice daily for 10 days	
Famciclovir	250 mg by mouth 3 times, i.e., 3 times/day for 5–10 days	
Herpes labialis (cold sores)		
Valacyclovir	2 g bid for 1 day	
Acyclovir	5% cream, apply the cream 5 times a day for 4 days	
Penciclovir (PCV)	1% cream every 2 hr during waking hours for 4 days	
Mucocutaneous HSV infections in immunocompromised patients		
Acyclovir	200–400 mg by mouth 5 times/day for 7–14 days 5–10 mg/kg iv over 1 hr every 8 hr for 7–14 days	For minor lesions only A maximum dose equivalent to 20 mg/kg every 8 hr should not be exceeded for any patient
	5% ointment, cover lesions every 3 hr, 6 times per day for 7 days	Limited infections
Valacyclovir	500 mg twice daily by mouth	
Famciclovir	500 mg 2 times/day by mouth for 7 days	HIV-infected patients
HSV encephalitis		
Acyclovir	10–20 mg/kg iv over 1 hr every 8 hr for 10 days	Pediatrics 3 month–12 yr 20 mg Adolescent > 12 years 10 mg
Neonatal HSV ^b		
Acyclovir	10 (20) mg/kg iv over 1 hr every 8 hr for 10 days SEM (skin eye mouth) 60 mg/kg/day for 14 days CNS or disseminated disease 60 mg/kg/day for 21 days	Birth to 3 month Current recommendation

Ocular herpes		
Acyclovir	3% eye cream	Drug of choice outside the United States
Trifluridine (TFT)	1% ophthalmic solution 1 drop every 2 hr while awake max daily dosage of 9 drops until the corneal ulcer has completely re-epithelialized. Following re-epithelialization, treatment for an additional 7 days of 1 drop every 4 hr while awake for a minimum daily dosage of 5 drops is recommended	Primary keratoconjunctivitis and recurrent epithelial keratitis (HSV) topical agent of choice in the United States
<i>Second-line therapy</i>		
Mucocutaneous acyclovir-resistant HSV infections		
Foscarnet (PFA)	iv 40 mg/kg (minimum 1 hr infusion) either every 8 or 12 hr for 2–3 weeks or until healed	Indicated for the treatment of acyclovir-resistant mucocutaneous HSV infections in immunocompromised patients
HSV infections		
Inosin [(1-dimethyl amino-2-propanol)-4-acetamidobenzoat]	6–8 tablets (500 mg) every 2–3 hr	Cell-mediated immunity; enhances T _H 1 immune response
Idoxuridine (IDU)	0.2% cream	HSV infections of the skin or eye
Vidarabine (Ara-A)	Topical (formally systemic use for HSV encephalitis)	It is used mainly today by health maintenance organization seeking cost-savings and in rare cases of hypersensitivity reaction to other drugs

^aDoses are for adults with normal renal function unless otherwise noted.

^bNot currently approved by the U.S. Food and Drug Administration.

Abbreviation: HSV, herpes simplex virus.

less effective than oral or intravenous treatment except for ocular herpes infections, where acyclovir (outside the United States) and trifluridine (14) ointments are also standard treatment options (15,16). Acyclovir ointment is approximately equipotent with trifluridine but blurs vision. ACV (400 mg tid (ter in die)) or equivalent prodrugs (VACV, FCV) may be administered as an alternative or supplement to topical therapy for the treatment of herpes epithelial keratitis. Ocular herpes or HSV infections of the eye are discussed in chapter 10 and treatment has been reviewed (15,16).

Severe disease like herpes encephalitis, neonatal herpes, visceral (hepatitis), or disseminated disease requires intravenous application of acyclovir. Intravenous ACV therapy reduces mortality from HSV encephalitis at 3 months from 70 to 19% and ~38% of treated patients regain normal neurological function. Mortality in neonates varies depending on the type of HSV disease and increases from 0% (localized neonatal skin disease) to 5% (encephalitis) and 25% for disseminated disease. The same holds true for morbidity. Ninety-five percent of the babies develop normally 2 years after skin infection, compared with 40–60% for babies with encephalitis and disseminated disease (37–39).

If response to first-line treatment after 3–5 days of therapy (Table 2) is poor and dose adjustments (e.g., 200 mg to 800 mg ACV five times a day or 500 mg to 1–2 g VACV bid) do not improve the situation by day 5–7, especially in immunocompromised patients, it is unlikely that the infection will respond to intravenous ACV. Differential diagnosis regarding fungal, bacterial, or other viral infection must be performed and ACV susceptibility studies of the clinical isolate should be ordered, if available. If the lesions are accessible for topical treatment, TFT (as ophthalmic solution) should be applied 3–4 times a day until the lesion is completely healed. If the lesion is inaccessible or the response to TFT is poor, therapy with intravenous foscarnet (PFA, 40 mg/kg tid or 60 mg/kg bid) should be given for 10 days or until complete resolution of disease symptoms. If foscarnet fails to achieve clinical clearing, consideration should be given to intravenous or topical cidofovir. Vidarabine is reserved for situations in which all of these therapies fail.

A generic immunomodulator (isoprinosine, Fig. 1) is licensed for treatment of chronic persistent viral infections; however, the product plays only a niche role for mild herpes infections due to its unspecific mechanism of action.

Finally, many nonprescription medications, which contain active ingredients, e.g., Zn, topical pain relievers, detergents (Docosanol®), or simply cover lesions cosmetically, are sold over the counter (a selection of web sites is provided, see Ref. 40). The obvious requirement for such symptomatic treatments underline the unmet medical need for novel, efficacious antiherpetic agents and professional care of patients. The standard treatment can abrogate progression of disease symptoms if therapy is initiated early during the disease course, whereas delayed treatment shows only

Table 3 Pharmacological Profile of Herpes Drugs (3–13,17,51 www.pdr.net, 18–25)

	Drug					
	Acyclovir (ACV)	Valacyclovir (VACV) prodrug of ACV	Penciclovir (PCV)	Famciclovir (FCV) prodrug of PCV	Foscarnet (PFA)	Investigational drug BAY 57-1293
<i>In vitro profile</i>						
Thymidine kinase (TK) (K_i) (μM)	172	See ACV	1.5	See PCV		
HSV DNA Pol (polymerase) inhibition constant K_i (μM)	0.07–0.08, competitive (dGTP), obligate chain termination	See ACV	8.5, competitive (dGTP), facultative chain termination	See PCV	0.4–0.6, non- competitive (dNTPs), un- competitive (DNA)	
Helicase ATPase IC_{50} (μM)						0.03 uncompetitive
Cell culture IC_{50} (HSV; μM)	0.5–1.0	See ACV	2–4	See PCV	~10–200	0.01–0.02
Correlation of IC_{50} with viral load	Strong	See ACV	Strong	See PCV	Strong	Weak

(Continued)

Table 3 Pharmacological Profile of Herpes Drugs (3–13,17,51 www.pdr.net, 18–25) (Continued)

	Drug					
	Acyclovir (ACV)	Valacyclovir (VACV) prodrug of ACV	Penciclovir (PCV)	Famciclovir (FCV) prodrug of PCV	Foscarnet (PFA)	Investigational drug BAY 57-1293
Mode of inhibition	Reversible (partial)	Reversible (partial)	Reversible (partial)	Reversible (partial)	Irreversible	Reversible
Selectivity index (SI)	~250	See ACV	~200	See PCV	~10	>2000
Half-life (hr) in cells	0.7–1.0, ACV-TP (triphosphate)	See ACV	~10, PCV-TP (triphosphate)	See PCV		
Target (genes containing mutations conferring resistance)	Thymidine kinase (TK), viral DNA polymerase	Thymidine kinase (TK), viral DNA polymerase	Thymidine kinase (TK), viral DNA polymerase	Thymidine kinase (TK), viral DNA polymerase	Viral DNA polymerase	Helicase, primase, U _L 5/U _L 52
Frequency of drug-resistant viruses	~1 × 10 ⁻³ (range 1 × 10 ⁻² –1 × 10 ⁻⁵)	See ACV	~1 × 10 ⁻³ (range 1 × 10 ⁻² –1 × 10 ⁻⁵)	See PCV		~0.5–4 × 10 ⁻⁶
<i>In vivo</i> potency ED ₅₀ (HSV-1/HSV-2) (ter in die mice)	22/16 mg/kg	17/15 mg/kg	See FCV	17/24 mg/kg		0.5/0.5 mg/kg

<i>Pharmacokinetic profile</i>						
Plasma half-life $T_{1/2}$ (hr) of drug in species	2–3 (man)	~2.9 (ACV man)	~2.0 (man)	2.0 (PCV man)	4.0 (man)	6 (mice)
Time to reach $C_{\max}$ of drug $T_{\max}$ (hr)	1.5–2.5 (man)	0.5–1.0 (VACV)		0.5–1.0 (FCV)		2.0–3.0
Peak concentration $C_{\max}$	200 mg tablet ACV	500 mg tablet VACV	See FCV	125 mg tablet FCV	Intravenous	
Dose (mg/kg)	2.7 man	6.7 man		1.7 man	60 man	1 mice
$C_{\max}$ (mg/L)	0.8	3.28 ACV		0.8 PCV	176	1.8
$C_{\max}$ (μ M)	3.5	14.6 ACV		3.2 PCV	589	4.4
Bioavailability (%)	10–20	54	<5	77	<20%	>60
Concentration in brain and spinal cord (CSF) (23–25)	0.83 μ M in CSF, ~20–50% of plasma concentration	~2 μ M ACV in CSF, ~20% of plasma concentration (dose 1 g VACV)			~80 μ M 13–68% of plasma concentration	
Protein binding	9–33%	13–18%	< 20%	See PCV	14–17%	>95%
Toxicity plasma concentration	>100 μ M	See ACV	>100 μ M	See PCV	>350 μ M	>100 μ M

Structural formulae are given in Figure 1.

marginal therapeutic efficacy. It may not make sense for the patient to arrange an appointment with a physician to receive a prescription and buy the drug at a pharmacy late during the disease course when the prescription drug does not offer significant efficacy compared to the over-the-counter medication. Treatment of disease retrospectively is futile. Patients must present themselves as soon as possible to a physician or ideally have the knowledge of self-diagnosis and the drug immediately available, meaning at hand or in store or at home, for optimal therapy.

MECHANISM OF ACTION

The precursor or prodrugs valacyclovir (8–10) and famciclovir (11–13) are converted to the respective nucleoside analogues acyclovir and penciclovir (Figs. 1 and 2) by esterases of the intestinal mucosa and first pass of the liver [esterases and aldehyde oxidase in the case of FCV to convert 6-deoxy-PCV (BRL42569) to PCV]. Acyclovir is transported into cells by passive diffusion or by a nucleoside transporter and subsequent phosphorylation traps the nucleosides in cells (Fig. 2). The intracellular half-life of ACVTP is ~ 0.7 (up to 2.5) hours versus 10–12 hours for PCVTP. In tissue culture, the relative potency of inhibition of herpesvirus replication is HSV-1 ($IC_{50} \sim 0.5$ – $1 \mu M$) < HSV-2 ($IC_{50} \sim 2$ – $4 \mu M$) < EBV ($IC_{50} \sim 0.5$ – $5 \mu M$) < VZV ($IC_{50} \sim 5$ – $20 \mu M$) $\ll$ HCMV ($IC_{50} \sim 20$ – $200 \mu M$) for ACV.

Acyclovir and penciclovir are prodrugs themselves, which are selectively phosphorylated by the viral thymidine kinase (TK) in HSV-, VZV-, or EBV-infected cells and subsequently converted to triphosphates by relatively nonspecific cellular kinases, in order to compete with deoxyguanosine triphosphate (dGTP) as a substrate for the viral DNA polymerase. Binding of ACVTP to HSV DNA pol (gene product of U_L30) is 30 times stronger than inhibition of cellular α DNA polymerase. While the phosphorylated penciclovir is incorporated into the growing DNA chain, acyclovir-triphosphate like 2',3'-dideoxynucleoside-triphosphates used for DNA sequencing is an obligate chain terminator, which lacks a 3' hydroxyl group and consequently immediately terminates DNA elongation as well as functionally inactivates the HSV DNA polymerase in a time-dependent manner by preventing dissociation from the DNA-ACVMP complex. Thus, initiation of DNA synthesis at an alternative extendible primer template is abrogated. If the virus does not express a functional TK (e.g., a resistant HHV1 or 2 strain or TK-negative viruses such as HCMV) or if the DNA polymerase does not have the optimal primary structure, the potency of the drug diminishes, the selectivity index is significantly smaller, higher doses have to be administered, and adverse effects are more likely to be associated with treatment. Since nucleosides are obligate or nonobligate chain terminators of DNA polymerization, they are potentially mutagenic, which is well documented regarding ganciclovir, idoxuridine, vidarabine, and trifluridine.

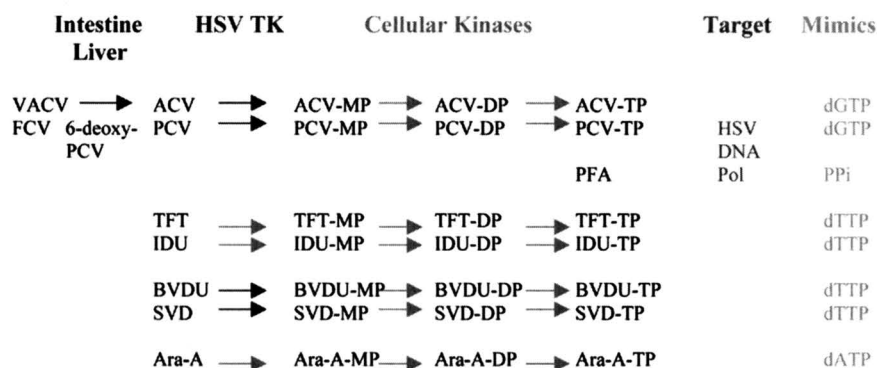


Figure 2 Mechanisms of action of antiherpes drugs targeted at the viral DNA polymerase. The precursor or pro-drugs (VACV or FCV) are converted to the respective nucleoside analogues (ACV and PCV) by the wall of the intestine (esterases) and first pass of the liver [esterases and aldehyde oxidase in the case of FCV to convert 6-deoxy-PCV (BRL42569) to PCV]. The applied (ACV) or released nucleoside analogues (ACV, PCV) are first activated by herpes simplex virus (HSV) encoded thymidine kinase (TK), and subsequent phosphorylation by cellular kinases. The inhibition of the viral DNA polymerase is competitive with regard to the natural nucleoside triphosphate dGTP, or noncompetitive in the case of pyrophosphate (PPi). In contrast to TFT, IDU, and Ara-A, the investigational drugs BVDU and SVD are monophosphorylated by the viral TK in infected cells resulting in a favorable selectivity index. TFT, IDU, BVDU, and SVD are dT mimetics, whereas Ara-A mimics dA. *Abbreviations:* ACV, acyclovir; FCV, famciclovir; PCV, penciclovir; VACV, valacyclovir; MP, monophosphate; DP, diphosphate; TP, triphosphate; PFA, phosphonoformic acid (foscarnet); TFT, trifluorothymidine; IDU, iododeoxyuridine; BVDU, brivudine; SVD, sorivudine; Ara-A, vidarabine.

Vidarabine and trifluridine (Fig. 1) are converted by cellular enzymes to the respective triphosphates, which inhibit the viral DNA polymerase by mimicry of adenosine and thymidine (Fig. 2).

The broad-acting foscarnet (Foscavir®) (17,51) is a pyrophosphate analog that inhibits DNA and RNA polymerases by mimicking the structure of pyrophosphate produced on extension of nascent DNA chains. The drug inhibits noncompetitively (with respect to dNTPs) and uncompetitively (regarding DNA, dNTPs saturating) all human herpes DNA polymerases but is indicated only as second-line therapy (iv and topical) for herpes disease refractory to nucleosidic drugs (e.g., acyclovir-resistant strains) due to its toxicity and or safety profile.

TOXICITY OF CHEMOTHERAPY

A continuous update on toxicity and side effects of the respective chemotherapy is provided online (www.pdr.net and www.rote-liste.de) and in

the current patient leaflets. Acyclovir, valacyclovir, and famciclovir therapies have few adverse effects. Reasons for the good safety profile of the above-listed nucleosidic drugs (ACV, PCV) are their selective conversion to the monophosphorylated drug (ACVMP, PCVMP) by the viral thymidine kinase (TK) in HSV-, VZV-, and EBV-infected cells, the poor formation of ACVMP/PCVMP in uninfected cells by the cytoplasmic 5' nucleotidase, the ~30 times higher affinity of ACVTP for the HSV DNA pol compared to cellular polymerases and the fact that ACV easily enters cells but phosphorylated nucleosides as polar compounds are trapped in cells and do not pass biological membranes in the absence of transporters. Toxicity of acyclovir correlates with serum concentration and occurs most commonly when it exceeds ~100 μM [Table 3, 22.5 $\mu\text{g/mL}$; parallels effects in cell culture due to low protein binding (10–30%)]. Renal dysfunction can occur, especially in patients given large doses of acyclovir by rapid intravenous infusion, but is rare and usually reversible. Risk of nephrotoxicity can be reduced by slow infusion of acyclovir and by ensuring adequate hydration. Due to its poor oral bioavailability (10–20%) oral acyclovir therapy, even at doses of 800 mg five times daily, does not cause renal dysfunction in normal patients. Valacyclovir, the prodrug of acyclovir, has an improved oral bioavailability, which translates into higher exposures in humans (a 1 g valacyclovir human dose leads to an exposure of $c_{\text{max}} \leq 5 \mu\text{g/mL}$ (acyclovir)). Consequently, overdosing of valacyclovir especially in severely ill patients resulted in a warning label such as “thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), in some cases resulting in death, has occurred in patients with advanced HIV disease and also in allogeneic bone marrow transplant and renal transplant recipients participating in clinical trials of Valtrex at doses of 8 grams per day.” Patients with renal dysfunction, geriatric patients, and patients on polypharmacy (receiving different drugs simultaneously) need more attention especially when the standard dose is modified.

Intravenous acyclovir has been linked with disturbances of the central nervous system, which include agitation, hallucinations, disorientation, tremors, and myoclonus. Continuous administration of acyclovir and famciclovir for suppression of recurrences has not caused long-term toxicity in the past decade.

Topical toxicity of trifluridine is mild and includes superfacial punctate keratitis, chemical conjunctivitis, punctual occlusion, and rare hypersensitivity reactions.

Idoxuridine is approved for topical use only due to its significant toxicity associated with systemic application.

Brivudin (BVDU) is metabolized in humans to bromovinyluridine (BVU), which possesses significant hepatic toxicity. Sorivudine is safer than BVDU, but was not approved based on the experience of the fatal drug interaction with 5-FU in cancer patients.

RESISTANCE

Resistant HSV can be selected *in vitro* in the presence of the drug, e.g., acyclovir or penciclovir at a frequency of $\sim 1 \times 10^{-3}$ (Table 3, range 1×10^{-2} – 1×10^{-5}). The mutation frequency of HSV-2 is somewhat higher compared to HSV serotype 1. Thus, up to one in a thousand HSV cases is already resistant to chemotherapy (41). By chance, this rate roughly reflects resistance in the clinic in immunocompetent patients (0.1–0.6%). The majority of resistance conferring mutations (95%) cluster in the thymidine kinase (TK, UL23) and a minority (<5%) of the mutations is found in the HSV DNA polymerase gene [DNA pol, UL30, (42,43)]. The mutations lead to three discrete classes of TK mutations:

1. TK-negative mutants: enzyme activity abolished, no phosphorylation of ACV
2. TK-low producers: partial TK activity
3. TK altered mutants: still phosphorylate thymidine but not ACV

They can also or cause expression of an HSV DNA polymerase with an altered primary structure that is no longer sensitive to competitive inhibition of dGTP by nucleotide mimetics such as acyclovir triphosphate (ACV-TP) (41–45). Acyclovir-resistant mutants caused by TK deficiency usually exhibit cross-resistance to all drugs that require the viral TK for activation; thus, ACV-resistant HSV isolates are usually also resistant to penciclovir. TK-negative mutants retain susceptibility to drugs that do not require phosphorylation but act directly on the viral DNA polymerase such as foscarnet (PFA). The HSV DNA polymerase mutants selected with ACV therapy may also be cross-resistant to other HSV DNA pol inhibitors like famciclovir, vidarabine, ganciclovir, and in some cases to foscarnet and cidofovir. TK and HSV DNA pol double mutants exhibit a broad degree of cross-resistance to most drugs used for HSV therapy. Recently, an update on drug resistance has been published (42,45). The particular mutations observed are summarized in Table 4.

The majority of the wild-type HSV strains isolated in the clinic displays an IC_{50} [ACV $\sim 0.2 \mu\text{g/mL}$ (~ 0.5 – $1 \mu\text{M}$) (HSV-1 ~ 0.15 , HSV-2 $0.4 \mu\text{g/mL}$), PCV $\sim 0.64 \mu\text{g/mL}$ ($\sim 2.5 \mu\text{M}$) (HSV-1 ~ 0.3 , HSV-2 $\sim 0.7 \mu\text{g/mL}$)] identical to those compared with laboratory strains *in vitro*. If resistance is defined for isolates with $ED_{50} > 2 \mu\text{g/mL}$ ($\sim 10 \mu\text{M}$ ACV), then resistance in the clinic is estimated to be $\leq 1\%$ among the immunocompetent patients (43). However, the resistance rate is 3–14% and higher among the immunocompromised patient population and correlates with the duration of chronic treatment. Resistance rates are lower for the immunocompetent patient population and acute treatment and delayed therapy show significantly higher resistance rates compared to prophylaxis and early treatment especially in the immunocompromised patient. Resistance correlates with

Table 4 Resistance Conferring Mutations of Drug-Resistant HSV

HSV TK mutations associated with ACV and in most cases PCV resistance		
	HSV-1	HSV-2
ATP binding site	R51W P57H K62N T63I	D55N G59P
Nucleoside binding site	A168T A175V R176Q	R177W
C-terminal active region	C336Y	C337Y
Other conserved regions	R216C R222C R222H	R217H+E39G T287M
Non-conserved regions	E83K Q104H Q104Stop H105P G144N T201P R281Stop L279S	E39G+Stop263 Q105P T131P S182D/R R223H R271V R272S D273R
Additions	G nt430- > Stop304	G nt433- > Stop227 GG nt433- > Stop183 G nt551- > Stop223
Deletions	A nt184- > Stop85 A nt227- > Stop85 C nt460- > Stop182 C nt548- > Stop408 C nt666- > Stop408 C nt1061- > Stop408 A nt1065- > Stop375	G nt180- > Stop69 C nt215- > Stop86 C nt463- > Stop183 C nt519- > Stop183 C nt551- > Stop223 C nt551- > Stop263 G nt779- > Stop263 T nt920- > Stop348 17 nt deletion- > Stop37/39
HSV DNA polymerase mutations associated with drug resistance		
	HSV-1	HSV-2
Acyclovir (ACV-resistant)	E597K/D A605V R700G A719V S724N/D/Q/E/A/K/T/H P797T V813M N815S/T/Q G841S/C R842S S889A F891C/Y V892M Y941H N961K	S729N D912V/A
Foscarnet (PFA-resistant)	S724N G841C P920S Y941H	A724T S729N L783M D785N L850I

Source: From Ref (42).

the immune status of the host, severe clinical manifestation, and start and duration of treatment; basically it correlates with the viral load that is selected for resistant virus in the presence of drug. While some of the in vitro selected drug-resistant viruses are impaired regarding viability, growth rate, or viral titers and not all of the mutants are pathogenic in animal models, resistant isolates identified in the clinic are pathogenic. Acyclovir-resistant HSV isolates have been identified as the cause of pneumonia, encephalitis,

esophagitis, and mucocutaneous infections in immunocompromised patients. Foscarnet is the only treatment indicated for therapy of acyclovir-resistant HSV disease in immunocompromised patients.

Methods for detection of drug-resistant HSV are either phenotypic or genotypic assays (44). Phenotypic assays are susceptibility tests such as cell-based assays [plaque reduction (PRA), cytopathic effect (CPE), or dye-uptake assays (DUA)], plaque autoradiography, flow cytometry, viral DNA inhibition assays, DNA:DNA hybridization assays, ELISA tests, immunofluorescence, or the more recently described AS-Assay (18,46).

Sensitivity/susceptibility of HSV to antiviral drugs is measured by PR, DU, CPE, or AS-Assays. Briefly, permissive cells are infected with a diluted viral inoculum in the presence of an antiviral agent. With a light microscope, the number of plaques and the extent of cytopathic effects or stained cells are analyzed after an incubation period and the drug concentration that reduces viral plaques or CPE by 50% or 90% is determined (inhibitory concentration IC_{50} or IC_{90}). More elegantly and broadly applicable, this method is performed with fluorescent dyes in anti-infective research and development (18,46). Resistance is defined by a breakpoint [e.g., multiples of the IC_{50} of the reference or set of wild-type strains, or simply IC_{50} (ACV) $>2 \mu\text{g/mL}$ (see above)]. Further refinements of the cell-based assay have been described in which virus DNA or viral antigens are used to determine the inhibitory effect; however, these techniques did not achieve widespread acceptance to date.

Genotypic methods are based on detection of viral mutations conferring drug resistance by DNA sequencing, polymerase chain reaction, restriction fragment length polymorphism, and single-strand conformation polymorphism. DNA sequences of specific amplified viral genes can be compared to those of a baseline specimen from the same patient or to a database of susceptible and resistant viruses.

Currently, both phenotypic and genotypic methods are pursued in parallel for assessing herpesvirus resistance to antiviral drugs.

Problems that could lead to incorrect estimation of drug resistance in clinical isolates are the cell type or species tissue of origin (nucleosidic compounds have different activity in different cell types), growth rate of cells (different response in culture if cells are multiplying or resting), media components (serum, thymidine reverses ACV inhibition), multiplicity of infection (IC_{50} correlates with m.o.i), mixture of drug-resistant and sensitive strains and finally, host factors (metabolism, latency are factors that may not be reflected in tissue culture) (44).

DRUG DISCOVERY

Acyclovir marked a breakthrough in antiviral research (3,4). Though numerous approaches and strategies were tested and considerable effort was

expended in the search for the next-generation antiherpetic therapy, it proved difficult to outperform acyclovir. The hurdle of efficacy, safety, and pricing set by a drug of the golden era of antimetabolite research in the late 1970s, for which the Nobel Prize was later awarded to Gertrude Elion and George Hitchings in 1988 (for the elucidation of mechanistic principles which resulted in the development of new drugs such as acyclovir), has hitherto blocked market entry for competitors. Vaccination against HSV has been evaluated since the 1920s (47–49), but a vaccine is not yet available for HSV infections. Experts question whether the development of an HSV vaccine per se is possible due to the frequent recurrences observed in infected patients, which can be regarded as a series of continuous immunizations with a live vaccine. An up-to-date review of the challenge to establish novel treatments for herpes simplex disease was published in 2005 (20). Among the investigational drugs, the most promising candidates are mentioned below; for details, see Ref. 20.

Currently launched drugs are approved for new indications and many companies are trying to relaunch generic drugs in a novel advanced formulation. Valtrex®, for example, was launched as a 1-day 2g bid therapy for herpes labialis in 2002 and for reducing the risk of transmitting genital herpes to heterosexual partners with healthy immune systems in 2003 (52). During an eight-month period of suppression therapy the rate of acquisition of HSV-2 among the susceptible partner was reduced by 48% and the incidence of clinically symptomatic HSV-2 infection was reduced by 75%. Valtrex XR, a controlled release formulation of valacyclovir, has entered phase I clinical trials in Dec 2003. Genvir® (formerly known as Viropump; Flamel Technologies), a twice-daily controlled-release microparticle formulation of acyclovir, is pre-registration, but approval is delayed.

The development of the second generation of nucleosidic drugs (pyrimidine analogues, which upon phosphorylation mimic dTTP; Figs. 1 and 2) such as brivudin (BVDU®, Helpin®, Zostex®) and its arabinosyl derivative sorivudine (BVAU®, BV-araU®, Brovavir®, Usevir®) looked promising. The compounds are equally potent against HSV-1 and show excellent activity against VZV when compared to acyclovir; however, these drugs are not or only weakly active against HSV-2. Moreover, these investigational drugs are converted by bacteria of the gut to the metabolite [(E)-5-(2-bromovinyl) uracil (BVU)] that interferes in cancer therapy with the degradation of 5-FU (fluorouracil). Several patients with stomach cancer receiving a combination of fluorouracil and sorivudine (SVD, 1-β-D-arabinofuranosyl-E-5-(2-bromovinyl) uracil; YN-72, SQ32,756) developed serious and, in a few cases, fatal bone marrow suppression. In view of this serious safety concern of a drug–drug interaction based on the inhibition of the dihydropyrimidine dehydrogenase, further development of sorivudine has been halted after nonapproval in the United States and elsewhere. Also brivudin though launched for VZV in

Germany has not been submitted to the FDA due to its similarity with sorivudine, its antiviral spectrum (not or only weakly active against HSV-2), its mutagenic potential, and the drug–drug interaction with 5-FU.

Research on targets such as TK or the transfer of knowledge regarding quinolone topoisomerase inhibitors to herpesviruses did not lead to more potent or safe compounds. The pharmacokinetics of ribonucleotide reductase inhibitors did not allow for systemic treatment and none of the herpes protease or uracil-DNA glycosylase inhibitors showed activity in animal models. The most promising compounds and development candidates (Fig. 1) are broad-spectrum herpes DNA polymerase [4-oxo-dihydro quinoline (PNU-183792)] and helicase primase inhibitors [thiazolylphenyl- (BILS 179 BS and BILS 45 BS)] especially thiazolylamide derivatives (e.g., BAY 57-1293).

In clinical trials are resiquimod (S-28463, R-848), A-5021 (AV-038), SP-303 (Virend[®]), a vaccine (see Chap. 2) and soon helicase primase inhibitors. A topical formulation (0.5%) of resiquimod (Fig. 1) has been developed as an immunomodulator of HSV infections. Phase III clinical trials were terminated due to lack of efficacy. Imidazoquinolines bind to cell surface receptors, such as Toll receptor 7, thereby inducing secretion of proinflammatory cytokines, predominantly interferon- α , tumor necrosis factor- α , and interleukin-12. This locally generated cytokine milieu biases towards a Th1 cell-mediated immune response with generation of cytotoxic effectors. The immunostimulatory effects have been exploited clinically in treatment of viral infections (HPV, HSV, and molluscum contagiosum) and nonmelanoma skin cancer.

A 15% SP-303 gel, a plant-derived product with novel antisecretory properties based on an inhibitory effect on cAMP-mediated Cl and fluid secretion, is tested in combination with ACV. Another nucleoside mimetic, A-5021, is comparable to ACV regarding its *in vitro* profile; however, for development of the prodrug of A-5021, AV-038 with better pharmacokinetics has been selected.

Recently, new inhibitors of the HSV helicase-primase with potent *in vitro* antiherpes activity, a novel mechanism of action, a low resistance rate, and superior efficacy against HSV in animal models have been discovered. The preclinical pharmacological profile of, e.g., BAY 57-1293 is superior to all HSV drugs (18–22). Thus, if the animal data translate into human trials, a new compound class might enter the market.

Finally, only one suitable therapeutic option to treat HSV infections does not match the medical standard of countries with a developed health care system. On the background of growing resistance, especially in the immunocompromised patient population (e.g., transplant patients), only one recommended treatment, nucleosidic drugs, for herpes simplex disease is clearly not in the public interest.

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Primary Herpes Simplex Gingivostomatitis and Recurrent Orolabial Infection

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INTRODUCTION

Herpes simplex virus (HSV) infection may cause a wide spectrum of illness in children and adults. Gingivostomatitis is the most common specific clinical manifestation of primary HSV infection in childhood. The peak incidence appears in the 1- to 3-years age group. HSV type 1 (HSV-1) is almost always the cause. The disease is a self-limiting but painful infection of the oral mucosa causing extreme discomfort that lasts for about two weeks. Herpetic gingivostomatitis has also been described in adults, with similar clinical manifestations (1,2).

PATHOGENESIS

In susceptible children or adults, the source of infection is mainly contact with infected oral secretions. Abraded oral mucosal membrane in the oral cavity is the predominant site of HSV-1 penetration. The incubation period ranges from 2 to 12 days (mean 4 days). In children, the lesions start in the gingiva and involve necrosis of infected cells and local inflammation, while in adolescents the primary infection often presents as ulcerative pharyngitis (3).

Attachment of the virus to cell-receptors, such as heparin sulfate proteoglycan, is mediated by the viral envelop encoded glycoproteins gB and gC, whereas gD is required for viral penetration into cells. Initial viral replication occurs at the portal of entry (4). It is not completely clear how the virus spreads to the whole oro-pharyngeal mucosal membrane. One assumption is that the penetrating virus migrates along the innervating axon to the trigeminal ganglia, where the virus replicates. Subsequently, progeny viruses return by the sensory nerve network to the oral mucosal membrane causing more degeneration of epithelial cells and multiple ulceration (5). The high titer of viruses in oral secretions may enhance penetration of normal mucosal membrane, as seen by the ongoing development of new lesions in the first week of gingivostomatitis.

The innate immune response that includes release of cytokines and activation of natural killer cells and macrophages prevents the spread of infection, thus significant viremia is absent (6). However, transient viremia could be detected by PCR in 34% of immunocompetent children with herpetic gingivostomatitis (Harel, Smetana and Amir-manuscript in preparation), and was present for two to four days (7). The adaptive immune response that develops after the first week is essential for clearance of the virus and resolution of the oral lesions. Disseminated clinical infection may occur in immunocompromised patients such as neonates or those in status post bone marrow transplantation (8).

Establishment of latency in the trigeminal ganglia occurs after primary orofacial infection, in latency, the viral genome is maintained in a repressed, non infectious static phase, a process which is not under adaptive immune control.

Reactivation of the virus and recurrent infection is triggered by factors such as UV light, febrile illness or emotional stress. The reactivated virus migrates via sensory nerves, in most cases to the lips, to develop either lesions or asymptomatic viral shedding. The adaptive immune response, mainly T-cell mediated immunity, is responsible for the clearance of the virus from the lesions.

EPIDEMIOLOGY

Primary HSV-1 infection is usually asymptomatic or associated with non-specific upper respiratory tract symptoms (9,10). Socioeconomic status has a profound influence on the epidemiology of HSV-1 infection. Approximately one-third of children in low socioeconomic status countries have serologic evidence of HSV-1 infection by five years of age and the prevalence rises to 70–80% by adolescence. In developed countries, in contrast, only 20% of middle- and upper-class individuals have HSV-1 infection by age five, and there is no substantial increase in adolescents (11,12). A recent study from the Middle East reveals a prevalence of HSV-1 antibody in

about 40% of children at five years of age, rising to 50% at 17 (13). The seroprevalence of HSV-1 rises to 40–70% between the ages of 20 and 40 years. Age-specific changes in HSV-1 seroprevalence in two U.K. surveys suggest that HSV-1 seroprevalence is declining in children and adolescents (14,15).

Only 16–30% of children with primary HSV-1 infection will have gingivostomatitis (9,10,16). The disease is very contagious, especially in close community settings such as day care centres or orphanage nurseries (17,18). In crowded nursery settings, more than 80% of susceptible children under three years of age became infected with HSV-1, and 93% developed severe herpetic gingivostomatitis (17).

Outbreaks of gingivostomatitis in adults are related to transfer of the virus by health care workers (19,20). The shift in age-specific HSV-1 seroprevalence is probably the reason for increased primary gingivostomatitis in adults (1,2,21,22).

Herpes labialis is the most common manifestation of recurrent oral HSV-1 infection. It is estimated to occur in 20–40% of the population despite an adequate immune response (23). The mean rate of recurrence after primary HSV-1 oral infection is approximately 0.1 episode per month (24). Data from children is limited. The occurrence of herpes labialis among children under the age of six years is low and averages some 6%, while the occurrence among children above the age of seven years is about 20%, which is constant until adolescence (25). It was also found that 28% of children who had been initially diagnosed with herpetic gingivostomatitis suffer from herpes labialis, as compared to the 6% of children without history of gingivostomatitis (25).

TRANSMISSION AND VIRUS SHEDDING

HSV-1 is spread through direct contact with lesions or oral secretions of infected individuals, therefore close interpersonal contact is usually required for effective transmission. In children with gingivostomatitis, the mean duration of viral shedding is seven days (range 2–12 days) and the virus can be isolated from 50% of affected children for up to seven days (26). Viral shedding of 10 days duration was seen in another study (27). The risk of transmission is increased when a large number of children are in close proximity, as in day care centres or nurseries (17,28). In such settings a high proportion of susceptible children become infected during an outbreak of gingivostomatitis. Data on intra-familial transmission of HSV-1 are lacking.

In patients with herpes labialis, during the vesicular stage, HSV-1 was isolated from 80–90% of oral lesions and was isolated less commonly from ulcers and crusts (29,30). The titer of virus shed varied also with the stage of lesions, maximal titers were measured in the first two days, and viruses were not isolated after the fifth day (31,32). Virus was also detected in 25% of saliva samples from patients with herpes labialis. Asymptomatic viral shedding was detected in 2–9% of individuals with history of recurrent herpes labialis

(32,33). The precise relationship between asymptomatic shedding and transmission is unclear.

CLINICAL MANIFESTATIONS

Patients with herpetic gingivostomatitis present with fever, *fetor oris*, irritability and painful oral vesicular lesions on the gingival and bucal mucosa and on the tongue and hard palate. The vesicles rapidly rupture to become shallow ulcers and persist for a mean of 12 days (range 7–18 days) (26). The lesions are located only on the oral mucosa and tongue in approximately a quarter of the children; and on the gums in about three-fourth of cases (Fig. 1A,B). The gums are usually edematous and frequently bleed on contact. Extraoral lesions around the mouth (lips, cheeks, and chin) are found in approximately two-thirds of affected children at day four (26). Whitlows are seen in children that auto-inoculate their fingers.

The painful oral lesions are the main cause of eating and drinking difficulties and hypersalivation that may last for about a week. Most children have a fever over 38°C for approximately four days, with enlarged cervical lymph nodes. Gingivostomatitis can occur in adults and may be more severe than in children (34).

The most common site of recurrent orolabial lesions is the lips (Fig. 2). Prodromal symptoms of tingling, pain or burning precede the papular eruption by one to several days. There is an orderly progression of the lesions to vesicles, pustules and finally to ulcers. As the ulcers begin to heal they form hard crusts that heal completely within 7 to 10 days. Pain is maximum at the vesicular stage and resolves within three to four days. Recurrence tends to take place at the same location on the lips or in closely related areas (Fig. 3). Most lesions develop on the outer third of the lips with the lower

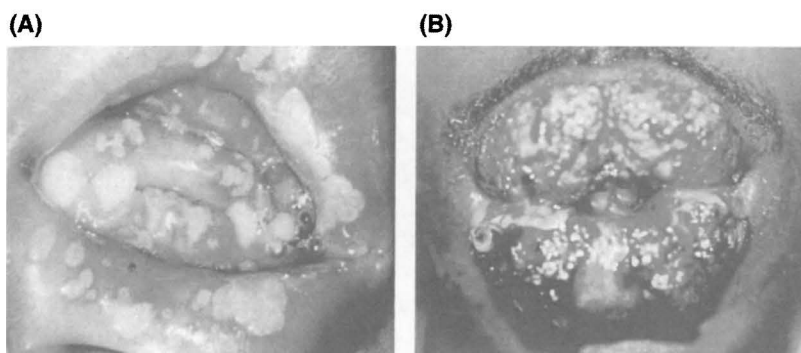


Figure 1 (A) An example of primary gingivostomatitis secondary to HSV type 1 in a child. (B) Such manifestations of primary infections are rarely seen in adults.



Figure 2 Herpes simplex labialis (type 1).

lip more frequently involved than the upper lip. Recurrence of intraoral lesions is rare, and tends to occur on the tissue adjacent to bone, the gums or the anterior hard palate (32,35).

COMPLICATIONS

The main complication observed in patients with herpetic gingivostomatitis is dehydration (9,26). Affected children experience extreme discomfort and dehydration resulting from poor fluid intake, saliva drooling and fever. Dehydration leads to hospitalization in less than 10% of affected children, and in one report accounted for 0.6% of all pediatric admissions (36).

Another complication of HSV-1 gingivostomatitis is secondary bacteremia caused by *Kingella kingae* (37) and *Streptococcus pyogenes* (38). Skeletal infections including osteomyelitis and septic arthritis (37,39), and endocarditis (40,41) are the most common pediatric manifestations of invasive *K. kingae* infection. These organisms, which are common components of the tonsillar flora of children, presumably invade the blood stream when the anatomical integrity of the mucosal surface is damaged by the herpetic infection, thereby providing a portal of entry.

Severe upper airway obstruction caused by ulcerative laryngitis was reported in children with herpetic gingivostomatitis (42–44). These children present with severe croup and are usually admitted to pediatric intensive care units for assisted ventilation.

Other complications of herpetic gingivostomatitis that occur include *Eczema herpeticum* (a serious disseminated HSV infection of the skin in



Figure 3 Recurrent herpes labialis with blisters located around the lips.

children with atopic dermatitis) (45) and various post infectious neurological diseases such as acute disseminated encephalomyelitis (46) or transverse myelitis (47).

DIAGNOSIS

The typical oral and extraoral lesions make the diagnosis straight forward and accurate in approximately 80% of children who are clinically suspected of infection (48). Culture of viral isolates is the most sensitive method for diagnosing an active HSV infection. A rapid enzyme immunoassay for the detection of HSV-1 antigen in children with gingivostomatitis has been found to have very high sensitivity and specificity (48).

Polymerase chain reaction (PCR) amplification of HSV DNA has been developed as a sensitive and specific diagnostic technique. HSV-1 can be rapidly identified in saliva from children with acute herpetic gingivostomatitis, by in vitro amplification using PCR and specific primers (49).

The differential diagnosis of herpetic gingivostomatitis includes herpangina and hand, foot and mouth disease, both of which are usually caused by coxsackie viruses. In herpangina the oral lesions are typically in the posterior portion of the oropharynx in contrast to the gingivostomatitis of HSV. In addition, unlike HSV infection, herpangina has a shorter duration and no extraoral lesions. Hand, foot, and mouth are characterized by typical vesicular eruption on the distal portion of extremities in addition to oral ulcers. Stevens-Johnson syndrome may mimic herpetic gingivostomatitis, but its common ocular and skin manifestations are not found in herpetic gingivostomatitis.

Herpes labialis is easy to diagnose clinically. Most adults who have suffered from recurrent herpes labialis recognize the prodromal symptoms, which are usually followed by the appearance of papules on the lip.

THERAPY

Supportive treatment of herpetic gingivostomatitis includes antipyretics, fluid administration and frequent feeding with soft food. The use of local anesthetics and mouthwashes is in practice but difficult in children and probably not indicated.

Acyclovir is the most commonly used drug for the treatment of various HSV infections including gingivostomatitis. Intravenous acyclovir is used by clinicians in hospitalized children with severe herpetic gingivostomatitis, although there are no data of the efficacy of this treatment. Experience indicates that the illness responds in a few days with defevescence and cessation of new oral lesions.

There are three randomized, double-blind and placebo-controlled studies on the use of oral acyclovir in the treatment of herpetic gingivostomatitis in children. The first study was performed in 1988 and included 18 children (Table 1) (50). Only pain and hypersalivation appeared to be alleviated in the acyclovir recipients. Outcome for the other parameters, such as fever and oral lesions, did not differ statistically between treatments. However, this study may have been too small for valid detection of differences between the two groups. In 1993 another study was presented at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy (Table 1) (27). In the children who received acyclovir, significantly shorter times before disappearance of the oral lesions (median 6 vs. 8 in the placebo group), gum swelling (5 vs. 7 days), saliva drooling (4 vs. 8 days) and cessation of viral shedding (4 vs. 10 days) were recorded. Compliance, as measured by the presence of acyclovir in the urine, was good. The third study was published in 1997 and included 61 children aged one to six years (Table 1) (51). Compared to placebo, acyclovir shortened the duration of all clinical symptoms: oral lesions (median 10 vs. 4 in the acyclovir group), fever (3 vs. 1), drooling (5 vs. 2), and eating difficulties (7 vs. 4). Duration of viral

Table 1 Summary of Randomized, Double-Blind, Placebo-Controlled Studies with Acyclovir for Treatment of HSV-1 Gingivostomatitis (HSV Was Documented by Viral Culture)

Study details	Cohort	Acyclovir dose	Results
Treatment started within 96 hours of onset of illness (50)	20	200 mg 5 × daily for 5 days	Pain and hypersalivation resolved significantly more rapidly with acyclovir ($p < 0.05$)
Treatment started within 96 hours of onset of illness (27)	68	20 mg/kg 4 × daily for 10 days	Duration of all symptoms (oral lesions, gum extraoral lesions, drooling) significantly shorter with acyclovir as well as viral shedding (4 vs. 10 days) ($p < 0.05$)
Treatment started within 72 hours of onset of oral lesions (51)	61	15 mg/kg 5 × daily for 7 days (maximal dose 200 mg)	Acyclovir shortens the duration of clinical symptoms (oral lesions, fever, extraoral lesions, eating difficulties, drooling) ($p < 0.01$) Viral shedding shorter with acyclovir treatment (1 vs. 5 days)

shedding was also significantly shorter in the group treated with acyclovir (1 vs. 5 days). The results of these studies clearly demonstrate that oral acyclovir shortens both the duration of all clinical manifestations of herpetic gingivostomatitis and the infectivity of affected children. Results seem to be better with early treatment. Thus commencing the treatment as soon as the diagnosis is made appears to enhance the efficacy of antiviral therapy. The suggested therapeutic dosage of acyclovir suspension is 15 mg/kg (maximum dose of 200 mg) five times daily for five to seven days.

Two prodrugs, valcyclovir and famcyclovir have been recently licensed for treatment of HSV infections. Although no controlled studies have been performed with these medications in children with herpetic gingivostomatitis, the pharmacokinetics of these medications would suggest superiority over acyclovir. Both drugs are unavailable as suspensions.

Prophylactic use of acyclovir in an outbreak of HSV gingivostomatitis in a closed community was reported in 1992 (28). In this single study, the incidence of symptomatic gingivostomatitis was significantly reduced in the children receiving prophylactic acyclovir. The practicality and value of oral acyclovir in similar settings remains to be determined.

Both topical and oral antiviral agents have been evaluated in the treatment of recurrent herpes labialis. Topical acyclovir and pencyclovir cream

treatment were modestly effective in reducing the duration of the lesion healing process (52–54). A variety of non-antiviral topical preparations are also available, but in most of the cases both the mechanism of action or efficacy were not profoundly studied. Docosanol cream was recently approved for use in the USA after trials that showed faster healing of lesions (55). Early treatment of herpes labialis with oral acyclovir or famcyclovir has marginal clinical value (56,57).

There are conflicting studies regarding the use of oral acyclovir for the prevention of herpes labialis. In one study significantly fewer subjects receiving acyclovir developed herpes labialis than did placebo recipients (58). The other study documented no significant differences in efficacy under similar trial conditions (59).

CONCLUSIONS

Gingivostomatitis is the most common specific clinical manifestation of primary HSV-1 infection in childhood. Gingivostomatitis occurs in adults also and may be more severe than it is in children. Only a quarter of children with primary HSV-1 infection will have gingivostomatitis. The infection is selflimiting and lasts for about two weeks. The main complications observed in patients with herpetic gingivostomatitis are dehydration and secondary bacteremia. Herpes labialis is the most common manifestation of recurrent oral HSV infection, and occur in 20–40% of the population. The results of few studies, clearly demonstrate that oral acyclovir shortens both duration of all clinical manifestations of herpetic gingivostomatitis and infectivity of affected patients. The suggested therapeutic dosage of acyclovir suspension is 15 mg/kg (maximum dose of 200 mg) five times daily for five to seven days. Both topical and oral antiviral agents in the treatment of recurrent herpes labialis have marginal clinical value.

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Herpesvirus Infections of the Skin

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INTRODUCTION

In addition to the more commonly known herpes genitalis and herpes labialis, both HSV type 1 and 2 can cause a variety of cutaneous manifestations in both children and adults. Cutaneous manifestations of the disease can vary significantly depending on immune status, predisposing skin conditions, genetic predisposition and history of recent procedures. Although most of these diseases are relatively mild in nature, some can lead to significant morbidity if not accurately diagnosed and treated.

HERPES WHITLOW

Herpes whitlow, characterized by a vesicular eruption in the digits, is commonly seen in children secondary to thumb or finger sucking (Fig. 1). As a result, the virus inoculates the cuticle after exposure to infected oropharyngeal secretions. HSV-1 was the most common etiologic agent; however, the incidence of HSV-2 whitlow has increased (1), as a result of digital-genital contact, resulting in greater number of HSV-2 related eruptions. Health care workers are also at the risk of acquiring the disease if



Figure 1 HSV type 1: herpes whitlow.

gloves and universal precautions are not implemented while in contact with infected secretions (2,3).

After an incubation period ranging from several days to weeks, patients typically complain of pain, tingling and burning in the affected digit. Within several days, erythematous clusters of vesicles appear, which may progress to ulcers during the course of the disease. Although the disease is self-limiting and precise treatment guidelines are not established, antivirals (e.g., oral acyclovir 200 mg 5 times daily 7–10 days) may be beneficial in shortening the course of the disease and in preventing transmission and recurrences (4,5).

HERPES GLADIATORUM AND HSV FOLLICULITIS

Herpes gladiatorum, another cutaneous manifestation of HSV-1, is seen in athletes involved in close contact sports, such as wrestling. The infection commonly affects the head or eye but involvement of the extremities and trunk can occur (6). Because the virus is acquired through skin-to-skin contact, it is recommended that infected wrestlers be diagnosed and excluded during an acute outbreak in order to prevent transmission (6). In addition, antiviral therapy (i.e., valacyclovir 500 mg every day) may be used to prevent recurrences in those with frequent outbreaks (7).

HSV-1 may also present as folliculitis, commonly described as painful, erythematous perifollicular vesicles on the body that are refractory to antimicrobial or antifungal therapy (8). Herpetic sycosis (viral folliculitis of the beard) is more common and is usually seen in individuals with recurrent perioral HSV. Treatment of lesions with antivirals usually shortens and aids in the resolution of symptoms.

ECZEMA HERPETICUM

Eczema herpeticum, also known as Kaposi's varicelliform eruption, is caused by HSV inoculation in patients with predisposing skin diseases. Although atopic dermatitis is the most common clinical condition associated with the disease, eczema herpeticum can also occur in patients with thermal burns and those with skin disorders like mycosis fungoides, pemphigus foliaceus, keratosis follicularis (Darier's disease), Sezary's syndrome, Hailey–Hailey disease, pityriasis rubra pilaris and congenital ichthyosiform erythroderma. In addition, the disease has been described in immunosuppressed individuals (9) and in patients with extensive sunburn (10). The disease occurs in individuals at any age, however, it tends to be more prevalent in young children. HSV-1 is a more common etiologic agent than HSV-2, but either virus can be acquired by auto-infection or through contact with an infected contact.

Patients with eczema herpeticum often present with extensive clusters of umbilicated vesicles and papules in susceptible areas (Fig. 2), which may be either localized in a dermatomal pattern or, more commonly, disseminated.

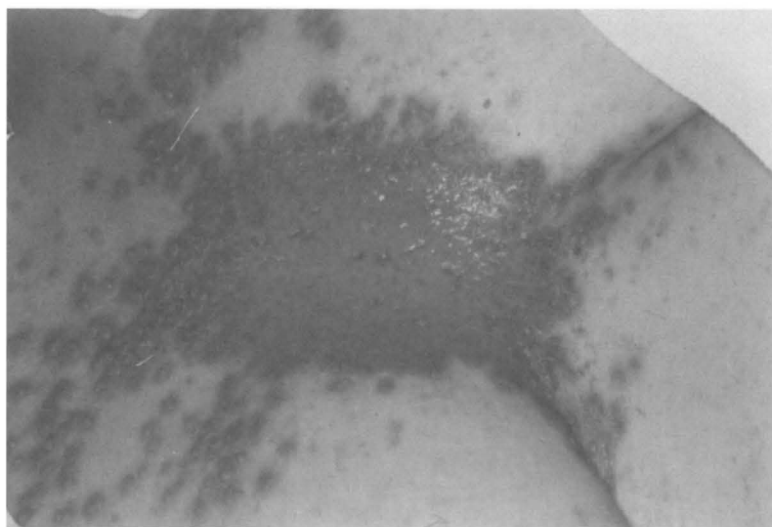


Figure 2 Eczema herpeticum.

Lesions may coalesce and become hemorrhagic erosions during the course of the disease. Patients are also susceptible to secondary bacterial superinfection (11) in addition to generalized symptoms like fever, chills and malaise. Clinical manifestations of the disease usually resolve in two to six weeks with appropriate oral antiviral therapy. However, if antiviral therapy is delayed secondary to misdiagnosis, the disease can be fatal (12). Prophylactic antiviral therapy can also be initiated in patients with recurrent HSV-1 or HSV-2 infection with pre-disposing skin conditions. Because precise dosing regimens are not established, it is appropriate to employ the same antiviral treatment regimens for eczema herpeticum as those used for genital herpes outbreaks.

ERYTHEMA MULTIFORME (EM)

EM minor is a condition that may develop in genetically susceptible individuals with recurrent HSV outbreaks in the orolabial, genital or extragenital regions of the body. Although some patients may not report a history of recent HSV infection, HSV DNA can still be detected by polymerase chain reaction in most skin lesions (13,14). Unlike EM minor, EM major is more commonly associated with adverse drug reactions than with HSV infection.

Expanding erythematous macules or papules are characteristic of EM minor. These symmetric lesions expand and usually develop into target lesions with central petechiae, vesicles or purpura (Fig. 3). The lesions are commonly found on the face, palms and hands and on extensor surfaces, but mucosal ulceration in the oral cavity may be noted. EM minor usually resolves in one week and is often associated with post-inflammatory hyperpigmentation.

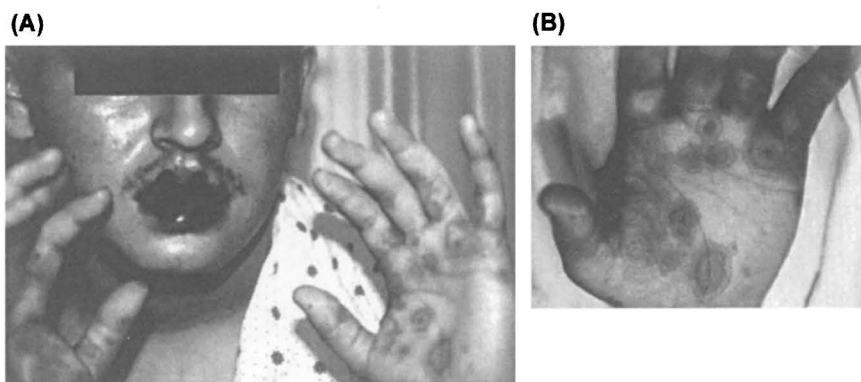


Figure 3 HSV 1 type lesions associated with erythema multiforme (EM). (A) Recurrent herpes labialis with EM. (B) Target lesions of EM of the palms.

Diagnosis for EM minor is usually clinical, although a biopsy may be preformed when in doubt. Pathologic findings include perivascular mononuclear infiltrate and epidermal necrosis. Treatment generally consists of antivirals and palliative care including cold compresses with saline, NSAID's, topical steroids and saline gargles. Recurrences are common, and antiviral therapy can be used to shorten the clinical course in individuals with recent history of HSV infection or for suppression therapy. As in other cutaneous manifestations of herpes simplex, the same antivirals (and their respective dosing regimen) used for the treatment and suppression of genital herpes can be used in patients with EM.

LOCALIZED CUTANEOUS HSV

HSV infections are commonly associated with the genitalia and oral mucosa; however, cutaneous manifestations of the disease can occur anywhere on the body in infected patients (Fig. 4). For example, patients with genital HSV-2 can have involvement of the buttock (34%), suprapubic area (15%) (Fig. 5) and thigh (7.5%) (Fig. 6) (15). HSV involvement of the buttock area tends to occur less frequently than genital lesions but it often lasts longer (16). Those individuals with HSV-1 genital infection have more frequent involvement of the hand and face rather than the trunk (Fig. 7) (16). It should be noted that the virus can occur without evidence of infection in the orolabial or genital region. Because the face, neck, shoulder and trunk region are possible HSV distribution sites, HSV infection can be misdiagnosed as herpes zoster infection, especially with the initial outbreak. However, recurrent episodes should aid clinicians in making the accurate diagnosis, as recurrence is more common with HSV infection than herpes zoster infection.

DISSEMINATED CUTANEOUS HSV

Individuals receiving immunosuppressive agents or those with HIV, leukemia, lymphoma, autoimmune diseases or transplants, are at an increased risk for recurrent cutaneous HSV disease. Depending on the degree of immunosuppression, patients may develop lesions ranging from recurrent herpetic vesicles, resembling those found in an immunocompetent host, to disseminated HSV infection. The latter is characterized by disseminated mucocutaneous vesicles, pustules and ulcerations (Fig. 8), which occur after reactivation of latent HSV infection. In addition, patients may also experience chronic herpetic ulcers that may enlarge and coalesce over time. Lesions can be found anywhere on the body including the face, trunk and genital region. Duration of the lesions can range from weeks to months, with bacterial or fungal superinfection of the lesions being common. Treatment of these lesions consists of antiviral therapy, and suppression therapy



Figure 4 Cutaneous HSV type 1 in a child.

can be employed to prevent recurrences. Acyclovir IV 5–10 mg/kg every eight hours for seven days can be used in children over the age of 12, and the dosage can be adjusted to 250 mg/m² every eight hours for seven days for children under the age of 12. However, as a result of frequent antiviral use in immunocompromised patients (such as those with HIV), resistance to these agents is increasing (17–19). Thus, Foscarnet IV 40 mg/kg every 8–10 hours for 10–21 days can be used as an alternative in these patients. Foscarnet, however, is associated with nephrotoxicity. Alternatively, cidofovir can be compounded (e.g., 1% cream) and can be safe and effective when used topically.

POSTOPERATIVE HSV-1 REACTIVATION

Since 60–90% of patients who undergo facial cosmetic procedures are seropositive for HSV-1, it is not uncommon for post-operative HSV-1 outbreaks

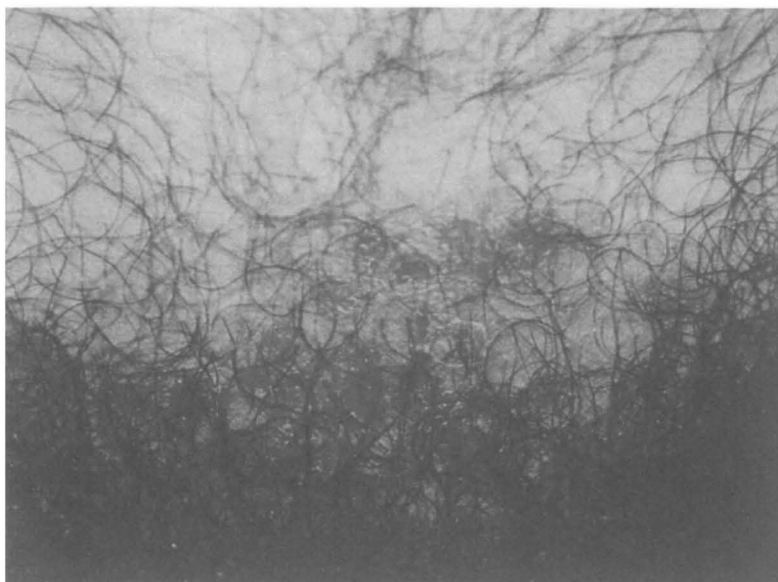


Figure 5 Primary herpes simplex in a man in the suprapubic region.

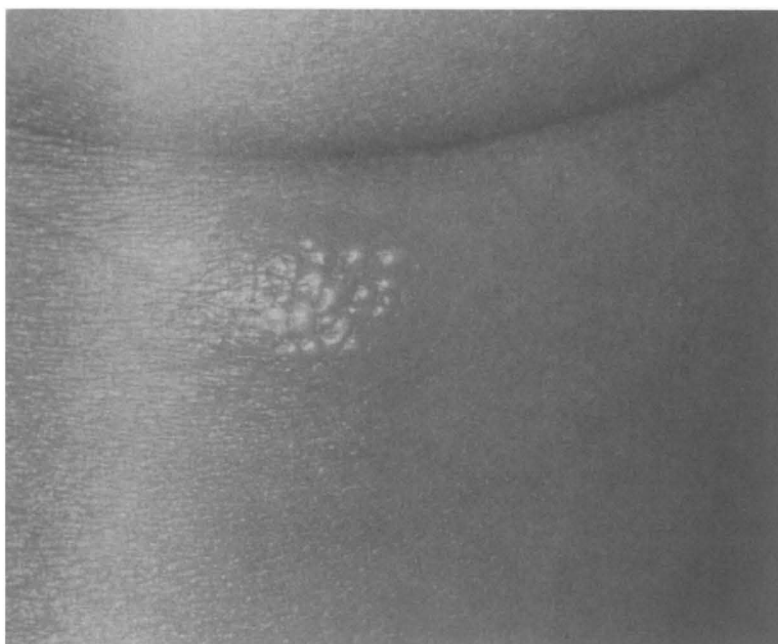


Figure 6 Recurrent herpes simplex of the upper thigh.

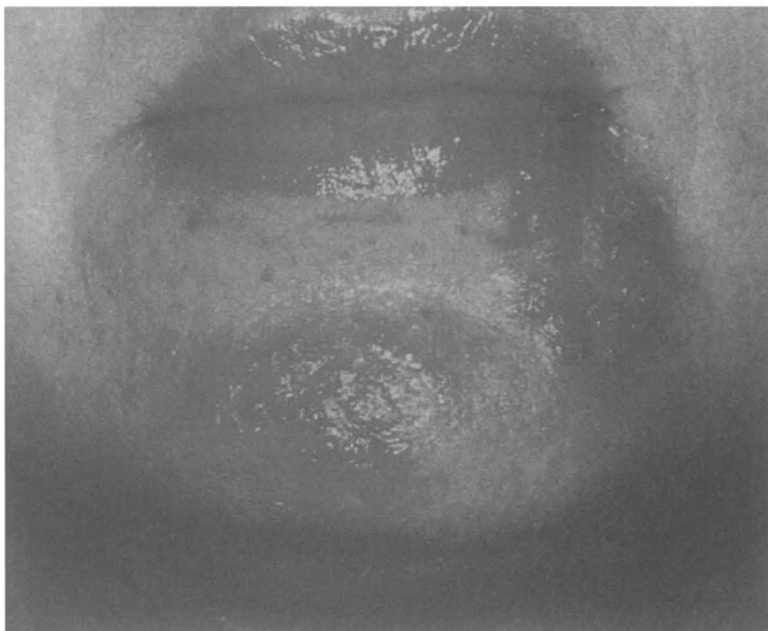
(A)**(B)**

Figure 7 HSV infection of the (A,B) faces and (C) ear. (*Continued*)

to occur. Reactivation and spread of the virus to adjacent areas can lead to increased postoperative pain, delayed healing and an increased risk for superimposed bacterial or fungal infections. Outbreaks can occur anywhere on the face as a result of reactivation of the virus from the sensory branches

(C)

**Figure 7(C)** (*Continued*)

of the trigeminal nerve. These outbreaks are not limited to facial cosmetic procedures, as they have been documented in variety of other procedures including trigeminal nerve decompression, corneal transplants and oral surgeries.

Some procedures like laser resurfacing, chemical peels and surgical removal of malignant lesions carry a higher risk of HSV-1 reactivation than other minimally invasive procedures (i.e., laser hair removal, microdermabrasion). However, recent studies have shown that prophylactic treatment with antivirals may help to decrease the frequency of postoperative HSV outbreaks (20,21). Because both valacyclovir (500 mg twice daily for 10–14 days) and famciclovir (250 mg or 500 mg twice daily for 10 days) have been shown to be equally effective, either can be started the morning of the surgery or 1–3 days prior to the surgery (22,23). Given the high percentage of individuals that are positive for HSV-1, antivirals are started in almost all individuals undergoing a high-risk facial cosmetic procedure. Those



Figure 8 Disseminated sacral HSV type 9 infection in an immunocompromised host.

undergoing minimally invasive procedures should be evaluated on a case-by-case basis to determine if prophylactic antiviral therapy is necessary.

CONCLUSIONS

Although herpes labialis and herpes genitalis are the more commonly known mucocutaneous manifestations of herpes simplex infection, one should be aware of other cutaneous manifestations of the disease. Manifestations of the virus can range from relatively benign conditions, such as herpes whitlow and folliculitis, to more severe and potentially fatal disease conditions like eczema herpeticum. Recognition of such diseases can be difficult, but knowledge of predisposing factors and medical conditions allows for more accurate diagnosis, treatment and prevention of outbreaks.

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Acute and Recurrent Genital Herpes Simplex Virus Infection

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INTRODUCTION

Herpes simplex viruses (HSV) infect a majority of the world population. Traditionally, the causative viral subtypes have been associated with different anatomical sites of infection. HSV-1 is usually associated with non-sexually acquired orolabial and extragenital conditions commonly encountered in childhood, while HSV-2 is generally viewed as the cause of sexually acquired genital infections seen in adults. This distinction is no longer valid because an increasing number of genital infections are now caused by HSV-1 (1).

Although it has formerly been regarded as a self-limiting, often trivial, condition, genital herpes is of increasing public health importance due to the physical and psychological morbidity associated with first episodes and frequent recurrences in adults, and serious, life-threatening neonatal disease resulting from vertical transmission (2–4). As the most common cause of genital ulceration, it is also a pre-eminent cofactor in the transmission of HIV infection (5). The annual health costs of incident genital HSV infections in the United States for 2000 were recently calculated at \$1.8 billion and the cumulative costs for the next 25 years have been estimated to amount to \$108 billion (6).

NATURAL HISTORY

The natural history of genital herpes is similar to that of other herpesvirus infections. Mucocutaneous exposure to the causative virus leads to infection that may be symptomatic or asymptomatic. During this initial infection, the virus is taken up by sensory neurones and transported to the dorsal root ganglia of the sacral nerves where a latent infection develops. Periodic reactivation results in axonal spread of the virus and initiates clinical or subclinical recurrent infection of epithelial cells in the anogenital dermatomes and shedding of virus. Severe, longer-lasting initial infections will result in a larger number of latently infected nerve ganglia and predispose to a pattern of more frequent recurrences. In many individuals, genital herpes is a chronic, recurrent, and lifelong condition.

EPIDEMIOLOGY

Causative Viral Types

Worldwide, HSV-2 remains the most commonly causative isolate from genital herpes lesions. Nevertheless, a rising trend of HSV-1 isolations from genital lesions in both sexes has been found in the United Kingdom during the past 20 years (7–11). In several recent studies, HSV-1 isolates have predominated in first episodes in women. In contrast, the proportion of HSV-1 isolates from recurrent genital herpes has generally been 20% or less.

A similar trend of increasing HSV-1 isolates from first episode genital herpes has also been observed elsewhere in Europe (12) and in Japan where HSV-1 was reported as the causal virus of genital herpes in 40% of women in Tokyo in 1976 (13). The increased frequency of primary genital HSV-1 infection in both the United Kingdom and Japan may help explain why HSV-1 is more commonly associated with neonatal herpes in these countries than has been reported in North America.

HSV-2 is as common as HSV-1 in clinical isolates in the extragenital regions, with the exception of the orofacial area in which HSV-2 is seldom detected (14).

In Sheffield, where the increasing prevalence of genital HSV-1 isolates was first reported in 1982 (15), viral typing using monoclonal antibodies was performed in a total of 605 consecutive women and 332 men presenting with culture-confirmed first episodes of genital herpes to a genitourinary medicine (GUM) clinic during the period 1990–1994 (16). The overall rates of HSV-1 isolations were 47% in females and 33% in males. HSV-1 infected individuals were more likely to be white and to have had no prior episodes of sexually transmitted infections (STI). HSV-1 isolates predominated among females aged less than 25 years and those without recent partner change. Although patients commonly reported recent orogenital sexual contact, active labial cold sores on their partners at the time of last sexual contact

were only reported in a minority of cases. Likewise in London, HSV-1 is far less commonly isolated from initial genital herpes lesions amongst blacks than in whites (17).

Recent anecdotal reports from North America have suggested both an increasing frequency of orogenital sex in young people and a rising proportion of genital herpes due to HSV-1 (18,19). HSV-1 remains the cause of an increasing number but still only a minority of cases. The proportion of HSV-1 isolates among initial genital herpes infections is higher in men who have sex with men and heterosexual women, and is associated with the white race and recent receptive oral sex.

Incidence

In developed countries, genital herpes has long been regarded as the most common initiating cause of genital ulceration, and is far more common than are syphilis, chancroid, and other sexually transmitted causes.

Between 1972 and 1999, the number of genital herpes diagnoses made at Genitourinary Med clinics in the United Kingdom (Fig. 1) increased 4 and 14-fold in males and females, respectively. This is reflected in the changing female to male ratio, from 0.4:1 in 1972 to 1.4:1 in 1999.

In the United States, where it is now estimated that over 50 million people are affected by genital herpes, the number of related physician visits has also multiplied in the past two decades and now exceeds half a million each year (20). From 1970 to 1985, the annual incidence of HSV-2 infection in HSV-2 seronegative persons increased by 82% to 8.4 (95% CI 7.7, 9.1) per 1000, and it was estimated that 1.64 million persons were being infected annually with HSV-2 (21).

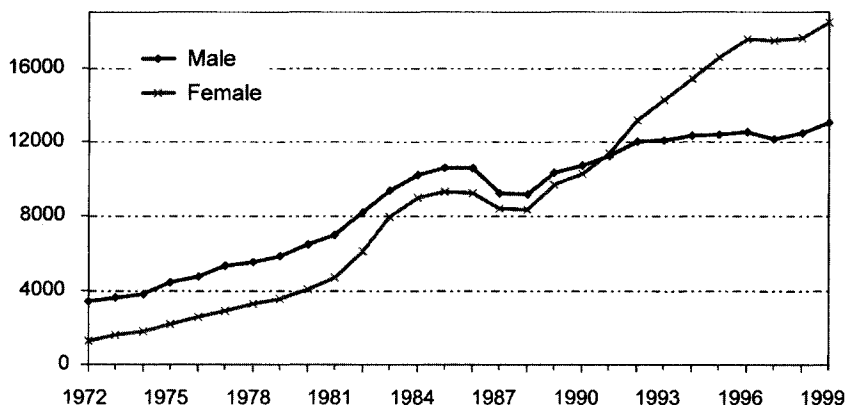


Figure 1 Diagnoses of genital herpes (first and recurrent episodes) in GUM clinics in England, Scotland, and Wales, 1972 to 1999.

Clinical diagnosis and conventional laboratory tests often give inaccurate indications of the etiology of genital ulcer disease. Recent studies in tropical and developing countries, which have used multiplex PCR diagnostic tests, now indicate that genital herpes has been underdiagnosed previously (22).

In a cross-sectional study in South Africa, HSV was identified as the most commonly identified infectious cause of genital ulcer disease found in 36% of patients and was significantly more common among HIV-positive as compared with HIV-negative men (23). Thus it now appears probable that genital herpes is the most common microbial cause of genital ulceration worldwide.

HSV Seroprevalence

The reported frequency of clinical presentations greatly underestimates the number of persons infected with both HSV-1 and HSV-2 (24).

Accurate type-specific serological tests can now reliably detect prior clinical and subclinical infections by either, or both, HSV types. The advent of these tests has led to better understanding of the epidemiology of genital herpes, its geographic variability, and have indicated that, while the prevalence of HSV-2 infections is strongly correlated with sexual behaviour, the most important correlate of HSV-1 seropositivity is increasing age (25).

HSV-1 Seroprevalence

It is generally the case in developing countries that HSV-1 seropositivity increases rapidly in childhood with a majority of individuals infected before adolescence. A recent serosurvey showed that 50% or more of the populations of Estonia, Morocco, India, Sri Lanka, and Brazil have become HSV-1 seropositive by the age of 10 years and in each of these countries 75% HSV-1 seropositivity was reached by those in their mid-teens (26).

This contrasts with the findings in developed countries where 50% HSV-1 seropositivity among women is not reached until around the age of 30 (27–30).

An age-stratified survey of HSV-1 and HSV-2 in the general population of England and Wales during 1994–1995, using type-specific monoclonal antibody ELISAs, showed that HSV-1 antibody was detected in 51% sera from infants under one year, usually from passively transferred maternal antibodies, and 23% of children aged 10–14 years (31). The latter had shown a significant decline since 1986–1987 when the HSV-1 seroprevalence in preadolescent children was 34%. Among adults, the HSV-1 seropositivity increased more sharply in teenage girls than boys, reaching 54% in women aged 25–30 years.

Studies of STD clinic attenders in the UK have recently shown that HSV-1 prevalence was linked with early age of first sexual intercourse, and may reflect an increase in orogenital sexual practices in teenagers (32).

In Sweden, similar results have been found. A longitudinal cohort study of 839 schoolgirls carried out between 1972–1987 showed that at

the age of 15–16 years, only 23% were HSV-1 seropositive, rising to 36% at the age of 19–20 years and 50% at the age 30–31 (33).

These results suggest that in both the United Kingdom and Sweden, less than one-quarter of adolescents have prior exposure to HSV and therefore have no acquired immunity to genital infection with either viral type. There is rapidly increasing HSV-1 seroprevalence rates between the ages of 15–30, the annual rate of increase being higher than for HSV-2. It seems likely that in the years of highest sexual activity, HSV-1 may be as likely to be acquired on the genitals as on the mouth and lips. Even after the age of 30, half the adult population in these countries remains susceptible to primary HSV infection.

HSV-2 Seroprevalence

Population-based surveys of adults in the United States have shown that the overall seroprevalence of HSV-2 in persons aged 12 years or over had risen by one third during the eighties from 16.4% in 1978 to 21.9% in 1990 (20). High seroprevalences are also found in African populations (34,35). In general, lower HSV-2 seroprevalences are seen in Western Europe (typically 5% to 10% prevalence in various populations studied in England, Scandinavia, Spain, and Italy), and are lowest in Asia.

However, a large population-based sample in Switzerland (36) has recently shown seropositivities of 18.9% for HSV-2 and 80.9% for HSV-1, a similar pattern to that in the United States, and a similar study in France showed comparable seroprevalence rates of 17.2% and 67%, respectively (37).

In the Netherlands, where 73% of neonatal herpes infections are caused by HSV-1, the HSV-2 seroprevalence in pregnant women varied between 11% and 35% and was related to the ethnic makeup of the population studied (38), being highest in those with the largest proportion of black women.

Because HSV-2 infection is sexually acquired, the major risk factor for HSV-2 seropositivity is the lifetime number of sexual partners. The seroprevalence is higher in men who have sex with men, in prostitutes, and those of lower socio-economic status. It increases with rising age. Within each age group, rates are higher in females, reflecting the increased efficiency of male to female transmission. The higher rates are also seen in black and Hispanic populations but whether this reflects differing racial susceptibilities to infection as well as patterns of sexual behavior is not yet clear. However, despite these higher seroprevalence rates, clinical episodes are less often reported in blacks than whites.

Seroconversion Studies

In Sweden, seroconversion to HSV-2 occurred in 22% of the population between the ages of 15–30 (33). A study of first-year students at an American university showed a very low seroprevalence rate of 0.4% during the students' first year, which had risen to 4.3% by the end of their third year (39).

Brown et al. (40) studied the acquisition of HSV among 7046 pregnant women in the United States. Of those women initially seronegative to both HSV-1 and HSV-2, the estimated risk of seroconversion to HSV-1 was 2.3% and to HSV-2 was 1.4%. Of those initially seropositive to HSV-1, 1.7% seroconverted to HSV-2 but there were no seroconversions to HSV-1 among those initially seropositive for HSV-2 alone. Symptomatic seroconversion was seen in a minority of cases, approximately one-third for each virus type. In those women with symptomatic seroconversion to HSV-1, 75% had genital lesions and only 25% had oropharyngeal lesions. Neonatal HSV-1 infection occurred in both infants born to two women acquiring primary genital HSV-1 at term as compared with two of seven infants born to women acquiring non-primary genital HSV-2 near term. This emphasizes the importance of preventing both genital HSV-1 and HSV-2 acquisitions in pregnant women near term.

Transmission

HSV-2 infection in adults is almost exclusively transmitted by sexual intercourse, while the predominant mode of HSV-1 genital infection is presumed to be orogenital transmission.

These epidemiological correlates have recently been confirmed in STD clinic populations in Denver, Colorado, U.S.A. (41). It was noteworthy that 85% of HSV-2 seropositive persons had never received a diagnosis of genital herpes. It is likely that this high rate of undiagnosed HSV-2 infection contributes to ongoing transmission.

Asymptomatic Shedding

Most sexual partners and neonates become infected during episodes of asymptomatic shedding from the source contact (42,43).

This is defined as presence of HSV on a mucocutaneous surface at a time where the patient does not experience genital symptoms nor reports genital lesions. Some may have unrecognized lesions of an area difficult-to-visualize or have microscopic external lesions, or there may be no detectable lesions.

Almost all HSV-2 seropositive patients have periods of asymptomatic shedding. It occurred in over 80% of 27 women with RGH followed for more than 50 days (44). Daily viral cultures indicated that HSV-2 was shed asymptotically on 1–2% of days sampled. The majority of episodes of shedding were of short duration, lasting for about 1 day. PCR is approximately three times more sensitive than culture in detecting asymptomatic shedding.

In women, shedding may occur from one or more of the external genital skin, the perianal area, or cervix. It shows a tendency to cluster just before and just after a clinical recurrence, and is more frequent in the recently infected (45).

In men, comparable results have been found (46). Over 80% of men studied shed virus on at least one occasion and the subclinical shedding rate was 2.2%. Shedding may occur from the penile skin that is normal in appearance, from the urethra, and can also be found in urine and semen.

Asymptomatic shedding is twice as likely within one year of acquisition of HSV than it is subsequently, is three times more common in those infected with HSV-2 than with HSV-1, and is more common in those who have a greater frequency of clinical recurrences. It can also occur in those who are apparently asymptotically infected with HSV-2 (47). There does not appear to be any association with age or with menstruation.

Effect of HSV-1 on HSV-2 Acquisition

It has long been believed that previous HSV-1 infection reduces the likelihood of acquiring clinical HSV-2 infection if exposed. There is also evidence that it may also reduce the rate of subclinical acquisition. In one study, the annual rate of seroconversion among discordant couples was more than three times higher in susceptible female partners who had no previous exposure to either HSV-1 or HSV-2 compared to those HSV-2 susceptible female partners who had pre-existing HSV-1 antibodies (43).

Recent large vaccine studies have also provided further opportunity to study transmission in large groups of patients in serodiscordant partnerships.

Spruance and his coworkers (48) followed 1171 persons at risk of acquiring HSV-2 infection within monogamous relationships. Over a period of 19 months, HSV-2 infection was acquired by 39 (14.4%) of 271 partners who were seronegative to both HSV-1 and HSV-2, but by only 39 (5.9%) of 678 persons who had prior infection with HSV-1 ($P = 0.001$). Those with prior HSV-1 infection also were more likely to have asymptomatic HSV-2 infection. The investigators concluded that prior HSV-1 infection not only ameliorates HSV-2 infection but also reduces the likelihood of HSV-2 acquisition.

Effect of HSV-2 upon HIV Acquisition

It has been postulated that genital HSV-2 infection may have more influence on HIV incidence worldwide than any other STD (49–51).

A meta-analysis of 18 cross-sectional and case-control studies collectively showed HSV-2 infection, determined by type-specific serology, to be independently associated with HIV infection; the odds ratio (OR) was 4.2, with a 95% confidence interval (CI₉₅) of 3.1–5.8. In 9 prospective cohort and nested case-control studies, prior infection with HSV-2 doubled the incidence of subsequent HIV infection (OR = 2.1; CI₉₅ = 1.3–3.2) (52).

In the Rakai district of Uganda, the risk of HIV transmission was analyzed in 174 HIV-discordant heterosexual monogamous couples who were followed with serial HIV serologies over a 2- to 3-year period (53). HSV-2 antibody in the HIV-uninfected partners was a substantially more potent predictor of HIV acquisition than was the HIV-positive partner's HIV viral

load, an association that was independent of the occurrence of symptomatic genital ulcer disease.

The association between HSV-2 infection and HIV acquisition has also been studied in men who have sex with men (MSM). Celum et al. (54) analyzed the association of incident HIV infection with prior HSV-2 infection in two large longitudinal cohort studies. Using a nested case-control design, the investigators compared 116 MSM who seroconverted to HIV with 342 who remained HIV-seronegative over 18 months of follow-up. By multivariate analysis controlling for demographic characteristics, sexual practices (e.g., unprotected anal sex), number of sex partners, health insurance status, bacterial STDs, and several other factors, serologic evidence of HSV-2 infection was one of several independent predictors of HIV acquisition (OR 1.8, 95% CI 1.1–3.0).

These studies highlight the importance that HIV prevention strategies should encompass genital herpes diagnosis, treatment, and prevention to limit the spread of HIV.

CLINICAL FEATURES

Spectrum

The majority of infected persons are unaware that they have acquired genital HSV infection and over 50% of transmissions occur without symptoms. Of HSV-2 seropositive persons, only about 20–25% have been diagnosed with genital herpes, about 50% have undiagnosed but clinically detectable disease, and 25% are truly asymptomatic (55–57).

The incubation period between transmission and symptom onset is generally short and between 1 and 7 days. Some authors claim longer incubation periods although these longer time between last sexual exposure and the appearance of lesions may reflect cases that are reactivations of subclinical disease rather than newly acquired infections.

Both HSV-1 and HSV-2 cause clinically indistinguishable genital lesions.

Based on the patient history, clinical episodes can be subdivided into first and recurrent episodes.

First Episode Genital Herpes

Classification

The first episode may be:

1. True primary infection with either HSV-1 or HSV-2 in a patient with no prior serological evidence of infection by either
 - a. non-primary first episode where there is typically prior evidence of infection by HSV-1 in a patient with a *first episode* HSV-2 infection, or

- b. first symptomatic episodes of recurrent genital herpes where there is typically serological evidence of prior infection by the causative viral isolate.

Primary Infection

Primary infection is predisposed by inflamed or damaged genital epithelial surfaces such as can be caused by infections, such as candidosis or bacterial vaginosis, trauma, or genital dermatoses.

Constitutional symptoms, consisting of headache, malaise, and myalgia, often precede the onset of genital symptoms.

Local itching and soreness are quickly followed by pain at the site of lesions and inflamed inguinal lymph nodes, usually bilateral. Many individuals also experience accompanying neuralgic pain or tingling paraesthesiae in the sacral dermatomes. The symptoms of first episodes tend to be worse in women, who often have more severe constitutional symptoms and dysuria. Excessive mucopurulent vaginal discharge occurs when there is associated herpetic cervicitis.

Lesions are typically bilateral and extensive, in contrast to recurrent infection where unilateral, localised lesions are more usual. Papular and vesicular lesions appear in the affected erythematous mucocutaneous surfaces, which rapidly ulcerate and often coalesce. Ulceration is associated with increased pain, especially in contact with urine. New crops of lesions can appear during the first 10 days of the illness. Thus papules, vesicles, and ulcers may coexist. On the external genital skin, crusting occurs prior to reepithelialization.

Lesions in males are most common on the penis, especially the glans and undersurface of the prepuce in uncircumcised men. Proctitis on homosexual men presents with perianal pain and rectal discharge.

Lesions in women are more widespread and affect the labia majora, the labia minora and introitus, the cervix, the perineum and perianal areas, and buttocks.

In untreated episodes, viral shedding continues for a median duration of 11 days, and the total duration of the illness may last for three to four weeks.

Acute Complications

Local acute complications include those of secondary bacterial or yeast infection of lesions, swelling and fibrinous adhesions causing phimosis in males or labial adhesions in females. Sacral radiculomyelopathy not only causes paraesthesiae in the lower limbs but also urinary retention, constipation, and impotence. This may take up to six weeks to resolve, long after the genital lesions have healed.

Extragenital complications include the risk of autoinoculation to adjacent epithelial surfaces, the fingers, oropharynx, and eyes.

Meningitis symptoms are common and both encephalitis and transverse myelitis are occasionally seen.

In neonates, pregnant women, and immunocompromised individuals, generalised systemic herpes infections with fulminant hepatitis may sometimes occur (58–60). These can be life-threatening.

However, for most individuals, primary genital herpes is an unpleasant illness causing incapacity and time off work for two to three weeks. Most patients can be managed as outpatients although hospital admission is required for those with urinary retention, neurological and systemic complications, and those with accompanying illness, such as diabetes where ketoacidosis may be precipitated.

Non-Primary Infection

In those with pre-existing infection by HSV-1, the illness is often modified and is usually less severe with fewer constitutional and local symptoms, a shorter duration of viral shedding, less extensive ulceration, and reduced new lesion formation. The total duration of the illness is correspondingly shorter than in those with true primary infections.

Superinfection with different HSV types in the same anatomic region can occur, albeit uncommonly (61,62). In one study among patients who first acquired genital HSV-1 infection, the subsequent acquisition of HSV-2 presented as a prolonged episode of genital lesions and a marked increase in frequency of subsequent recurrences from which both HSV-1 and HSV-2 could be isolated (63).

Recurrences

The clinical manifestations of first symptomatic episodes of recurrent infection like those of subsequent outbreaks are typically less severe, lesions are usually unilateral and localised. They may be accompanied by ipsilateral tender inguinal lymphadenitis.

After replication at the sites of initial inoculation, HSV establishes lifelong latent infections in the sensory and autonomic ganglia serving those sites. Periodically, the virus reactivates from these neurones and travels centripetally along the axon to cause recurrent epithelial infection. HSV-1 reactivates more efficiently from the trigeminal ganglia, while HSV-2 reactivates primarily from sacral ganglia.

Thus, the natural histories of genital HSV-1 and HSV-2 infections differ in their rates of symptomatic recurrence and asymptomatic shedding (64–66).

In a study of 39 adults with concurrent primary oropharyngeal and genital herpes, those with HSV-1 infections were six times more likely to develop orolabial recurrences than genital recurrences, and those with

HSV-2 were 400 times more likely to experience genital rather than oral recurrences (67). The mean monthly genital recurrence rates were 0.02 and 0.33 for HSV-1 and HSV-2, respectively.

In another longitudinal cohort study, HSV-2 genital infections were twice as likely to recur and recurrences were 8 to 10 times more frequent (68). By 180 days after resolution, 80% of HSV-2 infected individuals had experienced a symptomatic recurrence as compared with 40% of those with HSV-1. Asymptomatic shedding of HSV-1 occurred in a minority and was of shorter duration than in those infected with HSV-2.

In many individuals, there are characteristic provoking factors for reactivation of genital herpes. These may include physical trauma, such as that which results from sexual intercourse, exposure to UV light, menstruation, intercurrent physical illness or infection, and psychological stress.

Prodromal symptoms may precede the appearance of lesions by up to 48 hours. These are less commonly systemic, with fever and malaise, than local. Sacral neuralgia, manifested as low back, perineal, or sciatica-type pain in the legs, may be disabling. More typically, pain or itching in the area where lesions subsequently occur precedes the appearance of lesions.

The occurrence of prodrome can often be helpful to patients because it not only allows earlier treatment with appropriate episodic antiviral agents, but also can promote behavior modification that will reduce transmission to uninfected partners.

Not all prodromal episodes are followed by the appearance of clinical lesions but even aborted prodromes are likely to be associated with viral shedding.

Recurrent lesions have a typical evolution and resolution. There is initial erythema, followed by swelling, the appearance of vesicles, ulceration, scabbing, and then reepithelialization that indicates complete healing.

There may be one or more favored sites of recurrence for each individual located anywhere in the anogenital region, the natal cleft, or on the buttocks.

Atypical Lesions

It is also now clear that in many individuals atypical recurrences occur such that symptoms are easily mistaken by both patient and physician for other infective conditions caused by genital candidiasis, tinea cruris, folliculitis, urinary tract infection by hemorrhoids, skin sensitivities caused by condoms, spermicides, or clothing, trauma, and insect bites. Physical signs may be no less easy to recognize even by experienced clinicians and lesions may appear as perifollicular erythema and/or papules, skin fissures and tiny superficial erosions.

A high index of suspicion is necessary and diagnostic tests for HSV infection are indicated in a wide variety of presentations of minor genital conditions, especially where there is a history of preceding episodes.

Complications

While severity of recurrent genital herpes can be measured objectively in terms of the numbers of days on which a patient is affected by clinical lesions, their size, and/or the frequency of recurrences, the severity is often a matter of individual patient perception.

The most common complications of recurrences are psychological and emotional especially within, but not limited to, those with the most frequent recurrences.

Physical complications are uncommon although recurrent skin problems such as erythema multiforme and urticaria (69) have been precipitated by recurrent herpes episodes, and can be relieved by appropriate suppressive therapy.

Genital Herpes in Pregnancy

The range of clinical manifestations of genital herpes in pregnancy is similar to those in the non-pregnant woman and range from asymptomatic acquisition to florid primary genital herpes with occasional cases of life-threatening systemic infection. The greatest risk of vertical transmission occurs with infections acquired during the final trimester because, even after complete lesion healing, there is a 30–50% risk of asymptomatic shedding at term, and because there is insufficient time for the development of a complete maternal serological response that will provide the fetus with protective levels of passively transferred antibodies.

Genital Herpes in Immunocompromised Individuals

In severely immunocompromised patients, genital herpes may cause extensive giant indolent or necrotic lesions of the anogenital region. Systemic manifestations causing visceral disease are also more common.

Differential Diagnosis

The differential diagnosis of genital herpes (Table 1) includes a wide variety of sexually transmitted and other infective causes of genital ulceration, as well as a number of non-infective, dermatological causes.

Infective causes of genital ulceration may occur together, hence comprehensive investigation and follow-up of genital ulceration for syphilis and chancroid should always be considered. Moreover, patients with confirmed genital herpes should always be investigated for concurrent sexually transmitted infections, such as chlamydia and gonorrhoea. However, the timing of such testing will need to be determined by the severity of lesions, and it is often a kindness to the patient to defer vaginal examination until the lesions are resolving.

Table 1 Differential Diagnosis of Genital Herpes

Infective
Syphilis
Chancroid
Lymphogranuloma venereum
Granuloma inguinale
Herpes zoster
Pyogenic lesions, e.g., folliculitis
Erosive balanitis and vulvitis
Genital candidosis
Genital infestations, e.g., scabies, pediculosis pubis
Non-infective
Traumatic lesions
Dermatoses
Aphthous ulceration
Psoriasis
Lichen planus
Eczema
Intertrigo
Lichen sclerosis et atrophicus
Malignant
Intraepithelial neoplasia
Carcinoma
Other
Crohn's diseases of the vulva

The frequent atypical nature of genital herpes suggests that it should be considered in the differential diagnosis of any clinical presentation involving erythematous, vesicular, or ulcerative lesions of the anogenital region.

DIAGNOSIS

The clinical diagnosis of genital herpes, even by experienced clinicians, may be unreliable because of the high frequency of atypical cases. Accurate diagnosis is important for correct management and patient counselling.

In practice, the diagnosis can be made either by detection of HSV in clinical lesions or by serological methods.

Detection of HSV

Until recently, virus culture and typing has been the “gold standard” method. The quality of samples is critical and specimens should be collected using swabs directly from the base of the lesion. HSV is a labile virus and successful virus culture depends on maintaining the cold chain (4°C), rapidly transporting specimens to the laboratory, and avoiding freeze thaw cycles.

Antigen detection, by EIA or immunofluorescence methods, is less sensitive (70,71), but can be valuable particularly for samples taken late in a clinical episode or where there are not ideal circumstances for specimen transport and diagnostic laboratories are less accessible.

In recent studies, molecular diagnostic tests have been shown to be more sensitive and are becoming the new gold standard for tests of viral shedding. The amplified product may also be typed without further tests. In comparison with the LightCycler PCR, tissue culture detected 78% and HSV EIA antigen detection 56% of HSV in clinical specimens (72). In another study, the use of PCR increased sensitivity by 13% for vesicular lesions, by 27% for ulcerative lesions, and 20% for crusted lesions as compared with tissue culture (73).

Type-Specific Serological Tests

Until recently, most available commercial tests for HSV antibodies (for example, CFT and many EIAs) were not type specific and had no value in the management of genital HSV.

However, type-specific commercial assays have now become commercially available and are either EIAs based on glycoprotein G (gG1, gG2) or western blot (74,75).

Focus Technologies produces HerpeSelect-1 and HerpeSelect-2 enzyme-linked immunosorbent assay tests and the HSV-1 and HSV-2 HerpeSelect 1/2 immunoblot. Diagnology has marketed POCKit-HSV-2, a point-of-care test for HSV-2, which allows a fingerprick blood sample to be tested in clinic (76). This test relies on subjective visual interpretation of the result, and staff require adequate training as disagreement between readers has been found in 5–10% of tests (77).

The tests may be useful to confirm a genital herpes diagnosis, establish HSV infection with atypical complaints, identify asymptomatic carriers, and identify those at risk of acquiring HSV.

Full type-specific immune responses can take 8–12 weeks to develop following primary infection. Serological assessment of genital HSV in the United Kingdom requires access to both HSV-1 and HSV-2 type specific antibody assays because of the high proportion of genital herpes caused by HSV-1 infection (78).

The value of screening all genitourinary medicine clinic attenders or antenatal patients for HSV antibodies has not been established. The possibility of false positive test results should be remembered. A test with a sensitivity of 97% and specificity of 96% has positive and negative predictive values of 97% and 96%, respectively, when used in a study population where the prevalence of HSV-2 is 50% (for example, a STD clinic patients presenting with genital ulcers). However, in a population with a HSV-2 prevalence of 5% (for example, the general population of some European countries),

Table 2 Comparison of Serologic Tests for HSV-1 and HSV-2

Test type	Sensitivity	Specificity	Time to results	Earliest seroconversion
Western blot	100%	100%	2 weeks	2–7 weeks
Focus HSV-2 ELISA	96%	97%	1 week	3 weeks
POCKit HSV-2 Rapid test	96%	98%	6 minutes	2 weeks

while the negative predictive value is almost 100%, the positive predictive value declines to 63%.

MANAGEMENT

First Episode Genital Herpes

Antiviral Treatment

The management of genital herpes was revolutionized by the advent of nucleoside analogue treatment with aciclovir during the early 80s (79,80). Subsequently, the prodrugs valaciclovir and famciclovir became available and offer easier, less frequent dosing than required for aciclovir.

Patients presenting within five days of the start of the episode or while new lesions are still forming should be given oral antiviral drugs. Aciclovir, valaciclovir, and famciclovir are all effective in reducing the severity and duration of episodes (81–83).

The recommended regimens, all for five days are as follows:

- aciclovir 200 mg five times daily,
- valaciclovir 500 mg twice daily,
- valaciclovir 1000 mg twice daily,
- famciclovir 250 mg three times daily.

Although 10-day courses of treatment are recommended in North America, there have been no direct comparisons between 5- and 10-day courses of treatment and thus no evidence for benefit from the longer courses.

Topical agents are less effective than oral agents (84,85). There is no evidence to support the combined use of oral and topical treatment (86). The only indication for the use of intravenous therapy is when the patient is unable to swallow or tolerate oral medication because of vomiting.

At present, there is no evidence to suggest that any antiviral therapy alters the natural history of the disease.

Symptomatic Treatment

In first episodes, there are often severe constitutional symptoms and local pain.

Regular systemic analgesia is essential and must be of sufficient frequency and potency to control patient symptoms. In some cases, short-term opiate analgesia is required. Some doctors advocate the short-term use of topical anesthetic agents, e.g., 5% lignocaine gel applied to the affected area prior to urination or defecation, during early treatment. It does not achieve universal clinician support because of concerns over the risk of causing skin sensitization.

Although secondary bacterial infection may occur, this can usually be kept in check by frequent saline baths. Antibiotics have little place in routine management and do not usually enhance time to healing (87). Nevertheless, oral antifungal agents may have a place in reducing the discomfort associated with concurrent candidiasis.

Treatment of Complications

Inpatient admission is usually offered to those with severe constitutional symptoms or extensive lesions, especially if there is vomiting, or if there are deficient opportunities for rest and support at home. Those with local complications, such as incipient urinary retention, or systemic features such as meningism are also better observed and cared for within hospital. Urinary retention is far more common in women than in men. Control of pain is the most important adjunct to overcoming urinary retention and the need for catheterization. Some patients find it easier to void while taking salt baths. If catheterization is required, suprapubic catheterization is preferred. Once a urinary catheter is inserted, it will often be five days or more before this can be removed, which may prolong inpatient admission or expose the patient to the risk of secondary urinary infection.

Most patients can be discharged to home within 48 to 72 hours, but it is often recommended that they not return to work until their illness is resolving, which may take a further 7 to 14 days.

Counselling

Counselling of patients with first episode genital herpes should include a discussion of the following topics:

- possible source(s) of infection,
- natural history of genital herpes including the risk of subclinical viral shedding,
- future treatment options,
- risk of transmission by sexual and other means,
- risks of transmission to the fetus during pregnancy and the advisability of the obstetrician/midwife being informed,

sequelae of infected men infecting their uninfected partners during pregnancy,
the possibility of partner notification.

Recurrent Genital Herpes

Genital herpes recurrences are self-limiting and generally cause minor symptoms, so that many individuals require no more than supportive therapy. However, some individuals can be severely affected, especially when recurrences are frequent, are longer lasting, or disrupt their relationships. Decisions about how best to manage clinical recurrences should be made in partnership with the patient.

Management strategies include supportive therapy only, episodic antiviral treatments, and suppressive antiviral therapy and may vary over time according to individual patient circumstances.

Supportive Therapy

This includes saline bathing or covering the lesions with petroleum jelly and simple oral analgesics.

Episodic Antiviral Treatment

Ideally, treatment should be patient-initiated as early as possible after the onset of symptoms. Oral antiviral agents are effective at reducing the duration and severity of recurrent genital herpes in the following regimens: (88–90)

aciclovir 200 mg five times daily,
aciclovir 400 mg three times daily,
aciclovir 800 mg twice daily,
valaciclovir 500 mg twice daily,
valaciclovir 1000 mg once daily,
famciclovir 125 mg twice daily for five days.

The reduction in episode duration is a median of one to two days for most patients. Both famciclovir and valaciclovir have a twice-daily dosing regimen that is easier to take than five times daily dosing. Valaciclovir is no more or less effective than aciclovir. Famciclovir has not been compared with either aciclovir or valaciclovir.

More recently, multicenter, randomized, controlled trials have shown that episodic treatment of recurrent genital herpes with a 3-day course of valaciclovir, 500 mg orally twice daily, is as effective as a 5-day course, as measured by the frequency of aborted lesions, time to resolution of pain, and time to lesion healing. It is recommended that therapy should be extended to five days if lesions persist or if new lesions continue to form (91). Initiation of treatment within six hours of episode onset can also significantly increase the proportion of aborted episodes (92).

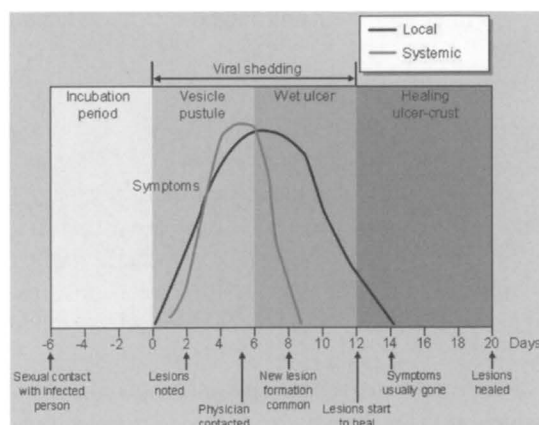


Figure 2 The clinical course of primary genital HSV infection.

Shorter courses of oral aciclovir have also been shown to be effective. In a randomized, double-blind, placebo-controlled trial, oral aciclovir administered as 800 mg three times daily for two days significantly reduced the duration of lesions (median duration four days vs. six days) and viral shedding (25 hours vs. 59 hours) as compared with placebo. It also increased the proportion of aborted episodes (93).

Topical 5% aciclovir cream, applied five times daily for five days, has also been used to treat recurrent genital herpes but is less effective than oral treatment (94).

Suppressive Antiviral Therapy

Experience with suppressive antiviral therapy is most extensive with aciclovir (95,96), which has been shown to be well tolerated in patients taking continuous medication for over 11 years. The drug is also approved for use in children. Extensive sensitivity monitoring of HSV isolates has shown a very low rate of aciclovir resistance among immunocompetent subjects (< 0.5%) and this remains low among immunocompromised individuals at around 5%. Valaciclovir enhances aciclovir bioavailability. It can achieve effective suppression when taken once daily (97).

All trials of suppressive therapy have been done in patients with a recurrence rate equivalent to more than six episodes per annum who are highly likely to experience a substantial reduction in recurrence frequency on suppressive antiviral therapy. However, it is likely that patients with a lower rate of recurrence will also reduce their rate of recurrence with treatment. The frequency of recurrence at which it is worth starting suppressive therapy is a subjective one. A balance between the frequency of recurrence against the cost and inconvenience of treatment should be sought for each individual.

Effective suppressive regimens are as follows:

- aciclovir 200 mg four times daily,
- aciclovir 400 mg twice daily,
- valaciclovir 250 mg twice daily,
- valaciclovir 500 mg once daily,
- famciclovir 250 mg twice daily.

The optimal daily dose of suppressive aciclovir therapy is 800 mg. Although a dose of 200 mg four times daily was shown to be clinically superior to 400 mg twice daily, the ability to comply with a four times daily regimen should determine prescribing decisions for individual patients. Once daily aciclovir does not suppress genital herpes recurrences.

Twice daily valaciclovir (250 mg twice daily) has been shown to be as effective as twice daily aciclovir (400 mg twice daily). Some patients with less frequent recurrences (<10 per annum) may be adequately suppressed on 500 mg once daily valaciclovir. Suppressive therapy using famciclovir (250 mg twice daily) has only been compared against placebo and not against aciclovir (98).

Therapy should be discontinued after a maximum of one year to reassess recurrence frequency. Twenty percent of patients will experience a reduction in recurrence frequency compared with pre-suppression symptomatic levels. The minimum period of assessment should include two recurrences. It is safe and reasonable to restart treatment in patients who continue to have unacceptably high rates of recurrence.

Short courses of suppressive therapy to prevent clinical symptoms may also be helpful for some patients to cover stressful life events.

Counseling Patients with Recurrent Genital Herpes

The CDC treatment guidelines in 2002 (99) offer some key counseling points for discussion with newly diagnosed patients. They are:

1. Patients should be educated about the natural history of the disease, i.e., potential for recurrences, asymptomatic shedding, and risk of sexual transmission.
2. Patients experiencing an outbreak should be advised that suppressive and episodic antiviral therapy are available and effective at preventing or shortening the duration of an episode.
3. All patients with genital herpes should be encouraged to tell their current and future partners.
4. Transmission can occur during asymptomatic shedding, and asymptomatic shedding of HSV-2 is more frequent during the first year after acquisition.
5. Latex condoms can reduce the risk of transmission if used consistently and correctly.

6. Sexual partners of persons with genital herpes should be advised that they might be infected even if they have no signs or symptoms.
7. The risk of neonatal herpes should be explained to both partners. Pregnant women with genital herpes should inform their doctors. Pregnant women who are not infected should avoid intercourse with HSV-infected partners during the third trimester and pregnant women not infected with HSV-1 should avoid oral sex in the third trimester.
8. Asymptomatic patients should receive the same counselling as those who are symptomatic.

Partner Notification

Although there is no definitive evidence that either antiviral treatment or patient education/counselling alters transmission rates of HSV at a population level, it seems logical to increase awareness of the diagnosis in partners when appropriate, with the aim of preventing further onward transmission. Asymptomatic shedding plays a major role in the transmission of HSV infection and partner notification is an effective way of detecting individuals with unrecognized disease. It has been shown that 50% of asymptomatic HSV-2 seropositive women can be taught to recognize genital herpes recurrences after counselling (100). Thus, there is at least the potential for prevention of transmission by educating patients to recognize symptomatic recurrences.

Despite the lack of evidence on which to base recommendations for partner notification, on an individual basis it may be appropriate to offer to see partners to help with the counselling process.

Management of Herpes in Pregnancy

The risk of transmission of HSV-2 from an infected mother to a neonate is between 30% and 50% in women who acquire herpes around the time of delivery, but the risk is low (c.1%) in women with recurrent genital herpes (101,102). Even so, since genital herpes is so common, the proportion of neonatal herpes cases that are acquired from HSV-2-seropositive women with recurrent disease remains high. For this reason, the new U.S. guidelines recommend counselling the mother to try to prevent acquisition of herpes during the third trimester and careful examination of the patient for presence of lesions at delivery (103).

The recommendations suggest that all patients should be questioned in detail regarding the presence of lesions, although only 50% of women who give a history suggestive of genital herpes will actually have herpes. Thus, the only way to be certain of the patient's status is to perform a type-specific serologic test on the patient before delivery, preferably in the first trimester, so that prevention strategies can be discussed.

Physicians vary in their method of management of patients who do acquire herpes around the time of delivery. Some recommend aciclovir (400 mg twice daily) to try to prevent transmission, some recommend cesarean section, and others recommend both (104–106). Clinicians should discuss the various options with the patient and her partner.

Management of Pregnant Women with First Episode Genital Herpes

When first episode genital herpes is acquired during the first and second trimesters, management of the woman should be in line with her clinical condition and will often involve the use of either oral or intravenous aciclovir in standard doses. Providing that delivery does not ensue, the pregnancy should be managed expectantly and vaginal delivery anticipated. Some clinicians advocate continuous aciclovir suppression in the last four weeks of pregnancy to prevent recurrence at term and hence the need for delivery by cesarean section. This is an unlicensed use of the drug so the risks and benefits need to be carefully discussed with each patient.

If genital herpes is acquired during the final trimester, cesarean section delivery should be considered for all women, particularly those developing symptoms within six weeks of delivery, as the risk of viral shedding in labor is very high. If vaginal delivery is unavoidable, aciclovir treatment of mother and baby may be indicated (107).

Management of Pregnant Women with Recurrent Genital Herpes

Recurrent genital herpes in pregnancy usually has no adverse effect upon the pregnancy or the delivery. Even symptomatic recurrences of genital herpes during the third trimester are usually brief and vaginal delivery is appropriate if no lesions are present. Studies has shown that sequential cultures during late gestation to predict viral shedding at term are not indicated, and there are no proven benefits of obtaining specimens for culture at delivery in order to identify women who are asymptotically shedding HSV.

For those women with genital lesions at the onset of labor, current practice in the United Kingdom is for delivery by cesarean section. There is evidence that the risks of vaginal delivery for the fetus are small and must be set against risks to the mother of cesarean section.

Management of Herpes in Immunocompromised Individuals

There is a significant interaction between HSV and HIV. HIV-induced immunodeficiency increases the frequency and severity of recurrent anogenital HSV shedding, disease, and risk of developing drug-resistant HSV infection (108).

All three antiviral agents (valaciclovir, aciclovir, and famciclovir) are considered safe for use in immunocompromised patients at the standard

doses recommended for the episodic and suppressive treatment of genital herpes (109).

Since the advent of highly active antiretroviral therapy for HIV infection, there have been fewer patients presenting with clinically refractory lesions caused by genital HSV infection. However these still occur in some AIDS patients and other severe immunodeficiency states.

A consensus symposium on management of aciclovir resistant herpes simplex led to the publication of guidelines in 1994 (110).

Initially, where a patient with genital herpes is failing to respond to standard therapy, it is recommended that a repeat culture be obtained for antiviral sensitivity studies. The dosage of aciclovir should be increased to 800 mg five times daily.

In the event of further deterioration, or where an aciclovir-resistant virus is demonstrated, the following treatment options are recommended.

1. Topical trifluridine every 8 hours until complete healing can be used for accessible lesions.
2. IV foscarnet 50 mg/kg twice daily until complete healing is used for inaccessible or systemic lesions.

In addition, there is evidence from a randomized, double blind, placebo-controlled trial that cidofovir gel (0.1% or 0.3%) applied once daily for five days achieves complete healing or >50% decrease in lesional area in up to 50% of patients. However, cidofovir has only been compared against placebo and not against current standard of care (foscarnet or trifluridine) (111).

New Drug Therapy in Genital Herpes

Immunomodulators

An alternative strategy in antiviral therapeutics involves enhancing the host's natural immune response to viruses.

The administration of exogenous cytokines, such as interferon- α , has been successfully employed in hepatitis C and other viral infections but requires parenteral treatment and is associated with unwanted systemic side effects.

Isoprinosine was hailed as an orally effective immune modulator; however, trials in first and recurrent episode genital herpes showed a lack of efficacy (112,113).

A new group of low molecular weight compounds, the imidazoquinolamines, have been shown to have properties of immune response modifiers *in vitro* and *in vivo*, and to demonstrate both antiviral and antitumor activity via endogenous cytokine production (114). Imiquimod induces alpha interferon and interleukin-12, and when applied to the skin affected by a recurrence in the presence of local herpes antigens, it can augment

HSV-specific cell-mediated immunity. In a randomized, controlled trial, no apparent effect was observed on the short-term natural history of herpes genitalis recurrences (115). However, the more potent resiquimod was reported in a phase 2 trial to delay the onset of recurrent genital herpes symptoms (116). Further trials are progressing.

Helicase-Primase Inhibitors

Recently, a new class of new anti-herpes drugs has been announced. The helicase-primase inhibitors have potent in vitro anti-herpes activity and a novel mechanism of action, a low resistance rate, and superior efficacy against HSV than nucleoside inhibitors of DNA polymerase (117). Reduction in the frequency and severity of subsequent recurrent disease has been also been found. Trials in human subjects are awaited.

Prevention

Strategies to limit the spread of genital herpes should be based on four components—education, increased detection of infected persons, improved clinical management, and the development of vaccines (118).

Education of the General Public

Heightening the awareness of the general public about genital herpes and other STDs must be an essential component of educational programs (119).

It is clearly important to expose the mythology of genital herpes. Like other infections caused by the herpes group of viruses, genital herpes is a common disease and affects many in the general population. It may present in mutually monogamous couples, is usually subclinical, and has effective treatment that controls symptomatic disease, albeit without eradicating the virus from its sites of latency.

Strategies for improving community education must take account of local circumstances and will differ between and within different countries. They may include the provision of telephone helplines and internet-based resources, use of the media, such as articles in newspapers and magazines, and support for the establishment of self-help groups. In many countries, integration of all sexual health matters in programs concerned with HIV/AIDS prevention in schools, colleges, and other community settings is desirable.

Education of Health Care Professionals

Ignorance of the true facts and negative attitudes among healthcare professionals are widespread (120). Besides raising knowledge of the epidemiology and clinical manifestations of genital herpes, clinicians should also be aware of the drawbacks of diagnosis in the poorly managed patient. These include stigmatization by a diagnostic label with the potential for profound

psychological and social sequelae. Doctors who make a diagnosis and simply prescribe antiviral treatment without educating and counselling do their patients few favors.

Several studies have reported that a high proportion of patients expressed depression, fear of rejection, fear of discovery, and self-instructive intent following their initial diagnosis. Patient dissatisfaction with the diagnosing health care professional was widespread. Many received inadequate advice about their emotional state, about their future sex life, or about STD risks. Almost half did not feel that their doctor was supportive, gave adequate information, or could answer questions effectively. These findings provide guidance for training of future doctors and nurses and for the content of continuing medical education programs.

Education of Patients

It is important to emphasize the importance of recognizing subclinical disease. All infected persons should be aware of the infectivity of orolabial herpes and the value of condom use in preventing sexual transmission, especially during periods of asymptomatic shedding.

Efficacy of Condoms

A history of using condoms during 25% or more of sexual encounters was associated with protection against HSV-2 acquisition for women but not for men in a randomized, double-blind, placebo-controlled trial of an ineffective candidate HSV-2 vaccine (121). A subsequent prospective cohort study involving 1862 HSV-2 susceptible persons at high-risk for STD, in whom the annual rate of HSV-2 acquisition was 5.2 per 100 person-years, has recently shown that condom use in at least two-thirds of all sexual encounters was independently associated with a significantly lower rate of HSV-2 acquisition in men (122).

Thus, consistent use of condoms can provide substantial protection against genital herpes in both sexes. The protection is incomplete because the male condom does not prevent all skin-to-skin contact during intercourse.

Increased Detection of Genital Herpes

Although viral culture remains the mainstay for herpes diagnosis in most clinical settings, false negative results are common in late lesions. New diagnostic tools can assist in the detection of those infections that would otherwise remain undetected. Identification and counselling of these individuals could help reduce the spread of HSV-2 infection.

PCR diagnosis has become the gold standard for diagnosis of herpes simplex infection of the central nervous system. It may also establish the cause of genital ulceration especially in those who present late or where antiviral medication has already been commenced. It has provided further

insights into the frequency of asymptomatic shedding, which can allow more informed discussion of transmission risks in individual patients and in pregnancy.

Type-specific serological tests are now commercially available. These assays are well documented to have clinical value in diagnosing genital ulcer disease and subclinical infection in the partners of persons with genital herpes. Testing the asymptomatic partners of patients with symptomatic HSV-2 infection will reveal that a proportion have already been infected subclinically. This finding usually alleviates considerable anxiety associated with future transmission risks.

Substantial disagreement remains about the role of HSV-2 serologic screening of asymptomatic persons because of concerns that the psychological morbidity engendered by uncovering subclinical genital herpes will outweigh any potential public health benefit. In the United States, the value of detecting pregnant women at risk of infection from a serodiscordant partner is being investigated as a possible means of preventing neonatal herpes (122). It is also recommended that all HIV-infected persons who may be at risk of the systemic consequences of reactivated HSV infections should be tested. Consideration should also be given for type-specific serologic testing of all HIV-uninfected persons at higher risk, such as men who have sex with men (MSM) and the sex partners of HIV-infected persons (123). Those found seropositive for HSV-2 should be counselled about their 2-fold elevated risk of HIV infection if exposed. This proposed strategy to change sexual behavior needs to be validated in controlled studies.

Improved Management of Genital Herpes

When genital herpes is first diagnosed, it is essential that the condition and its physical, psychological, and emotional consequences be discussed. The aim is to empower patients to deal with these and to reduce the risk of onward transmission.

A possible role for suppressive antiviral treatment in reducing asymptomatic viral shedding has been suggested (124). However, because neither viral shedding nor clinical outbreaks are entirely ablated, it was impossible to be certain that transmission would be curtailed (125). Recently, a multicenter, randomized, double-blind, placebo-controlled trial of valaciclovir to prevent sexual transmission of genital herpes has been reported (126). A total of 1494 monogamous heterosexual couples who were serodiscordant for HSV-2 infection were randomly assigned to treatment of the infected partner with valaciclovir 500 mg daily or placebo for eight months. Symptomatic, laboratory-confirmed genital herpes occurred in 17 partners (2.3%) of 741 placebo recipients and 4 (0.5%) of 743 partners of those given valaciclovir [relative risk 0.23, 95% confidence interval (CI) 0.1–0.7, $P = 0.006$]. Including additional cases documented only by HSV-2 seroconversion, the infection

rates were 3.8% in the placebo group and 1.9% in the valaciclovir group [relative risk 0.50, 95% (CI) 0.3–0.9, $P=0.04$]. Reduced acquisition risk was observed both for men and women, and time-to-event analysis showed that protection began immediately and was maintained as treatment continued.

Thus, once-daily valaciclovir markedly reduces the likelihood of sexual transmission to the uninfected partner within monogamous couples who were also counselled about other prevention strategies. Further research will be necessary to assess the role and efficacy of suppressive antiviral treatment in prevention of HSV-2 transmission in non-monogamous persons, in those who do not simultaneously receive repeated counselling about other prevention strategies, and in same-sex couples.

It will also be necessary to assess therapeutic compliance with long-term treatment, and the extent to which some infected persons might be less cautious about using condoms and avoiding sex when symptomatic, perhaps blunting the benefits of chemotherapy.

It seems likely that preventing transmission with valaciclovir sometimes will require higher doses than 500 mg daily. For persons with 10 or more symptomatic genital herpes outbreaks per year, 1.0 g daily is required for clinical suppression, and this should be the transmission-prevention dose for such persons. It may also be appropriate to raise the dose in persons with less frequent recurrences if they experience symptomatic outbreaks on the 500-mg dose.

Vaccines

Attempts to develop an effective HSV vaccine began in the 1930s (127) with formalin inactivated virus and were followed by other techniques of inactivating whole virus. None of these were proven to be significantly immunogenic. In recent years, a range of new vaccine formulations has been devised, largely as a result of rapid growth and knowledge in molecular microbiology and genetic engineering, including live and attenuated whole virus vaccines, and subunit vaccines consisting of recombinant viral glycoproteins in various adjuvants (128).

In randomized, controlled trials, a molecular subunit vaccine produced by the Chiron Corporation and consisting of recombinant HSV glycoproteins B2 and D2, combined with adjuvant MF59, failed to provide protection against acquiring HSV-2 infection (129,130).

More recently, a similar product based on glycoprotein D2, with an adjuvant consisting of alum and 3-*O*-deacylated monophosphoryl-lipid A, that induces stronger cell-mediated responses, produced by GlaxoSmith-Kline, has been found to offer partial protection (131).

The vaccine, which was administered in three doses at month zero, one, and six, was well tolerated with mild to moderate soreness at the injection site. The vaccine was effective only in women who were seronegative to both HSV types, who experienced a 75% reduction in symptomatic HSV-2

infection and 40% reduction in seroconversion. The vaccine was ineffective in HSV-1-infected women and in men.

The biological explanation for the differential effect in men and women is not yet clear. It has been suggested that this vaccine may not enhance the natural immunity to HSV-2 infection provided by previous HSV-1 infection.

Modelling of the results of this study have suggested that widespread administration of this vaccine to women seronegative to both HSV types could result in decreased spread of HSV-2 in the general population. The vaccine is to be entered into phase three trials, cofunded by the National Institutes of Health, in 7500 HSV-seronegative women.

New approaches to vaccine development are also undergoing extensive research. DNA vaccines, which allow for plasmid integration into host cells, are one such strategy undergoing clinical evaluation (132,133). In this approach, DNA encoding one or two viral proteins is inserted, allowing for cell-mediated and humoral responses. This method has recently seen success in animal models.

Vaccines administered through non-pathogenic vectors are also under investigation. This method employs the strategy of inserting HSV genes into a competent viral or bacterial vector. The vector replicates in the host and expresses the immunogenic HSV proteins, inducing immune responses through both the humoral and cell-mediated pathways. This novel approach has also seen success in murine models.

Despite these recent advances, a clinically useful vaccine against HSV-2 is still appears to be several years away.

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Herpes Simplex Virus Ocular Disease

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INTRODUCTION

Herpes simplex virus (HSV) is the most common cause of monocular infectious blindness in the industrialized world and is second only to trachoma in the developing world (1). During the initial infection in the ocular or facial area, HSV replicates in the mucous membranes of the mouth or the corneal epithelium, where sensory and autonomic nerve terminals take up the virus. The virus is transported in a retrograde direction to sensory trigeminal ganglion cell bodies. Although many ganglion neurons support a lytic infection, a subpopulation of neurons supports an HSV infection in which the virus remains latent. The second phase of corneal infection follows from viral reactivation in the latently infected trigeminal ganglion cells. After antero-graduate transport to the eye, the new virus can be found in tears and in the corneal epithelium and stroma. With repeated reactivation cycles, the corneal stroma becomes progressively scarred, with resulting decrease in vision and other ocular complications including glaucoma, iritis and cataract, and necrotizing retinitis. The burden of ocular HSV relates primarily to its recurrences that result in corneal scarring and glaucoma; the other complications or other ocular tissue involvement are less common. There is no effective therapy to prevent ocular recurrences and the treatment for active ocular HSV is limited.

The classification of this disease has varied in the literature. Ocular HSV disease may be classified as primary or recurrent and also by the tissue inflamed: blepharitis, conjunctivitis, epithelial keratitis, stromal keratitis, iridocyclitis, or retinitis. A simplified classification is presented to assist in understanding the disease entities discussed in this chapter (Table 1), and a diagram of the eye is provided to assist the reader in the ocular tissues involved (Fig. 1).

Table 1 Classification of HSV Ocular Disease

HSV blepharitis
HSV conjunctivitis
HSV epithelial keratitis
Dendritic keratitis
Geographic (amoeboid keratitis)
HSV stromal keratitis
Necrotizing stromal keratitis
Immune stromal keratitis
HSV corneal endotheliitis
Disciform or diffuse or linear endotheliitis
HSV anterior uveitis
HSV trabeculitis (secondary glaucoma)
HSV posterior uveitis
Retinitis
Acute retinal necrosis
HSV choroiditis
HSV retinal vasculitis
HSV optic disc papillitis
HSV neonatal ocular disease
Dermatitis
Conjunctivitis
Keratitis or corneal ulceration
Anterior uveitis
Cataract
Vitreous inflammation
Chorioretinal inflammation
Optic disc atrophy
Retinitis that may be associated with HSV of the CNS
HSV congenital ocular disease
Microphthalmos (small eye)
Retrolental masses (behind the lens in the vitreous)
Retinal dysplasia (retinal disorganization)
Retinal scarring
Cataracts
Optic nerve atrophy

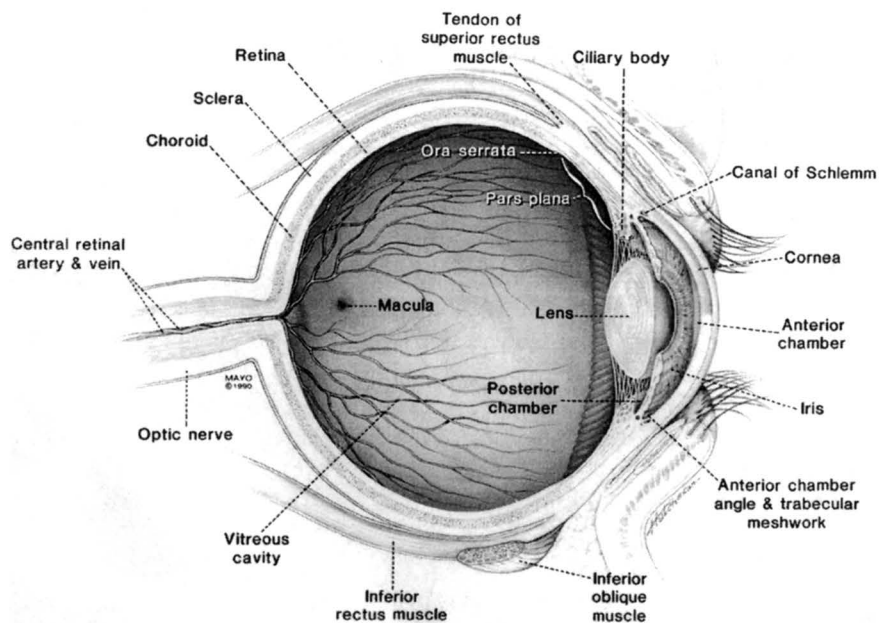


Figure 1 Cross-section and labeling of the eye and surrounding tissues to assist in understanding the tissues involved in HSV ocular infection.

PATHOPHYSIOLOGY

Transmission

The ocular area can be infected during the primary generalized infection with HSV; other cases of ocular herpes may result from a recurrence of HSV in the facial area and then spread to the ocular area, or ocular HSV may occur in isolation with no prior history of HSV. Once HSV infection occurs in the ocular area by any of these routes, the virus has access to, and remains in latency within, the nerve tissues in the trigeminal ganglion. It may then recur at any time; there is no therapy to remove the virus from the nerve tissue.

In laboratory animals, the most successful model of primary ocular infection mimicking the human infection involves a snout inoculation in the mouse with initial centripetal spread of virus towards the central nervous system (CNS), and later centrifugal flow of virus to involve the whole dermatome (2). This resulting model seems more reminiscent of recurrent ocular disease than direct corneal inoculation by scarification or partial thickness corneal trephination.

Identifiable and reproducible ocular lesions of distinctly different size, location, and shape result from infection with different strains of HSV (3–5).

Some HSV strains produce consistently mild corneal epithelial disease, some produce epithelial and stromal disease, and others produce uveitis (6,7). There is also a relationship between viral strain and their ability to produce latency, shedding, and recurrence. The specific nuclear-type sequence of DNA polymerase gene can contribute to differences in the capacity of HSV type 1 and 2 to replicate in the trigeminal ganglion and spread from the eye to the CNS (8,9). Ocular sensitivity (in the rabbit) is also strain-specific and can also be separated into sensitivity to endogenous (spontaneous) reactivation and exogenous (induced) reactivation tendencies (10). Whether humans manifest variation in genetic susceptibility has not yet been established.

Although it has been assumed that the same virus as the initial ocular HSV causes recurrent episodes of ocular HSV, a limited study demonstrated that recurrent HSV is frequently associated with corneal reinfection with a different HSV strain and suggests that a corneal transplant may be a risk factor for corneal HSV superinfection (11).

Role of Virus and Immune Response

Superficial corneal epithelial disease is primarily a viral infection. HSV keratitis and the other forms of ocular HSV are more complex ocular diseases with components of live viral infection, immune and inflammatory response, and damage to ocular structures (12,13).

Langerhans' cells migrate rapidly into the central cornea following HSV epithelial or stromal infection and act as antigen presenting cells for HSV. The presence of Langerhans' cells in the central cornea correlates with the onset and persistence of stromal keratitis (14). Activation of T-cells by viral antigens on the surface of Langerhans' cells initiates a chronic inflammatory response. There continues to be a debate about whether cytotoxic or helper T-cells play the predominant role in stromal keratitis and the role of Langerhans' cells and their migration into the central cornea.

Experimental data support the hypothesis that HSV stromal keratitis is an immune attack on HSV-infected corneas with delayed-type hypersensitivity directed at nonreplicating viral-encoded antigens or altered corneal antigens involving cytotoxic and helper T-lymphocytes. The source of the antigens has been debated; it may include residual viral antigen in the corneal stroma following previous corneal infection, concurrent or previous epithelial keratitis, smoldering lytic HSV infection in keratocytes, or recurrence from neuronal or keratocyte latency. The finding of immunopathogenic T-cells with specificity for corneal autoantigens is an indication that the cornea itself may be a direct target (i.e., autoantigen) (12). Abnormally expressed human leukocyte antigen (HLA)-DR antigens in HSV keratitis cause a cell-mediated autoimmune disorder to self-corneal antigens with destruction of host tissues (15). Other studies, however, cast doubt on the molecule mimicry hypothesis of HSV stromal keratitis and suggest that it

may be a bystander activation mechanism (16). Immune stromal disease may also have an active viral process in addition to the immunologic response (17).

The cellular infiltrate of the stromal inflammatory response of chronic HSV in humans is composed of polymorphonucleocytes (PMNs), macrophages, lymphocytes, and plasma cells. Cells isolated from HSV keratitis are capable of both HSV-specific cytotoxic activity (mediated by T-lymphocytes) and HSV-nonspecific cytotoxic activity (mediated by natural killer cells). HSV-specific T-cells mediate delayed-type hypersensitivity reactions, which eliminate infectious virus at the initial site, and the cytospecific T-lymphocytes prevent virus spread from the initial site. The action of T-lymphocytes has a dialectic role in stromal disease by not only preventing the spread of HSV, but also inducing the destruction of the corneal stroma (18,19).

HSV is not usually recovered directly from corneal homogenates of host corneal transplant buttons in patients with immune stromal keratitis, although it may be derived from organ culture on occasion (20). Nucleocapsids and capsids, or HSV DNA, have been observed in keratocytes and whole virions have been observed in the interstitium of some corneal buttons with immune stromal keratitis (20–24). Easty (25) demonstrated that HSV might enter stromal keratocytes where it could either be eliminated or escape the immune response and persist within this layer. The virus can also exist within the stroma as a whole virion shed into the stroma from neurons where it incites an inflammatory response; it can also behave as a slowly proliferating intracellular virus which can alter the antigenicity of the cell wall and elicit an immune response. Latent existence, during which the cell remains antigenically unchanged and there is no active clinical disease, also may occur. It is most likely the residual viral antigens within the stroma that incite the inflammatory response in immune stromal disease, subsequently involving lymphocytes, antiviral antibodies, serum complement, PMNs, and macrophages (12,19,26,27).

The role of live virus as a cause of endothelial infection in HSV endotheliitis (disciform keratitis) is supported by finding HSV-1 antigen, HSV DNA, or HSV virus in corneal endothelial cells (26,28–35). Sundmacher and Neumann-Haefelin (36) postulate that endothelial cells become productively infected with HSV and elicit a cellular and humoral immune response; the cells are occasionally lysed by either viral infection and/or immune mechanisms with release of infectious virus into the aqueous. HSV multiplication in the endothelial cells may lead to expression of viral antigens on the cell surface. Immunocompetent cells can then attack the endothelium, leading to enhanced damage by immunocytolysis. This may explain why corticosteroids are usually very effective and tends to confirm that the immunogenic cell destruction is more pronounced and more harmful than viral cytolysis.

Not all researchers are convinced of the possibility of HSV infection of the corneal endothelium. Alternatively, stromal edema may reflect a delayed-type hypersensitivity reaction to HSV antigens within the stroma or endothelium (19). Animal models of disciform keratitis have stressed the role of immune complex formation, cytotoxic antibodies, complement and antibody-dependent cellular cytotoxicity (37). The pathogenesis of herpetic anterior uveitis is complex and probably represents a combination of active viral replication in the anterior chamber and an immune response to viral antigens. Iris cell invasion by the herpes virus has been demonstrated by electron microscopy (38), and several have reported isolation of HSV from the anterior chamber of patients with HSV uveitis (29,30,36,39–43).

Shedding of Ocular HSV

The role and frequency of ocular shedding (either spontaneous or induced) in recurrent ocular disease are unclear. Animal models have been shown to demonstrate spontaneous asymptomatic HSV ocular shedding, but there are significant species differences (44,45). In humans, the data are conflicting; asymptomatic shedding has been found in up to 30% of humans (46), although a more recent study failed to detect any ocular shedding in the tear film (47).

Corneal Latency

There is increasing evidence for HSV latency in non-neuronal tissue, including the cornea (48–51). If confirmed, the cornea would be the first human tissue other than nervous tissue to harbor latent virus. This would have significant ramifications regarding the potential transmission of HSV infection or latency following corneal transplantation (48).

Easty and coworkers (52,53) isolated HSV by organ culture from corneal buttons obtained from patients with chronic HSV stromal keratitis. Several laboratories have demonstrated persistent HSV DNA in humans and animals during quiescent HSV stromal keratitis; HSV has been recovered from a limited number of explanted rabbit and mouse corneal tissues months after inoculation (19). Latency-associated transcripts (LATs), a sensitive marker for latency in neuronal tissue, have been detected by several techniques in the cornea, although they were not detected by the sensitive *in situ* hybridization technique (50,54), while simultaneously being detected in the trigeminal ganglion.

DNA of HSV has been identified in the corneal buttons of cases with primary graft failure, suggesting that it may be pathogenic (55–57). It remains very difficult to distinguish corneal latency from low-level persistent infection or low-level subclinical reactivations within the trigeminal ganglion.

EPIDEMIOLOGY

Etiology

Herpes simplex ocular disease is usually caused by HSV-1, and rarely by HSV-2; there has been no recent confirmation of the frequency of ocular HSV-2 in corneal disease. A clear HSV-type related tropism might be limited by the permissiveness of the orofacial region for HSV-1; both serotypes may readily establish infections below the neck.

Possible trigger mechanisms for HSV include fever, hormonal changes, ultraviolet exposure, psychological stress, and ocular trauma, although these were not confirmed in a recent ocular study (58); trigeminal nerve manipulation is an accepted trigger mechanism (59). The excimer laser (LASIK) has been shown to be an efficient trigger for reactivation of latent HSV in the cornea (60), but there has not been a recognized epidemic of HSV keratitis following the recent boom in refractive surgical procedures.

There have been several epidemiology studies of HSV ocular disease, although all remain limited in scope and confirmation. HSV, nonetheless, appears to be the most common infective cause of blindness in many developed countries (61), primarily because of its recurrent nature. Several studies have shown that recurrences were more frequent in males (61–63).

The reports that provide the most comprehensive information include the studies from Moorfields Eye Hospital in London, from the epidemiology studies in Rochester Minnesota, and from the Herpetic Eye Disease Study (HEDS) funded by the National Eye Institute.

Moorfields Studies

Investigators from Moorfields Hospital in London studied emergency room adult patients with acute follicular conjunctivitis and keratoconjunctivitis and reported 25 cases of herpes simplex. This emphasized for the first time the presentation of primary HSV as conjunctivitis in adults (64). In a further series of all patients with acute conjunctivitis in an emergency room setting, 21% were determined to have HSV as the etiology (65).

In a study of 107 patients with primary ocular HSV infections, the mean age for this first episode of ocular HSV was 25 years of age. An upper respiratory infection was present in 35%, and generalized symptoms in 31%. Conjunctivitis was present in 84%, blepharitis was present in 38%. Dendritic ulcers occurred in 15% and disciform keratitis in 2%. The disease was unilateral in 81% and bilateral in 19%. These same 107 patients with primary ocular HSV were then followed for 2 to 15 years to determine the pattern of recurrent disease (66). Thirty-two percent had a recurrence, and it was more frequent in patients under the age of 20. Of those with a recurrence, 49% had one recurrence, 40% had two to five recurrences, and 11% had six to 15 recurrences. This is the first study to demonstrate that recurrences occurred most commonly as either conjunctivitis or lid lesions. Of those with

a corneal infection during the primary episode of ocular HSV, 31% developed a recurrent infection.

The prognostic factors for recurrence of HSV were identified in a different series of 151 patients followed for five years after epithelial keratitis (67). Forty percent had a recurrence of epithelial disease within five years; 21% had more than one recurrence. Twenty-five percent developed stromal keratitis of which 63% had a disciform keratitis and 37% had an irregular stromal keratitis. Five percent developed ocular hypertension. On final evaluation, vision was 20/20 to 20/40 in 73%, 20/60 to 20/200 in 24%, and less than 20/200 in 3%. Only 6% of the total 151 eyes had poor vision related to stromal keratitis despite 22% of eyes having stromal scarring. There was no correlation of treatment with topical antiviral on the recurrence rate.

Mayo Clinic Studies

In the study at Mayo Clinic in Rochester, Minnesota, 121 patients with their first recognized clinical episode of ocular HSV were followed for up to 33 years (61). The initial episodes of ocular HSV involved the lids or conjunctiva in 54%, the superficial cornea in 63%, the deep cornea in 6%, and the uvea in 4%. The disease was bilateral in 12%, and the predominant form of recurrent disease was dendritic corneal involvement, although 20% had only recurrent lid involvement. Significant stromal disease developed in 20% of patients. Patients tended to get a recurrence of the same type of ocular disease that they had previously, that is, patients with epithelial disease tended to get recurrent epithelial disease; this was similar for conjunctival disease and stromal disease. Seventy percent of eyes maintained 20/20 vision; only three of 130 eyes had final vision that was worse than 20/100. In this series, no cases of retinal or neonatal herpetic disease were recognized.

Herpetic Eye Disease Study (HEDS)

The HEDS evaluated patients from a therapeutic perspective but yielded valuable epidemiological data. Patients with corneal disease were selected for the study, so they do not represent the entire spectrum of patients with ocular HSV disease. There were 703 immunocompetent patients who were followed after an episode of corneal epithelial HSV. There were 79% Caucasians, 9% African Americans, 8% Hispanics, and 3% Asians; other studies have also suggested that ocular HSV is more common in Caucasians. A HEDS publication addressed a cohort study within a randomized controlled trial that evaluated if variables such as psychological stress, systemic infection, sunlight exposure, menstrual period, contact lens wear, or eye injury could be triggering factors of recurrences. None of the variables mentioned was significantly associated with the triggering of ocular recurrences (58).

Another HEDS evaluated the role of gender, ethnicity, and previous history of herpetic eye disease as possible predictive factors for the

recurrence of herpetic eye disease (68). None of these factors were strong predictors for recurrence except previous herpetic eye disease.

Incidence and Prevalence

In the Mayo Clinic series, the incidence (age-adjusted) of HSV ocular disease was calculated at 8.4 new cases per 100,000 person-years. The incidence of all episodes was calculated at 20.7 cases per 100,000 person-years (61). The prevalence of ocular HSV disease in the community was calculated at 148 per 100,000 population. There was no evident seasonal trend. The recurrence rates for any form of ocular disease were 9.6% at one year, 22.9% at two years, 36% at five years, and 63.2% at 20 years.

The incidence of ocular HSV per 100,000 person-years has been estimated at 4 in Croatia (69) and 6–12 in Denmark (70,71), compared to 21 in Rochester, Minnesota (61). The incidence of HSV epithelial keratitis is six times (119 per 100,000 person years) higher in patients who have undergone corneal transplantation (for non-herpetic corneal disease) (72).

There is only one study on the incidence of adult ocular disease caused by HSV-1 compared to HSV-2; this was reported in 1978 from Germany (73). In a continuous series of 457 patients, virus isolation and typing revealed 153 patients with HSV-1 and three patients with HSV-2. There is at least one report of simultaneous HSV-1 and -2 infecting a cornea in a patient with AIDS (74).

Bilateral Ocular Disease

The frequency of bilateral HSV ocular disease varies in the literature, partially because the definition of bilateral disease varies; some report any form of lid, conjunctival, or corneal disease and others report only keratitis. Atopy, HIV infection, and other forms of immunosuppression predispose to prolonged or bilateral disease (59,75,76). In a series of 1000 patients with ocular HSV keratitis, 30 patients (60 eyes) or 3% had a history of bilateral corneal dendrites (77). Seventy percent were males and atopic disease was present in 40%. There was a recurrence in 41 of the 60 eyes (68%) and stromal disease occurred in 24 of the 60 eyes (40%). Patients with bilateral disease tended to be younger and had a higher proportion of ocular complications. In a series of 356 patients followed over 30 years in Japan, bilateral keratitis was found in 9% (78). It was more frequent in males and younger patients while 36% were atopic. In this series, HSV usually affected the epithelium only.

In the community-based series from Rochester, Minnesota, followed over 33 years (61,62), ocular HSV was defined as any form of herpes simplex, including lid, conjunctival, or cornea. Using this definition, 12% had bilateral ocular HSV and 28% were atopic. Most bilateral episodes represented lid and conjunctival disease at their first episode of ocular HSV. Simultaneous bilateral corneal involvement is uncommon.

Circannual Rhythm of Ocular HSV

Most studies report a more frequent occurrence of ocular HSV during the winter months. The circannual rhythms of ocular HSV were reported among 541 patients over a 14-year period in Israel (79). A peak was found in January, but only for epithelial keratitis and only in males; atopes, however, had a higher incidence in September. There were no association of rhythms and triggers for upper respiratory infection. In a study in the United States, November through February was found to have the highest frequency of ocular HSV recurrences (80); another study found the peak frequency in November (61,62). In a study from Japan, the highest number of episodes occurred in the December to February months (winter and spring in Japan) (78).

Epidemics of Ocular HSV

Most epidemics of HSV do not involve the eye but have been reported in wrestlers (*herpes gladiatorum*) (81). Sixty of 175 wrestlers (34%) attending a 4-week intensive training camp developed HSV disease; 8% developed ocular involvement, mainly with follicular conjunctivitis, blepharitis, and phlyctenular disease. Epidemiology studies confirmed the same strain of herpes simplex.

OCULAR DISEASE MANIFESTATIONS

In the ocular area, HSV causes disease of the lids (eyelid vesicles), conjunctiva (inflammation and vesicles), sclera (scleritis), cornea (keratitis), the anterior part within the eye (anterior uveitis), the retina (retinitis), or, less commonly, in the choroid (choroiditis) or optic nerve (optic neuritis) (Figs. 1–3). Although many of the manifestations begin as the result of the HSV viral infection itself, many manifestations are the aftermath of the body's response to the infection in terms of mounting an inflammatory response, an immune response, vascular leakage, scarring, and nerve damage. There is a complex interplay of these features such that each patient requires interpretation of the clinical findings and a tailored approach. It is necessary to limit the amount of inflammation and scarring since the function of the eye is then impaired.

HSV Corneal Epithelial Disease

The most commonly recognized clinical manifestations of epithelial keratitis are dendrites and geographic ulcers caused by viral replication. Most patients with this infectious epithelial keratitis complain of photophobia, pain, and a thin watery discharge. Immune mechanisms (18) and interferon production by the infected epithelial cells limit the spread of the virus and hasten resolution. Interference with this immune response (corticosteroid) favors the spread of the virus (82).



Figure 2 HSV facial, eyelid and conjunctival infection. HSV infection of the right cheek with simultaneous left eyelid and eyelid margin lid vesicles. There is an associated HSV conjunctivitis with redness but no corneal involvement at this time. This scenario can occur with either the first or recurrent episodes of HSV.



Figure 3 HSV of the lids, eyelid margins, and conjunctiva. Large confluent eyelid and cheek vesicles in a child with recurrent ocular HSV. Corneal and conjunctival involvement was also present at this time. HSV infection of the conjunctiva manifests with redness of the conjunctiva (hyperemia), lymphoid aggregates (follicles) and occasionally HSV vesicles visible.

The HSV dendritic lesion is a coalescence of infected epithelial cells that displaces fluorescein (negative staining) in a branching, linear lesion with terminal bulbs (swollen epithelial borders) that contain live virus. When it advances to a dendritic ulceration, it has extended through the basement membrane (Fig. 4).

A geographic (amoeboid) ulcer is a widened dendritic ulcer from desquamated viral-infected epithelial cells (Fig. 5). It extends through the basement membrane and has swollen epithelial borders with live virus. Geographic ulcers account for 22% of all cases of initial infectious epithelial keratitis and are usually associated with longer duration of symptoms and time to healing compared to dendritic ulcers (77,83). They may be associated with previous use of topical corticosteroid.

HSV Corneal Stromal Disease

HSV stromal disease accounts for approximately 2% of initial episodes of ocular disease (62,84), but about 20–48% of recurrent ocular HSV disease (62,70,85). The corneal stroma may be affected in HSV disease through a variety of mechanisms; it may be affected secondary to disease of the epithelium, endothelium, or from nerve damage. The tissue damage in the corneal

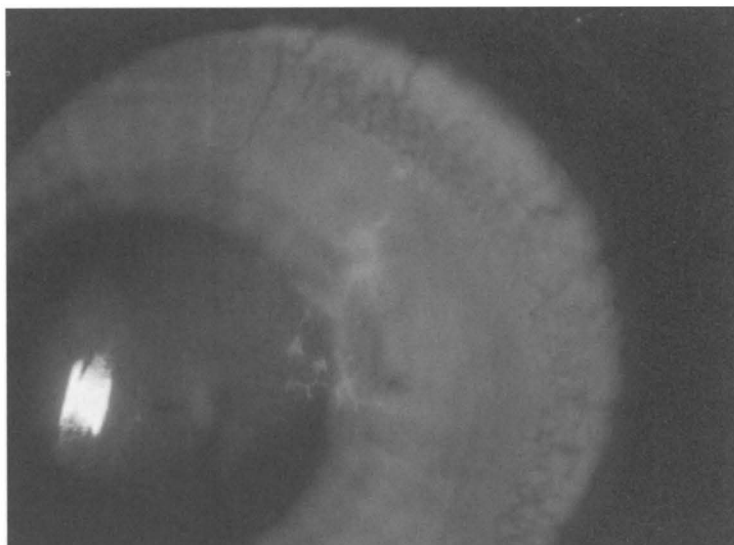


Figure 4 HSV corneal epithelial disease. HSV infection of the superficial cornea manifests as a branching lesion (dendrite) on the surface of the cornea. In this illustration, fluorescein dye has been instilled and the cobalt blue filter from the slit-lamp biomicroscopy highlights the dendrite. The dendrite or dendritic ulcer is the most common manifestation of primary or recurrent ocular HSV.

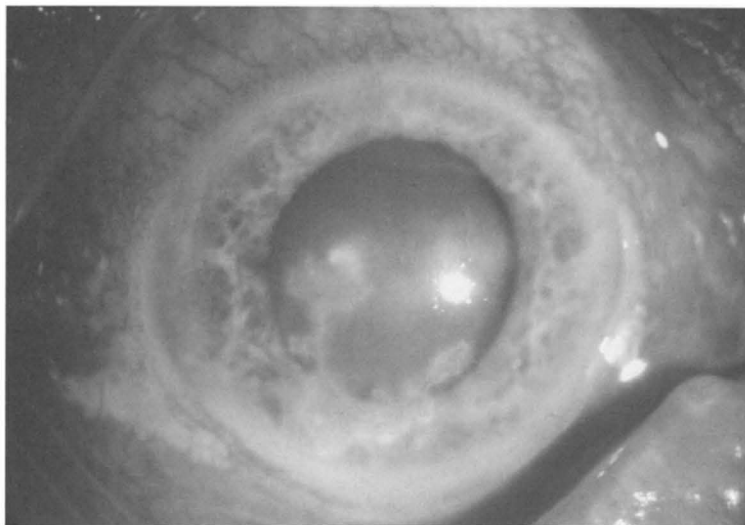


Figure 5 HSV dendritic and geographic epithelial disease. HSV infection of the superficial cornea demonstrated as a dendritic figure in some portions but as a wider geographic ulceration some portions. Fluorescein stain is utilized to better visualize the lesion.

stroma is more a consequence of an immunopathologic response to the virus than the direct result of virus replication; hence, the disease is controlled therapeutically by anti-inflammatory therapies.

The primary forms of stromal disease from HSV include necrotizing stromal keratitis (from direct viral invasion of the stroma) and immune stromal keratitis (from an immune reaction within the stroma to virus or viral particles). They are not mutually exclusive and probably form a continuum since some studies have shown systemic acyclovir to be partially effective in stromal keratitis (85–90), whereas others report no benefit (91–94).

Necrotizing Stromal Keratitis

Necrotizing stromal keratitis results from direct viral invasion of the corneal stroma. There is necrosis, ulceration, and dense infiltration of the stroma with or without an overlying epithelial defect. It is much less common than the immune stromal keratitis. Corneas may demonstrate single or multiple, gray or white, creamy, homogeneous abscesses with edema, keratic precipitates (KP), secondary guttate, severe iridocyclitis with hypopyon or hyphema, secondary glaucoma, and synechiae (Fig. 6). Replicating virus and the severe host inflammatory response can lead to destructive intrastromal inflammation that may become refractory to treatment with high-dose anti-inflammatory and antiviral medications. This can progress to thinning

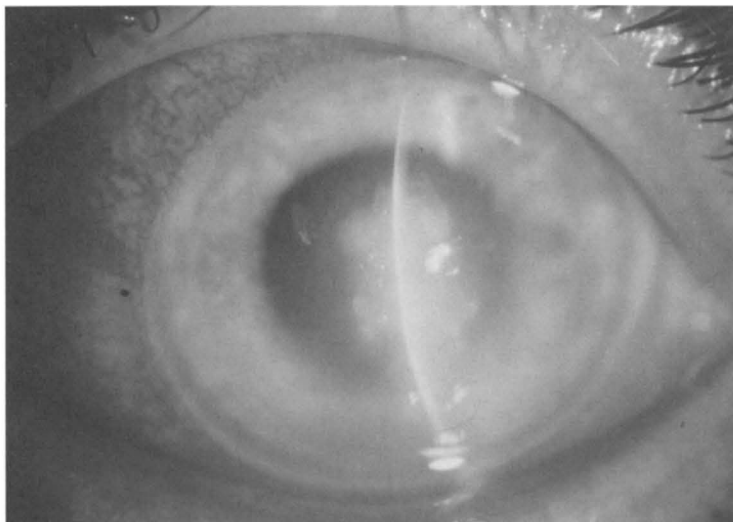


Figure 6 HSV necrotizing stromal keratitis. A severe inflammation of the deep cornea with inflammatory cell infiltrates or abscess, death of tissue (necrosis), pain, and redness of the eye. This may be caused by deep penetration of the HSV infection into the cornea. Relatively uncommon and usually seen following several prior episodes of recurrent HSV disease.

and perforation within a short period of time. A potentiating effect of corticosteroid in the absence of concomitant antiviral has been implicated (94,95). Intact virions have been detected in stromal keratocytes and lamellae on electron microscopic examination of pathologic tissue from patients with necrotizing stromal keratitis (17,26).

Immune Stromal Keratitis

Immune stromal keratitis is a common manifestation of recurrent and chronic HSV involving the corneal stroma. It occurs in 20% of patients with chronic or recurrent ocular HSV (62,70,84,95). It may present days after epithelial keratitis or months to years later, with or without prior infectious epithelial keratitis. Both the effectiveness of corticosteroid in HSV stromal keratitis (96) and the lack of benefit of oral acyclovir in preventing or treating stromal keratitis are confirmatory of the immune etiology.

Patients with immune stromal keratitis have a ground-glass subepithelial haze and later permanent scarring (Fig. 7). This pattern of stromal inflammation may be focal, multifocal, or diffuse (97). Stromal infiltration is often accompanied by anterior chamber inflammation, ciliary flush, and pain.

Immune stromal keratitis may be chronic, recurrent, or recrudescant for years; there may be constant low-grade inflammation with mild fluctuations in severity or intermittent bouts of inflammation with quiet stroma

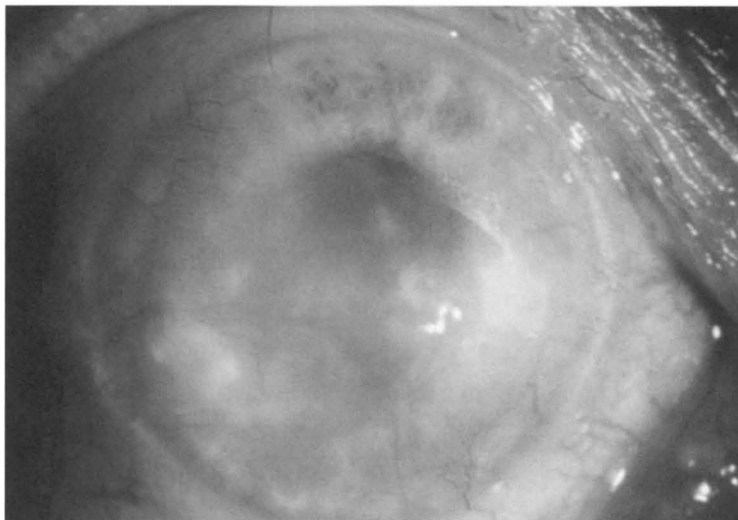


Figure 7 HSV chronic immune stromal keratitis. This is a persistent immune or inflammatory reaction of the cornea with abscesses, scarring, vessel ingrowths into the cornea (neovascularization), and either swelling (stromal edema) or thinning. It usually follows immune stromal keratitis that does not respond to therapy. There is probably no active HSV infection although viral particles may be seen.

between episodes. Untreated, undertreated, or nonresponsive inflammation can progress to stromal scarring, thinning, persistent neovascularization, lipid deposition, and severe loss of vision (Figs. 8–10).

HSV Endotheliitis

The corneal endothelium is the layer of the cornea adjacent to the anterior chamber and serves as a pump mechanism to keep the cornea dehydrated and clear. HSV endotheliitis is an inflammatory reaction of the endothelium with subsequent secondary stromal and epithelial edema. There is no stromal infiltrate or neovascularization. Patients characteristically have KP on the endothelium, overlying stromal and epithelial edema, and iritis. With persistence or untreated, secondary neovascularization and scarring may occur; chronic endotheliitis may also lead to endothelial decompensation and permanent intractable corneal edema (28).

Three forms of HSV endotheliitis are seen clinically: disciform, diffuse, and linear. Disciform endotheliitis is by far the most common form of endotheliitis. It is typically a round area of stromal edema overlying KP in the central or paracentral cornea (Fig. 11). Elevated intraocular pressure may occur from inflammatory cells blocking the aqueous outflow or because of a primary inflammation of the trabecular meshwork. Severe cases may

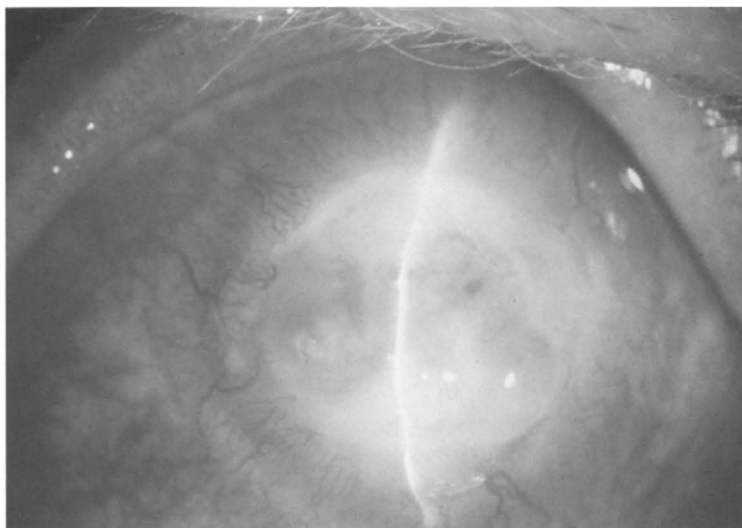


Figure 8 HSV chronic immune stromal keratitis. Persistence of chronic stromal keratitis with severe inflammation, abscess, and necrosis with loss of tissue resulting in thinning of the cornea and occasionally perforation requiring emergent surgical repair.

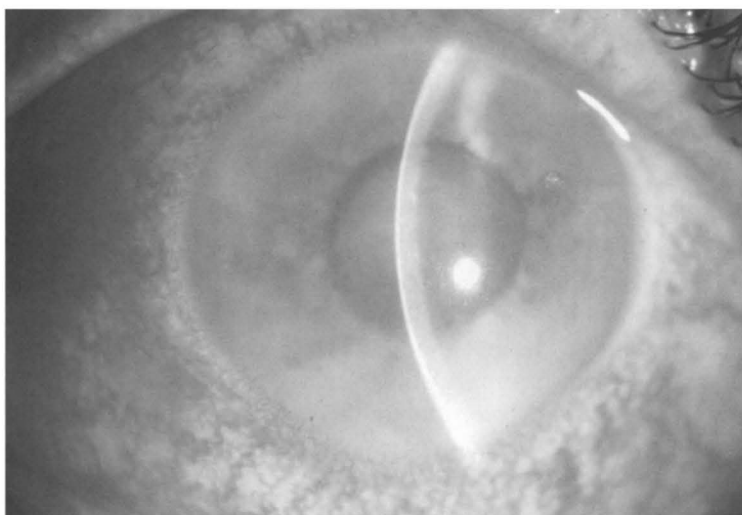


Figure 9 HSV immune stromal keratitis. HSV immune stromal keratitis manifest with corneal stromal edema, cellular reaction in the anterior chamber and ciliary flush (redness surrounding the peripheral cornea). This is an immune reaction to the viral infection or viral particles that can manifest as mild or severe. Therapy is directed at the immune and inflammatory reaction because persistence leads to scarring and poor vision. Topical steroids are usually employed.

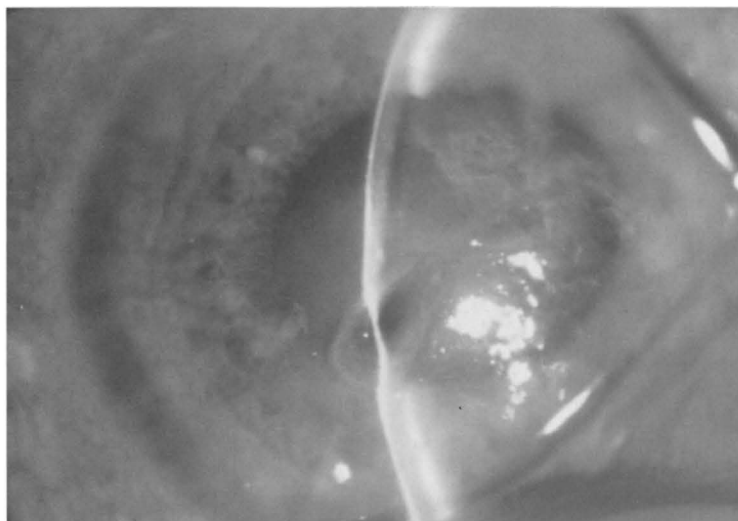


Figure 10 HSV stromal keratitis with perforation. Severe persistent and medical unresponsive HSV stromal keratitis lead to severe corneal thinning and a small central corneal perforation.

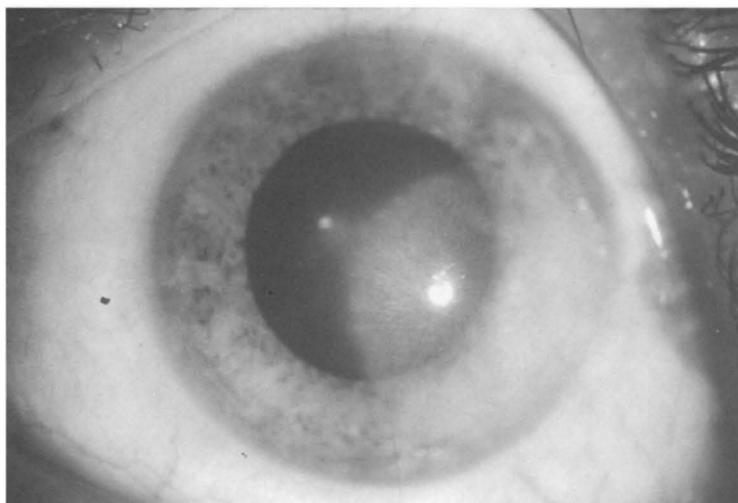


Figure 11 HSV disciform endotheliitis. HSV infection of the deepest layer of the cornea (the endothelium) or, alternatively, an immune reaction directed at this deepest layer. When this layer does not function well, the cornea swells. This edema may manifest as a disc-shaped corneal swelling (disciform endotheliitis, as shown here) or the whole cornea may be swollen (diffuse endotheliitis). The HSV infection may attack the endothelium from the inside of the eye, or may reach the endothelium after traveling from the surface of the cornea.

progress to immune interstitial stromal keratitis with permanent edema, scarring, and neovascularization.

HSV Anterior Uveitis and Trabeculitis

Anterior uveitis is an inflammation of the anterior eye, including the iris, the anterior chamber, the uveal tissue behind the iris, and the anterior vitreous. HSV anterior uveitis is an inclusive term encompassing iritis, iridocyclitis, and or anterior vitritis depending on which tissues are demonstrating inflammatory signs by biomicroscopic exam. Symptoms and signs of HSV anterior uveitis include photophobia, pain, and ciliary flush. Slit-lamp examination reveals fine KP and an anterior chamber cellular reaction that can range from mild to severe (Fig. 12). The uveitis usually accompanies immune stromal keratitis or endotheliitis but rarely can occur in isolation; uveitis may occur without a prior history of herpes simplex.

Trabeculitis is a peripheral variant of endotheliitis with precipitates and swelling of the anterior chamber angle. It presents with an acute elevation in the intraocular pressure. This usually responds quickly to topical corticosteroids. Chronic inflammation may lead to inflammatory cells blocking aqueous outflow and sometimes trabecular scarring with a chronic

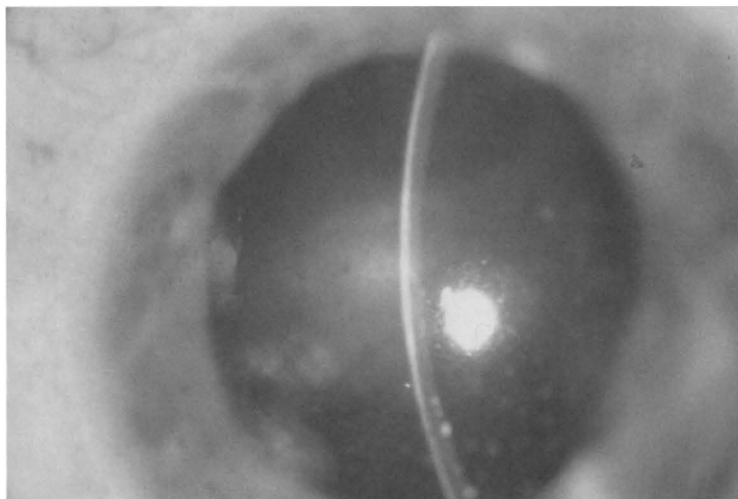


Figure 12 HSV uveitis. An infection in the anterior chamber of the eye from HSV that gained entrance through the vessels in the iris or, alternatively, an immune reaction directed at the virus or viral particles in the anterior chamber, iris, or endothelium. There may be a diffuse inflammation throughout the anterior chamber or there may be a focal reaction in the iris. Pigmented KP are noted on the corneal endothelium. This may be accompanied by glaucoma (HSV uveitis–trabeculitis).

persistent glaucoma. In HSV keratouveitis, it is postulated that HSV reaches the anterior segment through sensory innervation with the endothelium being involved secondarily (32,52,98,99).

HSV Posterior Uveitis

When the uveal inflammation is behind the lens, it is termed posterior uveitis and encompasses the various posterior segment complications of HSV infection such as retinitis, choroiditis, vasculitis, papillitis, and optic neuropathy. HSV-associated chorioretinitis has long been recognized in neonates and infants, but cases in adults are rare. Retinitis often occurs in association with encephalitis, although the eye disease may occur years after resolution of the CNS disease. The retinitis may be accompanied by exudative retinal detachments, flame-shaped hemorrhages, vitritis, optic disk edema, and vitreous opacities.

Acute retinal necrosis (ARN) is a severe form of necrotizing retinitis usually caused by HSV-1, HSV-2 or varicella-zoster virus (VZV) (100). VZV or HSV-1 tends to cause ARN in patients older than 25 years, whereas HSV-2 causes ARN in patients younger than 25 years (101). A history of CNS infection in a patient with ARN syndrome suggests that HSV is likely to be the viral cause. ARN is manifest more frequently in patients with AIDS (102).

Typical cases of ARN begin with retinal vasculitis and a diffuse uveitis and later peripheral retinal necrosis with discrete borders, rapid progression, circumferential spread, occlusive vasculopathy with arteriolar involvement, and inflammation in the vitreous and anterior chamber (Fig. 13). In the original definition of the ARN syndrome, immunocompetency was a requisite (103,104). The definition is now expanded to include immunosuppressed patients. In both healthy and immunosuppressed adults, HSV-1 has also been shown to cause other types of ocular inflammatory syndromes that differ from classic ARN syndrome (103).

The most frequent presenting sign of ARN in AIDS patients is a decrease of visual acuity, but signs related to a retrobulbar optic neuritis may also be present. The VZV causes most cases although HSV has been incriminated in a few (102).

Associations Between HSV and Other Ocular Diseases

HSV has also been detected in the aqueous humor of patients with the Posner-Schlossman syndrome (105). HSV has been implicated as a possible cause of Fuchs uveitis syndrome (38) and the iridocorneal endothelial syndrome (ICE) (24). Linear endotheliitis is a form of HSV infection of the endothelium and has been reported with a variety of names in the literature including keratitis linearis migrans, presumed autoimmune corneal endotheliopathy, progressive herpetic corneal endotheliopathy, and idiopathic corneal endotheliopathy (31,33,35,106–109). Several of these latter patients



Figure 13 HSV acute retinal necrosis. A montage fundus photograph of severe acute retinal necrosis with retinal hemorrhages, necrosis, vasculitis, and swollen optic nerve in a patient with herpetic encephalitis. *Source:* Courtesy of Hadden PW, Barry CJ. Images in clinical medicine: herpetic encephalitis and acute retinal necrosis. *Source:* N Engl J Med 2002; 347(24):1932.

have had an anterior chamber paracentesis that disclosed a positive antibody to HSV antigen or detected HSV by PCR (33,35). HSV has also been implicated in early corneal graft failure (55,56,110).

Pediatric Manifestations

The triad of skin vesicles, eye disease, and microencephaly or hydranencephaly characterizes congenital HSV infection. Typical findings of congenital ocular HSV infection include microphthalmos, corneal ulceration, anterior uveitis, cataract formation, vitreal inflammation, chorioretinitis, retrolental masses, retinal dysplasia, cloudy lenses, optic atrophy, and retinal scarring

(111). The retina may be infected by direct viral invasion (112), although retinal findings are usually not apparent until 1 month or later (113). Most cases of neonatal HSV retinitis are associated with HSV infection of the CNS, but 20% are associated with conjunctivitis, keratitis, or dermatitis (114).

HSV keratitis in children tends to be more severe and with a higher incidence of geographic ulcers compared to adults (115). There is generally more astigmatism, reduction of vision, and recurrences compared to studies in adults. In children with geographic or disciform keratitis, 89% had reduced visual acuity, 78% had induced astigmatism, and 87% had recurrences. A prior study of HSV keratitis in 21 children also emphasized the frequent recurrences and visual loss in children (116).

Manifestations in the Immunocompromised

Patients undergoing chemotherapy, organ or bone marrow transplant recipients, and patients with HIV infection can develop multiple and extensive lesions involving both the cornea and the retina, and in some cases visceral spread may occur (117–119). Recurrence rates of HSV keratitis are approximately two times higher in immunocompromised patients (119).

The incidence and clinical profile of ocular HSV were compared among patients who were positive and negative for HIV (119). Seven cases in the HIV-positive group were identified and contrasted with 27 cases in the HIV-negative group. There was no statistically significant difference between the groups for any of the outcome measures except for recurrence rates. The recurrence rate was 2.5 times more frequent among patients positive for HIV. Except for recurrence rate, the incidence and clinical course of HSV keratitis in this study were no different among patients positive and negative for HIV. Unlike cytomegalovirus (CMV) and VZV infections, ocular HSV does not seem to be a major problem among HIV-positive patients. This study did not confirm a lower incidence of stromal keratitis, as suggested in a prior study (120). There is at least one report of simultaneous HSV-1 and -2 infecting a cornea in a patient with AIDS (74).

ARN is a late event in the course of immunosuppression in patients with AIDS (102). VZV causes most cases although some have been associated with the HSV. There is no preventive or curative efficient treatment. ARN might be considered as another disease caused by an opportunistic infection because of its rapid clinical evolution and severe prognosis.

DIAGNOSTICS

HSV epithelial keratitis can usually be recognized by its biomicroscopic appearance and laboratory tests are not usually performed. The dendritic ulcer of HSV stains positive with fluorescein along the length of the ulcer base but the swollen epithelial borders are actually raised and stain negatively with

fluorescein. Rose Bengal and Lissamine Green, both of which stain devitalized cells, are typically taken up by the swollen epithelial cells at the ulcer's border and are seen on biomicroscopic exam.

Although most herpes virus infections can be diagnosed clinically, specific tests may be required when there is a dilemma. Scrapings from a vesicle can confirm the characteristic changes of herpes simplex infection in cytology (giant cells or intranuclear inclusions of HSV by Giemsa or Wright stains) or in immunologic exfoliative cytology (with direct or indirect immunofluorescent techniques, immunoperoxidase systems, or ELISA methods). The HerpChek is a 5-hour immunoassay designed to detect HSV antigen in *Chlamydia* transport media, allowing concurrent cell culture from the same specimen.

The HSV can be cultured from 70–80% of corneal or skin ulcerations (121). HSV antigens can be detected from skin or eye lesions with a variety of laboratory techniques including nucleic acid hybridization techniques and DNA amplification methods. The polymerase chain reaction (PCR) techniques are sensitive in detecting HSV DNA or RNA in corneal scrapings or in the anterior chamber or vitreous fluid. There are no simple PCR kits to make this useful in clinical practice at this time. One study reported that clinical exam alone was just as sensitive as any immunologic test and that the combination of clinical examination with HerpChek immunologic testing did not provide greater cumulative sensitivity (122).

The intraocular disease is also usually detected by clinical exam alone and occasionally with the aid of specific tests, such as fluorescein angiography. As intraocular fluids become available through surgery, they can be analyzed for HSV by the above techniques. The anterior chamber fluid may be assayed for HSV in idiopathic cases of intraocular inflammation, although this is not commonly performed in the United States because of the potential risks of the procedure and confidence in the clinical exam.

Acute and convalescent blood samples for HSV antibodies can confirm an acute primary infection with HSV but generally are not helpful in ocular recurrences, since serum antibody titers can fluctuate independently from clinical recurrences. The local production of anti-HSV antibody in aqueous has been measured and used to confirm the diagnosis of HSV in some cases of anterior uveitis. The Goldmann–Witmer coefficient, the ratio of antiherpes antibody in serum and aqueous humor compared with the ratio of total immunoglobulin G in serum and aqueous humor, can be used and appears to be specific for local antibody production (123).

TREATMENT

Medical Treatment

The historical treatment of epithelial HSV has been with topical antiviral agents. There have been three topical antiviral eye medications (idoxuridine,

vidarabine, trifluridine) for HSV in the United States, but only trifluridine is presently available. Acyclovir ophthalmic ointment is not available in the United States. These are all effective and nearly equivalent. Adding topical interferon speeds epithelial healing but remains a research drug. These antivirals are used to treat epithelial disease, but also as prophylaxis for recurrent epithelial HSV in high-risk situations such as with the concomitant use of topical corticosteroids or after corneal transplant surgery (Table 2).

The stromal disease and anterior segment inflammation (anterior uveitis, trabeculitis, endotheliitis) requires the addition of anti-inflammatory agents; NSAIDs have not been found to be effective in this disease (Table 3). Patients usually require continual topical corticosteroid with a very careful, prolonged reduction schedule, using different techniques (i.e., dose reduction or frequency reduction) varying with the patient or disease process.

Disciform endotheliitis and trabeculitis are usually exquisitely sensitive to topical corticosteroids, and early intervention leads to complete resolution.

Table 2 Indications for Antivirals in the Treatment of Different Forms of HSV Ocular Disease

Topical antivirals (only trifluridine is available in the U.S.A.)
HSV blepharitis
HSV conjunctivitis
HSV epithelial keratitis
Prophylaxis for corticosteroid treatment of stromal keratitis
Oral antivirals (acyclovir, valacyclovir, famciclovir are probably equivalent in ocular HSV)
Primary HSV infection
Intraocular HSV infection
Endotheliitis
Iritis/iridocyclitis/trabeculitis
Immunocompromised patients
Pediatric patients (where may be noncompliant with topical medication)
May be substituted for topical therapy if local toxicity or for ease of compliance
Prophylaxis for patients following corneal surgery in patients with history of ocular HSV (because of the high risk of HSV recurrence)
Prophylaxis for recurrent ocular disease in selected cases of ocular HSV (prophylaxis is of modest benefit but at substantial cost)
Intravenous antivirals (acyclovir, valacyclovir, famciclovir are probably equivalent in ocular HSV)
Any severe HSV intraocular disease
Acute retinal necrosis
Acute optic neuritis
Immunocompromised patients with severe disease
Any severe disease unresponsive to topical or oral antivirals

Table 3 Indications for Corticosteroids in the Treatment of Different Forms of HSV Ocular Disease

Topical ophthalmic corticosteroids

HSV immune stromal keratitis

HSV endotheliitis

Inflammatory keratitis caused by various HSV epithelial diseases

HSV Iritis/iridocyclitis/trabeculitis

Oral corticosteroids

May be helpful in selected cases of the above especially if more severe and/or bilateral disease

Usually used in conjunction with the topical corticosteroids

Avoid corticosteroid use

HSV conjunctivitis

HSV epithelial keratitis alone

In general, corticosteroid use in ocular HSV should be initiated and monitored by an ophthalmologist

Some milder cases may resolve spontaneously but some cases lead to smoldering recalcitrant inflammation.

A number of antiviral agents are available to treat intraocular forms of HSV including posterior uveitis, vasculitis, ARN, chorioretinitis, papillitis, and optic neuropathy. Oral acyclovir is the best studied and has been shown to reach therapeutic levels in the eye. Although not yet compared in HSV eye disease, valacyclovir and famciclovir appear to be equivalent to oral acyclovir in nonocular disease and provide a more convenient dosing schedule. Intravenous acyclovir is recommended in more severe intraocular involvement. ARN treated with intravenous acyclovir hastens resolution of retinal lesions but does not appear to prevent the development of retinal detachment. Adjuvant therapy with antithrombotic therapy (to prevent the vascular obstructive complications), corticosteroids (to suppress the intraocular inflammation), and prophylactic laser photocoagulation (to reduce the incidence of retinal detachment) usually accompanies the use of acyclovir.

The HEDS was supported by cooperative agreements between the National Eye Institute and the National Institute of Health. These multiple cohort and randomized clinical trials about ocular HSV of the anterior segment of the eye have generated a number of reports. Posterior segment involvement is uncommon and there are limited clinical trial results on treatment of ARN, posterior uveitis, papillitis, and optic neuropathy.

The HEDS hypothesis and results are as follows:

Is oral acyclovir useful in the prevention of herpetic eye disease in patients who had a history of HSV eye infection during the last year? Although acyclovir prophylaxis has a global impact on the reduction of herpetic eye disease, this oral therapy is only recommended in cases of previous

stromal keratitis. The treatment of seven patients during the year can prevent one episode of stromal keratitis recurrence, potentially reducing visual loss (124).

Can stromal keratitis and iridocyclitis be prevented in patients with epithelial HSV keratitis receiving trifluridine eye drops by the additional use of oral acyclovir? There was insufficient recruitment to reach statistical significance but it tended to show no significant differences between the groups (125).

What is the effect of adding topical steroids to patients with HSV stromal keratitis already receiving trifluridine? Although steroids improve healing times, they do not make a significant difference in the visual outcome (125).

What is the effect of adding oral acyclovir to patients with HSV stromal keratitis already receiving trifluridine and topical steroids eye drops? There was no statistical difference, suggesting that patients do not benefit from receiving oral acyclovir in that circumstance (91).

What is the role of oral acyclovir in patients with HSV iridocyclitis who were already receiving trifluridine plus topical steroids? The patients in the treatment arm seemed to show a marginal benefit with regard to recovery rates, as compared to those receiving placebo (126).

What is the effect of oral acyclovir therapy for recurrences of HSV epithelial keratitis and stromal keratitis? Long-term suppressive oral acyclovir therapy reduces the rate of recurrent HSV epithelial keratitis and stromal keratitis. Acyclovir's benefit is greatest for patients who have experienced prior HSV stromal keratitis (127).

Other studies prior to the HEDS reported that oral acyclovir was effective in prophylaxis of recurrent epithelial HSV, following a prior episode provided the acyclovir is continued (128). Because of the high incidence of HSV epithelial keratitis following corneal transplantation, prophylactic oral acyclovir is routinely administered for several months after transplantation with reduction in the rate and duration of recurrences of HSV (129).

Surgical Treatment

Since ocular HSV usually causes unilateral corneal scarring, corneal transplant surgery can be approached in an elective fashion in most situations, with foreknowledge of the increased risk of failure, rejection, and recurrence of HSV in these patients compared to other diseases that require corneal transplantation.

Surgical intervention is also indicated in selected circumstances once it is recognized that medical therapy has failed and progressive structural damage (including the threat of perforation) is continuing (Fig. 14). In this instance, the surgeon may be removing the nidus of viral antigen or infection, or removing an edematous cornea as a stimulus for advancing

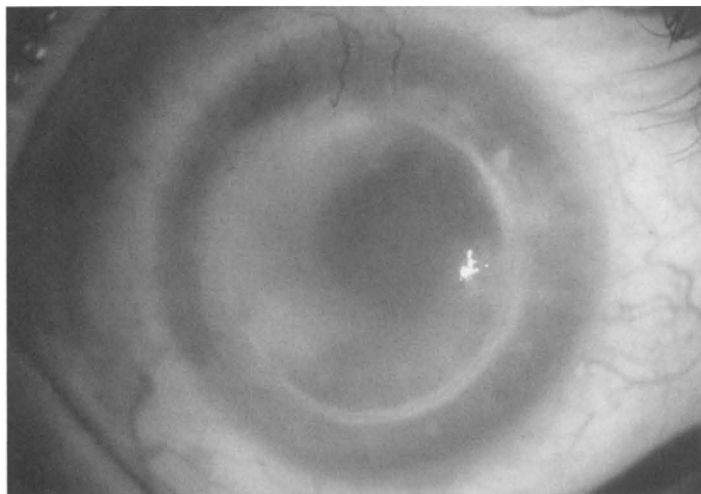


Figure 14 HSV-failed corneal transplant. Patient previously received a corneal transplant for severe corneal inflammation caused by HSV immune stromal keratitis. The graft remained clear for six months but then developed an immune rejection demonstrated here with medically unresponsive corneal edema.

vascularization, or eliminating the target of other forms of microbial keratitis. Allowing these conditions to smolder increases the degree of corneal vascularization and jeopardizes future successful corneal surgery.

Diffuse and chronic corneal edema (bullous keratopathy) may be the aftermath of repeated attacks of endotheliitis; it predisposes to corneal melting and corneal infection and may benefit from penetrating keratoplasty (39). Persistent trabeculitis and inflammatory response can lead to chronic glaucoma from structural damage to the trabecular meshwork. When it does not respond to medical measures, consideration should be given to glaucoma filtration surgery.

Although there are several reports on surgery in patients with HSV disease, there have been few studies of the incidence of the procedures. The prognosis for both elective and emergent corneal transplantation for HSV has improved over the past several decades. Transplantation immunologic reactions may still limit the number of successes. Ten percent of 3200 corneal transplantations in the United Kingdom between 1987 and 1991 were performed because of HSV keratitis (130). The expected long-term survival for first transplants in quiescent corneas with HSV keratitis was 70% compared to 45% in an earlier time period (131); the authors relate this improvement to prompt removal of loose sutures, concurrent antiviral treatment with immunosuppression during rejection episodes, and prompt treatment of recrudescant HSV disease.

Five-year survival data from the Australian Corneal Graft Registry indicate that grafts in patients with a history of HSV who remain recurrence free following transplantation enjoy surgical success (83%) equivalent to those grafts in patients with no history of HSV keratitis; it is viral recurrence which has a major effect on graft survival (132). Penetrating keratoplasty in patients with herpetic stromal keratitis in the quiescent stage results in fewer recurrences of epithelial and stromal HSV in the long term compared to controls with no penetrating keratoplasty (132). Oral acyclovir is now routinely being administered in the postoperative period by most corneal surgeons (133).

CONCLUDING COMMENTS

HSV ocular disease continues to cause significant visual disability in both developed and undeveloped countries. Even in this era of better hygiene and smaller families, HSV disease is still being transmitted to almost all individuals, with the first encounter frequently during adulthood. The ocular disease is complex since there are elements of viral replication and immune reaction that contribute to the severe manifestations, and multiple therapies must be directed at these diverse manifestations of the disease. The HEDS has significantly improved our knowledge of treatment but the epidemiology, recurrence rates, and morbidity from ocular herpes have not improved significantly since the introduction of potent antiviral drugs. Newer antivirals may improve the prognosis. More research is being performed to dissect the elements of the immune and viral response so that more effective therapeutic agents can be developed to counteract the various elements of the disease.

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Herpes Simplex Encephalitis and Other Neurological Syndromes Caused by Herpes Simplex Virus-1

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INTRODUCTION

Herpes simplex encephalitis (HSE) is a rare disease manifestation, although herpes simplex virus (HSV) infections are ubiquitous the world over. HSV is, however, the most common cause of nonepidemic, acute fatal encephalitis in the western world (1,2).

HSV has the ability to invade the central nervous system (CNS), and replicate in neurons and glia cells, and produce an acute, focal, necrotizing encephalitis localized in the temporal and subfrontal regions of the brain, often with a progressive course (3). The HSE diagnosis was established in 1941, when HSV was isolated from the brain tissue of an infant with acute necrotizing encephalitis for the first time (4). An adult with necrotizing encephalitis was diagnosed with HSE shortly thereafter (5).

HSE is a devastating disease; during the natural course, approximately 70% of patients die and only a tenth of survivors recover completely (1,6). After several antiviral trials performed in the 1970s, a breakthrough came

during the 1980s, when two independent trials showed that aciclovir was superior to vidarabine in attaining a significant improvement in the natural history in HSE with reduced mortality and morbidity (7,8). In the mid-1980s and early 1990s, the development and use of nucleic acid (NA) amplification-based techniques, mainly the polymerase chain reaction (PCR), revolutionized the diagnosis of HSE. Application on cerebrospinal fluid (CSF) made it possible to use this noninvasive technique to replace brain biopsy as a standard of diagnostics (9). Today, the high specificity and sensitivity of PCR allow initiation of early antiviral treatment on suspected cases and withdrawal of the therapy in cases not proven to be HSE (10). Furthermore, the progress in neuroimaging with magnetic resonance imaging (MRI) has contributed greatly to our ability to recognize HSE and to distinguish it from other processes in the brain (11). Despite the therapeutic and diagnostic advances made, HSE remains a difficult management problem with a significant mortality despite antiviral treatment (1,12) and with a majority of the surviving patients suffering from neurological sequelae (12,13). Expanding knowledge of the pathogenesis of HSE will hopefully lead to more effective treatment.

In this chapter, we consider the clinical manifestations of HSE in the non-compromised host (immunocompromised aspects are analyzed in Chap. 14) and describe the diagnostic procedures. Pathogenesis is briefly discussed (more detailed in Chap. 4), as are relevant differences between HSE in adults and children (except neonatal encephalitis, which is found in Chap. 15). Finally, the therapeutic aspects and current recommendations are summarized.

EPIDEMIOLOGY

HSE may occur at any age with an estimated incidence between two and four persons per million per year (7,14–16). There is no seasonal predominance of particular time of year (15,17). About one-half of HSE cases are older than 50 years of age, one-third less than 20 years of age, and more than 10% are between 6 months and 10 years old (17). In a pediatric study, 24/38 (63%) were between three months and three years of age, and 14/38 (37%) were 5–16 years of age (18). It is believed that both sexes are equally affected by HSE (7,17), but a slight predominance of males was found in an adult study (12), in a mixed material of adults and children (8), as well as in children alone (18).

According to seroepidemiological studies, approximately one-third of HSE cases were caused by primary infection and two-thirds resulted from secondary infection, i.e., either reinfection or reactivation (8,19). It has been speculated that primary infections should be more common in children than in adults, since patients with a primary infection had a lower mean age (mean 15 years) compared to patients with a prior infection (mean 50 years) (8). However, in the largest pediatric study dealing with this issue, the authors found a primary infection in 6/22 patients (27%) compared to

16/22 (73%) with a prior infection, i.e., detection of HSE antibodies in the CSF in early stage of the disease (18). Clinically, no differences have been observed between primary and recurrent HSE (3).

Person-to-person transmission has not been reported. Clusters of HSE occur rarely, and when studied, the viral isolates differed within a cluster (20,21). No particular risk factors have been identified in developing HSE, and a history of recurrent labial or genital herpes is not more common in HSE patients than in the general population (17,22). The disease often develops without any recognized triggering event.

HSE is caused by HSV-1 in the majority of cases, and HSV-2 etiology accounts for only approximately 2–6% of the cases (19,23,24). Although HSV-2 mainly causes meningoradiculo-myelitis when affecting the central nervous system in adults (25–27), it may cause encephalitis with clinical features similar to HSV-1 encephalitis (13,19,24). HSV-2 has seldom been isolated from CSF and brain biopsies from patients with meningoencephalitis (28) and encephalitis (19,29).

CLINICAL DISEASE

Herpes simplex encephalitis affecting children above neonatal age and adults is most often characterized by a focal, necrotizing process involving the temporal and subfrontal regions of the brain (30), but frontal, parietal, and occipital lobes and gyrus cingulae may be involved as well (3,31). It is a progressive disease and without antiviral treatment or after ineffective treatment, only 2.5% of patients recover to normal function (1). Forms with slow progression and fairly good recovery might exist (32), although the described cases often have received aciclovir (33,34), or have been immunosuppressed (35). HSV has been documented as one of the important etiologies of acute brain stem encephalitis (36,37). In studies describing the clinical picture of HSE, the symptomatology of the patients differs, which might be explained by the selection of cases due to the different diagnostic methods, e.g., brain biopsy with virus isolation (17) or mainly by PCR from CSF (12,13) or detection of intrathecal antibodies (7). In addition, the time to admission might influence the described symptoms since a more progressed disease leads to more severe symptoms, including decreased consciousness.

Clinical Presentation of HSE

Clinical symptoms found in HSE are indistinguishable from other encephalitides and it is not possible to base the diagnosis on clinical presentation only (17,38,39). A prodromal illness with unspecific symptoms, such as fever, headache, general malaise, in children often accompanied by gastrointestinal or respiratory symptoms, is present for less than a week in about half of the patients (40,41). The clinical features are acute onset with high

fever (90–100%), altered consciousness (24–97%), personality and behavior alterations (24–87%), headache (74–81%), disorientation (57–76%), seizures (33–67%), and focal neurological signs such as dysphasia (28–76%) and hemiparesis (24–40%). The biopsy-proven HSE cases in older studies (7,17) had, on admission, more decreased consciousness and higher frequencies of personality changes seizures than the patients in more recent studies (12,13,42), possibly reflecting earlier detection of HSE cases by PCR analysis of the CSF. The fever is high, 39–40°C, and the headache often starts during the prodromal phase. Usually, the symptoms progress over a course of hours to days and focal neurological deficits such as dysphasia and hemiparesis may develop (17,40). Seizures are mostly focal but may progress to generalization. Neuropsychiatric symptoms such as hallucinations and/or agitation are sometimes present on admission (43).

Children present with fever, alterations of consciousness, and often with seizures (38), the latter being more common in children than in adults (17). Small children (<4 years) are more prone to convulsions, and seizures have been noted to be brachiofacial in half of the cases. In contrast, older children more often present with consciousness disturbances and symptoms like meningeal irritation, behavioral changes, or speech disorders (18). Focal neurological abnormalities are less frequent (38) but may occur, and choreoathetosis is seldom reported during the acute stage (44). In rare cases, operculum syndromes in children have been diagnosed (45).

Brain Stem Encephalitis

This manifestation is rare and early case reports described untyped HSV brain stem encephalitis (46–50). PCR enabled diagnostics of HSV-1 (51,52) and HSV-2 (36,53,54) caused cases. The illness is characterized by myoclonic twitches, tremor, and ataxia. Cranial nerve palsies with symptoms of ptosis, anisocoria, nystagmus, ophthalmoplegia and gaze palsies, involvement of trigeminal, and/or facial nerves as well as cranial nerves IX–XII are frequently encountered. As the disease progresses, there is a risk of respiratory failure. Both adults and children may be affected (46–50,55). The course of the disease is usually monophasic although clinical relapsing/remitting cases, caused by either HSV-1 or HSV-2, sometimes occur (37,51,53).

Differential Diagnoses

A variety of CNS diseases may be confused with HSE, but a thorough history, CSF examination, and neuroimaging can be helpful in this regard. The main differential diagnoses to consider are CNS infectious diseases caused by virus other than HSV, bacteria, rickettsiae, or fungi, cerebrovascular disease, and toxic/metabolic encephalopathy (7,56). The spectrum of infectious diseases causing encephalitis varies between countries, and knowledge of the local epidemiology is required for making decisions about

adequate diagnostics. In case of mass lesion, a cerebral abscess, including tuberculoma, or brain tumors, can be mistaken for an encephalitic process if located in the temporal region (17). If a well-defined hemorrhage is revealed, vascular lesions such as intracerebral bleeding, subdural hematoma, and vasculitis have to be considered.

Toxic encephalopathy caused by alcohol or other drugs is another possible differential diagnosis (7), whereas MELAS syndrome (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) is more rare (57).

Prognosis

The prognosis of HSE has dramatically improved after the general treatment with aciclovir, which significantly reduces both mortality and morbidity after 12–18 months, as shown in the two multicenter trials of vidarabine/aciclovir performed in Sweden and the United States (7,8). However, despite such treatment, still up to a one-fifth of patients with HSE die and the surviving patients run the risk of long-term cognitive and behavioral impairments (12,13,58).

Mortality

Historically, the mortality of HSE was 70% in untreated cases and 50–54% with vidarabine (adenine arabinoside, Ara-A).

The introduction of aciclovir treatment decreased the mortality rate further, to 19–28% measured after 6 months and 18 months, respectively, when a 10-day course was given (7,8). In more recent studies, the mortality after six months has shown to be even lower, 6–14% (12,13,42), possibly reflecting identification of less severe cases (42), longer antiviral treatment periods (12,13), or a lower mean age of the patients (13,42). Death during HSE is either a direct consequence of the encephalitis or due to nosocomial infections.

Morbidity

In the era before effective antiviral treatment, 11% of survivors with HSE recovered their normal function (1). In the two multicenter antiviral trials of vidarabine/aciclovir in Sweden and the United States when a 10-day course of aciclovir was used, assessments six months after HSE revealed that 56% of the 27 patients who received aciclovir in the Swedish study had none or minor sequelae, while 11% had moderate and 15% severe sequelae (7). In the American study, 38% of 32 patients had no or minor sequelae, while 9% had moderate and 34% severe sequelae (8). Recent studies have shown similar results with good recovery in approximately a third to over half of the patients and moderate and severe disabilities in a third up to half of the patients (12,42). The most common disabling sequelae found were memory difficulties when long-term outcome was examined (13,58,59). Both verbal and nonverbal memory impairments were detected, and especially the

anterograde memory was affected (13,59), while remote amnesia was less common (59). Personality and/or behavioral abnormalities were found in almost half of the patients and psychiatric disorders such as depression, severe anxiety, insomnia, and emotional lability in up to a third. Obsessive-compulsive behavior, aggression, hyperphagia, and claustrophobia were uncommon. Anosmia, which was not always recognized by the patient, was found in two-thirds and epilepsy and mild nonfluent dysphasia were also relatively frequent (13). Psychiatric sequelae, with or without epileptic activity, have been treated with carbamazepine successfully in a few cases (60,61). Studies focusing on neurological outcome after pediatric HSE are scarce (62). In general, patients under age 30 years of age are associated with a better outcome than patients above 30 years of age (1).

Brain stem encephalitis is associated with severe neurological sequelae or death in children (47,48) as well as adults (37,51), although cases with minor neurological deficits or full recovery have been described (36,46,53).

Factors Related to Outcome

The four most important predictors in determining both mortality and morbidity are level of consciousness, duration of disease before therapy, age of the patient, and viral load. A Glasgow coma score of six or less at admission is usually associated with a poor outcome irrespective of the age of the patient (8). Patients who receive antiviral treatment within four days after onset of neurological symptoms are more likely to survive compared to patients where treatment is initiated after four days. Younger patients (<30 years) have better prognosis than patients older than 30 years of age (8). An association between a long time from admission to start of treatment and poor outcome was found in one study (13), and a delay of more than two days between admission and initiation of aciclovir was a predictor of poor outcome in another study (12).

In children, choreoathetosis, either as an early symptom or developing after discontinuation of antiviral treatment (or relapse), is associated with poor prognosis (44).

High quantities of virus cultured from the brain specimens obtained by brain biopsy predicted a poor outcome (19). Concerning the localization of lesions, MRI changes showing involvement of the medial limbic system, such as the hippocampus, and also bilateral involvement are most important for predicting sequelae in form of severe amnesia (63). A thorough neuropsychological evaluation is mandatory in the follow-up of a patient with HSE and a prerequisite for initiation of an individual rehabilitation plan.

Relapse

The incidence of relapse has varied in different studies between 3.8% and 9.5% (7,13,64) and up to 26% in pediatric HSE (65). In a study of Swedish

patients, the frequency of relapse was 13% (66). There is no established definition of relapse, and the underlying pathogenetic mechanisms of these secondary episodes of encephalitic illness are unclear. The patients often present with an acute onset of new or aggravated neurological symptoms and signs of focal encephalopathy with or without fever a time after acute HSE. The symptomatology differs between adults and children.

Relapse in Adults

A relapse of HSE in adults usually occurs between weeks to three months after the first HSE episode (7,64,67,68), but it may also develop several years after the initial disease (Sköldenberg, personal communication) (69). No particular risk factors for developing relapse have been identified, and cases are described after vidarabine (7,64,67,68) as well as after aciclovir treatment (69–71). Early relapse developing after a 10-day course of aciclovir raised the question of longer antiviral therapy (72). Neuroimaging findings at relapse have rarely showed new lesions or extended localization (66). The prognosis varies from recovery (66,70) to severe sequelae (71) and death (69,70). An ADEM-like syndrome, revealed by neuroimaging, with white matter changes developing eight months after HSE has been reported (73).

Relapse in Children

The clinical symptoms differ from adults often with choreoathetosis as a predominating symptom appearing 3–4 weeks after the onset of the initial HSE, as described mainly in children between 5 months and 11 years (44,74–80). Movement disorder seems to appear unrelated to the length of the course of the initial aciclovir therapy, as reviewed in 19 published cases (44), and may even develop during ongoing aciclovir therapy (76). Cerebral imaging often fails to show lesions of the basal ganglia (44).

In general, the outcome has been poor in children suffering from relapse with movement disorder, associated with high mortality and frequent neurological sequelae (44).

Relapses without choreoathetosis are more seldom reported in children. Fever and new neurological symptoms start days (early relapse) to several months and even years (late relapse) after initial HSE (65,78,80). Some authors have found new necrotic-hemorrhagic lesions in other parts of the brain in three patients (80), while others have observed late changes in white matter on MRI in two patients (78).

Acute Retinal Necrosis

Rarely, the retina is involved following HSE. Acute retinal necrosis (ARN) may develop months to years after the encephalitis, suggesting that retinal neurons may be a reservoir for HSV-1 (81,82). Genotyping of HSV-1 strains

in two patients with ARN following encephalitis showed identical strains in the brain and the eye indicating virus brain-to-eye transmission (83).

HSV-1-Induced Myelitis

Herpes simplex virus may occasionally cause infection in the spinal cord. In these cases, HSV-2 seems to be the causal agent more often than HSV-1 (84). Cases are described in both adults (84) and children (85). The onset of disease may be acute or insidious (84). The main features at neurological examination are decreased sensory functions, paresis of the extremities, bladder, and anorectal dysfunctions. Preceding symptoms from the gastrointestinal or the respiratory tract are usually seen the week before onset of myelitis similar to encephalitis (84). The HSV-1 myelitis involving the thoracic spine or cervical spine may be transverse or ascending (86,87). The HSV-2 myelitis may start in the lumbosacral region and progress further cranially (84,88). The CSF often shows inflammatory signs with pleocytosis and increased protein. The diagnosis can be established by the demonstration of HSV DNA in the CSF by PCR and intrathecal synthesis of HSV antibodies (84). Neurodiagnostic studies with MRI reveal enhancement of the spinal cord with low signals on T1-weighted images and high signals on T2-weighted images (Fig. 1) (84). The course is usually monophasic but may be recurrent (89) and fully develops within 1–3 months. The prognosis is often very poor, although favorable outcomes may occur (90). Rapid initiation of antiviral treatment is warranted to prevent death and sequelae.

HSV-1-Induced Meningitis

HSV-1 can occasionally cause aseptic meningitis (91–97), but the majority of cases of herpetic meningitis are caused by HSV-2 (see Chap. 12: Neurological Disease in HSV-2 Infection). Although the proportion of genital HSV-1 infections is increasing in Europe and the United States (22,98), this has not been reflected on increasing cases of HSV-1 meningitis during the same time. The clinical symptoms are similar to HSV-2 meningitis, but recurrences are usually not as frequent as compared with HSV-2 (99) (Aurelius, personal communication).

DIAGNOSTIC STRATEGIES

Early and precise diagnosis is imperative since HSE and myelitis are potentially devastating diseases if untreated. The suspicion of HSE is aroused by the history, the clinical observation of symptoms and signs, by CSF pleocytosis, and further support is provided by neuroimaging findings. Accurate diagnosis of HSE requires lumbar puncture with identification of viral genome in the CSF by PCR, which nowadays has replaced brain biopsy. Confirmative virological diagnosis can be made by serology. Lumbar

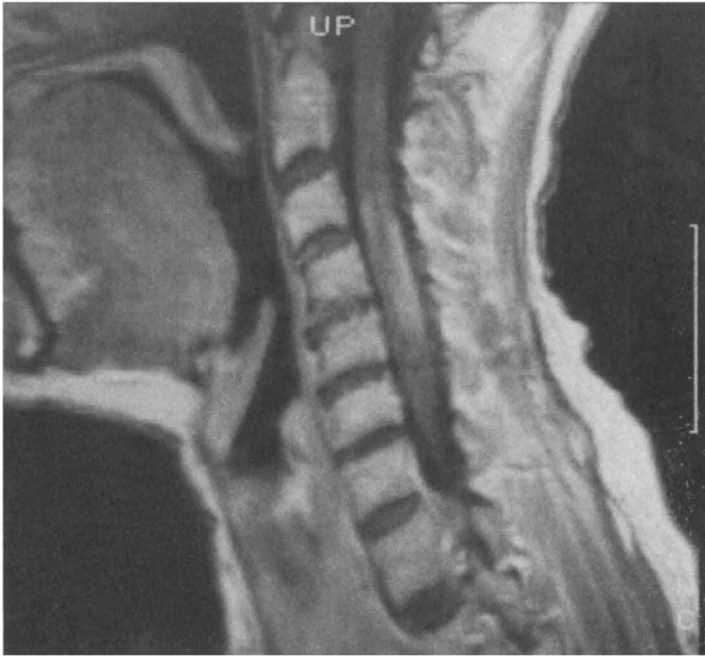


Figure 1 MR of the cervical spine from a 72-year-old woman with HSV-1-induced myelitis in the cervical spine with progressive course leading to tetraparesis. T1-weighted image showing pathologic contrast enhancement CIII-IV as a sign of myelitis.

puncture should be performed, unless the patient shows clinical signs of increased intracranial pressure. However, etiological diagnosis of the disease is most important and requires lumbar puncture. The mere suspicion of HSE warrants initiation of treatment with aciclovir as soon as possible, even before results from PCR CSF analyses are obtained.

Neuroimaging

Computed tomography (CT) scan and especially MRI have provided a better basis for the diagnosis of HSE. The specific pattern of lesions in the medial temporal lobe, which is affected in the majority of cases, gives cause to suspect HSE and renders a diagnosis of nonherpetic encephalitis less likely (12,100).

The encephalitis is initially unilateral, but if untreated, it frequently spreads to the opposite side, often with a less severe involvement (101). The insular cortex and orbital surface of the frontal lobes are frequently involved (102), as well as lesions in the basal or cingulate gyrus of the frontal lobes (12,100).

Involvement of the parietal and occipital lobes are more rare (12,100,103), as are lesions in basal ganglia, thalamus, brainstem, capsula interna, and cerebellum, which are seen only occasionally (104). If extensive lesions are found in these areas of the brain, differential diagnoses should be considered.

In children, the areas of the brain involved are usually the temporal lobes, but parietal, frontal, or occipital lesions are also found (18).

However, young children may present with radiological multifocal involvement including white matter changes, sparing the temporal and inferior frontal lobes (105,106), suggesting a vascular spread. Late developed white matter changes, which were self-limited, have been described in a 9-month-old girl (107).

Computed Tomography

CT scan of the brain is usually performed in the acute stage in order to exclude intracerebral bleeding, infarction, abscess, tumor, or to detect signs of raised intracranial pressure. The CT scan may reveal one or several major abnormalities: low-density lesions in the temporal lobe or insular cortex, mass effect, abnormal contrast enhancement, and sometimes hemorrhages (13,102). The first five days after onset of neurological symptoms changes typical for HSE may be lacking on CT (108) in a fifth to a fourth of the cases, as shown in a prospective study where 308 CT examinations from 50 HSE patients and 71 non-HSE patients were evaluated under blind conditions (100), as well as in a retrospective study of 91 HSE patients (12).

Repeated CT scans further increase the sensitivity and demonstrate focal areas of hypodensity (necrosis and edema), located in the temporal lobe (in almost 90%) (12,100) (Fig. 2A and B), in the inferior part of the frontal lobe and subjacent insular structures, and in the gyrus cinguli. Lesions are visualized before contrast is given, although intravenous contrast enhances the ability to differentiate HSE from other lesions (100). A gyriform pattern of enhancement can appear, which represents abnormal permeability of contrast material from damaged blood vessels and brain tissue (101,109). Brain shift of the midline structures, i.e., mass effect, parallels the increasing size of the low-density lesions, and the expanding area may also diminish the size of the lateral ventricle (40,100,109). If hemorrhages are present, they may appear as a well-defined mass or streaks of increased density and can develop later (102) (Fig. 3A and B). Follow-up CT may show atrophy, cystic necrosis of involved areas, and calcifications, the latter more commonly found in children (101).

Single Photon Emission Computed Tomography

This method visualizes the regional blood flow after intravenous administration of either ^{99m}Tc -hexamethylpropyleneamine oxime or ^{123}I -iodoamphetamine. Single photon emission computed tomography may

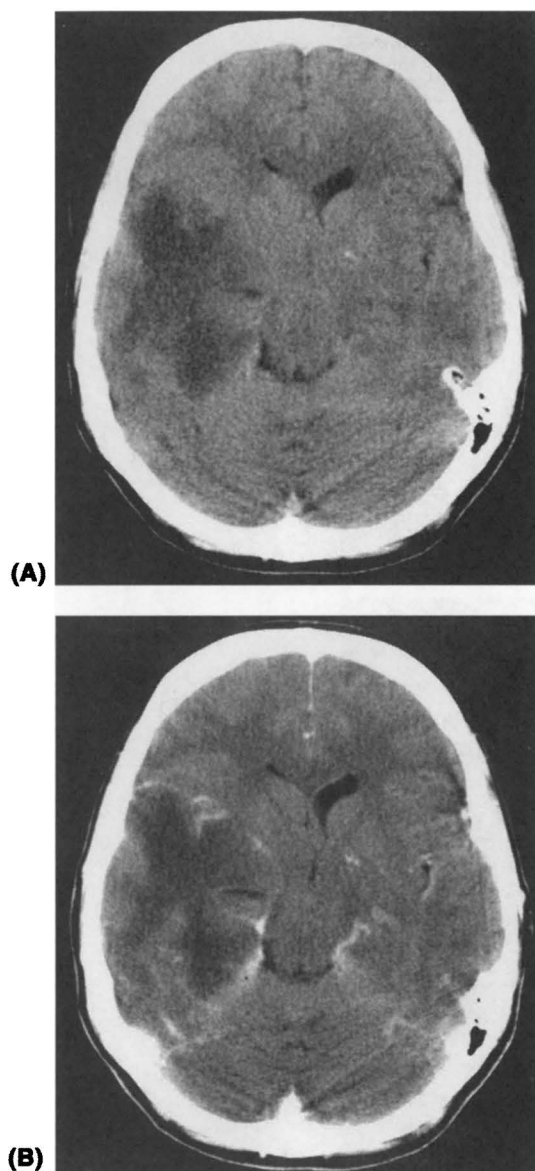


Figure 2 CT scans on the day of admission from a 58-year-old woman with herpes simplex encephalitis with fever, headache for five days, and disorientation at arrival to hospital. Before contrast (A), an expansive low attenuated lesion in the right temporal lobe with a finger-like oedema with a distribution in the temporal and parietal lobe is seen. After contrast (B), peripheral hyperemia is seen but no definite damage to the blood brain barrier. The appearance is concordant with herpes simplex encephalitis but could be mistaken for a low-grade astrocytoma.

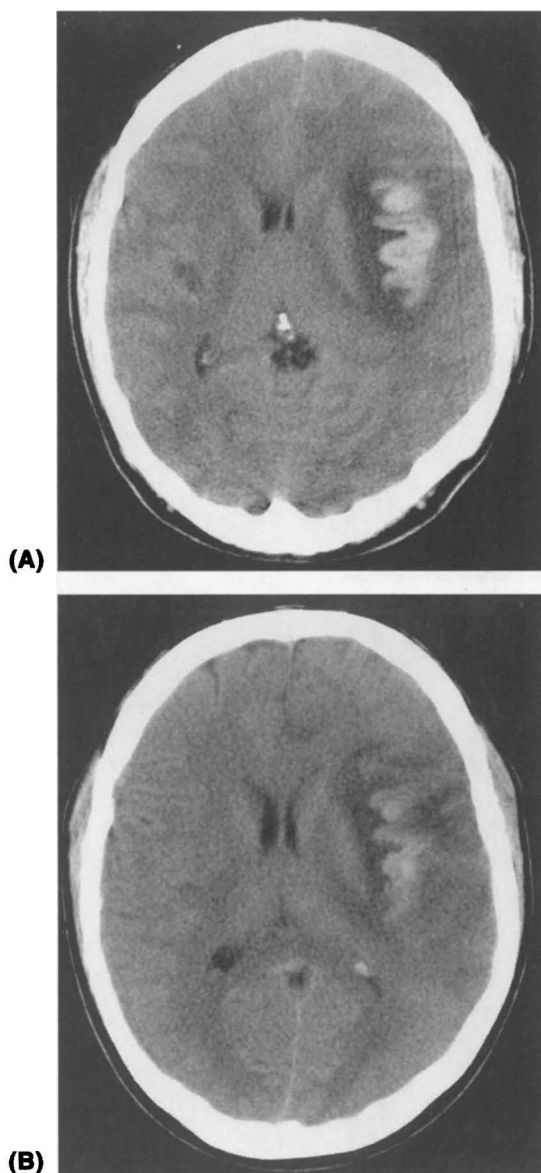


Figure 3 CT scans in the acute stage from a 27-year-old man with herpes simplex encephalitis with symptoms of fever, headache, and progressive disorientation during 10 days before admission to hospital. CT scan without contrast on the day of admission (A), reveal an expansive intracerebral hemorrhage in the insular region of the left temporal lobe with a large edema, causing compression of the left ventricle. CT scan without contrast after 6 days (B) shows gradual resorption of the hemorrhage and decreased edema.

be used to discriminate between HSE and other encephalitides in the early stage of disease, when hyperperfusion is seen in the temporal lobes in HSE (Fig. 4). Other encephalitides usually lack hyperperfusion (110–112). However, hyperperfusion can also be demonstrated in stroke and focal epilepsy though to a lesser degree than in HSE (110). The mechanism behind this focal hyperactivity is unknown, it may be secondary to inflammation-related hypermetabolism (111). At later stage of HSE, hypoperfusion is developed in the affected areas (110).

Magnetic Resonance Imaging

Cerebral MRI has greatly contributed to early diagnosis of HSE and has demonstrated superiority over CT as a neuroimaging method although extensive comparative studies are lacking (104,113–115). In early stages, edematous changes are demonstrated in the medial temporal lobes, orbital surfaces of the frontal lobes, insular cortex, and gyrus cingulate. The rapid increase of water content in the brain is visualized as hyperintense areas on T2-weighted images and as hypointense lesions on T1-weighted images (Fig. 5A and B) (112,113,115). Unilateral or bilateral inflammatory signs in the temporal lobes may be discernable on MRI before CT changes develop.

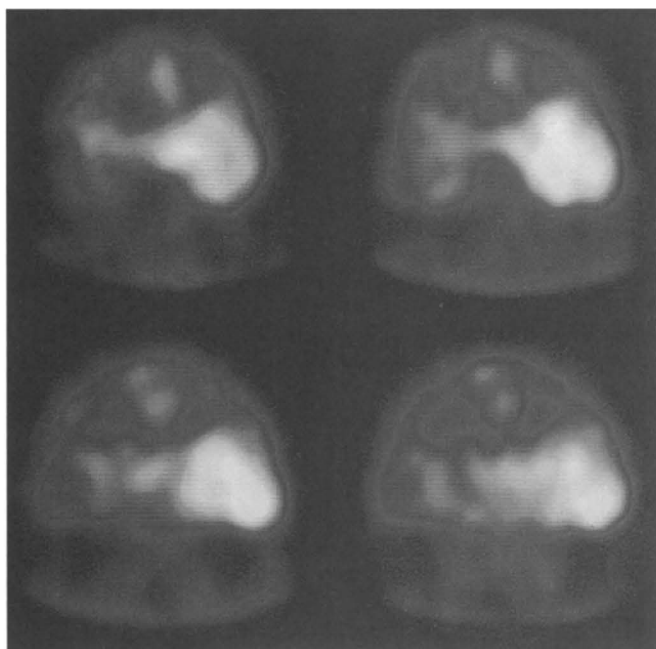


Figure 4 SPECT from a 52-year-old woman with herpes simplex encephalitis showing hyperperfusion in the left temporal lobe.

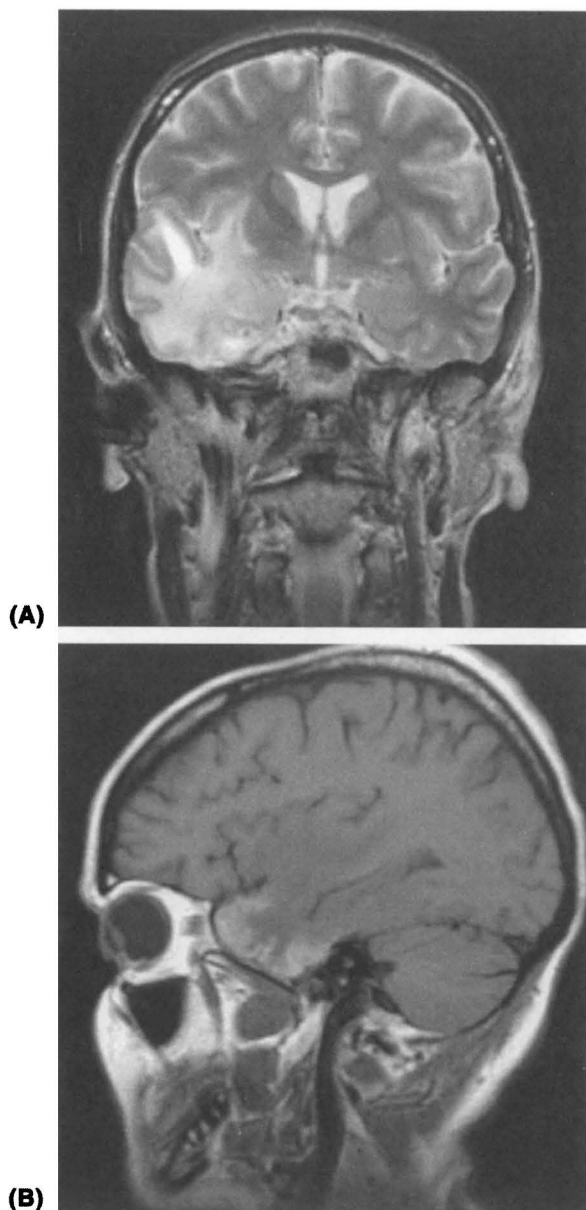


Figure 5 MR scans after two weeks from a 58-year-old woman with herpes simplex encephalitis and symptoms of fever, headache during five days and disorientation on the day of admission. T2-weighted coronal (**A**) and T1-weighted sagittal images (**B**) show subcortical edema and cortical hemorrhagic necrosis in cortex in the right temporal lobe.

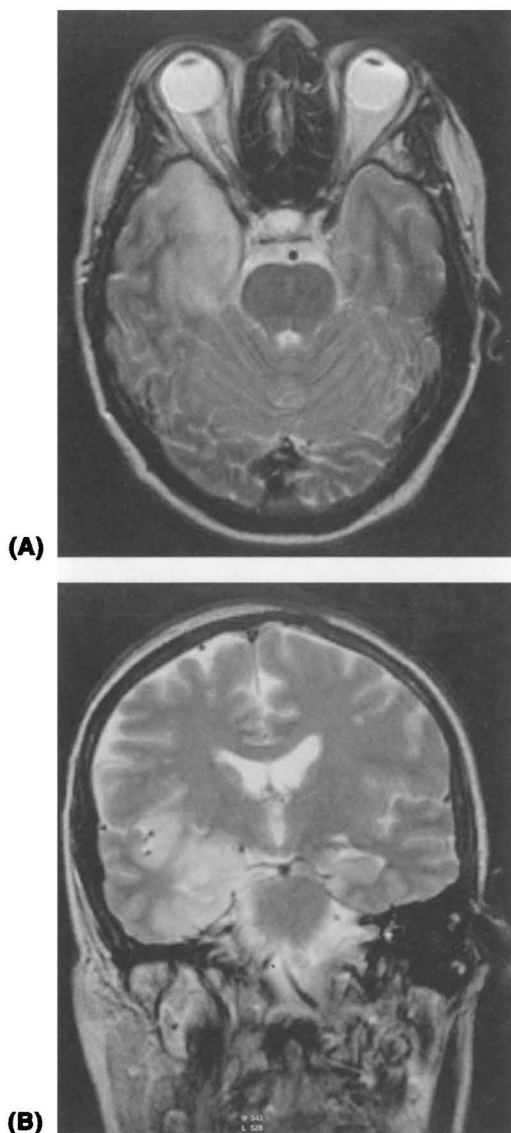


Figure 6 MR scans in the acute stage from a 53-year-old woman with herpes simplex encephalitis and symptoms of fever, headache for three days, and onset of disorientation on the day of admission. Six days after admission, axial (A), and coronal (B) T2-weighted images show a widespread edema in the ventromedial part of the right temporal lobe, dominating in the hippocampus region and in the right insula region. A minor edema is seen in the left hippocampus region. Typical picture of herpes simplex encephalitis. MR scans after three weeks and two years and nine months later, respectively, are shown in Figs. 9 and 10.

Involvement of both temporal lobes and gyrus cinguli strongly suggests HSE (Fig. 6 A and B) (113). Gyral contrast enhancement is visualized on T1-images, especially in coronal plane, and represents a disruption of the blood–brain barrier (115) (Figs. 7–9).

MRI without abnormalities during the course of HSE is unusual (116). Recently, diffusion-weighted MRI has been applied on HSE patients (117) (Fig. 10 A and B) with a differentiation between cytotoxic and hyperemic edema. After a few months of atrophy and encephalomalacia, cystic changes as well as calcifications may develop and persist for years (Figs. 10–11).

Electroencephalogram (EEG)

EEG is a relatively sensitive tool for recognizing encephalitis but demonstrates a low specificity. Pathological changes in the EEG develop during the course of HSE (40) and the pattern may fluctuate (118). Temporal abnormalities with EEG spikes, slow waves, asymmetric activity, and frontotemporal delta slowing or more nonspecific slowing can be demonstrated (42). This EEG pattern is suggestive of anatomical involvement rather than specific for the disease process and is also found in other conditions, such as infarction, abscess, tumors, and head injury.

Periodic lateralized epileptiform discharges (PLEDs) are associated with HSE and common if repeated recordings are made (118). PLEDs are not pathognomonic (119), but compatible with HSE (120). Less than a third of the patients present this EEG pattern (18,42). In children, the most common cause of PLEDs is CNS infection, and HSE is the most frequently detected among the CNS infections. However, PLEDs may also be seen in numerous other CNS diseases (121).

CSF analyses

The CSF routine analyses include the measurement of cell counts, glucose, and protein content. The CSF profile reveals pleocytosis with a slight to moderate increase of leucocytes in most cases. Lymphocytes and monocytes predominate, although polymorphonuclear leucocytes may be present as well. The pleocytosis is in the range of 20×10^6 – 300×10^6 /L with a median of 145×10^6 /L (122). Occasionally, the cell count may be normal or less than five leucocytes $\times 10^6$ /L, which is found in 3–8% of the patients (12,17,23). In children, low cell counts are more common in CSF samples drawn early (18). Erythrocytes are frequent (17) and xanthochromia may be present. The total amount of protein is elevated in the majority of patients with concentrations between 0.3 and 2.5 g/L, median 0.8 g/L, in the first CSF sample (2). The glucose level and CSF/plasma glucose ratios are seldom decreased in HSE, although a mild hypoglycorrachia is sometimes seen (17). CSF abnormalities, with signs of immune activation, persist for months or even years following HSE (123).

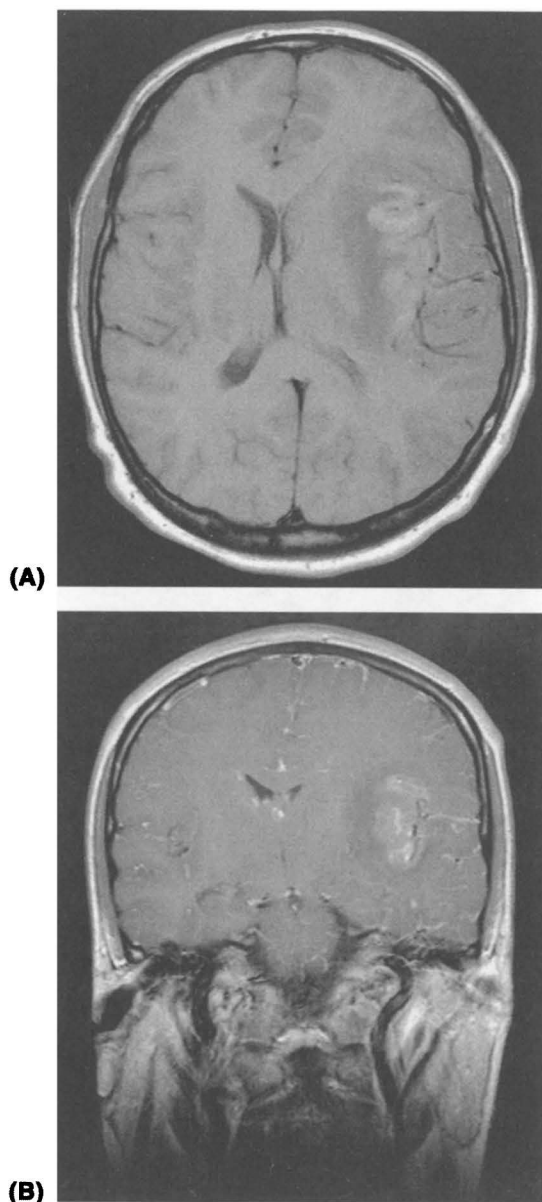


Figure 7 MR scans in the acute stage from a 27-year-old man with herpes simplex encephalitis with symptoms of fever, headache, and progressive disorientation during 10 days. After two days, T1-weighted axial (**A**) and coronal images (**B**) with gadolinium contrast enhancement show involvement of the left insular region. Hemorrhage with gyriform high signal intensity and a hyperemic reactive surrounding the bleeding. MR scans after 21 days are shown in Fig. 8.

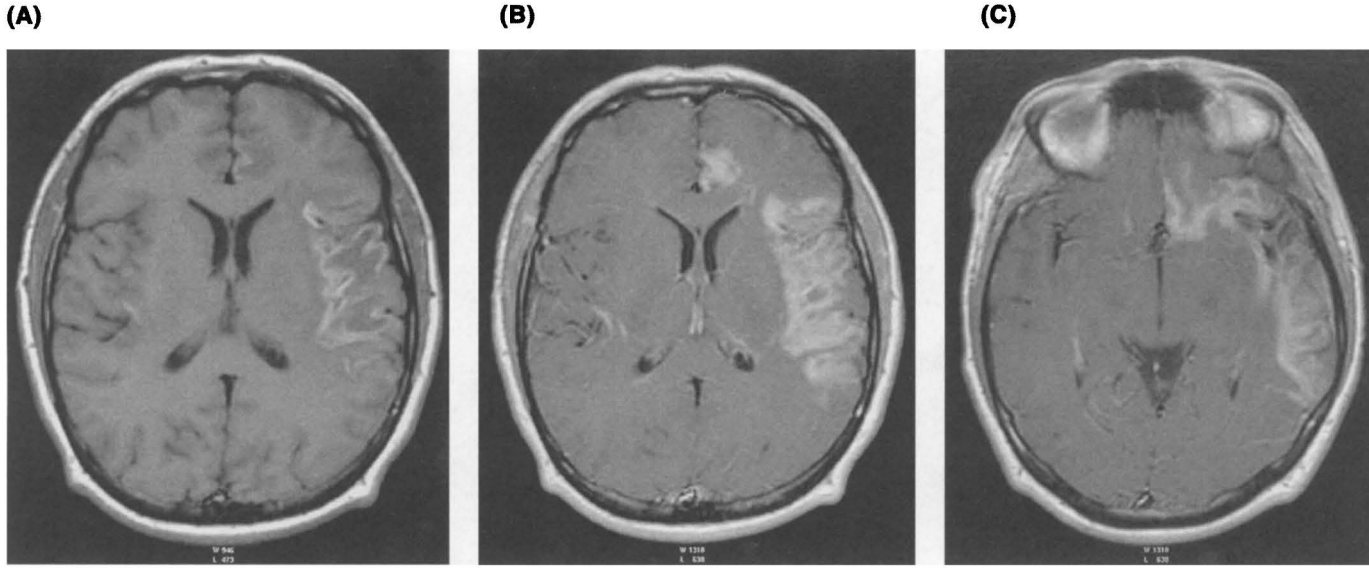


Figure 8 MR scans after three weeks from a 27-year-old man with herpes simplex encephalitis with symptoms of fever, headache, and progressive disorientation during 10 days before admission. Axial T1-weighted without (A) and with (B and C) gadolinium contrast enhancement reveal left-sided high-signal gyriform changes distributed in the cortical left frontal lobe, temporal lobe, insula and in gyrus cinguli representing cortical hemorrhagic necrosis accompanied by subcortical low signal lesions. Pathologic contrast enhancement indicate brain barrier damage (B and C).

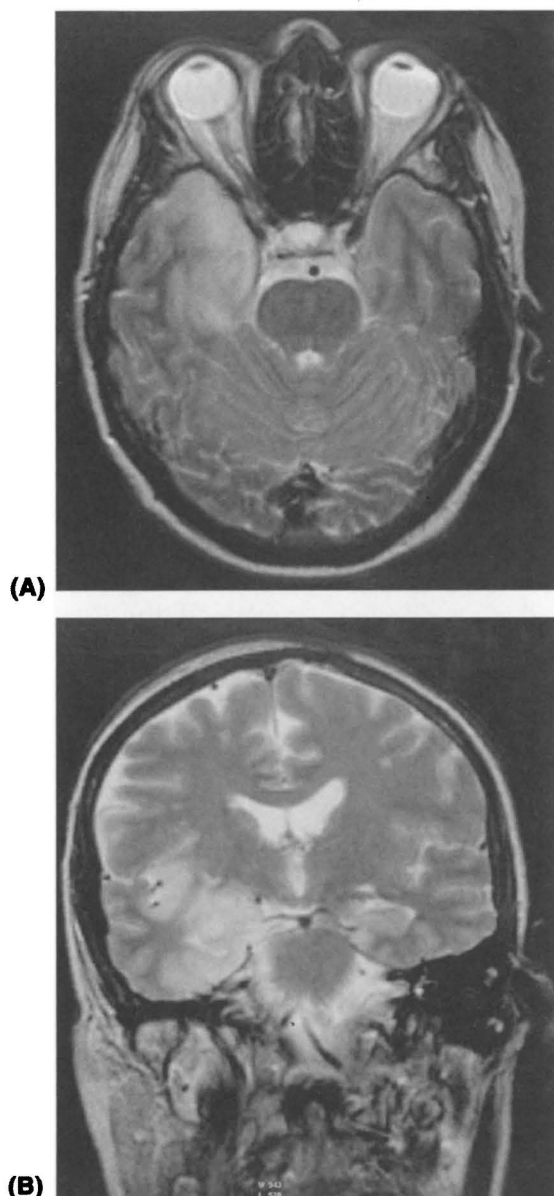


Figure 9 MR scans after three weeks from a 53-year-old woman with herpes simplex encephalitis and symptoms of fever, headache for three days and onset of disorientation on the day of admission. Axial T1-weighted without (A) and with (B) gadolinium contrast enhancement show gyriform hemorrhagic necrosis including blood brain barrier damage. MR scans in acute stage and 2 years and nine months later, respectively, are shown in Figs. 6 and 10.

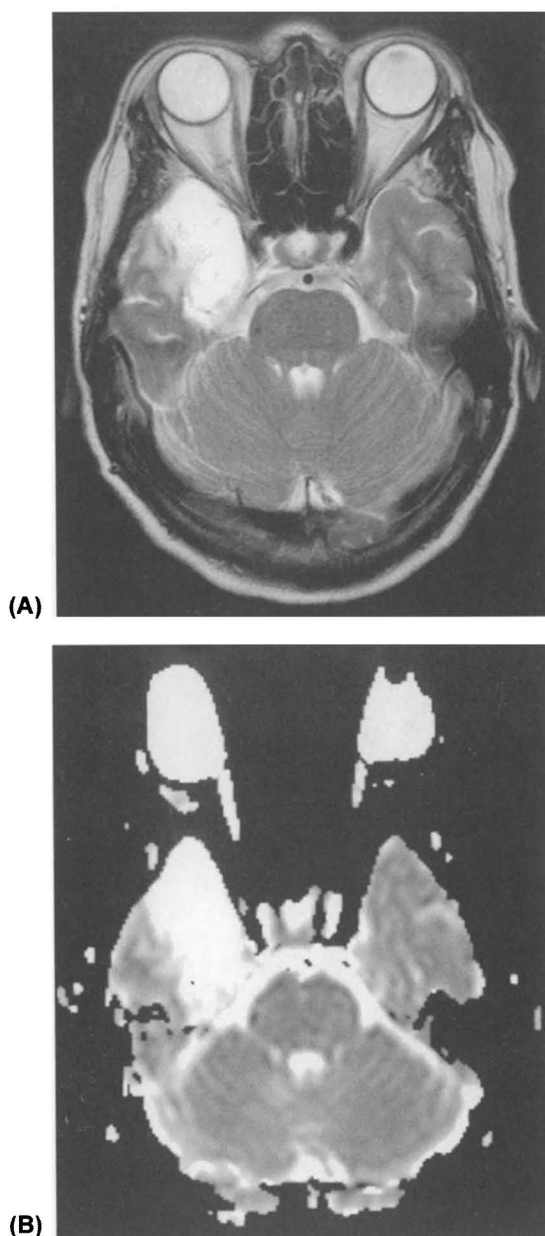


Figure 10 MR scans from a 53-year-old woman with herpes simplex encephalitis and symptoms of fever, headache for three days and onset of disorientation on the day of admission. After two years and nine months, axial T2-weighted (A) and diffusion weighted image (ADC-map) (B) show malacia in the ventromedial part of the right temporal lobe.

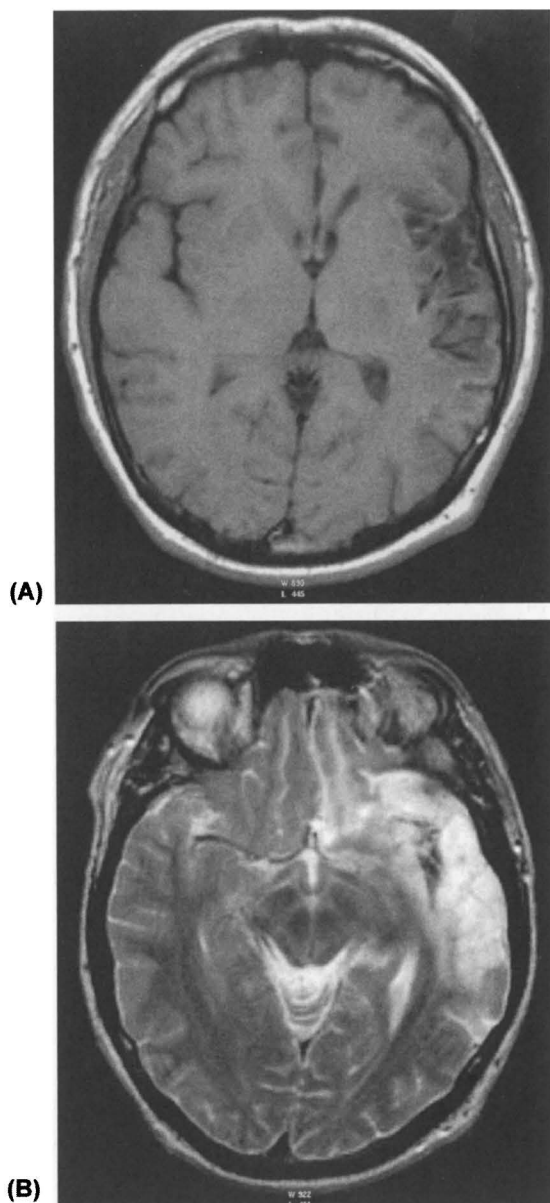


Figure 11 MR scans after three months from a 27-year-old man with herpes simplex encephalitis with symptoms of fever, headache, and progressive disorientation during 10 days before admission to hospital. Axial T1-weighted (A) and T2-weighted (B) images show atrophy and encephalomalacia in the major parts of the left temporal lobe and in the gyrus recusus of the frontal lobe. MR scans after three weeks are shown in Fig. 8A–C.

Polymerase Chain Reaction

The availability of PCR has dramatically improved the diagnosis of HSE. The advantages of PCR are several; it possesses an excellent specificity and sensitivity and is noninvasive. In addition, it is a rapid method, providing an answer from the virology department 1–2 days after the sample has arrived at the laboratory. PCR is the method of choice for diagnosing HSE and has replaced brain biopsy with virus isolation, which used to be the standard method of identifying HSV in encephalitis.

The sensitivity and specificity of different PCR assays have been investigated in several retrospective and prospective studies with a demonstration of 90% sensitivity and almost 100% specificity (9,124–134). The more widespread use of PCR has facilitated the identification of more uncommon forms of HSV infections, e.g., less severe forms of HSE (33,34), or other localizations such as occipital and parietal regions (105) or brain stem encephalitis (36,51). However, it is important to note that amplification of viral DNA may fail in CSF samples collected during the first days after onset of neurological symptoms (9,34,134). The reason for this might be a lower degree of leakage of viral DNA from the encephalitic lesion to the CSF during the first days of disease. A negative PCR test in the initial stage of illness after onset of neurological symptoms should therefore be repeated. If a second PCR test is negative, alternative diagnoses should be considered. In children, the sensitivity of PCR has been reported to be lower than in adults, with results of 79% sensitivity in early phase of the disease, and only 74% on the day of onset of CNS symptoms. An initial negative PCR result has also been associated with a low level of protein and $<10 \text{ WBC mm}^3$ in the CSF (18).

Persistence of viral DNA in the CSF occurs in virtually all cases of HSE throughout the first week after initiation of antiviral therapy and usually disappears from the CSF during the first 10–20 days of the illness (Fig. 12). However, viral DNA may sometimes still be detected even after a 10-day treatment with aciclovir has been given (9,62,128,134,135). Whether this prolonged presence of viral DNA in CSF is associated with poor neurological outcome has not been proven (128,135). However, if a positive PCR is found upon completion of 14–21 days aciclovir therapy, longer treatment should be considered (10).

Quantitative PCR

Soon after PCR diagnostics was introduced, an attempt to quantitate HSV viral DNA in CSF samples from HSE patients was made first by a semi-quantitative method (132). During the aciclovir treatment HSV-1 DNA in the CSF decrease (136,137). The amount of HSV DNA correlated with age and higher amount of viral DNA was found in comatose ($n = 4$) than in noncomatose patients ($n = 12$) (132). By a competitive PCR, the amount

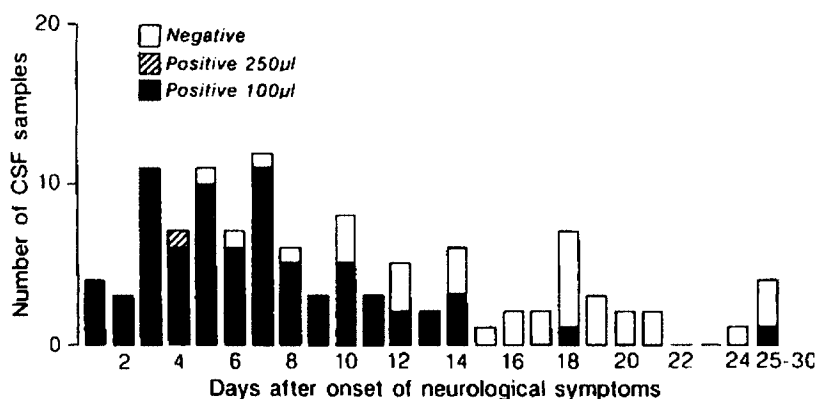


Figure 12 PCR results in CSF samples from 42 PCR-positive patients with HSE in relation to time onset of neurological symptoms. None of 36 samples taken at 30 days or more was positive.

of HSV DNA was associated with longer periods with symptoms, old age, reduced consciousness at presentation, and with focality on CT in 16 patients with HSE (136). In contrast, two other studies with eight and 23 patients, respectively, failed to correlate the amount of DNA in CSF to primary or recurrent infection, severity of symptoms, degree of brain imaging findings, and outcome (135,137). In neonates and young children, absence of correlation between amount of HSV DNA in CSF, measured by semiquantitative PCR, and outcome has been reported (138). The disparate results in these studies may be explained by a lack of a clear correlation between the amount of viral DNA copies/mL CSF and amount of virus within the brain lesions and/or by amplification differences between the various quantitative or semiquantitative PCR methods used. The new automated PCR techniques, e.g., TaqMan and Lightcycler, are promising and might answer these questions since they exhibit accuracy, are easy to handle, and enable more exact quantitation of DNA in a wide range as well as standardization between laboratories (139). The quantitative technique is certainly going to be valuable using repeated CSF samples during different treatment trials in the future.

Antibody Detection

Serological CSF analyses are valuable in confirming the diagnosis of HSE in retrospect during later phases of the disease, but of less use in the acute stage (140–144) (Fig. 13).

Detection of intrathecal virus-specific antibody response is possible after the first week of illness (2), as the antibodies gradually develop in parallel with the disappearance of HSV DNA from the CSF (Fig. 14). Simultaneously drawn serum/CSF samples must be used to detect intrathecally

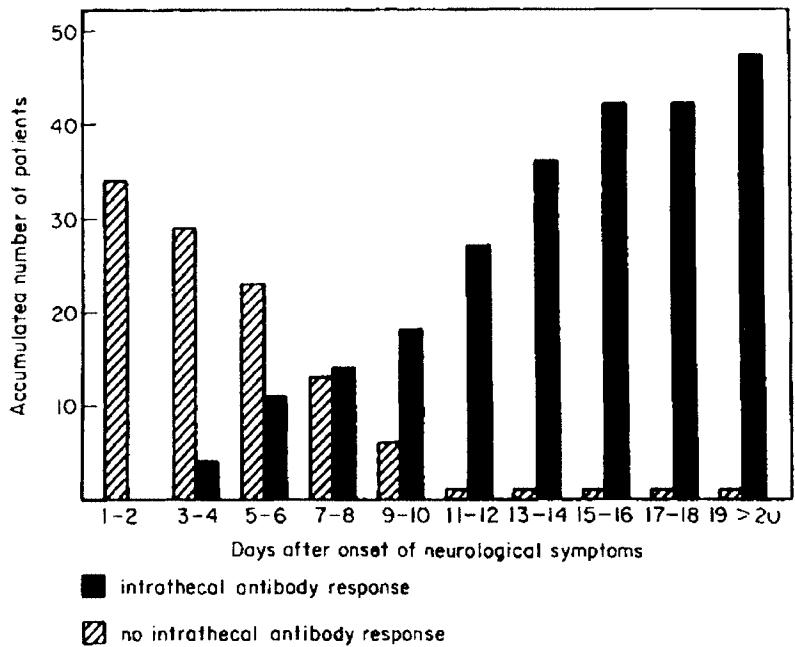


Figure 13 Intrathecal synthesis of HSV antibodies in relation to time onset of neurological symptoms in HSE.

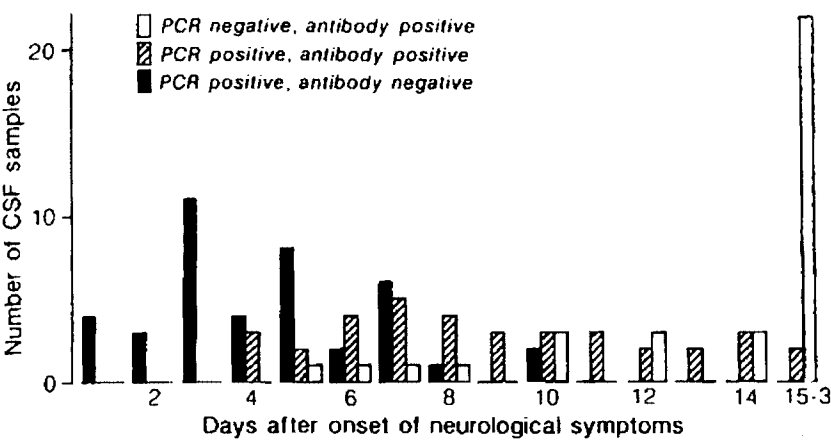


Figure 14 Time relation between PCR results and demonstration of intrathecal production of HSV antibodies in samples from 42 patients with HSE. None of 36 samples taken at 30 days or more was PCR-positive; all 36 were HSV-positive.

produced antibodies, and to exclude increased CSF HSV antibody levels due to damage of the blood–brain barrier, a non-HSV antibody (e.g., morbilli or adenovirus) and/or CSF/serum albumin ratios preferably can be analyzed in parallel. Usually, a four-fold or greater increase of CSF/serum HSV antibody ratio compared to the reference is required for diagnosis (2,10,141). IgM antibodies against HSV are rarely detected in the CSF (145). Long-term persistence of intrathecally produced antibodies has been demonstrated 4.5 to 15 years after onset of disease (2,123,141). The CSF levels of HSV-specific antibodies in the late convalescent phase correlate with poor outcome. Whether persistence of high titers of antibodies reflects continuous viral replication in the CNS or an immune reaction initially triggered by HSV replication remains obscure (123). Presence of CSF antibodies may be delayed or not develop when antiviral treatment is initiated early (146).

Antibody detection in serum is a less reliable method. In primary infection, seroconversion is demonstrable after one to several weeks, and in about one-fifth of the patients (brain-biopsy positive) seroconversion cannot be detected during the first month. Furthermore, in reactivated cases, 30% (brain-biopsy positive) do not exhibit a four-fold antibody titer rise in serum (19).

Brain Biopsy

The need for brain biopsy in HSE diagnostics has substantially been reduced as a result of the introduction of the less invasive PCR technique. Brain biopsy is associated with complication risks in approximately 3% (30). Still, there are clinical circumstances where diagnostic uncertainty remains. A brain biopsy remains an option to be considered if no alternative noninvasive method can be used to determine whether a patient is suffering from a potentially treatable disease. Sensitivity and specificity depend on the neurosurgical technique and the biopsy site chosen (147,148). When brain biopsy is performed, routine histology and specific staining for viral inclusions, fungi, bacteria, and mycobacteria should be undertaken as well as viral DNA/RNA PCR analyses, virus isolations, fungal, bacterial, and mycobacterial cultures. It is important that the biopsy is taken from the affected regions, i.e., usually the temporal lobes, and stereotactic techniques should be used.

PATHOGENESIS

The pathogenesis of HSE remains incompletely understood. How can a widespread virus give rise to a fatal CNS disease with such a low incidence and why are primarily the frontotemporal regions involved? The role of the immune system, both the cell-mediated and the humoral parts, in preventing HSE in healthy carriers, in eliminating virus during HSE, and in

contributing to further brain damage is still obscure both in primary and relapsing HSE.

Entry of HSV into the Brain

Several theories of HSV routes of access to the CNS have been suggested. In primary infection, animal models has indicated that the olfactory tract can provide a way for the virus to reach the CNS with further spread axonally into the limbic structures, including the temporal lobes (149,150). Esiri (31) found HSV antigen by immunocytochemistry in brains from patients with HSE in the olfactory tract, cortex, temporal lobes, hippocampus, amygdaloid nucleus, insula, and cingulate gyrus.

In reactivated HSE, a retrograde spread of the virus may occur from the olfactory and/or trigeminal ganglions where HSV is harbored (151). Another possibility is in situ reactivation of virus, which is supported by the fact that HSV genomes are known to be present in the medulla, pons, and olfactory bulb of patients without known neurological disease (152). A comparative study of virus isolates from brains and from cold sores in patients with HSE has demonstrated that the two isolates were different in 3/8 and identical in 5/8 of the cases; this also strengthens the possibility of reactivation inside the CNS (153).

Autopsy Findings

The neuropathological picture of HSE is characterized by an acute necrotizing encephalitis in the orbitofrontal and temporal lobes with involvement of the cingulate and insular cortex. The major study performed consisted of a material collected from patients treated with idoxuridine or cytosine arabinoside, over a 20-year period, and dying within three weeks after onset of HSE (31). The macroscopical picture of the brain at autopsy showed an acute inflammation, congestion, and softening dominating in the temporal lobes. The meninges were cloudy and congested. The inferior frontal lobes, parietal and occipital lobes may also be involved. After two weeks, necrosis and liquefaction appeared. The areas involved microscopically extended the areas that appeared macroscopically pathological. In initial stages, the changes were nonspecific, whereas necrosis and inflammation dominated later on. The capillaries and other small vessels in the cerebral cortex and subcortical white matter were congested and perivascular cuffing and hemorrhagic necrosis were present. Intranuclear inclusion bodies (Cowdry type A inclusions), surrounded by an unstained zone, were seen mostly during the first week. There were signs of acute neuronal degeneration. Both neurons and glial cells stained positive for HSV antigen with immunoperoxidase technique during the first weeks and then disappeared. Antigen was found on both sides of the brain but more abundant on one side than the other. Later on the inflammation persisted but was gradually diminished.

Reactive gliosis and fibrillary glial scarring appeared. The necrotic lesions changed into cystic spaces and the meninges were thickened by deposition of collagen (31).

Host Immunity

The immune system plays an essential, but still largely unclear, role in the pathogenesis of HSE. HSE affects mainly nonimmunocompromised patients. In these individuals, the local and lytic intracerebral HSV infection provokes an intense cytotoxicity and a hyper-reactive immune response. In immunosuppressed patients, HSV causes a subacute, often progressive, encephalopathy lacking temporal necrotizing lesions.

Genetics

The role of increased genetic susceptibility in HSE is not well investigated. A few familial cases of HSE have been reported (154–156), but in the majority of patients with HSE, there is no such association. The familial cases could possibly be attributed to an exceptionally neurovirulent or neuroinvasive strain but more probable is a genetic susceptibility for invasive HSV infection. The genotype of Apolipoprotein E (Apo E) is a host genetic factor that has been studied in HSV infection. This lipoprotein is produced within the brain and is involved in nerve regeneration. In recurrent facial herpes, one study has shown higher frequency of carriage of the type 4 allele of the gene than in controls. In contrast, in HSE patients, the frequency of carriers was similar to controls and does not seem to influence the risk for developing HSE (157).

CSF Studies on Immune Activation and Astroglial and Neural Destruction

In HSE, the CSF shows signs of extensive brain cell destruction and an immediate and vigorous immune activation restricted to the CNS, which has been proved to be long lasting. Markers of immune activation such as neopterin and beta 2-microglobulin are found early during the course of HSE and high levels correlated with severe clinical outcome as well as with mortality. These elevated neopterin and beta 2-microglobulin levels may persist >13 years after acute HSE, indicating chronic intrathecal inflammation (123). In the acute phase of disease (during the first week) pronounced increased levels of IFN-gamma and IL-6 and moderate elevation of TNF-alpha levels were found while IL-1 levels were not elevated (158). These cytokines decline rapidly, whereafter IL-2R and sCD8 increase in the CSF, and high levels persist from three to four weeks and during several years, reflecting the presence of activated T cells in the CNS. The kinetics of several proteins that reflect the disintegration of astroglial cells (glial fibrillary acidic protein, S-100-beta protein) and neurons (neuron-specific

enolase, neurofilament protein) have been investigated in HSE (159,160). Glial fibrillary acidic protein, S-100-beta protein, and neuron-specific enolase are extremely increased in early HSE and decline gradually within 45 days, while neurofilament protein peaks after a month and remains pathological up to 10 months (160).

Pathogenesis in Children

The distribution of lesions in older children with HSE is quite similar to adult HSE (115), although a higher proportion of children than adults show parietal, frontal, or occipital lesions (117). However, in smaller children multifocal involvement is sometimes seen (105). Signal abnormalities in the cortex and adjacent white matter have been described in young children, aged 4–13 months, differing from the pattern in neonates as well as in older children and adults. This vascular distribution of lesions has been suggested to be secondary to a hematogenous spread (106).

Pathogenesis in Relapse

Adult Relapse

Active virus replication in relapse has been documented in only a few adult cases, where virus was isolated from brain biopsies after 10 days aciclovir treatment (72), after cytosine arabinoside treatment (161), and after adenine arabinoside treatment (68). Failure to isolate virus from brain biopsy at relapse has also been described (67). When HSV DNA PCR diagnostics has been applied on relapse cases, viral DNA has not been found in adults (66,71,162) except for single cases (13).

During relapse of HSE, sCD8 increased while IL-10 was undetectable and sCD8/IL-10 ratios were higher than in acute stages of disease. Furthermore, the protein markers of neuron and astroglial destruction did not deviate from those in patients without relapse (66). Few adult autopsy cases of relapse have been reviewed, but signs of early cell-mediated demyelination with perivascular and parenchymal infiltration of plasmacytes and mononuclear cells without viral inclusions are described (67,68). However, one recent case with relapse five years after initial HSE showed intranuclear inclusions in the brain supporting active virus infection (69).

Immunological cytotoxicity is more likely to dominate in relapse over the viral cytotoxicity found in initial HSE. However, a continuous local or low-grade viral replication in the brain tissue is possible and might even be a prerequisite for the immunological course of events.

Pediatric Relapse

Different mechanisms are proposed behind relapse with choreoathetosis and early and late relapse without movement disorder (80). The relapse with

choreoathetosis often appears between three and four weeks after the initial HSE and an immune-mediated mechanism has been suspected due to lack of isolated virus from brain biopsies (74,76,163) and the occurrence of relapse irrespective of length of aciclovir treatment (44). In children, viral DNA has been detected in the CSF (62,65,79), as have negative PCR cases (65). In some cases, viral DNA was found later on during relapse (day 4 and 9 and 69 after relapse) (76). In children, brain biopsies ($n = 2$) and autopsy ($n = 1$) at relapse have shown diffuse edema, gliosis, and vasculitis in the meninges, cortex, and subcortical white matter changes supporting an immune-mediated pathogenesis. In addition, extensive neuronal necrosis in the temporal lobe and lack of inclusion bodies has been demonstrated (76).

TREATMENT

A rapid initiation of antiviral treatment when HSE is suspected is of utmost importance for a positive outcome. In addition, supervision of vital functions in the intensive care unit, with special attention to development of raised intracranial pressure and seizures, is necessary. Corticosteroids and anticonvulsants may be necessary and even surgical decompression in severe cases.

Antiviral Treatment

In the late 1960s and early 1970s, several antiviral drugs were compared to placebo, i.e., idoxuridine, cytosine arabinoside (Ara-C), adenine arabinoside (vidaribine, Ara-A), and found to be ineffective or associated with severe side effects. In the 1980s, two independent trials showed superiority of aciclovir over vidarabine in treating HSE. The mortality was reduced from 70% in untreated cases to approximately 50–54% with vidarabine and to approximately 20% with aciclovir, as measured after six months. With aciclovir treatment the morbidity, measured after 12 and 18 months respectively, was reduced and 56% and 38% of the patients returned to normal life in the two studies (7,8).

For effective treatment, aciclovir must be initiated as early as possible during HSE. Aciclovir should be administered intravenously at a dose of 10–15 mg/kg every eight hours as soon as the presumptive diagnosis can be made on the basis of the clinical picture, the presence of CSF pleocytosis, and neuroimaging findings (1,7). The compound is virostatic and has few side effects, the most common being rash, nausea, thrombophlebitis, reversible nephropathy, and, rarely, encephalopathy.

Previous trials of aciclovir have included 10 days of treatment (7,8), but the optimal duration of treatment length is not established and prolongation of the aciclovir treatment is discussed. Since the majority of survivors still suffer from neurological sequelae, longer antiviral therapy are used in clinical practice and now recommended, up to 14–21 days (164). Ongoing

trials will probably resolve whether the use of valaciclovir orally for an extended period (3 months) after the intravenous aciclovir will be beneficial.

Anticonvulsants

There are no separate studies on seizures during HSE, but convulsions are common during its course and often treated intravenously with phenytoin or phenobarbital and later with orally administered preparations. There are no data concerning the optimal duration of antiepileptic treatment. Usually, the anticonvulsant therapy is kept up for at least 6–12 months in patients who have had one or several seizures.

Management of High Intracranial Pressure

A continuous clinical monitoring of consciousness and neurological status to detect deterioration with signs of high intracranial pressure (ICP) is mandatory.

If a patient shows clinical signs of raised ICP, corticosteroids are used in addition to fluid restrictance, osmotherapeutics, and hyperventilation if needed.

Decompressive craniotomy is a therapeutic option in cases when conservative anti-edematous therapy appears to be insufficient.

Corticosteroids

In practice, the patient is given corticosteroids if clinical signs of elevated ICP develop. Studies to assess the effect of corticosteroids on HSE in humans are lacking and the role of corticosteroid treatment is unclear. One question is if the use of corticosteroids hampers the antiviral effect of aciclovir or if it influences the inflammatory reaction in the brain with beneficial result for the patient. After the two antiviral trials in the 1980s, this question remains unsolved (7,8). In experimental HSE in rabbits, the use of corticosteroids has resulted in a slight delay in clearance of virus and a minimal reduction in the inflammatory reaction to the viral lesion (165). In another animal model of mice, the viral load was measured and did not increase during combination therapy of aciclovir and corticosteroids (166).

Intracranial Pressure Monitoring and Surgical Intervention

The role of ICP monitoring in HSE is uncertain. Approximately one-third (15/46) of patients with HSE had initially elevated ICP (40). Continuous measurement of ICP investigated in a study of eight HSE patients, showed that ICP peaked on approximately the twelfth day of the disease. An initially high ICP and mean ICP >20 mmHg was associated with higher mortality (167). However, the role of high ICP as a predictor of outcome remains uncertain.

If a large hematoma develops within the necrotic lesions, it may cause impending uncal herniation. In some cases where patients had deterioration

with clinical signs of elevated ICP and a space-occupying lesion with or without hemorrhage, decompressive craniotomy has been performed and in a few cases resection of the temporal lobe with successful result (168–170).

Treatment of Relapse

The optimal treatment of relapse is not established. Since the pathogenesis in relapse probably is dominated by immune mediation rather than viral cytolysis, although a slow viral replication in the brain cannot be excluded (66), most patients receive a new course of aciclovir 10–15 mg/kg $\times$ 3 iv for 14–21 days concomitantly with corticosteroids in de-escalating doses.

CONCLUSION

To reduce mortality and morbidity in HSE, it is important to raise the suspicion of HSE in patients with headache, fever, and neurological symptoms, perform diagnostics with PCR, and promptly institute antiviral treatment. Quantitative PCR may be valuable as a prognostic marker and will certainly be useful in assessing virological response during future treatment trials. Intriguing questions are the pathogenesis of relapse and the role of the immune system reactions during HSE. There is a need of more efficient treatment protocols. The optimal treatment length with aciclovir/valaciclovir is currently under investigation and in future trials more potent antiviral substances as well as anti-inflammatory and antiapoptotic agents in acute HSE as well as in relapse should be investigated.

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Neurological Disease in Herpes Simplex Virus Type 2 (HSV-2) Infection

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Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) usually cause mucocutaneous lesions but may also induce a wide range of neurological symptoms. Although the two subtypes are genetically highly homologous, their respective tropisms differ and they give rise to different clinical pictures, especially after the neonatal period. HSV-2 causes vesicular lesions mainly in the genital or lumbosacral region, but the virus may also induce a broad spectrum of neurological manifestations with or without preceding or concurrent mucocutaneous lesions. The neurological HSV-2 manifestation most often reported among adult immunocompetent individuals is aseptic meningitis, sometimes with transient mild-to-moderate encephalitis, and/or myelitis and radiculitis. Neurological HSV-2 disease carries a risk of recurrences. Considerable neurological morbidity may follow with recurrent episodes of meningitis and myeloradiculitis and also a wide variety of less distinct neurological symptoms. Thus, patients with HSV-2 meningitis and/or myeloradiculitis should be identified by means of thorough history taking, careful examination, and specific viral diagnosis in order to enable adequate counseling and to aid decision-making regarding antiviral therapy. Rarely, HSV-2 induces serious neurological diseases such as classical encephalitis located in the temporal lobe, brain stem encephalitis, and necrotizing myelitis.

ASEPTIC MENINGITIS

Epidemiology

Infection with HSV-2 is common all over the world although the prevalence varies. There is an age-related increase in HSV-2 seroprevalence starting with the onset of sexual activity. In adults, the reported seroprevalence of HSV-2 is 4–40% (1). Female sex, more advanced age, and lower socioeconomic status are associated with a higher seroprevalence (2–6). When different frequencies of HSV aseptic meningitis are compared, such background data concerning the population studied have to be taken into consideration. According to serological studies made in Sweden, about 20–30% of the population are infected with HSV-2 (4,5). About a third of those who are HSV-2 seropositive are aware of mucocutaneous symptoms. Our knowledge of the extent of HSV-2-induced neurological disease, most often aseptic meningitis, is incomplete.

Meningitis is known to be a frequent complication of primary genital herpes infection (7). Corey et al. found symptoms suggestive of aseptic meningitis in 36% of women and 13% of men in a population of 268 patients with primary-episode genital herpes type 2 infection. Primary as well as recurrent meningitis may, however, appear without mucocutaneous symptoms, now known to be the most frequent presentation (8–14). The overall incidence of aseptic meningitis is reported to be about 10–20 per 100,000 adults. The variation reflects the difference in incidence as regards enteroviruses, which dominate numerically (15–19).

The frequency of aseptic meningitis due to HSV-2 has probably been underestimated hitherto due to under-reporting and underdiagnosis (i.e., the etiology of aseptic meningitis is often undetermined) and to previous limitations of the diagnostic methods. In older studies, HSV appeared to account for 0.5–3% of all cases of aseptic meningitis (15,19). In later (Swedish and German) studies of consecutive cases of acute meningitis of suspected viral origin, HSV-2 DNA was detected by PCR of the CSF in 5–8% (16,17,20). With novel improved molecular and serological diagnostic methods consistently applied in the diagnosis of aseptic meningitis, the proportion of cases of aseptic meningitis confirmed to be caused by HSV-2 is likely to increase even more. In a recent 5-year survey, an HSV-2 etiology was confirmed in 16% of 374 cases of aseptic meningitis and was strongly suspected in another 3% (21).

Being caused by a sexually transmitted virus, most cases of HSV-2-induced neurological disease after the neonatal period occur in young adults (8,7,10,22). In a large retrospective study, 82% of the patients were 50 years old or younger and only 9% were over 60 (14). The incidence shows no seasonal variation. A female predominance is striking in HSV-2 meningitis with a female-to-male ratio of about 2:1–6:1 reported (7,10,18,21,22). In a U.K. study on CSF samples received in the laboratory for diagnosis of viral CNS infection,

the relative incidence of HSV-2 was high and HSV-2 was the virus most frequently detected among young adult women with aseptic meningitis (18).

Pathogenesis

A temporal association of genital herpes and benign aseptic meningitis was noted as early as 100 years ago (23). Since then the HSV etiology of aseptic meningitis has been established unequivocally. The pathogenesis of HSV meningitis is still not fully understood, however. Hematogenous or neuronal spread from the portal of entry in the genital mucosa has been suggested. Viremic spread was known to occur in other viral infections and was suggested early for HSV-2 (24). Isolation of the virus from the blood (buffy coat leukocytes) of two adult meningitis patients, reported by Craig and Nahmias (25) in 1973, indicated a hematogenic spread. Later attempts to isolate the virus from the blood of 30 patients with clinical meningitis after primary genital herpes were, however, unsuccessful (7). Nor could HSV DNA be demonstrated in sera from 20 patients with primary HSV-2 meningitis (Aurelius, Forsgren, unpublished).

Genital lesions preceding meningitis with an interval of about a week, as well as focal neurological signs associated with the meningitis, support the suggestion of a neuronal spread. The fact that genital lesions are missing in many cases might well be explained by the fact that asymptomatic genital HSV-2 infections are common. Animal studies have shown that the virus reaches the meninges from infected sites in the periphery via neuronal routes. The virus is transported along peripheral sensory nerves, spinal ganglia, and nerve roots to the meninges (26,27) and neuronal spread is now thought to be the most important pathogenic pathway (13).

An important characteristic of all members of the alpha-herpesvirus group is the ability to establish latency in neuronal ganglia from where it can be reactivated. HSV-2 generally resides latently in the sacral ganglia, where it has been demonstrated in autopsy cases (106). After reactivation, the virus is believed to migrate along axons of sensory neurons towards the skin or mucosa, causing peripheral lesions or asymptomatic shedding. In some cases, the virus is thought to be transported towards the meninges where it may cause an inflammatory response. Extragenital peripheral lesions appearing late in the course of primary genital infection (7), after the primary meningitis, or in connection with recurrent episodes of meningitis, but not in the primary meningitis (10), may be the result of spread within the nervous system. Spread of virus to other ganglia may have occurred, even if some cases may be explained by autoinoculation. Spread within the CNS may be an early event indicated by focal neurological signs accompanying primary meningitis (10).

Attacks of headache without overt signs of meningitis and without CSF pleocytosis have been reported after herpes meningitis (8,10,22,28).

In many instances, viral reactivation does not lead to overt clinical symptoms, as shown by findings of asymptomatic shedding in genital herpes (29). Whether viral reactivation regularly leads to migration to the meninges is not known, nor if such migration necessarily leads to clinical meningitis. Viral reactivation may pass unnoticed but may, for instance, also be the cause of recurrent limited attacks of headache without other findings of meningitis, either clinically or in the CSF (10,22).

Crucial for maintenance of latency is certainly immune competence. In the otherwise immune competent individuals, several stimuli are known to trigger reactivation such as physical stress caused by trauma, other infectious diseases, hormonal changes, and psychological stress, suggesting that neuronal disturbances are important in the reactivation process (13). Such stimuli leading to reactivation of latent HSV infection are most probably relevant also for recurrences of neurological disease, for instance, recurrent attacks of meningitis are reported to follow periods of psychological stress as well as lower back trauma.

Although pathogenetic factors of decisive importance for symptomatic neurological HSV-2 disease in the otherwise immunocompetent individual have not been clarified, some properties associated with both the virus and the host have been suggested. Neuropathogenic and neuroinvasive characteristics of viral subtypes may be involved (30). The traditional locations of primary HSV-1 and HSV-2 infection to the oral cavity (HSV-1) and the genitalia (HSV-2) are no longer maintained in most populations, but the biological adaptation of these two viruses is reflected in relation to recurrences, i.e., oral recurrences are most likely to be due to HSV-1 and genital recurrences to HSV-2 (13,31–33). Furthermore, primary and recurrent aseptic meningitis is much more common in HSV-2 than in HSV-1 infection (20,21) despite the fact that HSV-1 genital herpes is increasing (34).

Among host factors, female sex is known to be a risk factor. The reason for the much more frequent neurological complications seen in women than in men is not clear. A larger total surface of infection, from where the viral load is forwarded to a larger number of neuronal ganglia, has been suggested (7,13). Furthermore, a prior HSV-1 infection indicated by a pre-existing humoral immune response to HSV-1 might be a marker of protection and, vice versa, a lack of HSV antibodies constitutes a risk factor for developing neurological disease induced by HSV-2 infection. HSV-1-specific seroprevalence in a group of young adult women with HSV-2-induced meningitis was significantly lower than in a matched control group and than was expected from seroepidemiological studies in the same geographic area (Aurelius, Bergstrom, unpublished).

Clinical Picture

Clinical symptoms of HSV-2-induced meningitis are mainly the same as those found in aseptic meningitides caused by other viruses. Headache,

usually described as intense, develops during 2–3 days (10), together with varying degrees of other signs and symptoms of meningeal irritation such as neck stiffness, photophobia, nausea, and vomiting. Fever is common but not an obligatory finding [present in 63% of 71 patients in one study (14)]. In most cases, the acute symptoms of primary meningitis resolve spontaneously within a week, although sometimes only after a protracted illness (8,7,10,19,25). Neurasthenic symptoms such as mild headache, lability, concentration disabilities, and fatigue may, however, last for several weeks (8,22). One case of chronic meningitis with predominantly headache and meningism lasting for 4 weeks has been reported (35).

Cerebrospinal fluid typically shows a mild to moderate, predominantly monocytic, pleocytosis of 400 (mean) leukocytes $\times 10^6/\text{mL}$ (range 5–1100) in primary meningitis (10,7), slightly increased protein 1.6 g/L (range 0.4–3.0 g/L) (10), a CSF:serum glucose ratio of more than 0.5, and normal lactate. Hypoglycorrhea has been reported in a number of cases (28,35–37) and occasionally a slightly increased lactate concentration can be found.

Meningitis due to HSV-2 has some characteristics that distinguish it from meningitis of other origins: the association with mucocutaneous herpetic lesions and with additional neurological symptoms, along with the appearance of recurrent disease.

ASSOCIATED MUCOCUTANEOUS LESIONS

Herpetic lesions in the skin or mucosal membranes may precede the meningitis (7,8,10,23,24,38). When present, genital herpes usually precedes meningitis by a few days to 2 weeks, and usually by about 1 week (7,8,10). The first clinical episode of meningitis may, however, also follow a previous asymptomatic acquisition of the HSV infection, as indicated by HSV-2 antibodies found in the acute phase serum of individuals with acute aseptic meningitis without a history of symptomatic herpes disease. Cases of primary meningitis in connection with peripheral lesions dominate in the literature, but with improved diagnostic methods; an increasing number of cases without mucocutaneous symptoms have been recognized (21).

ASSOCIATED NEUROLOGICAL COMPLICATIONS

Another distinctive trait in HSV-2 meningitis is the relatively high prevalence of associated neurological complications. Urinary retention, constipation, dysesthesia, radiating pain or weakness in the lumbosacral area and/or lower limbs, indicating sacral myeloradiculitis, are frequent associated findings in primary HSV-2 meningitis. Additional neurological symptoms in the acute phase of primary meningitis are found in approximately half of the patients in larger series (10,22). The prognosis is generally good with gradually decreasing and disappearing symptoms, usually within weeks up

to 1–2 months (8–10,39). In one study, zero out of ten patients had residual symptoms at 6 months (10).

RECURRENT HERPETIC DISEASE

Reactivation of the virus may give rise to new episodes of aseptic meningitis and/or mucocutaneous symptoms. Benign recurrent lymphocytic meningitis has been found to follow herpetic meningitis for a long time as described by several authors (8,24,38). However, verification of the etiology posed diagnostic difficulties because of difficulties in isolating HSV from CSF in patients with recurrent meningitis. HSV-1 was isolated in a single case, but culture of HSV-2 has never been successful in recurrent meningitis. With improved diagnostic techniques, more cases were verified. HSV-2 etiology was confirmed by demonstrating intrathecal HSV-2 antibody synthesis by immunoblot (10). Subsequently, application of PCR techniques allowed identification of HSV DNA in the CSF of a large number of patients with recurrent aseptic meningitis, predominantly HSV-2. By this method, it has now been established that HSV-2 is a major cause of benign recurrent lymphocytic meningitis and that HSV-2 cases by far outnumber those caused by HSV-1 (11,12,22,40–46).

If present, the mucocutaneous symptoms may recur either preceding or concurrently with the neurological symptoms or on separate occasions. Herpetic eruptions may also appear for the first time after the episode of meningitis. Peripheral lesions are, however, more often unrecognized or absent. Even in recurrent HSV-2 meningitis in patients with a known history of genital herpes, coincident peripheral herpetic lesions are seldom documented (8,10,14,22).

In recurrent meningitis, as in recurrent genital herpes, the subsequent episodes vary in intensity but tend to come with milder clinical and laboratory symptoms and be of shorter duration (2–5 days) than the primary attack (10,37). This may be explained by partial protection by the specific immune defense. The risk of recurrent bouts of meningitis is not fully known, but it seems to be large and new attacks are reported to occur in at least 20–30% (8,10,22). Recurrent attacks of meningitis may appear more or less frequently and after a shorter or longer period of time without symptoms. They may occur once or twice and up to 20 times or more. The symptom-free interval may vary from months to several years and even decades. Whether a shorter interval and a higher frequency of recurrences are correlated with the severity of clinical symptoms in primary infection remains unclear.

A syndrome characterized by repeated episodes of aseptic meningitis, separated by symptom-free intervals, and by a distinctive CSF cytology, was described by a French neurologist named Mollaret in 1944 (47). After an initial neutrophilic dominance, lymphocytes and monocytes predominate in the cerebrospinal fluid. A hallmark is the finding of characteristic

monocytoid cells in the CSF throughout the course of meningitis. These so-called Mollaret cells are large and fragile monocytes with indistinct cytoplasm in which vacuoles may be found and also large irregular nuclei, which are characteristically kidney-shaped or resemble footprints.

Mollaret's syndrome may be of heterogeneous, infectious, and noninfectious origin. It has been suggested to be associated with allergic, autoimmune, or chemical induction, and with dermoid cysts. A microbiological etiology of Mollaret's meningitis has been suspected for a long time. Mollaret himself suggested that the disease in some patients might be caused by a virus (48) and the disease entity has been associated with EBV and borreliosis (49,50).

Alpha-herpesviruses are ideal candidates because they are neurotropic, establish latent infection, and cause recurrent illness. Mollaret cells were demonstrated in the CSF by Picard et al. in two out of three patients with recurrent meningitis of HSV-2 origin. Most reported cases of recurrent meningitis of verified HSV origin, often termed Mollaret meningitis, have a clinical course in line with the one stated in Mollaret's syndrome, but the characteristic CSF cytology is either not found or, more often, not sought.

Whether Mollaret's syndrome is an entity separate from benign recurrent lymphocytic meningitis is a matter of debate. The author suggested an endothelial origin of the Mollaret cells. Later, ultrastructural and immunocytochemical studies have found features suggesting them to be of monocyte/macrophage lineage (51–53) and it has been proposed that these cells represent functionally activated macrophages responding to antigenic stimuli (53). Furthermore, as in Mollaret's syndrome, a transition from polymorphonuclear to monocytic dominance in the CSF is not seldom found in the initial phase of aseptic meningitis of mainly enteroviral origin, but occasionally also in herpes meningitis (15).

In any case, a search for an HSV-2 etiology should be in the first line of action in the differential diagnosis in all cases of benign recurrent meningitis. It seems reasonable that the diagnosis of Mollaret's meningitis should be made only after other recognized causes of recurrent lymphocytic meningitis have been excluded.

Encephalitic symptoms may accompany a clinical picture otherwise dominated by symptoms of aseptic meningitis. Transient encephalitis symptoms are documented in connection with primary as well as recurrent attacks of HSV-2 meningitis (10,22). In one case, attacks of meningitis as well as recurrent bouts of clinical encephalitis were seen. All attacks followed a benign course with rapidly resolving symptoms and recovery (49).

Not only episodes of meningitis, sometimes with transient encephalitis or myeloradiculitis, may follow an episode of meningitis but also a variety of less clear-cut conditions, for example attacks of headache and general malaise that influence everyday life, have been reported (8,10,22,28). These symptoms may not necessarily be thought of as possible manifestations of

the herpetic infection, neither by the patient nor the doctor. Unwillingness of the patients to seek medical advice or even denial of symptoms for psychosocial reasons may also contribute to underestimating the real impact of the disease (54).

Diagnosis

Diagnosis of herpetic meningitis begins with an awareness of its clinical presentation with and without concurrent or preceding mucocutaneous lesions and its ability to cause recurrent symptoms. A thorough history of previous herpetic manifestations, such as mucocutaneous herpes and/or bouts of aseptic meningitis, should be taken in every case of aseptic meningitis/myelitis/radiculitis.

Isolation of HSV in the CSF may be done and has been reported in several cases in primary meningitis of both types (8,10,19,55). With few exceptions, attempts to isolate HSV in the CSF in recurrent meningitis are unsuccessful and have been documented in only a single case with HSV-1 (49). In a large series, HSV-2 was recovered from the CSF in 21 of 27 patients with a primary infection, but from zero of ten with recurrent disease (10).

Viral culture from genital or lumbosacral lesions preceding or concurrent with an attack of recurrent meningitis may be employed to support the diagnosis, although a positive finding does not exclude that peripheral reactivation could be a concurrent phenomenon and thus be of no relevance to the etiology of the meningitis.

In the absence of peripheral lesions, serum and CSF antibodies have been used to establish the diagnosis. A seroconversion to type-specific HSV antigen demonstrated by ELISA may verify the diagnosis in primary infections. It is, however, noteworthy that seroconversion to HSV-2 occurs on the average after about 3 weeks, but not seldom after 4–5 weeks (10) or even later (own unpublished data). Significant rises in serum titers are usually not observed in recurrent meningitis (10).

The intrathecal antibody response in HSV meningitis has not been thoroughly studied, since successive lumbar punctures are not frequently performed for practical and ethical reasons. HSV-2 immunoblotting analysis of serum and CSF may be useful to demonstrate an intrathecal antibody response (10,46). The method may help in the diagnosis of recurrent meningitis, where the intrathecal response seems to be enhanced with increasing numbers of episodes (10). It seems to be specific [as no CSF antibodies to the type 2 selective antigen (gG2) have been found in controls with normal CSF, serous meningitis of other infectious origin ($n = 26/4$ HSV-2 seropositive) or other neurological diseases with known high HSV-2 titers in serum ($n = 11$)], but it has not been sufficiently evaluated regarding sensitivity. In contrast to serological data available for HSV encephalitis, kinetics of the intrathecal antibody response in HSV meningitis is not fully known.

An intrathecal response is probably not detectable in the very initial phase of the attack (10). In complicated, PCR-negative cases where an etiological diagnosis is needed, a later lumbar puncture to allow for CSF analysis together with serum for the detection of specific intrathecal antibody synthesis may be of value.

With the use of PCR, rapid detection of even minute amounts of HSV DNA is possible. HSV DNA, almost always of HSV-2 (although HSV-1 is found occasionally), has been detected in the CSF of several patients with primary and recurrent aseptic meningitis, both with and without mucocutaneous lesions (11,12,22,40–46,56,57).

In primary meningitis, PCR for the detection of HSV-2 DNA in the CSF seems to be very sensitive. In a comparison between isolation and detection of DNA by PCR in the CSF in the first episode of HSV-2 meningitis (verified by seroconversion to gG-2 antigen in ELISA and/or virus isolation), all isolation-positive samples were PCR-positive, whereas only seven of 16 PCR-positive were isolation-positive. The first available CSF specimen (sampled 1–16 days after the onset of meningitis) was positive in 27 of 32 patients. In samples drawn within the first week of disease, 23 of 26 specimens were positive. Negative samples were drawn on days 1, 3, 9, and 16. At follow-up, DNA was demonstrated up to day 16 (56).

The diagnostic sensitivity of PCR for detection of HSV-2 DNA in CSF in cases of recurrent meningitis has not been documented in larger-sized series, but is thought to be less than that for primary meningitis. In an investigation of nearly 100 patients with verified HSV-2 meningitis, HSV-2 DNA was found in more than 95% of patients with primary meningitis. In recurrent meningitis episodes in the same patients, HSV-2 DNA was found in approximately 75% by routinely used PCR methods (own unpublished data). In a number of cases where an HSV-2 etiology is strongly suspected on clinical grounds, e.g., in recurrent episodes in individuals having had previous episodes with a confirmed HSV-2 etiology and where extensive searches for differential diagnoses are negative, current PCR assays fail to detect HSV-2 DNA (12). This limitation should be kept in mind.

Different reasons for these “false negatives” can be suggested: (a) DNA might be present in the CSF but inhibited. The risk of inhibition by, for instance, the hem group of hemoglobin in red blood cells or other factors is well known. Proper amplification of DNA should be ascertained by adding cellular or other DNA for parallel amplification. (b) DNA might be absent. CSF may be drawn too late. DNA may have been present in the CSF but may already have disappeared; the sensitivity decreases with time. Furthermore, the DNA level in the investigated sample may be around the level of detection and the PCR assay is negative due to a random distribution, a so-called sampling effect. Minute amounts of DNA might be picked up in one sample but not in another, as shown by analysis of a large number of portions of CSF drawn on the same occasion (58).

The specificity seems to be excellent, provided precautions to avoid contamination are taken. Recurrent infection may be provoked by a variety of stimuli, including stress and exogenous infection and the HSV DNA detected might simply be a “bystander.” This is unlikely, as the presence of HSV DNA correlates with symptomatic disease and is not found in follow-up samples or in samples drawn in symptom-free intervals (12,42,45,46). HSV DNA is accompanied by intrathecal antibodies to the same serotype (12,22). Analyses of CSF samples from patients with inflammatory disorders of other known infectious or noninfectious origin or of unknown cause have yielded negative results and HSV DNA is not found in HSV-2-seropositive controls (12).

Nevertheless, although a positive finding of HSV DNA correlates well with a definite diagnosis of viral CNS infection in studies of large groups (57,59), its clinical significance has to be judged individually. In unclear cases, additional virological analyses, such as virus isolation of peripheral lesions and serology, may be helpful.

Since their introduction, PCR assays have been developed further and, with real-time PCR, quantification of the viral copies is possible. Such methods have been found to be at least as sensitive as previously used qualitative PCR assays (60–62). These methods are easily adapted for simultaneous analyses to detect other presumptive agents and they are rapid, yielding a result in hours. With a rapid virologic diagnosis, additional patient investigations may be avoided for the good of the patient and the reduction of costs. When confirmation of a result is required, as with unexpected results, this can be done within the same working day (18,62). Thus, PCR-analysis of acute phase CSF for the detection of HSV DNA provides a sensitive, reliable, rapid, and cost-effective (63) diagnosis, and should be used in the first-line routine diagnoses in all cases of primary or recurrent aseptic meningitis.

Treatment

Acute HSV-2 meningitis is a self-limiting disease that heals without specific antiviral treatment in immunocompetent individuals. In genital herpes, antiviral therapy with acyclovir was shown to be effective both in the short-term treatment of primary episodes (64) and in the suppression of recurrences by continuous prophylactic medication (65). Treatment of HSV-induced meningitis with acyclovir has been documented since the beginning of the 1980s, but there have been no controlled studies.

PRIMARY MENINGITIS

A case of meningitis after primary genital herpes apparently responding to intravenous (iv) acyclovir was described by Levy and Sagar in 1984 (66). In 1990, Bergström and Alestig (67) described therapy with IV acyclovir in one

patient with meningitis and another with ascending myelitis in connection with meningitis after a primary genital HSV infection. A few other uncontrolled reports subsequently described acyclovir treatment in cases of meningitis after genital HSV infection, where acyclovir was thought to be capable of shortening the duration of symptoms (11,40). Acute-stage treatment of primary meningitis without severe genital herpes has not been reported in more than a few sporadic cases in uncontrolled studies. It was suggested that the duration of meningitis symptoms was shortened. An immunocompetent woman with chronic meningitis was given acyclovir and glucocorticoid therapy and improved rapidly after being unwell for about 5 weeks and CSF inflammatory parameters returned to normal (35). It is not known whether the long-term course is affected compared with the course of the individuals who do not undergo antiviral intervention during the acute phase of the disease.

Although data from controlled studies in the setting of acute meningitis are lacking, initiation of antiviral therapy may be considered in the early phase of HSV-2 meningitis and severe symptoms that appear not yet to have culminated. In primary HSV-2 infection, this seems justified in light of the long duration of symptoms in untreated patients.

The bioavailability of orally administered acyclovir is limited (68) and the concentration in CSF has been found to be only 13–52% of that found in plasma (69). Enhanced bioavailability is achieved by valacyclovir, which is converted to acyclovir on the passage through the liver (70). A comparison of acyclovir pharmacokinetics following administration of oral valacyclovir (vacv) and oral and intravenous acyclovir (acv) has shown comparable plasma acv concentrations ($C_{\max}$ and daily AUC) with vacv 1000 mg 4×/day and acv 5 mg/kg q8h iv, and with vacv 2000 mg 4×/day and acv 10 mg/kg q8h iv (70,71); CSF concentrations of acv after vacv 1000 mg tid have been found to be three times higher than those achieved after acv po 800 mg tid (72).

Thus, in patients with nausea and vomiting initial IV acyclovir may be administered, otherwise PO therapy may be given. According to the approximate distribution found in a recent publication, oral administration of valacyclovir at 1000 mg tid should ensure CSF concentrations of acyclovir in the range required for the treatment of herpes simplex virus infections (72).

RECURRENT MENINGITIS

Antiviral treatment in acute episodes of recurrent meningitis is sparsely documented. Intermittent treatment or continuous suppression by acyclovir was administered in three patients with recurrent meningitis of probable HSV-2 etiology (67). Some possibly beneficial effects of the treatment were seen, although the authors state that the results should be interpreted in relation to the unconformity of drug administration, lack of placebo

controls, and limited numbers of the patient study group. All of the three patients with several previous meningitides per year were free of recurrences during acyclovir suppressive medication. Interestingly, in one patient a neuropathy lasting for five years also disappeared and she had fewer episodes of headaches than before treatment. Another report described three cases of recurrent meningitis associated with genital herpes in which therapy with acyclovir apparently decreased or prevented recurrences of meningitis (73).

As the episodes of recurrent meningitis often are less severe, a decision to avoid antiviral medication may be arrived at in some patients, in view of the self-limiting property of the disease. In recurrent episodes when the physician and patient are aware of the diagnosis, treatment may be instituted promptly, thereby increasing the potential clinical effect including shortening of the duration of symptoms. Patients with frequent attacks would rather benefit from prophylactic treatment with antiviral agents such as acyclovir, valacyclovir, or famcyclovir. The dosage needed to achieve freedom from symptoms may vary from individual to individual and also from one period of time to another. It may be helpful to thoroughly go through the history of herpes attacks in the patient in order to identify features and patterns in the course of the disease, including possible precipitating factors and periods of increased risk. When antiviral prophylaxis is deemed to be required, the regimen has to be tailored and the doses titrated individually with the aim of arriving at the minimal effective dose for preventing a breakthrough of symptoms. Analogously to the management of genital herpes, the prophylaxis should be interrupted on a regular basis to evaluate the possibility of discontinuing the medication. Although the toxicity of these antivirals is known to be low in otherwise healthy immunocompetent subjects (74), patients should be followed up clinically and with laboratory assessments of side effects.

MENINGITIS WITH ASSOCIATED NEUROLOGICAL SYMPTOMS

In patients with encephalitic or myelitic symptoms in addition to the meningitis, the prompt institution of antiviral therapy is recommended. Intravenous administration of acyclovir seems advisable at least initially and as long as the severity of the condition is unclear. For further information on the subject, please see the chapter on HSV encephalitis in this book.

RADICULOMYELOPATHY

Sacral radiculomyelopathy, mainly with symptoms of autonomic nervous system dysfunction, such as urinary retention and constipation, as well as sacral paraesthesia, radiating pain in the buttocks, perineum, and lower limbs, and muscle weakness in the lower limbs, may appear associated with

primary genital herpes and is, as mentioned, a frequent associated finding in primary HSV-2 meningitis. The condition may also present without other obvious symptoms or signs of herpetic infection. Sacral radiculomyelopathy occurs predominantly in women (7). In men, HSV-proctitis seems to predispose to this complication, as reported among homosexual men (75). The most common manifestation is urinary retention, which should be kept in mind in the clinical management of genital herpes and herpes meningitis. Conversely, HSV infection should be borne in mind in the differential diagnosis in all cases of urinary retention, especially in young adults (76–79). Cystometric findings indicate a lower motor neuron neuropathic bladder (13,75,76). Catheter à demeure, or preferably, self-administrated intermittent catheterization is often necessary for some time. In cases of muscle weakness, electromyographic abnormalities, such as slowed nerve conduction and fibrillation potentials, are found. Severe radiculitic pain may appear, and HSV has been reported in cases of recurrent sciatica syndrome (80).

NEURITIS

Similar to prodromes of VZV reactivation, a local discomfort or pain at the site of a developing mucocutaneous lesion or, less often, at a site at some distance but in the area innervated by the same ganglion, occurs in HSV infection. Such prodromal symptoms are very frequent and are reported in approximately 90% of cases. Neuralgia, such as leg and thigh pain, is frequently experienced in association with lesions, but also as remitting episodes in the absence of lesions, the so-called nonlesional prodromes (13). Prodromal discomfort in HSV infection may last for hours to days, but is not usually associated with a neurological deficit (81) and only very rarely reported to become chronic (13).

MYELITIS

On rare occasions, HSV of both types may cause myelitis. The majority of reported cases are due to HSV-2. HSV as a cause of myelitis was first identified in fatal cases of ascending necrotizing myelitis in patients with various kinds of immunosuppression. The HSV-2 etiology was verified at autopsy (82,83). In a single report, HSV was identified by isolation from the CSF in a surviving patient (84). With improved diagnostic techniques, HSV myelitis has been verified during life in a number of nonimmunocompromised patients (85–89). An expanded clinical spectrum of HSV-induced myelitis has been recognized and also a few cases of recurrent herpetic myelitis (57,88–90). Clinical courses vary, ranging from a mild form with total recovery to acute lethal ascending necrotizing myelitis. Nakajima et al. reported nine cases of severe myelitis in immunocompetent patients, due to HSV-2

($n = 7$) or HSV-1 ($n = 2$). The neurological disease may develop rapidly, within days to be fully developed at 2 weeks (acute myelitis), or more slowly during 1–3 months (subacute course) (87). The neurological onset is sometimes preceded by a febrile illness with respiratory or gastrointestinal symptoms. Typical initial neurological symptoms are weakness of the lower limbs (mostly symmetrical), sensory derangements (such as hypoalgesia, hypoesthesia, reduced vibration, and joint position senses, sensory loss for all modalities), urinary disturbances (overflow incontinence), and reduced anal sphincter tone. A transverse myelitis develops which may remain at the same level (nonascending pattern) or ascend cranially in the spinal cord. With few exceptions, the CSF shows a mild to moderate pleocytosis and slightly increased protein.

Viral reactivation with intra-axonal spread from the sensory ganglion into the spinal cord is suggested to be the most likely pathogenic mechanism (89). Most cases due to HSV-2 show clinical symptoms in keeping with reactivation in dorsal root ganglions at a lumbosacral level and neuronal spread into the spinal cord and further upwards within the spinal cord, while the HSV-1-induced myelitis predominantly appears at a thoracic or cervical level (87,90–93).

The diagnosis has been made in sporadic cases by isolation of HSV in the CSF (84). Peripheral herpetic lesions may point toward the HSV etiology but are rarely seen in connection with the myelitis episode, although a history of genital herpes is very frequent. As mentioned before, most reported cases are diagnosed by the demonstration of HSV DNA in the CSF by PCR. Intrathecal synthesis of specific antibodies has been demonstrated (94). Neither diagnostic method has been systematically evaluated regarding myelitis, although the specificity of PCR seems good; herpetic DNA was not detected in the CSF of any of 16 controls with nonherpetic inflammatory CNS disorders (87). MRI typically discloses partial swelling of the cord and sometimes the nerve roots with a low signal on T1 and a high signal on T2-weighted images. In the acute phase, more or less extended hyperintense intramedullary lesions and myelomeningeal enhancement of gadolinium are seen, sometimes with extension along posterior nerve roots (85,87,88,93).

Although mild cases with recovery without treatment are also seen, prompt therapy is generally instituted, as a severe and potentially lethal disease may result. Antiviral therapy with intravenous acyclovir along with corticosteroids has been given in order to prevent further development of an ascending pattern of the myelitis. The duration of antiviral therapy has mostly been 2–3 weeks IV, sometimes with a switch to oral valacyclovir during the third week. With early initiation of this treatment, cessation of disease progression has been reported and even recovery in a few cases. Most reported patients are left, however, with severe sequelae like paraplegia or tetraplegia. Some few cases of recurrent myelitis have been reported to be treated successfully with valacyclovir (1 g tid) together with decreasing

doses of corticosteroids followed by long-term antiviral suppressive prophylaxis (88).

ENCEPHALITIS, BRAINSTEM ENCEPHALITIS

In addition to meningoencephalitis with transient encephalitic symptoms accompanying a clinical picture otherwise dominated by symptoms of meningitis, HSV-2 may, rarely, cause classic herpes simplex encephalitis (HSE) primarily with temporal lobe involvement clinically indistinguishable from the classic HSV-1-induced encephalitis (94–96). Hence, methods able to detect both types should be used in the diagnosis of HSE. Rarely, encephalitis is located primarily or exclusively in the brainstem. Brainstem encephalitis due to HSV, untyped in earlier reports (93,94,97,98) and later confined to an HSV-1 (99,100) or HSV-2 origin (86,101,102), has been reported. Single cases of recurrent brainstem encephalitis have been documented (86,99,102).

The virus has been suggested to reach the brainstem through the cranial nerves from a source of latent infection in the cranial ganglia (103–105). Patients develop symptoms such as diplopia, nystagmus, ataxia, and sensory or motor changes in the face and limbs. The diagnosis, although not systematically evaluated, can be verified by detection of HSV DNA in the CSF (86,101,102) and by demonstration of intrathecal synthesis of HSV-specific antibodies (102). MRI reveals hyperintense lesions in the midbrain, pons, and medulla, sometimes along with changes in the cerebral cortex. The prognosis of reported cases is diverse; fatal cases due to respiratory failure, among other things, have been reported, but also benign courses resulting in only slight disability or total recovery. Cases with a favorable outcome apparently responding to antiviral treatment with intravenous acyclovir (86,101,102), and also with oral valacyclovir given as an alternative in a relapse of the disease and difficulties in venous access, have been reported (102).

CONCLUSION

The spectrum of neurological HSV-2 disease is broad. Fortunately, the most serious manifestations in the brain, brainstem, and spinal cord are relatively rare but should be recognized at an early stage and antiviral therapy instituted promptly. The much more common and less severe HSV-2 meningitis nevertheless carries a risk of considerable neurological morbidity, not least through its tendency to recur. Although the condition could be readily diagnosed when thought of, it is probably still underdiagnosed. For the patient, the mere diagnosis may be of considerable medical and psychological benefit as well as time and cost saving. Antiviral therapy is available and may be considered for acute and prophylactic use.

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Herpes Simplex Virus and Bell's Palsy

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INTRODUCTION

The seventh cranial nerve or facial nerve traverses, at its periphery, the temporal bone and parotid gland. Peripheral facial paralysis may be caused by a range of disorders including otitis media, temporal bone fractures, and carcinoma of the parotid gland. Viral infections can also be associated with facial paralysis. For example, the reactivation of varicella-zoster virus (VZV) can result in Ramsay Hunt syndrome, the second most common form of acute peripheral facial paralysis. Zoster around the ears or in the oropharynx and dysfunction of the eighth cranial nerve accompany this syndrome. However, in the majority of patients (about 70%) (1) with peripheral facial paralysis, the cause of the disease remains unknown and a diagnosis of “idiopathic” peripheral facial paralysis or Bell's palsy is made (Table 1). Bell's palsy is the most common form of acute peripheral facial paralysis.

The pathology associated with Bell's palsy is still unknown, but ischemia and viral infection of the facial nerve have been proposed (2–4). The reactivation of herpes simplex virus type 1 (HSV-1) has been reported most frequently and this is one of the most likely causes (reviewed in Refs. 5–9). It is noteworthy that VZV reactivation can cause acute peripheral facial paralysis in the absence of zoster, a condition termed *zoster sine herpette*. This form of paralysis should be distinguished from Bell's palsy

Table 1 Major Causes of Peripheral Facial Paralysis

Idiopathic
Bell's palsy
Inflammation
Ramsay Hunt syndrome (varicella-zoster virus)
Otitis media (acute, chronic, cholesteatoma, tuberculosis)
Lyme borreliosis
Acquired immunodeficiency syndrome (AIDS)
Tumor
Facial nerve and acoustic nerve schwannoma
Carcinoma of parotid gland
Injury
Iatrogenic (parotid gland surgery, middle ear surgery, neurosurgery)
Temporal bone fracture
Facial injury
Systemic disease
Diabetes mellitus
Guillain-Barré syndrome
Sarcoidosis

by appropriate serological and/or molecular assays (10). This chapter describes our current knowledge of Bell's palsy in general and its diagnosis and its treatment. The literature describing investigations of HSV infection and facial paralysis is also reviewed.

ANATOMY OF THE FACIAL NERVE

The facial nerve consists of two roots: the motor division, and the nervus intermedius containing parasympathetic and sensory fibers. The two roots enter the caudolateral aspect of the pons in the cerebellopontine angle. The periphery of the nerve traverses the temporal bone and parotid gland. Within the temporal bone, the facial nerve is surrounded for around 30 mm by the bony wall of the fallopian canal. The motor fibers arise from the facial nucleus in the pons and end in the facial muscles. The cell bodies of the sensory fibers are located in the geniculate ganglion and some of the afferent fibers supply the skin of the external auditory meatus and that surrounding the ear. The sensory fibers also include taste fibers, which supply the mucus membranes of the soft palate and anterior two-thirds of the tongue. The parasympathetic fibers have postganglionic connections to the submandibular and sublingual glands, the lacrimal glands, and the glands of the palatal and nasal mucosa (Fig. 1). Therefore, besides weakness of facial movement, patients with peripheral facial paralysis exhibit loss of taste, and decreased lacrimation. The facial nerve has numerous connections with cranial nerves V, IX, X, and with the cervical plexus.

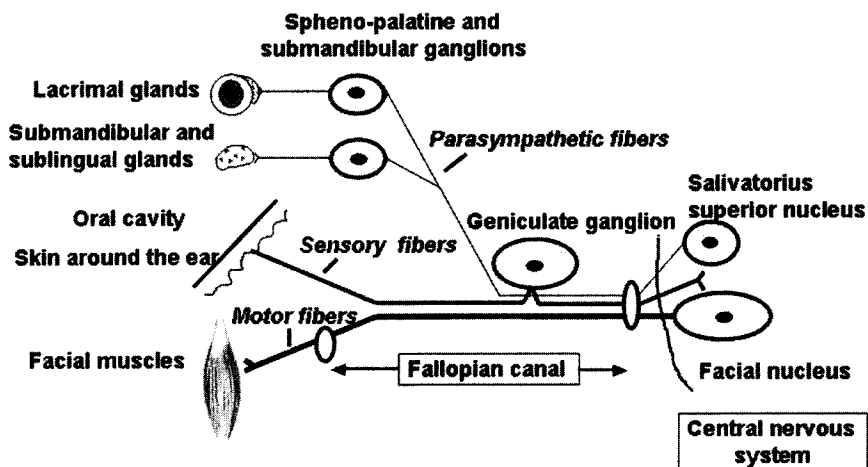


Figure 1 Schematic diagram showing the anatomy of the facial nerve.

GENERAL ASPECTS OF BELL'S Palsy

Bell's palsy accounts for about 70% of all cases of facial paralysis. Patients suddenly become unable to wrinkle their foreheads, raise their eyebrows, close their eyes, or puff out their cheeks. At rest, their eyebrows droop and nasolabial fold becomes flattened (Fig. 2). Course of the paralysis is acute and progressive, reaching maximal weakness within 1 week from onset in most patients. Its overall prevalence is about 20 cases per 100,000 person-years, but it becomes more prevalent with increasing age (1). The condition is distributed equally among men and women and there is no seasonal variation in incidence. Although the left and right sides of the face are involved equally, bilateral cases are rare (0.3%). Approximately 9% of patients with Bell's palsy have had previous attacks involving the contralateral or ipsilateral facial nerve (1). However, simultaneous bilateral facial paralysis is a characteristic unique to the acute peripheral facial paralysis associated with Guillain-Barré syndrome, Lyme borreliosis, and sarcoidosis. The majority of patients with Bell's palsy recover uneventfully, but in approximately 10–20% of patients recovery is incomplete and synkinesis, contracture, and spasm occur as sequelae.

REACTIVATION OF HSV AS A CAUSE OF BELL'S Palsy (HYPOTHESIS)

Following primary infection, HSV exists in a latent state within the peripheral-nerve-cell axons and nerve endings, subsequent trauma or metabolic damage causing the virus to reactivate at a later date. During reactivation, HSV particles infect the Schwann cells of the nerve, eventually spreading



Figure 2 A patient with right-sided facial paralysis.

throughout the nerve itself. McCormick (3) hypothesized in 1972 that such HSV reactivation causes inflammation and edema of the facial nerve. When the inflammation reaches the bony fallopian canal (see “Anatomy of the Facial Nerve”) compression of the nerve may result in peripheral facial paralysis (Fig. 3). This hypothesis was based on the observation that patients with Bell’s palsy often have prodromal symptoms, such as upper respiratory infection, emotional stress, and physiological fatigue, common predisposing factors to HSV reactivation. McCormick was also influenced by several clinical and experimental observations describing HSV reactivation after neurosurgical manipulation (11) and animal models of HSV infection and reactivation (12,13).

Evidence in support of McCormick’s hypothesis has increasingly accumulated since it was first proposed (reviewed in later sections). These data support the existence of an association between HSV (especially HSV-1) and Bell’s palsy. Several additional findings have suggested that inflammation and edema can cause mechanical compression of the facial nerve within the rigid bony fallopian canal and result in facial paralysis.

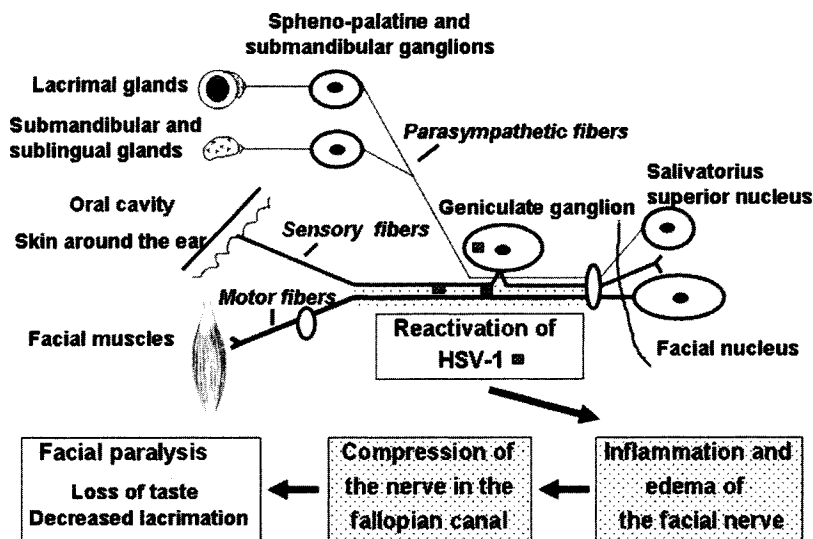


Figure 3 Hypothetical mechanism of Bell's palsy.

Firstly, evidence of inflammation and edema within the facial nerves has been found in the temporal bones obtained during postmortems of patients with Bell's palsy (14). Secondly, increased vascular permeability in the fallopian canal, caused by inflammation and edema, is observed on magnetic resonance imaging (MRI) of paralyzed facial nerves using gadolinium enhancement (Fig. 4) (15). Thirdly, inflammation and edema of paralyzed facial nerves in the fallopian canal have been demonstrated during decompression surgery in patients with Bell's palsy (16). The uniqueness of the anatomical features surrounding the facial nerve (see "Anatomy of the Facial Nerve") may play a functional role in the development of facial paralysis (17), since this is the most frequently paralyzed of all the cranial nerves.

PRIMARY HSV INFECTION AND FACIAL PARALYSIS

Occasionally, facial paralysis may develop during a primary HSV-1 or HSV-2 infection. Smith et al. (18) described an 8-year-old boy with primary herpetic gingivostomatitis who developed facial paralysis on the 10th day of the illness. The involvement of HSV-1 was confirmed by its isolation from the oral lesions and an increase in antibody titer, confirmed by complement fixation test. A similar case of facial paralysis following primary HSV-1 gingivostomatitis was described by Nasatzky and Katz (19). A further case of primary HSV-2 stomatitis in a 24-year-old man was described by Santos and Adour (20). The stomatitis appeared 5 days after oral sexual contact with his female partner who had active genital HSV-2 lesions. Between 1 and 2 weeks after the

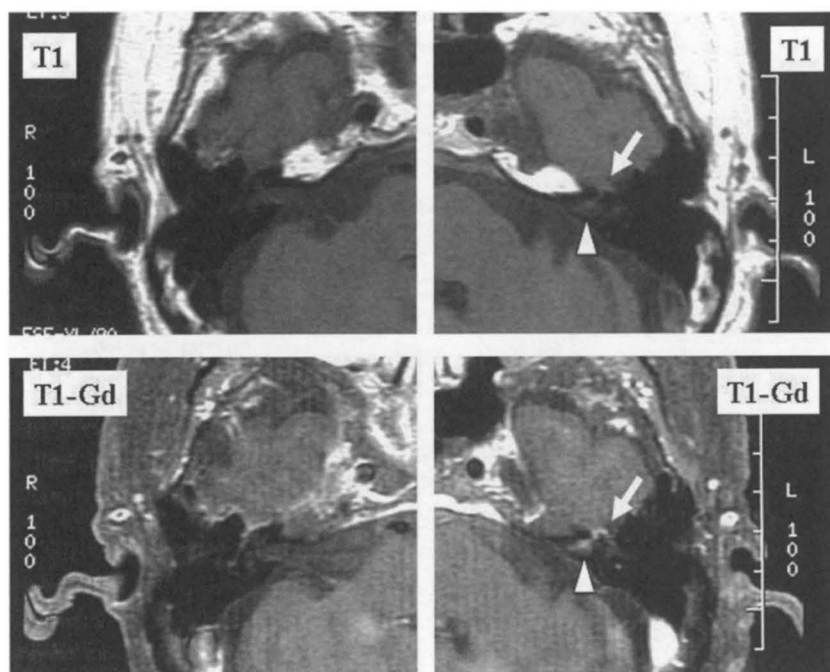


Figure 4 Axial magnetic resonance imaging (MRI) in a patient with left-sided Bell's palsy. T1-weighted gadolinium-enhanced MRI (T1-Gd) shows marked enhancement at the left geniculate ganglion (*arrows*) and meatal fundus (*arrowheads*), indicating inflammation and edema in these regions. No enhancement is observed at the right facial nerve.

onset of the illness, he developed bilateral facial paralysis. These observations clearly indicate that primary infection with HSV can cause dysfunction of the facial nerve.

ANIMAL MODELS OF FACIAL PARALYSIS BY HSV INFECTION

Several animal models have been established to prove that HSV infection causes facial paralysis. Kumagami (21) showed the development of facial paralysis in 16 of 19 rabbits following the inoculation of HSV through the stylomastoid foramen into the facial nerve canal. Paralysis did not improve in all rabbits and regeneration of the nerve was extremely poor. Hill et al. (22) inoculated HSV-1 or -2 into the ear skin of mice and observed paralysis of the ear in 6–7% of the animals. In addition, a mouse model of acute and transient facial paralysis was created by Sugita et al. (23), who inoculated HSV-1 into the auricle or tongue. The mice developed facial paralysis between 6 and 9 days after inoculation. Paralysis continued for 3–7 days, and was followed by spontaneous recovery, simulating Bell's palsy. Although

the incidence of facial paralysis was higher in the auricle inoculation group (56.7%) than in the tongue inoculation group (20%), the latter seems to be a more appropriate model of the natural HSV-1 infection route. Studies using these models have demonstrated a mixed lesion of the facial nerve involving demyelination and degeneration (24). In contrast, Thomander et al. (25) failed to create a model of facial paralysis by the direct inoculation of HSV-1 into the tongues of mice. Inoculation of HSV-1 into the nasal or oral mucosa of guinea pigs by Ishii et al. (26) also failed to cause facial paralysis. The frequency of facial paralysis probably differs in each model due to genetic differences between animals, virus strains, or the inoculation routes used (Table 2). Furthermore, it has been demonstrated that the age and the immunologic potency of animals are closely related to the onset of facial paralysis (27).

Recently, an animal model of recurrent facial paralysis was developed in mice latently infected with HSV-1 (28). In this model, 4-week-old Balb/c AJcl mice were inoculated in the auricle with HSV-1 KOS strain. As described previously (23), 58.2% of mice exhibited transient facial paralysis. Eight weeks after recovery from facial paralysis, these mice underwent an auricular skin scratch at the site of the previous inoculation and an intraperitoneal injection of anti-CD3 monoclonal antibody. A total of 20% of the mice showed transient facial paralysis and HSV-1 DNA was detected in the facial nerve tissue. The cause of recurrent facial paralysis is thought to be reactivation of latent HSV-1 by local skin irritation and general immunosuppression. Although the frequency of recurrent facial paralysis is low, this animal model may be useful in research aiming to determine the pathogenesis of Bell's palsy.

Table 2 Animal Models of HSV Infection in the Facial Nerve

Animal (strain)	Age	HSV strain	Site of inoculation	Facial paralysis	Ref.
Rabbit	50–60 day-old	Not specified	Stylomastoid foramen	Present (84%)	21
Mouse	4 week-old	HSV-1/Lab, HSV-2/Lab	Ear skin	Present (6–7%)	22
Mouse (Balb/c)	4 week-old	HSV-1 KOS strain	Auricle, tongue	Present (20–57%)	23
Mouse (Swiss albino)	12 day-old suckling	HSV-1 strain KJ 502, strain F	Tongue	Absent	25
Guinea pig (Hartley)	Not specified	HSV-1 Tomioka strain	Nasal or oral mucosa, auricle	Absent	26

LATENCY OF HSV IN THE GENICULATE GANGLIA

In his 1972 hypothesis, McCormick (3) proposed that HSV became latent within the peripheral-nerve-cell axon. Around this time, latent HSV was isolated from the human trigeminal ganglia of unselected cadavers by a cocultivation method (29,30). This led Adour et al. (17) to propose two possibilities. Firstly, that latent HSV in the trigeminal ganglia may reactivate, traveling along the axon, to cause a secondary infection of the facial nerve through trigeminal facial nerve communication. Or secondly (and more straightforwardly), that reactivation of latent HSV in the geniculate ganglia could cause facial paralysis.

Attempts to isolate HSV from the geniculate ganglia of humans by cocultivation of material from autopsies have been unsuccessful (31,32). Through the use of immunohistochemistry or the polymerase chain reaction (PCR), several experiments have employed animal models to demonstrate that the facial nerves, geniculate ganglia, and facial motor nucleus can be infected with HSV (23,25,26,33,34). We have shown by *in situ* hybridization and PCR that HSV-1 latency-associated transcripts (LATs) (35) are expressed in the human geniculate ganglia obtained from autopsy cadavers (Fig. 5) (36,37). The HSV-1 genome was detected in 71% of the geniculate ganglia by *in situ* hybridization and in 88% by PCR. These findings have been confirmed by several studies, although the percentage of infected ganglia differs somewhat in each (ranging from 43% to 70%) (38–42). Interestingly, HSV-1 DNA was detected in the geniculate ganglia of archival temporal bone sections obtained from a patient who had suffered Bell's palsy and died six weeks after the onset of facial paralysis (43). VZV DNA was also positively identified in 69% of the geniculate ganglia (44), whereas HSV-2 DNA was undetectable (37). These findings prove that HSV-1 establishes latency in the geniculate ganglia after primary infection. Because the geniculate ganglia contain the cell bodies of the sensory fibers, inflammation and edema of the sensory fibers, which are caused by HSV-1 reactivation in the ganglia, may spread directly to the motor fibers or indirectly compress the motor fibers within the fallopian canal, causing weakness of facial movement.

VIRUS ISOLATION IN PATIENTS WITH BELL'S PALSY

Virus isolation in cell culture is the gold standard for the diagnosis of viral infections. Attempts have been made in several studies to isolate HSV from patients with Bell's palsy. For example, a case of Bell's palsy in which HSV-2 was isolated has been reported in association with genital and pharyngeal herpes by Lewis and Morris (45). Vahlne et al. (46) isolated HSV from a patient who had a small lip vesicle. However, such active herpetic lesions are rarely observed at the onset of Bell's palsy, distinguishing it from Ramsay

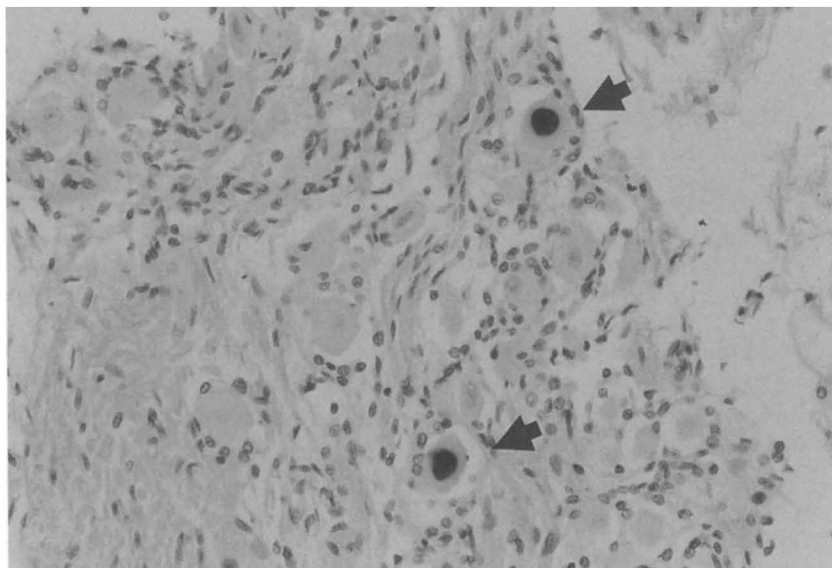


Figure 5 Latent HSV-1 in the geniculate ganglion. In situ hybridization using HSV-1 latency-associated transcript as an antisense probe. Positive hybridization is shown in the nuclei of the ganglion cells (*arrows*).

Hunt syndrome (facial paralysis with VZV reactivation). Of the 202 patients we examined, only three (1.5%) had HSV lesions around the lip at the onset of Bell's palsy and HSV-1 DNA was detectable in their saliva samples by PCR (Fig. 6). However, it is difficult to conclude whether or not the HSV-1 reactivation was associated with the onset of Bell's palsy.

HSV frequently reactivates without causing herpetic lesions, and can be cultured from the saliva and tears of healthy people (asymptomatic shedding) (47). Such viral shedding without mucocutaneous disease has also been documented in patients with Bell's palsy. Djupesland et al. (48) isolated HSV from the nasopharynx in two of 51 patients with Bell's palsy. Mulkens et al. (49) reported that HSV-1 was isolated from the epineurium of a patient obtained during decompression surgery. Conversely, several authors have reported patient samples that were culture negative. For example, Vahlne et al. (46) assessed 36 patients with Bell's palsy; no virus was isolated from nose, throat, or conjunctival secretions or fecal samples. Palva et al. (50) were unable to isolate HSV from the geniculate ganglion of two patients who had undergone total decompression surgery. Finally, Korczyn et al. (51) studied 63 throat smears, 40 fecal and 18 blood samples, detecting no virus in any of the specimens. Thus, the results of attempts to isolate HSV from patients with Bell's palsy have been equivocal, probably due to the inherent insensitivity of virus isolation techniques. Furthermore, the detection of HSV reactivation in only a small percentage of

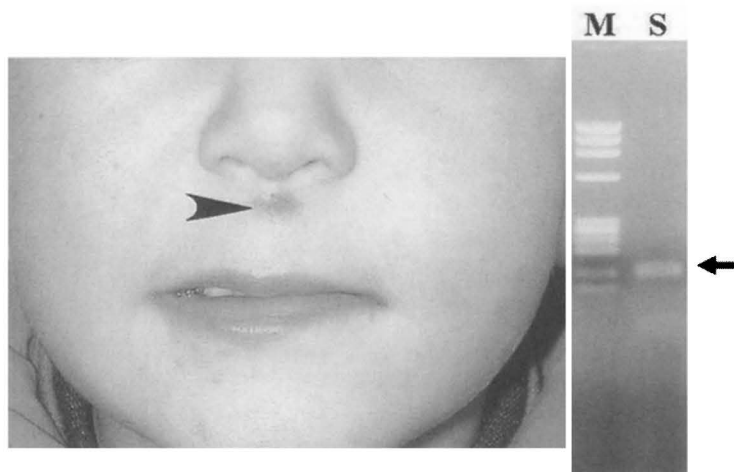


Figure 6 A 3-year-old boy with incomplete facial paralysis on the left side. A herpetic lesion occurred below the nose (*arrowhead*) at the onset of paralysis and HSV-1 DNA was detected in his saliva sample by PCR. *Abbreviations:* M, DNA marker; S, saliva.

patients, if any, appears to be weak evidence of a causal association with Bell's palsy.

SEROLOGICAL STUDY OF HERPES VIRUS INFECTIONS

Serological assays are not useful for the detection of HSV-1 reactivation in patients with Bell's palsy since reactivation per se is rarely accompanied by a rise in antibody titer (52) and only very few Bell's palsy patients will have such a rise. For example, Morgan and Nathwani (53) reviewed 21 methodologically distinct serological studies and reported that only 3.7% of patients with Bell's palsy showed significant changes in their anti-HSV antibody titers. In our own work, only two out of 202 patients (1%) with Bell's palsy showed significant changes in the anti-HSV IgG antibody titer, as measured by enzyme-linked immunosorbent assay (ELISA). Both patients lacked HSV lesions at the onset of illness, although HSV-1 DNA was detected in the saliva of one patient (Fig. 7). HSV was strongly suspected to be the causative agent of facial paralysis in these two cases. Interestingly, several serological studies (17,46,54,55) have shown that the prevalence of antibodies to HSV among patients with Bell's palsy is higher than that among healthy control subjects. These findings may provide indirect evidence of an association between HSV and Bell's palsy.

It is difficult to distinguish the acute facial paralysis caused by *zoster sine herpete* (VZV reactivation without zoster) from Bell's palsy if clinical

Days after onset	Day 2	Day 16	
ELISA			
HSV IgG	20	72	(<1.0 negative)
HSV IgM	1.2	1.8	(<1.0 negative)
PCR (saliva)			
HSV-1 DNA	+		
Western blot			gB – 116
			– 97
			– 66
			gD – 55
			– 36.5

Figure 7 A rare case of Bell's palsy in which HSV-1 reactivation was demonstrated by serology. A significant increase in the level of anti-HSV IgG antibody was observed and the IgM antibody was weakly positive by ELISA (Enzygnost Anti-HSV/IgG and IgM, Dade Behring Marburg GmbH, Marburg, Germany). Changes in immunoreactivity against the HSV-1 antigens were confirmed by Western blot analyses (gB and gD, glycoprotein B and D). Molecular weights are indicated on the right in kDa. HSV-1 DNA was detected in the saliva obtained at the initial visit (day 2).

manifestations alone are used. The purpose of serological diagnosis is to distinguish these two groups of patients by using appropriate protocols. *Zoster sine herpete* has been diagnosed by the complement fixation (CF) test in about 10% of patients who were previously diagnosed clinically as suffering from Bell's palsy (53). In our own study, we identified 254 patients over 4 years of age diagnosed clinically with Bell's palsy within 7 days of the onset of facial paralysis. Serological assays (ELISA) of paired sera allowed us to detect either a significant increase in anti-VZV IgG or the presence of anti-VZV IgM antibody in 37 of the 254 patients (15%). In addition, saliva samples were examined by PCR and VZV DNA was detected in 15 of the 37 patients (41%) on at least one occasion within 7 days of onset, while VZV DNA was not detected in the remaining 22 patients. VZV DNA was also detected in five patients with either a constantly high level of anti-VZV

IgG antibody (no significant increase from the first to the second of the paired sera) or a weakly positive IgM antibody. Furthermore, VZV DNA was detected in an additional 10 patients in the absence of a VZV antibody response. Therefore, a revised diagnosis of *zoster sine herpete* was made in 52 of the 254 patients (20%) who had an initial diagnosis of Bell's palsy (Fig. 8). Our data indicate the usefulness of serological assays (ELISA) and PCR for the differential diagnosis of *zoster sine herpete* and Bell's palsy.

Finally, it is worth noting that HSV is not the cause of facial paralysis in HSV-seronegative patients. In particular, reactivation of VZV should be considered in patients lacking anti-HSV IgG antibody detectable by ELISA (55). Studies of HSV-seronegative patients who also have no detectable evidence of VZV reactivation may help us to further elucidate the etiology of Bell's palsy. In conclusion, serological assays are an important tool for the differential diagnosis of Bell's palsy, but not for the detection of HSV reactivation.

DETECTION OF HSV GENOMES IN PATIENTS WITH BELL'S PALSY

During the 1990s, sensitive molecular techniques were developed, which led to the publication of several remarkable reports describing HSV-1 reactiva-

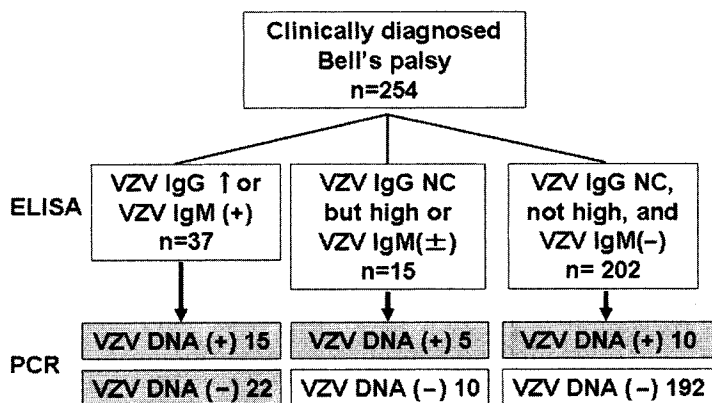


Figure 8 Detection of VZV reactivation in patients with clinically diagnosed Bell's palsy. VZV IgG ↑, a significant increase (more than two-fold) in anti-VZV IgG antibody level; VZV IgG NC, no significant change; VZV IgG high, ELISA value more than the mean +3SD of the healthy controls (50≤); VZV IgM-, negative (<1.0); ±, weakly positive (1.0–1.9); +, positive (2.0≤). Enzygnost Anti-VZV/IgG and IgM (Dade Behring Marburg GmbH) were used. The shaded cases were diagnosed with *zoster sine herpete*.

tion in patients with Bell's palsy. In 1996, Murakami et al. (56) provided new evidence suggesting that the two conditions were closely associated. They investigated whether HSV-1 DNA was detected by PCR in facial nerve endoneurial fluid and posterior auricular muscle obtained during decompression surgery. HSV-1 DNA was detected in 11 of 14 patients (79%). The decompression surgery was performed 15 to 60 days after the onset of illness; therefore, the association between HSV-1 DNA in clinical samples and the onset of paralysis was not definitively proven by these findings. However, HSV-1 DNA was not detected in nine patients with Ramsay Hunt syndrome or in 12 controls. Instead, VZV DNA was detected in eight of nine Ramsay Hunt syndrome patients. Furthermore, the detection of HSV-1 DNA in the endoneurial fluid and muscle indicates the presence of reactivated virus as latent infection cannot be established in these locations. Taking all these data into account, Murakami et al. (56) concluded that HSV-1 infection in the facial nerve is related directly to the pathogenesis of Bell's palsy. Attempts to reproduce the Murakami data using PCR to detect HSV-1 DNA in posterior auricular muscle, orbicularis oris muscle, or epineurium (obtained by biopsy or during decompression surgery) from patients with Bell's palsy have met with limited success, detection rates ranging from 0% to 5% (57,58).

If Bell's palsy is caused by HSV-1 reactivation in the geniculate or, occasionally, in the trigeminal ganglia, the virus may be shed into saliva or lacrimal fluid through the sensory branch of the facial nerve or the trigeminal nerve, and therefore detectable at the onset of illness. Interestingly, Bonkowsky et al. (59) reported five patients with delayed facial paralysis following uneventful middle ear surgery. HSV-1 DNA was detected by PCR in tongue swabs obtained from four of the five patients. They concluded that stimulation of the facial nerve during surgery could trigger the reactivation of HSV-1 in the geniculate ganglion, causing delayed facial paralysis. We investigated HSV-1 shedding into the saliva of 47 patients with Bell's palsy (60). By nested PCR, HSV-1 DNA was detected in the saliva of 40% of HSV-seropositive patients on at least one occasion during acute phase of the disease. The frequency of HSV-1 shedding in patients with Bell's palsy was significantly higher than that in patients with Ramsay Hunt syndrome (7%). Abiko et al. (61) reported that saliva or tears from five of 16 Bell's palsy patients (31%) contained HSV-1 DNA, but other studies found both these fluids and cerebrospinal fluid (CSF) negative for HSV-1 DNA (58,62,63). Differences in the frequency of sample collection (either at every visit or only once at the onset), method of DNA preparation, and PCR conditions may explain these discrepancies.

Since HSV-1 can be shed asymptomatically in the saliva and tears of healthy people (47), the detection of HSV-1 DNA in clinical samples from patients with Bell's palsy does not indicate that HSV-1 is a direct cause of facial paralysis. Therefore, a definitive diagnosis of HSV-1-induced facial

paralysis is still difficult. We recently measured the copy numbers of HSV-1 DNA in saliva samples obtained from patients with Bell's palsy using a real-time quantitative PCR. We observed two characteristic patterns of HSV-1 loads in PCR-positive patients: firstly, a high HSV-1 DNA copy number during the acute phase of the disease (within 7 days of onset), and secondly, an increase in HSV-1 DNA copy number following the acute phase of the disease (Fig. 9). The former pattern of HSV-1 load indicates a close association between HSV-1 reactivation and the onset of facial paralysis, while the later pattern suggests that stress due to the facial paralysis or steroid therapy causes HSV-1 reactivation. This new approach may be useful for the diagnosis of HSV-1-induced facial paralysis and more data are required to determine the percentage of facial paralysis cases induced by HSV-1.

MECHANISM BY WHICH HSV CAUSES FACIAL PARALYSIS

Inflammation and edema leading to compression and ischemia of the facial nerve in the fallopian canal may be the pathology underlying Bell's palsy (see section "Reactivation of HSV as a Cause of Bell's palsy" and Fig. 3). However, the mechanism by which the reactivation of HSV-1 causes this inflammation remains to be elucidated. Adour et al. (17) hypothesized that reactivated HSV-1 infects the Schwann cells. Lymphocytic infiltration of the facial nerve fiber then causes edema and demyelination, resulting in abnormal nerve conduction. In fact, HSV can cause demyelination of the peripheral nerve (64) and the facial nerve; the latter has been demonstrated in animal models of facial paralysis (24). A significant increase in the myelin basic protein levels contained in CSF (a useful indicator of active demyelinating disease) has been reported in patients with Bell's palsy (65). Honda and Takahashi (66) analyzed the activity of the myelin-associated enzyme 2', 3'-cyclic nucleotide 3'-phosphohydrolase (CNP) in CSF and found that its activity was raised significantly in patients with Bell's palsy. Also, HSV-seropositive patients had higher CNP activity than those who were HSV-seronegative. It has also been shown that the serum levels of tumor necrosis factor- α , a cytokine associated with demyelination, are elevated in patients with Bell's palsy (62). These results support the hypothesis that inflammatory demyelination of the facial nerve contributes to the pathogenesis of Bell's palsy.

A high level of similarity in CSF findings and peripheral blood lymphocyte subpopulations has been identified among patients with Bell's palsy and those with acute inflammatory demyelinating polyneuropathies, such as Guillain-Barré syndrome (66,67). Abramsky et al. (68) also demonstrated a similar cellular immune response to peripheral nerve P1L basic protein among patients with Bell's palsy and Guillain-Barré syndrome. In Guillain-Barré syndrome, the immune response to a preceding bacterial or viral infection

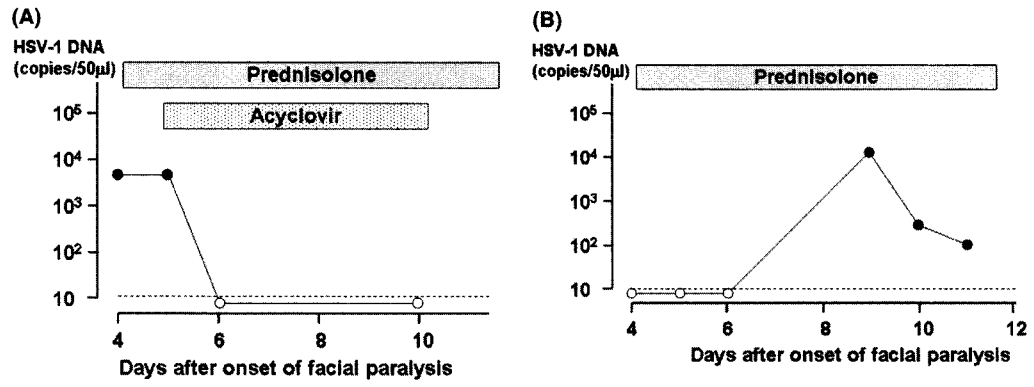


Figure 9 Changes in HSV-1 DNA copy numbers in saliva samples obtained from patients with Bell's palsy using a real-time quantitative PCR. ●, HSV-1 copies expressed as the copy number/50 μ L of saliva (logarithmic scales); ○, HSV-1 DNA was not detected. Dotted lines indicate the minimum detection level of HSV-1 DNA (10 copies). **(A)** A 10-year-old girl with Bell's palsy who visited our clinic 4 days after the onset. In addition to prednisolone (30 mg daily), acyclovir treatment (800 mg in tablets daily for 5 days) started on day 5 because HSV-1 DNA was detected in saliva obtained on day 4. The HSV-1 DNA levels decreased markedly after the initiation of antiviral therapy. A high HSV-1 DNA copy number during the acute phase suggests a close association between HSV-1 reactivation and the onset of facial paralysis. **(B)** An 82-year-old man with Bell's palsy who visited our clinic 4 days after onset. Prednisolone (45 mg daily) was administered. HSV-1 DNA was not detected until day 9 when HSV-1 DNA copy number showed a peak. An increase in copy number after the acute phase suggests that stress due to the facial paralysis or steroid therapy induced HSV-1 reactivation.

leads to the production of antibodies, which are coincidentally cross-reactive with human gangliosides. This autoimmune response is the cause of nerve damage in this condition (69). However, HSV-1 is not one of the major viruses involved in Guillain-Barré syndrome and anti-ganglioside antibodies are rarely detected in patients with Bell's palsy (70). In addition, bilateral and multiple peripheral nerves are generally involved in Guillain-Barré syndrome. These findings suggest that the pathogenesis of Guillain-Barré syndrome and Bell's palsy may involve a similar immunological pathway. However, the causative agents and antigenic loci on the peripheral nerve, which are involved are more likely to be distinct. Further studies of the antigenic epitopes of HSV-1, the components of facial nerve, and host factors are needed to clarify the immunological processes, which induce demyelination of the facial nerve.

CONFLICTING ISSUES AGAINST HSV ETIOLOGY IN BELL'S PALSY

As described previous sections, evidence in support of McCormick's hypothesis has accumulated since it was proposed originally. However, several issues conflicting with the hypothesis have been identified. Firstly, patients with Bell's palsy rarely have mucocutaneous HSV-1 lesions, such as cold sores, at the onset of facial paralysis. In contrast, Ramsay Hunt syndrome is usually accompanied with mucocutaneous zoster. The zoster is mostly observed in the skin around the ear (the geniculate zone), where HSV-1 lesions are rarely seen. Therefore, it might be reasonable to speculate that HSV-1 reactivation in the geniculate ganglia rarely produces mucocutaneous lesions in the geniculate zone but causes inflammation of the facial nerves. In general, mucocutaneous HSV-1 reactivations are rarely associated with motor impairment. However, the anatomical uniqueness of the facial nerve may result in facial paralysis induced by compression of the facial nerves in the fallopian canal. In addition, the paresthesia and pain around the ear commonly observed in patients with Bell's palsy may be caused by HSV-1 reactivation and inflammation of the sensory fibers. Such HSV-1 reactivation without mucocutaneous lesions may be characteristic of the pathology of Bell's palsy.

Secondly, despite the frequent recurrence of cold sores throughout a patient's life, Bell's palsy rarely recurs more than twice. There is also a large difference in the prevalence of cold sores and Bell's palsy, the former affecting many more individuals than the latter. This could be explained if HSV-1 reactivates frequently in the geniculate ganglia, but rarely causes the inflammation, edema, and compression in the fallopian canal, which lead to Bell's palsy. These theories remain to be confirmed and additional data are required to further confirm the McCormick hypothesis.

TREATMENT OF BELL'S PALSY

It has been reported that approximately 71% of patients with Bell's palsy experience complete recovery of facial movement without treatment (71). Therefore, about 30% of patients with Bell's palsy recover poorly if appropriate therapy is not provided. They suffer sequelae such as synkinesis, contracture, and spasm. Steroids have been proven to be an effective treatment for Bell's palsy and are widely used, resulting in good recovery of facial movement [grades I and II according to the House and Brackmann (72) facial function grading system] for 94–100% of patients (73,74). The purpose of steroid therapy is to reduce inflammation and edema of the paralyzed nerve in the fallopian canal and to prevent the progression of nerve degeneration. Meta-analyses of clinical trials show that the cure rate is significantly better among patients in the steroid-treated group than among those in the placebo-treated group; the difference being 17% (75). In addition, the guidelines published in *Neurology* in 2001 recommend the use of steroids for the treatment of Bell's palsy (level B) (76).

The facial paralysis associated with Ramsay Hunt syndrome is more severe and has a lower recovery rate than Bell's palsy (77). In Ramsay Hunt syndrome, the early administration of antiviral agents and steroids after the onset of paralysis has been recommended (78). Antiviral agents may inhibit the replication and spread of VZV in the facial nerve and steroids may reduce inflammation and edema of the paralyzed facial nerve. These synergistic effects can also be achieved in patients with *zoster sine herpette* if early diagnosis is made by PCR (79).

Adour et al. (80) conducted a double-blind, controlled study to test the hypothesis that reactivation of HSV-1 is the major cause of Bell's palsy. Their aim was to evaluate the effectiveness of treatment with an antiviral agent in combination with conventional steroids. Acyclovir in 2000 mg tablets/day for 10 days in combination with prednisolone in 1 mg/kg/day for 5 days was administered within 3 days of the onset of paralysis. They reported that the cure rate of the group receiving acyclovir–prednisone therapy (92%) was significantly higher than that of the group receiving placebo plus prednisone (76%). Interestingly, De Diego et al. (74) reported that acyclovir therapy alone resulted in a worse recovery than prednisone therapy alone. These reports, however, did not determine whether or not the patients actually had HSV-1 reactivation. Furthermore, it is not clear whether patients with *zoster sine herpette* were excluded from the studies following identification by appropriate virological assays. The possibility exists that HSV-1 does initiate nerve damage, but reactivation is already over by the time facial paralysis begins. The use of antiviral agents is of no benefit in such cases. Further clinical trials involving a larger patient population, and employing virological assays to distinguish *zoster sine herpette* from Bell's palsy, would establish more clearly the efficacy of combination therapy with steroids and

antiviral agents for the treatment of Bell's palsy. Large, multi-institutional, randomized studies are presently ongoing in Scandinavia and Japan to address this issue.

SUMMARY

Since McCormick (3) first proposed the hypothesis that HSV reactivation causes Bell's palsy by inducing inflammation and edema in the bony fallopian canal, supporting evidence has increasingly accumulated. It is now accepted that HSV-1 reactivation is one of the causes of Bell's palsy. However, diagnosis of HSV-1-induced facial paralysis is still difficult because serological assays are unhelpful in the detection of HSV-1 reactivation and healthy people can shed HSV-1 asymptomatically. Until it becomes possible to reliably diagnose HSV-1-induced facial paralysis, accurate differential diagnosis remains important. The benefits of combination therapy with steroids and antiviral agents for the treatment of Bell's palsy will be clarified by ongoing clinical trials. Additional studies are needed to further elucidate the mechanisms by which HSV-1 reactivation causes facial paralysis.

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Herpes Simplex Virus Infections in Immunocompromised Patients

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INTRODUCTION

Immunosuppressed patients are at increased risk of developing infections because of immune system impairment. Infections caused by viruses are of particular concern in patients in whom the defect of immunity involves the cytotoxic T cells, i.e., the cells primarily involved in immune defense against viruses. A deficit of T-cell-mediated immunity is encountered in a number of conditions dominated by immune suppression, including transplanted and oncology patients, as well as patients with other congenital or acquired immune deficiencies.

The number of patients exposed to immune suppression has increased dramatically over the second half of the last century. This increase reflects the increasing availability of immunosuppressive agents used for treatment of tumors, the progress with transplantation, and treatments to prevent graft rejection and, finally, the onset of the human immunodeficiency virus (HIV) epidemics starting in the 1980s. This chapter will review the most relevant issues and clinical manifestations of herpes virus infections in the setting of transplantation and HIV infection.

HSV INFECTIONS IN TRANSPLANTED PATIENTS

Human transplants include hematopoietic stem cell transplants (HSCT) and solid organ transplants (SOT). HSCT refers to the infusion of hematopoietic stem cells from a donor into a patient who has received bone marrow ablative chemotherapy. SOT defines several different conditions, including lung, heart, lung, kidney, liver, and pancreas transplantation.

Herpesvirus Infections in Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation consists today of allogeneic and autologous stem cells derived from bone marrow, peripheral blood, and cord blood. Indications include leukemias, lymphomas, and solid tumors as well as nonmalignant disorders, such as aplastic anemia, congenital immunodeficiencies, metabolic disorders, and autoimmune diseases (1). Following transplantation, patients are at a great risk for infections, reflecting the immune suppressive effects of both bone marrow ablation and treatments used to prevent graft-versus-host disease (GVHD). Depending on the mechanisms, which are predominantly impaired, there are three phases of immune recovery in HSCT patients. Phase 1, or pre-engraftment phase (0–30 days post-transplantation), is characterized by infections resulting from both neutropenia and breaks in the mucocutaneous barriers, e.g., by the use of vascular catheters. Herpes simplex virus (HSV) reactivation usually occurs in this period, in addition to bacterial and fungal infections, e.g., those caused by *Candida* and *Aspergillus* species. Phase 2, or post-engraftment phase (30–100 days post-transplantation) is featured by impairment of cell-mediated immunity, which is dependent on the extent of immunosuppressive treatment given to avoid GVHD. All herpesviruses are important pathogens in this phase, although cytomegalovirus (CMV) infections are dominant. Phase 3, or late phase (>100 days post-transplantation), is observed in allogeneic transplant patients with chronic GVHD or unmatched donor transplants and is also characterized by persistent deficit of cell-mediated immunity. Although infections caused by CMV and varicella-zoster virus (VZV) are typical in this phase, HSV infections are infrequently observed (2).

In patients undergoing HSCT with an HSV-positive pretransplant serostatus, reactivation of latent HSV infection is reported to be as high as 70% in absence of antiviral prophylaxis. Overall, about 50% of HSCT recipients are HSV seropositive before transplantation, which is consistent with the rate of reactivation of 40% reported in these patients. On the contrary, only 2% of infections following HSCT have been estimated to be due to primary infection (3,4).

Herpesvirus Infections in Solid Organ Transplant Recipients

HSV disease is an important potential complication of lung and heart–lung transplants, whereas it is less frequent in other SOT recipients, including kidney, liver, heart, and pancreas recipients. As in HSCT patients, SOT patients are at high risk of developing viral infections during the peri-transplant period. Differently from HSCT patients, however, the risk factor associated with infections in the SOT setting is represented mainly by the duration and nature of immunosuppressive drugs used to prevent graft rejection. SOT recipients more frequently develop HSV infection as a result of virus reactivation during the first weeks post-transplant. Latently infected donor organs can transmit the infection and cause severe disease in the context of an impaired immune system because of immunosuppressive regimens. However, and differently from other herpesviruses such as CMV and Epstein–Barr virus (EBV), transmission of HSV by allograft is extremely rare. HSV primary infection is more commonly seen in seronegative recipients of a seropositive donor graft as documented in renal transplant recipients (5–7).

Clinical Manifestations

Clinical manifestations of mucocutaneous HSV disease in transplant patients do not significantly differ from those of immunocompetent hosts, but as a consequence of impaired immune competence the evolution of the disease appears to be more prolonged in this setting, with persistent viral shedding and slower healing (8). Furthermore, HSV disease in transplant patients has the potential to disseminate and cause severe disease.

Mucocutaneous Manifestations

Before the introduction of aciclovir for prophylaxis against HSV infections, more than 70% of HSV-seropositive HSCT recipients would develop HSV mucositis during the initial period of chemotherapy-induced neutropenia, consisting in painful ulcerative lesions of the oropharynx and perineum (1). The introduction of short courses of aciclovir prophylaxis during the initial post-treatment period has dramatically reduced the incidence of early symptomatic HSV infection to <5% in the first month after transplantation (9). However, reactivation of HSV can occur quickly after prophylaxis is stopped (1,9).

Primary and recurrent orolabial disease: Recurrent orolabial disease represents the most common manifestation of HSV infection following transplant and account for 85% of HSV-related diseases in the early post-transplant setting (10). Furthermore, up to 50% of all oral lesions in the post-transplant early phase are due to HSV. Herpetic lesions may involve lips, tongue, oral mucosa, pharynx, and perioral skin. The presence of

HSV gingivostomatitis because of very frequent severe mouth pain can reduce oral intake of food and medications, and when it occurs during neutropenic phase can be difficult to differentiate from mucositis due to chemotherapy. In this case, laboratory diagnosis is recommended. Oral HSV infection can predispose the patient to bacterial superinfection and is a risk factor for esophageal herpes and pneumonia.

Acute and recurrent genital herpesvirus infection: Anogenital herpes accounts for 10–15% of all HSV-related diseases in the transplanted patient. In the majority of cases, this manifestation is due to a reactivation of latent HSV-2. Although the lesions are usually self-limiting, these may become chronic, lasting even for months. This manifestation can predispose to herpes lymphocytic meningitis. Although this complication is usually self-limiting, prompt pharmacological treatment is recommended.

HSV infections of the skin: On intact skin, HSV disease begins as painful erythematous lesions evolving in clusters of vesicles that are fragile and easily ruptured resulting in ulcerations. Intact vesicles evolve toward pustules. In immunocompromised hosts, healing can take 4–6 weeks compared to the 5–10 days of normal hosts (11–13). Large chronic ulcerations due to HSV are occasionally observed (14,15).

Gastrointestinal Manifestations

HSV esophagitis: Esophageal HSV disease in transplant patients can either follow HSV reactivation of latent virus in the vagus ganglia or direct spread of oral infection. The clinical picture is difficult to differentiate from that caused by CMV or *Candida* infections. The most common symptom is dysphagia and the endoscopic examination reveals superficial erosions and often confluent ulcerations of the mucosa (16). Diagnosis of HSV esophagitis is achieved by biopsy followed by virus identification through culture, immunohistochemistry, or molecular methods.

HSV colitis: HSV colitis, although less frequent than CMV colitis, has been reported in both SOT and HSCT recipients. This disease is often described in association with CMV- and GVHD-related colitis (17).

HSV Pneumonia

HSV pneumonia is more frequently reported in HSCT and heart and heart–lung transplants than in other SOT transplant settings. In lung transplant recipients, HSV pneumonia is an early complication after transplantation and is either due to viral spread from oral cavity or by aspiration from an upper respiratory tract infection. The presence of medical devices as a tracheal tube is considered a risk factor to develop pulmonary involvement during oral HSV mucocutaneous infection. These cases are mainly caused by infection with HSV-1. Less frequently, the pulmonary involvement can occur as a result of

HSV viremia and in this case the disease can be due either to HSV-1 or to HSV-2. Most of the patients (85%) diagnosed with HSV pneumonia have a recent or concomitant history of mucocutaneous disease (18).

A diagnosis of HSV pneumonia should be considered in every early post-transplant patient that presents lower respiratory tract infection symptoms, especially if associated with mucocutaneous herpetic lesions and if patient received HSCT, lung, or heart transplantation. Patients with HSV pneumonia present fever, cough, dyspnea, hypoxemia, and chest radiograph abnormalities that are often indistinguishable from that of a bacterial pneumonia. The pneumonia resulting from an extension of disease initially involving the upper respiratory tract is similar in the radiological finding to that of a bacterial pneumonia with focal or multifocal infiltrates. Pneumonia resulting from a viremic spread of infection is more commonly seen as diffuse infiltrates (18). The bronchoscopic examination usually shows large mucosa ulcerations with a tracheal and bronchial diffuse involvement. The diagnosis of HSV pneumonia is made relying on histopathology or identification of the virus in lung specimens obtained by transbronchial biopsy.

Herpes Simplex Encephalitis

Herpes simplex encephalitis does not occur in transplant recipients with increased frequency when compared with normal hosts and is seen only rarely as post-transplant complication (19,20).

Disseminated HSV Disease

HSV viremia and disseminated HSV disease are well known in neonates in whom these are associated with high morbidity and mortality (21,22). However, HSV infection can be followed by viremia also in the transplant setting, resulting in multiple organ involvement, including skin, liver, lung, adrenal glands, and gastrointestinal tract. This dramatic syndrome has been described in HSCT as well as in SOT recipients, especially renal- and liver-transplanted patients (13,15,23). The mortality of HSV disseminated disease is high, of over 60%; therefore, prompt diagnosis is essential. This syndrome usually occurs within 1 month post transplant. Symptoms include fever, often accompanied by severe abdominal pain and abnormal liver function tests. Skin and mucosal HSV lesions are not necessarily present. HSV hepatitis is generally seen at a median of 18 days after transplant. However, late onset of HSV disseminated disease with hepatitis has also been described up to 4 years after liver transplant (24). This form of hepatitis needs be distinguished from CMV hepatitis, which is usually less aggressive, and from graft rejection in hepatic transplant recipients. Disseminated intravascular coagulopathy is a very sensitive predictor of poor outcome with an associate mortality that is near to 100%. Diagnosis is achieved by positive histological and virological findings on a liver biopsy sample. Because of the severity of this syndrome, however, it is advisable to start antiviral treatment in patients in

the early post-transplant phase presenting a rapid alteration of liver function tests, fever, and abdominal pain.

Prevention and Treatment

Preventing Exposure

Determination of HSV IgG serostatus is recommended in all transplant candidates. Usually, it is not necessary to discriminate between antibody specificity for HSV-1 or HSV-2. The presence of HSV IgG indicates the probability of post-transplant viral reactivation and is an indication for starting antiviral drugs as prophylaxis. HSV-seronegative recipients should be informed about the mode of transmission of HSV and be advised of behavior that will decrease the likelihood of exposure, e.g., avoiding potentially infectious secretions such as cervical secretions and saliva. Since some cutaneous lesions caused by HSV, e.g., herpes simplex lesions of lips or fingers, can be transmitted by direct contact, the affected personnel should be restricted from patient contact and temporarily reassigned to other duties. Testing of donor serostatus is currently not routine in most HSCT centers since HSV transmission by marrow or stem cell products has not been documented (25).

Preventing Disease and/or Disease Recurrence

During the past 20 years, major advances have been achieved in the prevention of herpesvirus infections and disease by use of antiviral drugs. Both aciclovir and valaciclovir have been evaluated in randomized controlled studies in SOT and HSCT recipients, showing to be very effective for the prevention of HSV infections.

The majority of the studies have evaluated HSCT patients. Early trials showed that intravenous aciclovir given at 5 mg/kg or 250 mg/m² every 8 or 12 hours during the early neutropenia phase following allogeneic transplant was effective in preventing the disease (3,26,27). Subsequent studies with oral instead than intravenous aciclovir, however, were more disappointing, as they were associated with breakthroughs of HSV disease, likely to result from suboptimal adherence to medication because of vomiting or diarrhea (9,28,29). A number of studies conducted in the 1980s confirmed that prolonged administration of aciclovir was highly effective in reducing the frequency of HSV disease, whereas early discontinuation after transplantation was associated with disease recurrence. More recently, aciclovir levels following valaciclovir administration were shown to be similar to those achieved following intravenous administration of 5 mg/kg aciclovir (30).

In contrast, only few clinical trials have evaluated the effect of prophylactic administration of aciclovir to SOT recipients. These have involved kidney (31,32), heart (33), and liver (34) transplant recipients. In general, these studies showed aciclovir to be effective for prevention of HSV disease. There are no specific trials evaluating the effect of valaciclovir in preventing

HSV disease in SOT recipients. However, a recent study evaluating the effect of valaciclovir in preventing CMV disease in kidney transplant patients showed that this drug was also effective to control HSV reactivation (35). The importance of specific HSV prophylaxis diminished since the introduction of the practice of prophylaxis of CMV infections. All CMV prophylactic antiviral regimens also prevent HSV recurrence and no additional measures are needed if CMV prophylaxis is used. With the recent advent of pre-emptive treatment of CMV infection, which is used instead of CMV infection prophylaxis in most transplant centers, the issue of HSV prophylaxis has again been addressed.

The above-mentioned studies on aciclovir represent the basis for treatment strategies that are still in use in most clinical centers (Table 1). Prophylaxis with aciclovir, valaciclovir, or famciclovir is indicated in all HSV-seropositive allogeneic HSCT recipients and in some hematopoietic autologous bone marrow recipients at high risk to develop mucositis. Oral administration is

Table 1 Summary of Major Recommendations for Prevention and Management of HSV Infections in Transplanted Patients

I. Prevention of exposure
Testing of recipient IgG serostatus
Counseling of seronegative recipient on transmission mode of HSV
Use of latex condoms during sexual contacts in sexually active seronegative recipients (not in long-term monogamous relationships)
Counseling of seronegative recipient of behavior that will decrease HSV transmission
Contact isolation for persons with disseminated, severe, or severe mucocutaneous HSV disease
II. Prevention of disease or disease recurrence in HSV-seropositive recipients
HSCT ^a
Aciclovir: oral, 400 mg tid or intravenous, 5 mg/kg tid
Valaciclovir: oral, 1g tid
In HSV-seropositive recipients
SOT ^b
Aciclovir
Valaciclovir (same as above)
In HSV-seropositive recipients
III. Treatment
Aciclovir: oral, 400 mg 5 times/day or intravenous, 5 mg/kg tid
Valaciclovir: oral, 1g tid
Aciclovir: intravenous, 10 mg/kg tid in severe or visceral disease
Foscarnet: intravenous, 40 mg/kg tid in HSV-resistant infection

^aSince start of conditioning until engraftment or resolution of mucositis.

^bSince transplant and for 4–8 weeks.

Abbreviations: HSCT, hematopoietic stem cell transplants; SOT, solid organ transplants.

Source: From Refs. 2, 25, 36, 37.

preferred, although intravenous administration may be required in the presence of specific conditions associated with low drug adsorbance. Prophylaxis should be continued throughout the aplastic phase and adjusted individually. Most centers administer aciclovir from the onset of conditioning until engraftment and resolution of mucositis. This may occur before day 30 after transplant, which is the time period that was used in the original randomized studies of aciclovir prophylaxis. Routine extension of HSV prophylaxis beyond day 30 is not recommended; however, longer prophylactic courses are recommended in patients with GVHD or a history of frequent HSV reactivation before transplantation (2,26). It has been observed that the frequency of HSV reactivation after stopping prophylaxis is lower among patients who received long-term prophylaxis but that, on the other hand, reactivation episodes occurring after the aplastic phase are usually mild (27,38).

In the SOT setting, prophylaxis should be considered when CMV prophylaxis is not used. In this case, HSV-seropositive recipients should receive 4–8 weeks of aciclovir, valaciclovir, or famciclovir to prevent HSV recurrence. Prophylaxis should also be used during periods of intensified immunosuppression or during concomitant non-CMV severe illness (37).

Treatment of HSV Infection

If HSV disease occurs, it can be treated orally, using aciclovir, valaciclovir, or famciclovir, or intravenously, with either aciclovir or foscarnet. Recommended regimens for the various manifestations are shown in Table 1.

HSV Resistance to Antiviral Drugs

Following the first cases of isolation of aciclovir-resistant HSV in 1982 (39,40), aciclovir resistance has increasingly been reported in transplanted patients. Nevertheless, the reported rate of aciclovir resistance in case series indicates that this phenomenon has remained stable despite the fact that this drug has now been used for more than 20 years in prophylaxis of transplanted recipients. In contrast to immunocompetent hosts, in whom aciclovir resistance is reported to be around 0.1% (41,42), resistance is detected in 2–3% of SOT and in 10–18% of HSCT patients. In the latter, prevalence of resistance has recently been estimated to be of 2% in autologous and 29% in allogeneic transplant patients (42,43). In a recent survey, 25% of HSCT centers reported cases of proven or suspected HSV infections with resistant virus. In addition, there are recent reports of an increase of aciclovir-resistant HSV infections in unrelated and HLA-mismatched family transmission and patients with GVHD (44,45).

HSV INFECTIONS IN HIV-INFECTED PATIENTS

Patients with HIV infection are at increased risk of developing HSV-1 and HSV-2 infections compared to other immune-suppressed patients because of

the higher rate of previous infection and common via acquisition of HSV and HIV. HSV infections were among the first opportunistic infections to be described in immunocompromised homosexual men in the early times of the AIDS epidemic (46). Severe and persistent mucocutaneous HSV infection defines the status of AIDS according to the 1992 revised Centers for Disease Control (CDC) diagnosis definition criteria, as well as the surveillance case definition for AIDS in resource-poor countries (47,48).

Inter-Relationship Between HSV and HIV Infection

In addition to the elevated morbidity due to HSV infection in people with HIV infection, there are several inter-relationships between the two viruses. First, there is strong evidence that HSV infection may increase significantly both HIV transmission and acquisition. The importance of HSV-1 and HSV-2 as cofactors for HIV transmission is corroborated by a number of epidemiological studies and by the results of a recent meta-analysis (49). Among these, a recent prospective observational study carried out in India showed that HSV-seropositive persons had a significantly higher risk of acquiring HIV infection than those who were HSV-seronegative (7.5% vs. 3.6%). In addition, the risk of acquiring HIV was substantially higher during the first 6 months of HSV seroconversion (50). In line with these observations, studies of discordant couples have documented a decreased risk of HIV transmission when the HIV-seropositive patient was receiving HSV suppressive therapy (51). These observations are plausible from a biological point of view. On the one hand, HIV shedding is increased through ulcerative herpetic lesions, thus facilitating transmission—HIV being consistently detected in genital ulcers caused by HSV-2 with no association with plasma HIV RNA levels (52). In addition, HSV ulcerative lesions harbor a high concentration of activated CD4 lymphocytes, which are the target of HIV itself (53).

Another connection between HIV and HSV relates to the response of HIV infection to aciclovir. In early studies, aciclovir in association with zidovudine, a nucleoside analog anti-HIV drug, was associated with increased survival compared to zidovudine alone (54,55). This effect was observed both in prospective controlled trials and in retrospective surveys and at both high and low aciclovir dosages (56,57). Several mechanisms have been hypothesized to explain these observations, including the reduced immune activation of CD4 cells induced by HSV, as well as the suppressive effect of aciclovir on HSV replication and reactivation, leading to a reduction of morbidity and mortality from herpesvirus infections.

Finally, there is evidence that HIV and HSV mutually interact both *in vitro* and *in vivo*. *In vitro*, it has been shown that some HSV proteins can upregulate the replication of HIV by interaction at a molecular level (58–62). In addition, HSV infections seem to affect HIV replication *in vivo*. High plasma HIV-1 load has been found to correlate with high rate of HSV

shedding and, conversely, patients receiving aciclovir show lower HIV-1 RNA levels than those found in untreated patients (63,64).

Epidemiology

Seroprevalence of HSV infection is higher in patients with HIV infection compared to the general population, likely to reflect the common sexual mode of acquisition of HIV and HSV-2 and, to a lesser extent, HSV-1. Whereas seroprevalence of HSV-2 in the general population has been estimated of 8–15% in Europe and of approximately 20% in the United States (65), the seroprevalence in HIV-infected persons in the same geographic areas may be as higher as of 80% (66,67). In Africa, where seroprevalence in the general population is up to 50%, it may be over 80% among seropositive individuals (68–71), with figures close to 100% in selected populations, as female prostitutes (72).

Clinical Manifestations and Their Treatment

Clinical manifestations of HSV infection in HIV-positive persons vary according to the degree of immune suppression. When this is moderate, e.g., with a CD4 cell count of above 200 CD4/ μ L, the principal manifestations consist of mucocutaneous lesions, including both atypical lesions and particularly frequent relapses of typical genital or oral herpes. In patients with advanced infection and a very low number of CD4-positive cells, severe mucocutaneous lesions are observed, together with involvement of visceral organs, such as the gastrointestinal tract, lung, eye, and central nervous system (CNS). Similar to other HIV-related opportunistic infections, the incidence and prevalence of severe HSV manifestations have dramatically declined in the developed countries since the introduction of combination anti-HIV treatments (highly active antiretroviral therapy, HAART). However, HSV infections remain a frequent complication of HIV infection in countries where access to HAART is limited and, as mentioned above, a factor associated with increased risk of HIV transmission. On the other hand, frequently relapsing HSV infections remain a problem in HIV-positive treated people because the rate of virus shedding from genital lesions does not seem to be affected by HAART (73). In addition, atypical HSV mucocutaneous manifestations have been described as temporarily associated with HAART-induced immune reconstitution.

Mucocutaneous Manifestations

HSV mucocutaneous infections are usually self-limited in the early phases of HIV infection and in people successfully treated with HAART. Once significant immune suppression occurs, however, lesions tend to become persistent. HSV lesions usually involve the genital and perianal areas, primarily caused by HSV-2, or the lips and oral cavity, more frequently caused by HSV-1.

Lesions are typically vesicular, but tend to become ulcerative in patients with advanced immune suppression. The presence of mucocutaneous HSV infection persistent for more than 1 month defines the status of AIDS (47).

Genital herpes: Compared to HIV-negative individuals, HIV-infected persons show a higher rate of recurrences of HSV genital infections. Whereas HIV-negative individuals usually experience less than 12 episodes a year, the rate of recurrences in HIV-positive people has been estimated to vary between 0 and over 20 episodes per year. Frequency of recurrences is higher in those with less than 50 CD4 cells/ μ L (74). Also the frequency of asymptomatic HSV-2 shedding, as detected by daily viral culture of the mouth, genital, and rectum, is higher in HIV-positive than in HIV-negative persons. In addition, the rate of asymptomatic shedding is higher in patients with lower CD4-positive cells (73,75) and high HIV-1 RNA levels (64).

Also presentation and course of genital herpes in HIV-infected persons differ from those observed in HIV-negative subjects. First, while episodes tend to be self-limited in the latter, these often persist in subjects with HIV-Infection, especially those with low CD4 cell counts (76). This is especially true for primary HSV infections. Primary genital infection may not resolve spontaneously in HIV-infected patients, but cause progressive disease characterized by multifocal and coalescing mucocutaneous lesions localized in the anogenital area (77). In addition to severe mucocutaneous lesions, both primary and recurrent episodes may be associated with systemic systems, such as fever and malaise. A typical manifestation in these patients is the chronic ulcerative mucocutaneous perianal herpes, which is characterized by tender, often painful, ulcerative lesions, which tend to enlarge peripherally if untreated.

Other cutaneous lesions: In addition to the above-described mucocutaneous lesions, a spectrum of skin HSV manifestations is observed in HIV-infected individuals, including periungual lesions (herpes whitlow), follicular facial lesions (herpes folliculitis), and multiple scattered lesions in one or more skin areas (78). Compared to same localizations observed in immunocompetent individuals, lesions can be larger and become chronic in HIV-infected people. In addition, atypical herpes lesions have been reported in patients with moderate immunosuppression, for example pseudotumor of the tongue or genital verrucous lesions (79,80).

Diagnosis: The diagnosis of mucocutaneous lesions is based on characteristic clinical presentation and is confirmed by the laboratory. In a study in the pre-HAART era, 58% of all ulcerations and 67% of all perianal ulcerations contained HSV in patients with less than 50 CD4 cells/ μ L (74). As for mucocutaneous lesions of non-immunosuppressed people, the etiological diagnosis relies on the identification of the virus in preparates obtained by lesions scrapings or viral swab by Tzanck smear, direct fluorescent antibody

staining, culture, or polymerase chain reaction (PCR) (81). In case of negative test results, a diagnosis of etiology may be achieved upon skin biopsy from the ulcer margin of the lesion, followed by histological examination, immunocytochemistry, or culture. Serological tests are of no use in the diagnosis of these infections.

Treatment: Because clinical course and presentation of HSV orolabial and genital herpes in HIV-infected people may differ from that of immunocompetent individuals, different management strategies are often required (81,82). Several antiviral drugs are currently available for treatment of these infections, including aciclovir, valaciclovir, famciclovir, foscarnet, ganciclovir, and cidofovir.

In general, oro-labial and genital herpes are thought to respond more slowly to antiviral therapy in HIV-infected than in HIV-negative individuals and thus higher doses may be required in the former (83). Although no published studies have compared directly the effects of antiviral treatments between HIV-positive and HIV-negative patients, the results from studies in HIV-positive patients treated with aciclovir or famciclovir suggest that time to complete healing of lesion is usually longer in the former (median 7 days).

Randomized clinical trials have been performed with aciclovir, valaciclovir, and famciclovir, showing these drugs being effective and safe in the treatment of genital HSV infections in HIV-infected people and helping establish dosages and times of administration (84–91). An overview of more recent clinical trials involving valaciclovir and famciclovir in HIV-infected patients with genital herpes is presented in Table 2. These drugs, together with foscarnet, are all registered for treatment of HSV infections in HIV-positive people. On the other hand, evidence supporting the systemic use of other drugs, such as ganciclovir and cidofovir, does not appear sufficiently robust.

Aciclovir, valaciclovir, and famciclovir are all well tolerated. Rarely, patients receiving aciclovir might experience renal dysfunction and monitoring of renal function is recommended. Thrombotic thrombocytopenic purpura/hemolytic uremic syndrome resulting in death was reported in initial trials in HIV-infected patients receiving high-dose valaciclovir, but is extremely rare at recommended doses (91). Current recommendations for treatment are shown in Table 3. Dosages and times of administration are same or similar to those recommended in HIV-negative patients (see chapter on orolabial and herpes genitalis); however, it is advisable to administrate the drugs for 5–10 days and in any case not less than 5 days.

In case of limited lesions, low-dose oral aciclovir or equivalent valaciclovir or famciclovir regimens are sufficient for complete healing of the lesions. In patients with more severe lesions, higher dosages and longer treatments may be required. If lesions persist or recur despite adequate

Table 2 Clinical Trials Evaluating Valaciclovir and Famciclovir in HIV-Infected Patients With Genital Herpes

I. Treatment of genital herpes	
1. Famciclovir versus aciclovir (86)	
Drug regimen:	Famciclovir 500 mg bid versus aciclovir 400 mg 5 times/day, for 7 days.
Patients:	293 (1:1 randomization)
Results:	Same time to resolution of lesion-associated symptoms, cessation of viral shedding, and complete lesion healing between the two groups; no different rate of new lesion formation. Similar safety profile
2. Valaciclovir versus aciclovir (88)	
Drug regimen:	Valaciclovir 1000 mg bid versus aciclovir 200 mg 5 times/day, for 5 days (with additional 5 days if necessary)
Patients:	476 (1:1 randomization)
Results:	Same length of episode and time of lesion healing. Similar safety profile
II. Suppression of genital herpes	
1. Famciclovir versus placebo (85)	
Drug regimen:	Famciclovir 500 mg bid for 8 weeks
Patients:	48 (1:1 randomization)
Results:	Lower rate of HSV isolation and lower frequency of days with HSV-2 shedding in famciclovir group
2. Valaciclovir versus aciclovir (88)	
Drug regimen:	Valaciclovir 500 mg bid versus 1000 once daily versus aciclovir 400 mg bid, for 48 weeks
Patients:	1062 (1:1:1 randomization)
Results:	Similar time to first recurrence of either valaciclovir regimen compared to aciclovir. Higher efficacy of valaciclovir 1000 mg once daily over 500 mg bid in preventing or delaying recurrence
3. Valaciclovir versus placebo (90)	
Drug regimen:	Valaciclovir 500 mg bid for 6 months
Patients:	293 (1:1:1 randomization), antiretroviral-treated.
Results:	Lower frequency of disease recurrences and longer time to first recurrence in valaciclovir group.

Note: bid, every 12 hours.

treatment, infection with a resistant strain should be suspected. Ideally, a viral isolate should be obtained for susceptibility testing; however, these tests are not performed routinely in all laboratories. HSV strains resistant to aciclovir are also resistant to valaciclovir and likely to famciclovir. Intravenous foscarnet should be administered in these cases. Mucocutaneous lesions due to foscarnet-resistant strains are also described. Intravenous cidofovir® has also been suggested for treatment of lesions caused by aciclovir-resistant strains (92). Use of this drug, however, is limited by frequent cross-resistance

Table 3 Recommended Antiviral Treatment and Suppressive Regimens for HSV Infections in HIV-Infected Patients**Treatment**

Mucocutaneous infection (initial or recurrent, mild)

Aciclovir: oral, 200–400 mg 3–5 hr times/day 5–7 days

Valaciclovir: oral, 1000 mg bid for 5–7 days

Famciclovir: oral, 500 mg bid for 5–7 days

Mucocutaneous infection (moderate to severe)^a

Aciclovir: oral, 800 mg 5 times/day

Aciclovir: intravenous, 5–10 mg/kg tid until lesions begin to regress, then oral regimen

Valaciclovir: oral, 1000 mg tid

HSV keratitis

Trifluridine® 1% ophthalmic solution, 1 drop into the cornea every 2–3 hours (up to 9 drops/day and until 21 days)

Encephalitis, and other visceral organ disease^a

Aciclovir: intravenous 10 mg/kg tid

Foscarnet: intravenous, 120–200 mg/kg/day divided into 2–3 doses

Aciclovir: intravenous 10 mg/kg tid + foscarnet 60 mg/kg tid

Aciclovir-resistant HSV infection^a

Foscarnet: intravenous, 120–200 mg/kg/day divided into 2–3 doses

Cidofovir: 5 mg/kg once weekly for 2 weeks, then every second week^b

Topical trifluridine or cidofovir (1% cream OD)

Suppression of recurrences^c

Aciclovir: oral, 200 mg tid or 400–800 mg bid or tid

Valaciclovir: oral, 500–1000 mg bid

Famciclovir: oral, 250–500 mg bid

Foscarnet (aciclovir resistance): intravenous, 90 mg/kg once daily

Notes: tid, every 8 hours.^aDuration of treatment established based on patient response.^bWith oral probenecid 2 g, given 3 hours before cidofovir administration.^cIncluding suppressive treatment of genital herpes and maintenance regimens following treatment of severe mucocutaneous or visceral disease.*Source:* From Refs. 81, 82.

with aciclovir. In the case of aciclovir-resistant HSV infection, topical cidofovir gel (93) or topical trifluridine (94) might also be effective.

In general, treatment should not be completed until complete lesion resolution is attained. Long-term suppressive prophylaxis with oral aciclovir, valaciclovir, or famciclovir, or intravenous foscarnet in case of aciclovir-resistant strains, is recommended. Before HAART introduction, suppressive treatment was to be continued life long. In patients presenting with extensive mucocutaneous lesions but who subsequently respond to

HAART, interruption of suppressive therapy can be considered when the CD4 cell count increases above 200 CD4/ μ L.

Gastrointestinal Manifestations

HSV esophagitis: In the pre-HAART era, approximately one-third of AIDS patients developed symptoms of esophagitis (95). These were mainly due to *Candida* infections, which accounted for 50–70% of the cases, but also to CMV (10–20%) or HSV (2–5%), or were idiopathic (aphthous ulcers, 10–20%). Symptoms of herpes esophagitis are not specific, including dysphagia, odynophagia, and focal pain. However, ulcers are frequently recovered in the oral cavity in HSV esophagitis.

Diagnosis is usually achieved through endoscopic examination, followed by histologic and microbiologic tests. HSV esophagitis needs to be distinguished not only from the above-mentioned causes of esophagitis but also from other less common causes, including mycobacteriosis, cryptosporidiosis, isoplasmosis, Kaposi's sarcoma, and lymphoma. Other noninfectious causes of dysphagia should also be considered in the differential diagnosis of esophageal ulcers, especially in patients with CD4-positive cells higher than 200/ μ L, including drugs (e.g., anti-HIV agents), food, and gastroesophageal reflux. Treatment consists of intravenous aciclovir or foscarnet (Table 3).

Ocular Manifestations

HSV keratitis: Corneal infections are an infrequent complication of HIV infection, occurring in less than 5% of the patients. HSV, together with VZV, is the most frequent cause (96). Incidence of HSV keratitis in patients with HIV infection is not different from that of HIV-negative patients. Similarly, clinical presentation and course do not seem to differ substantially between the two groups in terms of type (epithelial vs. stromal) and location (central vs. peripheral) of the lesions, duration of treatment required, and time to first recurrence. However, a higher rate of recurrences has been observed in patients with HIV infection compared to HIV-negative subjects (97).

The diagnosis of keratitis can be established by ophthalmologic evaluation. A diagnosis of etiology, or HSV subtyping is achieved by virus culture or PCR on tissue or fluid obtained by corneal scraping, anterior chamber aspirate, or biopsy. Recommended treatment does not differ from that of HSV keratitis occurring in immunocompetent patients and must be aggressive and initiated promptly to prevent further complications. The medical treatment of choice of epithelial keratitis is topical trifluridine. Vidarabine 3% ointment five times/day may also be effective. Use of oral aciclovir is debated, as it has been shown that oral administration did not prevent active herpes lesions of the corneal epithelium from moving deeper into the stroma (98). In cases of frequently recurrent disease, oral aciclovir, valaciclovir or famciclovir may be administered to suppress the risk of recurrence (9).

HSV retinitis: Retinal manifestations include the acute retinal necrosis (ARN) and the progressive outer retinal necrosis (PORN), also named rapidly progressive herpetic retinal necrosis (RPHRN). These entities are usually considered as two distinct syndromes, although they might represent different expressions of a spectrum of HSV manifestations. Both ARN and PORN can be caused by either HSV-1 or -2 or VZV, the latter being however much more frequent. HSV and VZV manifestations are clinically undistinguishable and PCR of aqueous or vitreous fluid is used to define their etiology (100).

ARN is characterized by focal well-demarcated areas of necrosis, initially involving the areas outside the temporal vascular arcades and progressing circumpherentially. Occlusive vasculitis and vitreous inflammation can also be observed (Fig. 1). Although ARN responds well to antiviral treatment in immunocompetent patients, the prognosis can be severe in HIV-infected persons. In HIV-related ARN the lesions are larger, often bilateral, and progress rapidly to retinal detachment left untreated.

PORN or RPHRN is a fulminant form of retinitis, characterized by rapidly progressive multifocal lesions usually involving both the eyes. Initial lesions consist of multifocal areas of opacification in the outer layers of the retina, often involving also the macula. Lesions coalesce rapidly, resulting

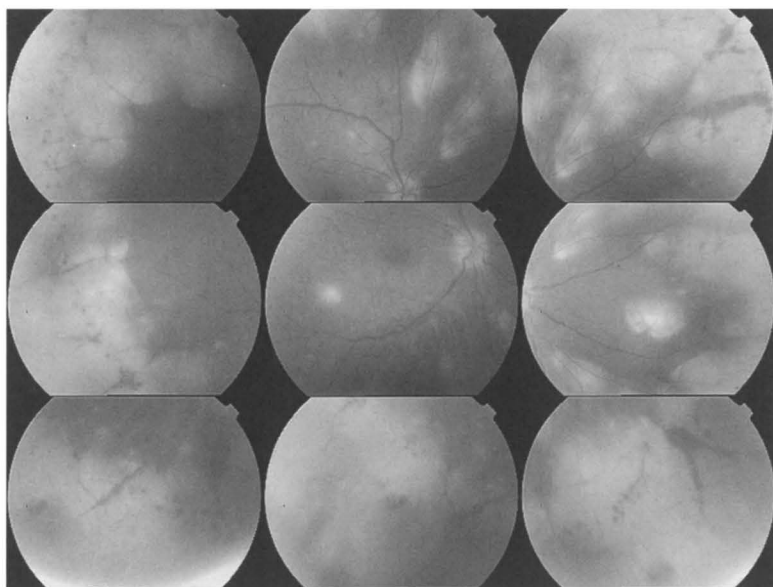


Figure 1 HSV-2 acute retinal necrosis in an AIDS patient. Ophthalmological examination shows typical peripheral necrotic lesions with both hemorrhagic lesions and ischemic vasculitis. *Source:* Courtesy of Dr. Giulio Modorati.

in large white-yellowish patches of full-thickness retinal necrosis. Vitreal inflammation is minimal or absent. This syndrome is poorly responsive to systemic antiviral treatments, including aciclovir, foscarnet, and ganciclovir, used as single drugs or in combination. In the majority of the patients, PORN progresses rapidly to retinal detachment and blindness (101). The PORN syndrome differs from ARN for several aspects. In PORN, the initial lesions involve the posterior pole and the periphery and subsequently spread outwards. In addition, retinal vasculitis and optical neuritis are less common. In general, PORN is a more severe disease as it progresses more rapidly and is less responsive to treatment.

Diagnosis of HSV-associated ARN or PORN can be achieved by PCR on aqueous or vitreous fluid or by the demonstration of an intraocular synthesis of a specific antibody. Although the combination of both techniques can make a valuable contribution to the diagnosis of HSV ARN of immunocompetent patients, PCR analysis is preferable above local antibody production in detecting the inciting agent of retinitis in patients with AIDS. PCR is usually positive in samples obtained within 2 weeks after the onset of disease (102).

There are no controlled studies on treatment of retinal manifestations by HSV that can guide clinical management of these manifestations. Current recommendations are done based on clinical practice and anecdotal cases. In general, treatment has to be initiated early and be aggressive. Intravenous aciclovir or foscarnet at high dosages is recommended. Local intravitreal administration of antiviral drugs has also to be advocated in association with systemic treatment. Since HSV-1 and HSV-2 are susceptible to ganciclovir, and this drug has been shown to be safe and effective for local treatment of CMV retinitis both by intravenous injection and implantation of sustained-release reservoirs (103,104), intravitreal ganciclovir has been suggested as a treatment option for PORN. Foscarnet represents an additional choice, as this drug can also be administered intraocularly, this drug may represent an additional choice (Table 2). Theoretically, intravitreal treatment would also prevent problems relating to delivery of systemically administered antiviral drugs to the retina, which is further complicated by the presence of arteriolar occlusion and retinal necrosis. There are anecdotal reports of patients with PORN responding to intravitreal injection of ganciclovir following failure to respond to systemic treatment (105).

Central Nervous System (CNS) Manifestations

HSV infection of the CNS has been reported in postmortem examination series in 2–3% of cases (106). Most of these cases were not recognized *in vivo* because of atypical clinical courses and difficulties in obtaining a positive viral isolation from the CSF. Following the advent of molecular approaches

for the diagnosis of viral infections, CNS infections caused by HSV-1 or -2 started to be recognized, which led to a better definition of epidemiological, pathological, and clinical features of these manifestations.

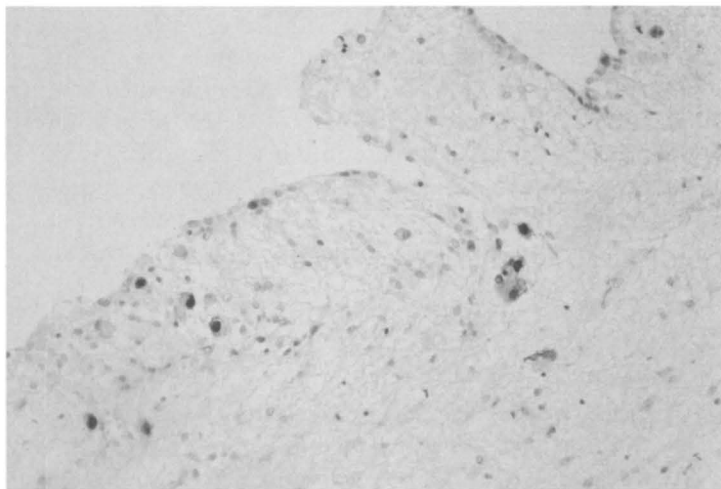
In a minority of the cases, HSV infection of the CNS in patients with HIV infection may show the same clinico-morphologic features of classic herpes simplex encephalitis (HSE), i.e., acute, focal necrotizing encephalitis involving the temporal lobes and adjacent areas. These typical HSE manifestations are usually observed in patients with a relatively conserved immune function, e.g., high CD4 cell counts. In patients with severely impaired immunity, the better-recognized clinicopathological picture is subacute focal encephalitis or ventriculo-encephalitis (106,107). More recently, cases presenting with clinical features of meningitis or meningoencephalitis and mainly caused by HSV-2 have also been reported (108).

The great majority of the cases of encephalitis occur in concomitance with CMV encephalitis, which also involves preferentially the periventricular areas. In a postmortem study of 82 cases of CMV encephalitis, 16% of these were actually found to be mixed HSV and CMV encephalitis (106). These mixed forms are not distinguishable from pure CMV encephalitis, both histologically and clinically. Histopathological examination shows necrotic lesions in the periventricular areas and, occasionally, foci of necrosis deep in the brain parenchyma. Inclusion-bearing cells are always present within or peripheral to the lesions, although cannot be attributed to either virus. The diagnosis of coinfection is usually established by immunocytochemistry (Fig. 2). The relative contribution of either virus to the clinical picture is not clear, although immunohistochemical studies show a greater or equal prevalence of CMV-positive cells compared to HSV-positive cells (106,109).

Clinically, both mixed HSV-CMV and pure CMV encephalitis patients present with a picture characterized by subacute onset of drowsiness, confusion, or lethargy, often associated with focal signs, including cranial nerve palsies and motor deficits (107). Contrast-enhancing magnetic resonance imaging may show low signal intensity on T1-weighted images, with high signal intensity on T2-weighted images in the periventricular white matter or in the brain parenchyma. Ill-defined periventricular enhancement is often observed following gadolinium injection.

The etiological diagnosis is achieved by the identification of HSV-1 or HSV-2 DNA in the CSF by nucleic acid amplification. CSF PCR has been shown to be highly sensitive and specific, whereas other methods, including virus isolation from CSF or detection of specific intrathecal antibody synthesis, are low sensitive for diagnosis in HIV-positive patients (107,110). The disease is usually progressive despite aggressive treatment with antiviral drugs, including aciclovir, foscavir, ganciclovir, or a combination of these drugs, with death ensuing within weeks from onset of symptoms (107,110).

(A)



(B)

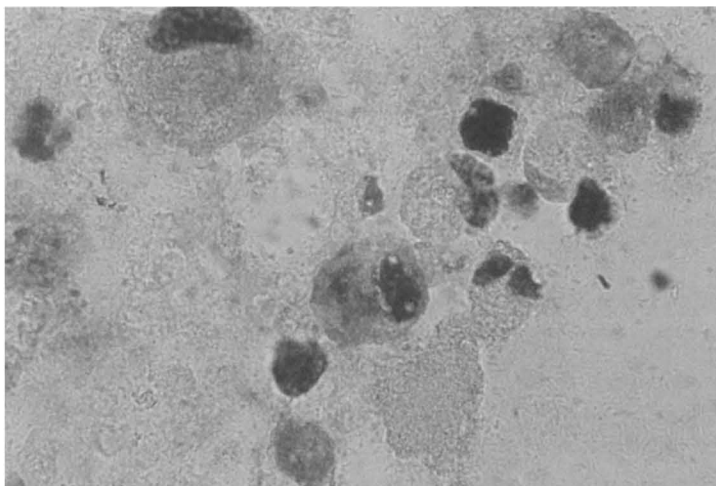


Figure 2 (A) Mixed HSV-1 and cytomegalovirus (CMV) ventriculo-encephalitis. Hematoxylin–eosin staining followed by immunocytochemistry for HSV-1 identifies HSV-positive cells in the context of necrotizing subependymal lesions. (B) Mixed HSV-1 and cytomegalovirus encephalitis. Double immunohistochemical staining shows the presence of reactivity against both HSV-1 and CMV antigen. HSV staining is mainly cytoplasmatic, whereas CMV staining involves both cytoplasm and nucleus. *Source:* Courtesy of Dr. Luca Vago.

Immune Reconstitution HSV Disease

HSV infections have been observed in the context of HAART-related immune reconstitution. Immune reconstitution disease (IRD) defines a spectrum of conditions characterized by symptomatic and paradoxical inflammatory response to a pre-existing infection that is temporally related to the recovery of the immune system (111). IRD has been described in a variety of conditions, including transplanted patients following withdrawal of immunosuppressive agents and HIV-infected patients starting HAART. More specifically, HAART-induced immune recovery is coincident with an increased CD4 cell count, reduced viral load, or both, with an interval between the start of HAART and IRD variable between days to months (111). IRD varies in its clinical manifestations, including lymphadenitis, pneumonitis, uveitis, CNS disease, and other organ diseases, and has been linked to a wide variety of infectious agents, such as *Mycobacterium avium complex*, *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, *Pneumocystis carinii*, CMV, VZV and HSV (112,113). The disease manifestations have been attributed to paradoxical worsening or reactivation of previously quiescent disorders following HAART-induced immune reconstitution.

IRD associated to HSV infections has included skin and mucosal lesions, as well as CNS disease. Perianal, genital, or perioral herpes have all been observed (112,113). Cases have been reported of chronic erosive HSV genital infection presenting weeks to months after starting HAART and characterized by florid erosions and copious exudate and, histologically, by plasma cell and eosinophilic infiltrates (114). CNS disease varied in presentation, including temporal lobe encephalitis, encephalomyelitis, or myelopathy, and was often observed in concomitance with mucosal or skin reactivation of HSV (113). In general, the clinical features of immune reconstitution HSV manifestations do not differ from those observed in typical HSV infections and the diagnostic approach is not different. Treatment is based on antiviral drugs. Corticosteroids may be considered in individual cases.

HSV Resistance to Antiviral Drugs

HIV-infected people, especially those with a low number of CD4 cells, are at high risk of developing resistance to antiviral drugs used to treat herpesvirus infections. This is due to the prolonged and severe immune suppression that prevents virus clearing from the lesions, leading, as a consequence, to prolonged periods of treatment. This situation of a highly replicating virus in the presence of viral inhibitors creates the background for the emergence of naturally occurring drug-resistant variants.

Infections with aciclovir-resistant HSV strains have frequently been reported in HIV-infected patients, often in association with treatment failure. The occurrence of aciclovir-resistant HSV infections was first described in the end of the 1980s in patients with ulcerative mucocutaneous herpes failing

to respond to aciclovir (115–117). Recent case series reported a prevalence of aciclovir resistance among HIV-infected patients with genital herpes of between 4% and 6% (113,118–120). Of note, these figures are usually lower than those reported in bone marrow transplanted patients. For instance, one study reported a prevalence of aciclovir-resistant HSV in 4% of HIV-infected patients but in 11% of bone marrow transplanted patients (119). This difference may be explained by the fact that the tested HIV-positive population was likely heterogeneous, also including people with a relatively preserved immune function, and thus less at risk for development of antiviral drug resistance.

Emergence of aciclovir resistance is clinically suspected when lesions fail to improve following 4–5 days of adequate therapy. Ideally, in these cases, a susceptibility test should be performed on viral isolates from the lesions and treatment modified. Increasing the dosage of aciclovir is not useful because most of the resistant strains are characterized by the lack of thymidine kinase (TK-deficient mutants or TK-), thus preventing drug phosphorylation into the active form. In addition, these strains will be cross-resistant to famciclovir and ganciclovir, which also require this phosphorylation step for activation. On the other hand, susceptibility will be conserved towards cidofovir, because this nucleoside drug is not activated by viral, but by host-specific enzymes, and to foscarnet, which does not require phosphorylation to exert its antiviral effect (see chapter on antiviral drugs).

Other viral-resistant mutants characterized by altered TK (TK-altered) or DNA polymerase activity show different susceptibility to antiviral drugs other than aciclovir. TK-altered mutants may be susceptible to penciclovir and thus to famciclovir. On the other hand, DNA polymerase mutants may be resistant to both nucleoside analogs and foscarnet, since this enzyme is the ultimate target in both cases (see chapter on antiviral drugs). Of note, patients harboring aciclovir-resistant strains often develop resistance to foscarnet following switching to this drug. HSV infection caused by foscarnet-resistant HSV was also initially described in the end of the 1980s (121) and remains an important concern in HIV-infected patients with severe HSV disease (42). Recently, it has been observed that as many as 61% of patients who excrete ACV-resistant strains also develop resistance to foscarnet (119).

Despite the figures being much higher than those observed in immunocompetent individuals, prevalence of aciclovir-resistant HSV among HIV-infected people has remained stable through the 20-year period of aciclovir usage, as observed in transplanted patients. Such a stable prevalence figure is likely the result of a number of virus and host factors (120). Principally, the majority of aciclovir-resistant strains appear to be less biologically competent than wild-type virus and this seems to impact significantly on normal infection, reactivation, and transmission. Even if a resistant virus emerges during reactivation, peripheral virus will unlikely

replenish the reservoir of latent virus. Thus, subsequent reactivations will likely be caused by pre-existent virus, i.e., that first establishing latency. In addition, aciclovir-resistant virus is unlikely to be transmitted. Although a few case reports suggest potential transmission, this remains a theoretical possibility since no proof has ever been shown. Since the advent of HAART, the likelihood of emergence of resistance to antiviral drugs has further decreased, mainly as a consequence of reduced frequency of severe HSV manifestations requiring prolonged antiviral treatments. However, and although it appears not to be a public concern on a population basis, the problem of HSV resistance to antiviral drugs remains an important issue in individual patients.

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Neonatal Herpes Simplex Virus Infection

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INTRODUCTION

Herpes simplex virus (HSV) infection in the neonate is of great concern because of its significant morbidity and mortality. Over the last few decades, there have been significant advances in diagnosis and treatment, but further improvements are needed.

The first effective antiviral therapy for neonatal HSV was demonstrated in a randomized placebo-controlled trial of vidarabine given for 10 days by Whitley et al. (1) in 1980. Mortality decreased with vidarabine therapy from 74% to 38% in infants with disseminated disease and central nervous system (CNS) involvement and from 85% to 57% in infants with disseminated disease alone (1). Currently, with the use of high-dose acyclovir® for 21 days, studies demonstrate a 24-month mortality of 31% in infants with disseminated disease and 6% in infants with CNS disease alone (2).

Despite these improvements in mortality, a significant amount of morbidity remains among survivors. Among surviving patients, only 83% of patients with disseminated disease and 31% of patients with CNS disease had normal development at 12 months of life (2). Even in patients with disease apparently limited to the skin, eye, or mouth (SEM), 6% had subsequent neurologic impairment, and this was significantly correlated to the frequency of recurrence of lesions in the first 6 months of life (3).

In this chapter, we will discuss the epidemiology and clinical presentation of neonatal HSV infection. Current techniques in diagnosis and management will be discussed, along with current areas of investigation.

EPIDEMIOLOGY

The reported incidence of neonatal HSV infection varies in different countries. In the United States, the incidence is estimated to range between 1 per 3000 to 20,000 live births, with most estimates around 1 in 3000 to 1 in 3200 (4-8). HSV-2 predominates 3:1 over HSV-1 as the cause, reflecting the prevalence of HSV-2 as the major cause of genital herpes in the United States (4,9). In contrast, in Japan, 70% of cases of primary genital herpes are caused by HSV-1, and two-thirds of isolates from neonatal infections are HSV-1 (10). Tookey and Peckham (11) have reported an incidence of 1.65 per 100,000 live births in the British Isles. Over a 10-year period in the 1980s, the incidence of neonatal HSV-2 infection in Sweden was estimated to be 1 per 20,000 live births (12).

The incidence of adult HSV-2 genital infection is increasing. In a study defining the seroprevalance of HSV-2 in the United States, from 1988 to 1994 the prevalence was 21.9%, a 30% increase from the incidence measured between 1976 and 1980 (13-15). The majority of these people are asymptomatic, with fewer than 10% of seropositive individuals reporting a history of genital herpes (11). In mothers with HSV-infected newborns, at least 80% are asymptomatic and deny both a history and presence of genital herpes in their sexual partners (16).

Two studies evaluated the seroconversion rates of women during pregnancy and found that between 16 and 20 per 1000 seronegative women acquired HSV at some point during pregnancy (15,17). This is of significance since the risk of HSV infection in an infant born by vaginal delivery to a mother with a primary genital HSV infection is much higher than an infant born to a mother with reactivation of infection (4,8). Epidemiologically, primary HSV infection has been defined as either isolation of virus from a seronegative woman, or seroconversion from the first prenatal sample to a repeat sample at time of delivery. Non-primary, first episode infections have been defined as isolation of HSV-2 from a woman who is HSV-1 seropositive and HSV-2 seronegative, or isolation of HSV-1 from a woman who is only HSV-2 seropositive. Reactivation occurs when the woman is seropositive for the virus isolated (8,17). In a recent study, Brown et al. (8) demonstrated a transmission rate of 54 infants per 100,000 live births in HSV mothers who experienced either primary or first episode genital disease, compared to only 12 per 100,000 live births in mothers seropositive for both HSV-1 and HSV-2.

ACQUISITION OF INTRAUTERINE AND NEONATAL INFECTIONS

Neonatal HSV infection is acquired during one of three periods: in utero, intrapartum, or postpartum. Intrauterine infection can occur by an ascending infection, or by a transplacental route. However, even though intrauterine infection has been documented to occur with both primary and recurrent maternal HSV infection, it is uncommon (approximately 5–9% of neonatal HSV infections and 1 in 300,000 live births) (6,9,14,18). Risk factors associated with intrauterine infection have not been identified (6,18).

Intrapartum transmission of HSV to the neonate is the most common mode of infection (85–90%) (6). Several factors have been shown to influence the risk of transmission of HSV to the neonate (Table 1). Primary maternal HSV infections result in higher transmission rates of HSV to the neonate than recurrent infection. In one prospective study following 15,923 pregnant women, the rate of transmission was 10 times higher in women with primary infection (33%) than in women with reactivation of genital herpes (3%) (7). In a more recent study, Brown et al. isolated HSV from the genital tract of 202 women (out of 40,023 cultures obtained). Neonatal transmission occurred in 5% of culture-positive women. Based on their data, the neonatal HSV infection rate per 100,000 live births was 54 in primary infection, 26/100,000 among women seropositive for HSV-1 only and 22/100,000 among all HSV-2-seropositive women (8). Other studies have shown that the risk of neonatal HSV infection is at least 50% in primary infection, approximately 20–30% in women with non-primary HSV-2 infection, and less than 3% in recurrent HSV-2 infection (7,14,17,19). This agrees with the observation that primary genital infection is associated with larger quantities of virus replicating in the genital tract for longer periods of time (3 weeks in primary infection compared to an average of 2–5 days in recurrent infection) (6). Furthermore, primary infection, particularly during the third trimester of gestation, is associated with a delay in the full maturation of both humoral and cell-mediated immune responses (6). The risk of primary infection is highest in seronegative women with seropositive partners (discordant partners).

Table 1 Risk Factors Associated with Neonatal Infection by HSV

-
- Primary maternal HSV infection
 - Low titers of neutralizing antibodies
 - Invasive obstetrical procedures
 - Prolonged rupture of membranes (>6 hr)
 - Discordant partners
-

Preterm birth is common among babies with neonatal HSV with approximately half of the babies born prematurely (16). These data suggest that HSV may be a cause of premature labor. One case-control study demonstrated that asymptomatic genital shedding of HSV during a subclinical primary infection was associated with preterm delivery, but no association with recurrent shedding was found (20).

Studies have shown that antepartum viral cultures are not predictive of maternal viral shedding during labor. When cultures were obtained serially at 2-day intervals, a positive culture had a 59% predictive value of the next culture being positive, but if the interval between cultures was greater than 2 days, only 19% of subsequent cultures were positive (21).

Lower neutralizing antibody titers in the infant and primary infection later in pregnancy have also been associated with an increased risk of infection, as suggested above (17,19). One study compared a population of infants exposed to recurrent HSV-2 infection without neonatal disease with a group of infants with neonatal HSV infection. The infants with neonatal HSV were significantly less likely either to have neutralizing antibody to HSV-2 ($P < 0.001$) or to have titers above 1:20 ($P < 0.001$) (19). This agrees with the finding that mothers with primary infection during pregnancy who complete seroconversion prior to the time of labor were not associated with increased incidence of neonatal HSV infection (17). Furthermore, it is suggested that the titer of neutralizing antibody is related to the disease presentation, with higher titers in disease isolated to the skin, eyes, and mouth (SEM) and lower titers in CNS and disseminated disease (22,23).

Invasive obstetrical procedures increase the risk of neonatal HSV infection. In particular, fetal scalp electrodes have been associated with increased risk of infection, presumably by disrupting the skin and providing a site of inoculation for the virus (6,8,24).

Prolonged rupture of membranes (>6 hours) also increases the risk of an ascending infection and subsequent development of neonatal HSV disease (6). Brown et al. have also published data supporting the use of cesarean delivery to reduce neonatal HSV transmission. The transmission rate was 1/85 (1.2%) in infants born to mothers with positive genital cultures for HSV and delivered by cesarean section, compared to 9/117 (7.7%) of these infants born vaginally (8).

Postnatal acquisition of HSV accounts for approximately 8–10% of cases. These infections are primarily caused by HSV-1. Infants who come in contact with individuals with orolabial herpes are at risk for postnatal infection. These contacts may include relatives, health care workers, child care workers, or even contact with maternal orolabial or breast lesions. It is of great importance that individuals with active lesions avoid close contact and maintain good hand-washing procedures when near infants.

CLINICAL MANIFESTATIONS

Recognizing the clinical manifestations of neonatal HSV disease is not only important in correctly diagnosing and treating this infection but the classification of disease is also important in predicting long-term outcomes.

Intrauterine Infection

Intrauterine infection is the least common mode of acquisition of neonatal HSV, but is associated with among the most severe disease. Typically, intrauterine infection causes multisystem disease, but in a small subset of patients may only cause skin or eye lesions. This most often represents the result of prolonged rupture of membranes with skin or eye lesions present at the time of delivery (6).

Most infants with intrauterine infection have involvement of the skin, eyes, and CNS. In one study population of 13 infants with clinical manifestations of intrauterine infection, 92% had abnormalities of the skin present either at delivery or by 72 hours of life. These skin findings included vesicles and/or bullae, with or without skin scarring of the scalp, face, trunk, or extremities (18). Chorioretinitis with or without keratoconjunctivitis also occurs, as does microphthalmia (6,18,25). Central nervous system manifestations occurred in 92% of infants, with microcephaly or hydranencephaly as the most common findings (18). Overall, almost all infants (12/13) had findings of at least two organ systems. Approximately 25% of infants in this series had hepatomegaly or hepatosplenomegaly. No risk factors for intrauterine infection were identified (18).

Intrapartum and Postnatal Infection

In intrapartum and postnatally acquired neonatal HSV disease, three major patterns of clinical disease are recognized: (1) disease localized to the SEM (40%); (2) encephalitis, with or without SEM disease (CNS disease)(35%); (3) disseminated infection (25%) (4,6,9). The incidence of signs and symptoms in each of the presentations and the mean age in which the presentation occurs are summarized in Tables 2 and 3, respectively (9).

Disseminated Disease

Disseminated neonatal HSV disease has a significant mortality and morbidity, with death in greater than 80% of untreated infants. HSV pneumonitis and/or disseminated intravascular coagulopathy (DIC) are the most common causes of death in disseminated infection (6).

While signs of disseminated disease may present as early as 4–5 days of life, children present for medical treatment on average between 9 and 11 days of life. The presentation of disease can mimic bacterial sepsis, with the nonspecific signs and symptoms of irritability, poor feeding, respiratory

Table 2 Incidence of Signs and Symptoms in Different Disease Presentations

Disease classification	SEM (%) (<i>n</i> = 64)	CNS (%) (<i>n</i> = 63)	Disseminated (%) (<i>n</i> = 59)
Skin vesicles	83	63	58
Lethargy	19	49	47
Fever	17	44	56
Conjunctivitis	25	16	17
Seizure	2	57	22
DIC	0	0	34
Pneumonia	0	3	37

Source: From Ref. 9.

distress, temperature instability/fever, jaundice, seizures, DIC, and shock. The characteristic vesicular rash is useful in making the diagnosis when it is present, but in one recent study population, the rash was absent in 39% of patients with disseminated disease (9).

Because of the nonspecific nature in which the disease may present, it is important to always keep HSV infection in the differential diagnosis of neonates presenting with suspected sepsis, especially if there are risk factors for infection present. While the liver and adrenals are the most common organs involved, infection can involve almost any organ system. Ocular involvement occurs in approximately 10–17% of neonates with disseminated infection (9,27). CNS infection secondary to viremia and crossing of the blood brain barrier occurs in 60–75% of patients with disseminated disease.

As mentioned, HSV pneumonitis is a significant cause of mortality (relative risk of 3.6) with the chest X ray demonstrating a diffuse interstitial pattern, which progresses to a hemorrhagic pneumonitis (3,6). Infants with gastrointestinal disease may demonstrate pneumotosis intestinalis on abdominal films.

Laboratory studies to evaluate the extent of systemic disease are indicated. Liver enzymes and bilirubin measurements can indicate hepatic involvement. Cytopenias can be seen on complete blood counts (particularly neutropenia and thrombocytopenia). Evidence of disseminated intravascular

Table 3 Mean Age of Presentation of Neonatal HSV Disease

Disease type	Age of presentation (days)
Skin, eye, mouth (<i>n</i> = 3)	8–11
CNS (<i>n</i> = 24)	16–17
Disseminated (<i>n</i> = 6)	9–10

Source: From Refs. 6, 26.

coagulation (elevated PT/aPTT, increased fibrin degradation products/D-dimers, decreased fibrinogen level, thrombocytopenia) should be sought.

Mortality in disseminated disease at 24 months of life was 31% with high-dose acyclovir (60 mg/kg/day) for 21 days, 57% with intermediate dose for 21 days (45 mg/kg/day), and 61% with standard dose for 10 days (30 mg/kg/day). In particular, infants with significant hepatitis demonstrated by AST elevation ≥ 10 times the upper limit of normal had markedly increased survival rates with high-dose acyclovir compared with intermediate and standard doses (2).

Development at 12 months of life is normal in 83% of patients with disseminated disease treated with high-dose acyclovir, compared with 60% of patients treated with standard dose (2). This also compares with 58% of patients treated with vidarabine developing normally at 1 year of life (28).

Encephalitis With or Without SEM Involvement (CNS Disease)

The mean age of presentation in infants with CNS infection ranges from 1 to 3 weeks of life (6,26). In one study, all infants with disease due to HSV-1 presented with skin lesions compared to 60% with HSV-2 (26). Most patients had a history of lethargy for 2–3 days prior to the development of seizures (26). The frequency of signs and symptoms are listed in Table 4.

CNS infection can occur as a component of disseminated infection (discussed previously), or it can occur as an isolated encephalitis. One-third of infants with CNS disease have encephalitis only. Infection of the CNS most likely occurs by retrograde axonal transmission of the virus. The infant can present with lethargy, irritability, poor feeding, seizures (both focal and generalized), temperature instability, bulging fontanelle, and/or pyramidal tract signs (29).

Laboratory findings in CNS disease are outlined in Table 5. The cerebrospinal fluid (CSF) is typically abnormal with a lymphocytic predominant pleocytosis and proteinosis. Electroencephalogram abnormalities and abnormalities of the brain on imaging by computed tomography (CT) scan

Table 4 Clinical Signs and Symptoms in CNS Infections

	HSV-1 (%) (n = 9)	HSV-2 (%) (n = 15)
Mucosal lesions	100	60
Fever or temperature instability	22	40
Conjunctivitis	33	44
Apnea/cyanosis, bradycardia	11	33
Lethargy/seizures	11	66

Source: From Ref. 26.

Table 5 Laboratory Findings in HSV CNS Disease

	HSV-1 (n = 9)	HSV-2 (n = 15)
CSF pleocytosis	100%	100%
Mean CSF WBC count (cells/ μ L)	20 (range 7–45)	113 (range 9–545)
Mean % lymphocytes	85 (50–100)	88 (40–100)
Mean CSF RBC count (cells/ μ L)	87 ($\pm$ 127)	383 ($\pm$ 1038)
Mean CSF protein (mg/dL)	55 (40–66)	104 (82–396)
Mean CSF glucose (mg/dL)	45 (39–52)	46 (< 20–50)
Electroencephalogram abnormalities	33%	33%
CT scan abnormalities	0%	36%

Source: From Ref. 26.

can be seen. Serial CSF evaluation will show increasing protein concentrations. Viral cultures of the CSF will grow HSV in 25–40% of cases (29). More recently, PCR of the spinal fluid has been employed as a diagnostic test. The sensitivity and specificity of HSV PCR in the diagnosis of CNS infection have been high, when performed by an experienced laboratory.

Most infants with CNS disease have a poor outcome. High suspicion for the diagnosis and early initiation of treatment are critical to improve outcomes. Historically, 50% of untreated infants with CNS disease died. Treatment with high-dose acyclovir for 21 days (60 mg/kg/day) decreases the mortality rate to 6%, compared with a rate of approximately 20% with intermediate dose (45 mg/kg/day) or standard dose (30 mg/kg/day) acyclovir. In the same study, only 30% of patients were developing normally at 12 months of life regardless of the treatment group (2). This is similar to other studies demonstrating normal development in only 36% of treated children at 12 months of life (3). Surviving children have a spectrum of disease severity with a variable degree of developmental delay, which may be associated with microcephaly, hydranencephaly, porencephalic cysts, spasticity, persistent seizures, blindness, and/or chorioretinitis (3,6).

SEM Disease

By definition, infants with disease clinically localized to the skin, eyes, and/or mouth have no mortality. Of major concern, however, are reports of between 6% and 30% of these infants eventually developing neurologic impairment (3,5).

The classic vesicular/ulcerative skin lesions typically appear first on the presenting part of the infant. Lesions may also appear at breaks in the skin, such as the site of a scalp electrode. The vesicles may appear in clusters, and progress to other areas of the body. These infants will typically present at 8–11 days of life (6,9). Less commonly, lesions may be bullous, zosteriform, or necrotic (14).

Eighty-three percent of patients with SEM have skin vesicles during some point of their disease (9). Recurrence of lesions is not uncommon. In a trial comparing vidarabine with acyclovir therapy, between 20% and 35% of infants with SEM disease had recurrence of lesions in the first month, and this increased to 46% 6 months after therapy ended (28). The frequency of recurrences is important. Infants with fewer than three recurrences in the first 6 months of life will most likely be normal neurologically at 1 year of life. Only 79% of infants with three or more recurrences of HSV-2 skin vesicles were normal at 1 year of life in the same study (3). The major neurologic abnormalities noted include spastic quadriplegia, microcephaly, and blindness (6).

One retrospective study examining the application of PCR to detect HSV DNA in cerebrospinal fluid (CSF) found that HSV DNA was present in seven of 29 infants with disease localized to the skin, eyes, and/or mouth based upon clinical and other laboratory parameters (30). All seven infants had normal white blood cell (WBC) counts, normal glucose and normal protein chemistries. One of these patients had severe neurologic impairment on follow-up. It is not known if the neurologic impairment in these children occurred secondary to otherwise unrecognized CNS damage during the initial infection, or by subclinical reactivation of HSV in the CNS. The correlation of neurologic outcome with the frequency of recurrences of skin vesicles suggests that subclinical reactivation of HSV in the CNS may play a significant role in causing damage to a developing brain. Of note, in a Phase I/II trial of suppressive acyclovir for SEM disease, one patient who was on bid acyclovir developed recurrent skin lesions and had HSV DNA detected in their CSF. This child's development was normal at 1 year of age (31).

Ocular manifestations of HSV disease occur in approximately 25–33% of infants with localized infection (9,27). HSV infection can result in conjunctivitis, keratitis, chorioretinitis, and/or cataracts. Conjunctivitis occurs in approximately 5% of cases. The onset is anywhere between 3 days and 2 weeks, with the mean close to 7 days. Erythema of the bulbar and palpebral conjunctivae is noted. Involvement may be unilateral or bilateral with or without a serosanguinous discharge. No apparent follicular hypertrophy is noted (27). When present without any other signs or symptoms, HSV conjunctivitis is difficult to discern from bacterial etiologies.

Keratitis is present in around 10% of cases. In most cases, conjunctivitis is also present. Diffuse epithelial involvement may be noted with punctate staining with fluorescein, or dendritic ulcers may be noted. Larger irregular geographic ulceration can also be seen (27).

Keratoconjunctivitis can progress to chorioretinitis. Chorioretinitis is usually seen at 1–3 months of life, but if detected much earlier (first week of life) suggests intrauterine infection. Typically extensive areas of the retina are involved with perivasculitis. Yellow-white exudates and severe vitreous

Table 6 Relative Risk of Prognostic Factors in Neonatal HSV Disease

	Relative risk	
	Mortality	Morbidity
Disease classification		
SEM	1	1
CNS	5.8	4.4
Disseminated	33	2.1
Level of consciousness		
Alert or lethargic	1	NS
Semicomatose/comatose	5.2	NS
DIC	3.8	NS
Prematurity	3.7	NS
Viral type		
HSV-1	2.3	1
HSV-2	1	4.9
Seizures	NS	3
Pneumonitis (disseminated disease)	3.6	NS

Abbreviation: NS, not statistically significant.
Source: From Ref. 3.

reactions are noted. Cataracts have been reported on follow-up visits of infants with a history of HSV chorioretinitis (6,27).

Predictors of Morbidity and Mortality

The most important predictor of outcome in neonatal infection is the disease classification. As previously stated, with high-dose acyclovir for 21 days, mortality rates are 6% in CNS disease and 31% in disseminated disease, with no mortality associated with SEM disease (2).

The risk of death is higher in infants who present with coma, develop pneumonitis or DIC, or were premature. Morbidity in surviving infants is highest with encephalitis, DIC, seizures, or with infection caused by HSV-2 (Table 6) (3).

VIROLOGICAL DIAGNOSIS

It is important to remember that delays in initiation of therapy in neonatal disease may increase morbidity and mortality so that if neonatal HSV infection is a significant possibility, therapy should be started while the diagnosis is pursued.

The gold standard for diagnosis is the isolation of HSV in an infected individual. Viral culture of HSV will show a cytopathic effect within 2 days

in 80% of cultures, and in 99% of cultures by 4 days. The location and technique of specimen collection can influence the sensitivity of the culture. Skin lesions are the most frequent sites of virus isolation (16). The mouth or nasopharynx, eyes, urine, blood, stool or rectum, and CSF should also be cultured. Isolation of virus from any site at greater than 48 hours of life should be considered active infection, and not transient colonization after maternal exposure (4,6,14).

HSV DNA detection by PCR is a useful tool when performed by an experienced laboratory. In one small study of four neonates, HSV DNA was detected by PCR in all four neonates with CNS disease, compared to one of four by viral culture. The sensitivity and specificity was 100% in this study. Of note, serial samples in one patient receiving acyclovir therapy were still positive after 2 weeks of treatment (30 mg/kg/day) (10).

Another study found the sensitivity and specificity of HSV PCR of CSF in neonatal HSV encephalitis to be 75% and 100%, respectively, with a positive predictive value of 100% and a negative predictive value of 98% (32). In a Swedish study by Malm and Forsgren (33), a similar sensitivity of 78% was found. Kimura et al. (34) demonstrated a sensitivity of 100%. A study by Kimberlin et al. had a lower sensitivity and specificity of 80% and 71%, respectively. However, the investigators felt that this was falsely low with the specificity decreased by the finding of HSV DNA in patients with clinical SEM disease, and the sensitivity decreased by the number and timing of the specimens available for testing (30).

In adults with HSV encephalitis confirmed by brain biopsy, HSV PCR has been shown to have a sensitivity of 98%, and a specificity of 94%. The positive and negative predictive values were 95% and 98%, respectively (35).

Since the test detects HSV DNA and not necessarily live virus, it must be interpreted accordingly. This is beneficial when CSF or serum is tested after therapy has been started, and has in one study been shown to detect HSV DNA in CSF after 2 weeks of acyclovir therapy (10). This has been confirmed in other studies with one demonstrating a worse outcome with persistence of HSV DNA in the CSF after completion of treatment, suggesting that PCR predicts neurologic outcome in neonatal disease (33).

More recently, Kimura et al. performed quantitative PCR for HSV DNA on serum and CSF. The highest serum viral load was found in disseminated disease, and viral load was significantly higher in the serum of patients who later died. In CSF, the highest viral load was found in CNS disease, with high levels in patients who died or had neurologic impairment at follow-up, but this was not shown to be statistically significant compared to patients with normal neurologic development at 12 months of life (34).

Of note, in their population, infants with HSV-2 had greater CNS involvement and subsequent neurologic impairment. These infants with HSV-2 had higher CNS viral loads in CSF, but not serum, compared to infants with HSV-1 infection (34).

Further investigations are required to assess the utility of quantitative PCR in determining long-term outcome in neonatal HSV disease.

Monoclonal antibodies used with enzyme-linked immunosorbent assays to detect antigen, or with immunofluorescent staining, can be used with lesion scrapings, or in conjunction with cultures with acceptable sensitivity and specificity (4,14).

Traditional staining for multinucleated giant cells with intranuclear inclusions (Tzanck smear) has a poor sensitivity and specificity, and should not be used in light of improved diagnostic methods. Maternal type-specific serologies may help suggest the diagnosis. Infant serology is not useful in the acute diagnosis of neonatal HSV infections. IgG antibodies can be acquired across the placenta from the mother (4,6,14). The presence of IgM antibody in the infant is strongly suggestive of infection. However, in a study by Sullender et al. (22), early IgM antibody synthesis was not prominent in the immune response of infants with HSV. Infants may require 2–4 weeks to produce detectable IgM (6).

TREATMENT

Vidarabine (adenine arabinoside) was the first antiviral agent shown to be effective in treating neonatal HSV infection. Previously, idoxuridine and cytosine arabinoside were used as therapy in individual cases, but never underwent a controlled study secondary to significant toxicity. In 1974, a double-blind, placebo-controlled collaborative study of vidarabine was started to examine its use in the treatment of neonatal HSV disease. Vidarabine at 15 mg/kg/day for 10 days or placebo was administered. Fifty-six infants were included in the study, with 13 with SEM disease, 16 with CNS disease, and 27 with disseminated disease. Mortality decreased from 74% to 38% in infants with disseminated disease. In disseminated disease without CNS involvement, mortality decreased from 85% to 57%. Mortality in localized CNS disease improved from 50% to 10%. Improvement in morbidity was also noted, but a significant number of infants had residual neurologic abnormalities at 1 year of age. In infants with disseminated disease only 14% treated with vidarabine were normal at 1 year of age, compared to 8% of infants that received placebo. In CNS disease, 50% were normal with treatment compared to 17% of infants that received placebo (1).

When acyclovir was developed, a study comparing this more targeted inhibitor of viral replication with vidarabine was undertaken. Infants were randomized into treatment with vidarabine at 30 mg/kg/day for 10 days, or acyclovir at 30 mg/kg/day for 10 days. There were no differences in outcome of either morbidity or mortality between either groups (28).

Because of its comparable efficacy, ease of administration (every 8-hour dosing of acyclovir compared to a 12-hour continuous infusion with vidarabine), and fewer side effects, acyclovir has replaced vidarabine as

the treatment of choice, and was approved in 1998 by the United States Food and Drug administration for the treatment of neonatal HSV infection.

More recently, in an effort to further reduce mortality, and the remaining substantial morbidity, an open-label study using higher doses of intravenous acyclovir for longer periods was undertaken. Infants received either intermediate-dose acyclovir (45 mg/kg/day divided q8h) or high-dose acyclovir (60 mg/kg/day divided q8h) for 21 days. Treatment with high-dose acyclovir was shown to have a significantly higher survival rate compared to standard dose therapy in disseminated disease. Survival rates between high dose and standard dose in CNS disease were statistically similar. Only a small number of patients received intermediate-dose acyclovir, and the statistical interpretation of the data for this group was limited by its size. There were no statistically significant differences in morbidity at 12 months of age between the groups, but in infants with disseminated disease, 83% of patients receiving high-dose acyclovir were normal compared to 60% in the standard-dose group. This suggests that future studies with a larger population may show a statistical significance (2).

The major toxicity reported in this study was neutropenia, which occurred in 21% of infants with localized disease (SEM or CNS) treated with high-dose acyclovir. No adverse outcome of the neutropenia was reported, and all recovered with continuation of the acyclovir, or at the end of therapy (2). It is reasonable to monitor serial neutrophil counts in infants receiving a course of high-dose acyclovir treatment. With close monitoring of neutrophil counts, the risk/benefit ratio favors the use of high-dose acyclovir for the treatment of neonatal HSV infection.

Currently, the recommendation for treating disseminated disease or disease involving the CNS is 21 days of intravenous high-dose acyclovir (60 mg/kg/day divided q8h) (4). SEM disease is treated with intravenous high-dose acyclovir for 14 days (4). Relapse of SEM and CNS disease can occur after treatment. The best management of relapsed disease has not yet been determined.

Because of its poor bioavailability, the use of oral acyclovir is contraindicated in the treatment of neonatal HSV infection. Therapeutic plasma and CSF levels are not obtained (6).

Infants with ocular disease should be treated with a topical ophthalmic agent in addition to parenteral therapy. Agents that have been used include 1–2% trifluridine, 1% iododeoxyuridine, or 3% vidarabine. Consultation with an ophthalmologist is appropriate to follow disease course and monitor for complications and treatment efficacy (4,6).

As previously mentioned, the frequency of recurrences in infants with SEM disease is correlated with neurologic outcome. A Phase I/II trial of suppressive oral acyclovir therapy was completed in order to determine an oral dose to be subsequently evaluated as suppressive treatment. Infants with confirmed HSV-2 SEM disease were treated with oral acyclovir at

300 mg/m²/dose, given twice or three times a day for 6 months. Treatment with oral acyclovir was shown to prevent recurrences of skin lesions. In the group on three times a day dosing, 81% of patients had no recurrence of skin lesions while on therapy. After completing treatment, 44% in this group experienced recurrence of skin lesions. Seventy-two percent of patients were available for follow-up at 12 months of life, and all were neurologically normal. However, this study was uncontrolled and thus was not able to determine the effect of treatment on neurologic outcome (31).

The major side effect of suppressive therapy is neutropenia, which occurred in 46% of patients (31). Eighty-three percent of these patients with neutropenia had recovery of counts with continuation of therapy at the same dose. In one patient, an acyclovir-resistant HSV-2 mutant was isolated, but repeat cultures grew acyclovir-sensitive virus. There are few cases in the literature reporting acyclovir-resistant isolates (36).

Currently, two ongoing studies being conducted by the Collaborative Antiviral Study Group are investigating suppressive therapy. The first study is a Phase III trial examining the long-term neurologic outcome of infants with SEM disease on suppressive therapy and the second study is a Phase III trial looking at the effect of suppressive therapy in infants following treatment for CNS disease on long-term outcome.

In addition to ongoing studies investigating treatment of neonatal disease, efforts are also focused on preventing transmission. Researchers are investigating maternal acyclovir, valacyclovir, and famciclovir prophylaxis to reduce neonatal HSV transmission by decreasing shedding at time of delivery (37,38). Studies are also investigating maternal vaccine development for genital herpes (39).

CONCLUSION

Even with the advances made in the diagnosis and treatment of neonatal HSV infection in the past few decades, significant morbidity and mortality remains. Education of both pediatricians and obstetricians in the risk factors associated with transmission and a high index of suspicion for neonatal HSV disease are paramount in prevention and early treatment of disease.

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Future Outlooks

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The contributions within this volume clearly show the increasing burden of disease for which HSV-1 and HSV-2 have been identified as responsible pathogens. These two viruses are of concern to almost every medical specialty, and hopefully, the work of the authors presented herein will aid the clinicians to diagnose and to treat patients that they previously might not have recognized as suffering from HSV. Probably, additional diseases of hitherto unknown etiology will be linked to HSV in the future. But why do these viruses, which almost may be considered as a normal flora in most subjects, play such a growing role for human health?

One likely answer is that HSV-1 and HSV-2 always have caused a plethora of medical conditions that have been difficult to identify on clinical grounds, due to lack of visible mucocutaneous lesions. Advances in diagnostics have previously enabled new understanding, as for example, cell culture techniques that elucidated the pathogenesis of herpes simplex encephalitis. Today, polymerase chain reaction (PCR) broadens the scope of HSV disease, not in the least regarding neurological conditions. But is any presence of viral DNA in body fluids such as cerebrospinal fluid abnormal?

We foresee that studies using standardized quantitative PCR on all kinds of patient sample materials will be needed as a basis of diagnosis, and that a clinical evaluation will remain pivotal. A question that can be addressed is whether the viral load at primary infection will determine the severity of the first attack, as well as frequency of clinical recurrences.

To add to the complexity, the medical consequences of a defined HSV DNA quantity will vary with location and immune status of the patient. Moreover, simultaneous detection of HSV together with other herpesviruses will be more common as a result of growing diagnostics and will have to be interpreted. Given the recent advancements in proteomics enabling a highly increased sensitivity, antigen detection might again be developed as a useful complement in the future. Diseases of several organ systems might thus be better explored for HSV as pathogens or co-factors, as for example, ulcers and inflammations in internal organs such as the gastrointestinal and respiratory tracts.

Another reason for the increased medical importance of HSV as pathogens is the growing number of immunocompromised patients in the world, not least due to the HIV pandemic in poorer countries. As if driven by an immunological clock, HSV and other herpesviruses take turns in making matters worse, often in an environment where antiviral treatment or prophylaxis is ill afforded. The role of HSV-2 as a facilitator of HIV spread must clearly be more emphasized, and this pathogenetic property remains a strong driving force for the development of an effective vaccine against genital herpes. A successful control of spread of HSV-2 (and other pathogens causing genital ulcers) might well be a prerequisite for a successful combat of AIDS in the third world. In more developed countries, the ever-increasing activity regarding organ and bone marrow transplantation as well as more aggressive treatment of hematological malignancies will lead to increasing numbers of patients being prone to recurrences of herpesviruses. Clearly, effective vaccines against HSV are highly needed.

But how should an effective HSV vaccine be constructed? There is a disturbing discrepancy between the increasingly global use and relative success of the live, attenuated varicella-zoster virus (VZV) vaccine against both primary and recurrent disease and the current lack of similar immune protection against HSV in, for example, Africa. However, for HSV the task may be vastly more difficult than for VZV, given that both HSV-1 and HSV-2 readily and frequently reactivate in the face of full active immunity in large proportions of our populations. Thus, a vaccine that successfully hinders HSV infection and recurrences may require a substantially increased understanding of the pathogen–host interplay involved, which seems to be an important future task for the HSV research community.

Regarding antiviral therapy, the emergence of new anti-HSV drugs with novel mechanisms of action seems promising. Resistance to nucleoside analogues, today practically limited to the growing population of immuno-

compromised hosts, may become an even more important challenge in the future. But should the aim be placed higher, toward pharmaceutical viral clearance rather than virustatic treatment? Is there a place for immunomodulating therapy in recurrent HSV disease? These prospects are unclear. In the near future, it seems encouraging that antiviral treatment trials are again being attempted to optimize the use of nucleoside analogues to well-known HSV diseases, as well as to attempt to prove or disprove association of drug-sensitive viruses to some neurological conditions.

We live in a dynamic world, and HSV is constantly changing its epidemiology, as are all viruses. Today, we witness a shift in the etiology of genital herpes in that HSV-1 is increasingly becoming the most common pathogen, at least during primary infection. What are the consequences? Can we expect a parallel shift in neonatal HSV toward HSV-1 with better outcome of treatment, or will HSV-2 continue to be a devastating agent that partly defies therapy? Epidemiological studies will continue to be of clinical importance, and may shed light on transmission and course of the lesser studied asymptomatic infections as well.

HSV-1 and HSV-2 are two intensely studied viruses, subjected to immense experimental work and clinical investigations. Still, large gaps of knowledge exist regarding HSV genetics and proteomics during chronic infection in man. The future HSV world, although its evolution will be difficult to predict, will continue to be a source of excitement and engagement to researchers and to physicians, which will ultimately be of benefit to the patients.



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