CHAPTER III

REVIEW OF LITERATURE

History

The *Herpesviridae* is a large family of viruses that infect virtually all man including vertebrates more than 100 different *herpesviruses* (63). *Herpesviruses* are readily identified by the distinctive architecture of the virus virion. The *herpesviruses* contain several members that affect humans. This family was divided into three subfamilies of *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*, base on biological properties. For the human *herpesviruses*, there are eight human *herpesviruses* have been identified to date: Herpes simplex virus type-1 (HSV-1), Herpes simplex virus type-2 (HSV-2), Varicella-Zoster virus (VZV), Epstein–Barr virus (EBV), Human cytomegalovirus (HCMV), Human herpesvirus-6 (HHV-6), Human herpesvirus-7 (HHV-7), and Human herpesvirus-8 (HHV-8) corresponding to Kaposi's sarcoma associated herpesvirus (KSHV) (Table1).

Herpes simplex virus (HSV) is a virus that has affected human beings for thousands of years. HSV infections of humans have been documented since ancient Greek times (6,64). Description of the condition it causes date back to the Hippocrates (65). The Greek translation of the word "herpes" aptly describes the tendency of the virus "to creep or crawl" which refers to the spreading nature of the visualized skin lesions (64,66). The other reports from Roman scholar Herodotus (67), and many of these original observations of Galen's deduction are descriptions of skin lesions until 1893 that vidal specifically recognized human transmission of HSV infections from one individual to another (64).

One significant advance in HSV is the detection of antigenic differences between HSV types. In 1962, Schneweiss KE (68), demonstrated that there were in fact two serotypes of HSV, HSV-1 and HSV-2, whose formal designations under International Committee on Taxonomy of Viruses (ICTV) rules are now human herpesviruses-1 and -2. In 1968, the medical literature by Nahmias and Dowdle (69) demonstrated the antigenic and biologic differences were demonstrated between HSV-1 and HSV-2 (69-70). These

investigators demonstrated that HSV-1 was more frequently associated with nongenital infection (infection above the waist), whereas HSV-2 was associated with genital disease (infection below the waist). The observation was pivotal for many of the clinical, serologic, immunologic, and epidemiologic studies (64). However, either type can occasionally be found in either area or at other sites (71). Interestingly, infection with HSV-1 and HSV-2 account for a significant amount of morbidity each year, especially in immunocompromised patients (11).

Table 1: Members of the family *Herpesviridae* that infect humans (1).

Subfamily	Colloquial name and abbreviation	Numerical designation
Alphaherpesvirinae	Herpes simplex virus type-1 Herpes simplex virus type-2 Varicella-Zoster virus	HSV-1 HSV-2 VZV
Betaherpesvirinae	Human cytomegalovirus Human herpesvirus-6 Human herpesvirus-7	HCMV HHV-6 HHV-7
Gammaherpesvirinae	Epstein–Barr virus Kaposi's sarcoma associated herpesvirus (Human herpesvirus-8)	EBV KSHV (HHV-8)

Properties of herpes simplex viruses

Herpes simplex viruses are the membership of the family *herpesviridae*. They have been classified in subfamily *alphaherpesvirinae*, genus *simplexvirus*. This subfamily usually replicates fast and spreads rapidly killing infected cell by lysing, occasionally establishes latent infections in sensory nerve ganglia (3,72). There are two antigenic types, designated HSV-1 and HSV-2, which share antigenic cross-reactivity but different neutralization patterns (73,74). The hybridization study has revealed that HSV-1 and HSV-2 had 50% sequence homology (75). The herpesviruses are widely separated in terms of genomic sequence and proteins but in terms of virion structure and genome organization are similar.

The HSV virion is 200-300 nanometer (nm) in diameter and consists of four structure elements: an electron-opaque core, an icosahedral capsid surrounding the core, an amorphous tegument surrounding the capsid. The tegument contains at least two proteins of known function: Alpha-TIF (Alpha trans-inducing factor, also known as VP16 and vmw65) and VHS (virion host shut off), and an outer envelope exhibiting spikes on its surface (63,76) (Figure 1).

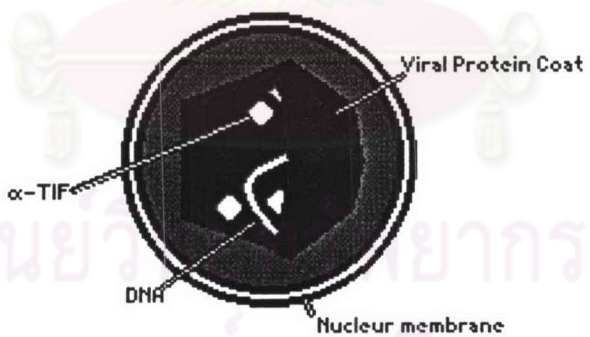

Figure 1: A typical HSV particle that the outer membrane is derived from the host cells nuclear membrane (63).

The core contains the viral DNA genome. HSV DNA is linear and double stranded (77-79) and packaged in the form of a toroid (80) or a spool (81). The physical characterization of HSV genome was originally estimated to be about 150 kilobase pairs (Kbp), molecular weight of 96 x 10^6 kilodatons, with a G + C content of 68% for HSV-1

and 69% for HSV-2 (77,78). The complete DNA sequencing of HSV-1 and HSV-2 is now known: the HSV-1 and HSV-2 genome described the genome as 152,260 bp (accession number x 1412) (82); minor updates altered this sequence to 152,261 bp (83) of HSV-1 strain 17 genome and 154,746 bp (accession number 286099) of HSV-2 strain HG 52 (83).

The HSV genome can be viewed as consisting of two covalently linked components, designated as L (long) and S (short) (Figure 2). Each component consists of unique sequences bracketed by inverted repeats (84). The repeats of the L component (long unique sequences; U_L) *ab* and *b'a'*, whereas those of the S component (short unique sequences; U_S) *a'c'* and *ca*. Because of this sequences arrangement, the L and S components can invert relative to each other, giving rise to four possible genome populations of unit-length DNA from wide-type virus-infected cells consist of equimolar concentrations of four predicted isomers (85,86). The isomers have been designated as P (prototype), I_L (inversion of the L component), I_S (inversion of the S component), and I_{SL} (inversion of both S and L components) (86,88).

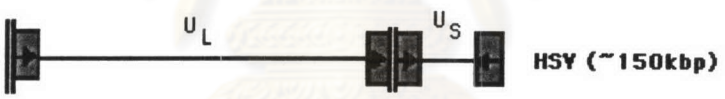

Figure 2: The prototype of the HSV has been consisted of two covalently linked components, designated as L (long) and S (short) (89).

The core is surrounded an icosahedral nucleocapsid. The structural features of the capsid, 125 nm in diameter, is composed of 162 capsomers (90) arrange in a T=16 icosahedral symmetry, and 150 elongated hexons (9.5 x 12.5 nm) comprising the faces and edges (91). There are three capsid structures in infected cells: type A; that lacks DNA and was never enveloped, type B; that contains DNA and was never enveloped, and type C; contains DNA and was obtained by deenveloping intact virions (92,93). The space between the undersurface of the envelope and surface of the capsid was designated as the tegument. The tegument is largely unstructured, except for some icosahedral structure around the pentons (81). It consists of virally encoded proteins and enzymes involved in the initiation of replication such as enzymes, which are needed

to tack control of the cell's chemical processes and subvert them to virion production, and some of which defend against the host cell's immune response.

Finally, the envelope consists of a lipid bilayer with about 12 different viral glycoproteins embed in it. It contains numerous protrusion of spikes, which are more numerous and shorter than those appearing on the surface of many other enveloped viruses. The spikes are composed the major components; gB, gC, and gD which three morphologically distinct spikes are 8 mm, 14 mm, and 24 mm, respectively (94).

Viral replication

Attachment and penetration

Herpes simplex virus genome must enter the cell for the initiation of infection by attachment and penetration. Five viral glycoproteins are dispensable for virus growth in culture (gC, gE, gG, gI, and gJ). Three glycoproteins (gB, gD, and gH) are essential and represent the minimal set of surface proteins necessary to sustain and carry out the dominant flow of events. The initial association, heparin sulfate proteoglycans appear to be the receptor molecules which are recognized by either gB or gC and which permit initial attachment of the virus (63). This is followed by a specific interaction with one of several cellular receptors collectively termed "HVEM" for "herpesvirus entry mediators". These are related to receptors for nerve growth factors and tumor necrosis factor. The association requires the specific interaction with the gD with one of several cellular receptors. When the viral envelope and the plasma membrane fuse to release the capsid tugument. Fusion of the envelope with the cellular membrane rapidly follows the initial attachment. This requires the action of a number of viral glycoproteins including gB, gH, gI, and gL (95) (Figure 3).

The deenveloped tegument-capsid structure is then transported to the nuclear pores. The viral capsid with some tegument proteins then migrates to nuclear pores along cellular microtubules utilizing cellular transport machinery. This "docking" is thought to result in the viral DNA being injected through the pore while the capsid remains in the cytoplasm. Some tegument proteins, such Alpha-TIF, also enter the

nucleus with the viral genome. Transcription of the viral genome, replication of viral DNA, and assembly of new capsids take place in the nucleus (95).

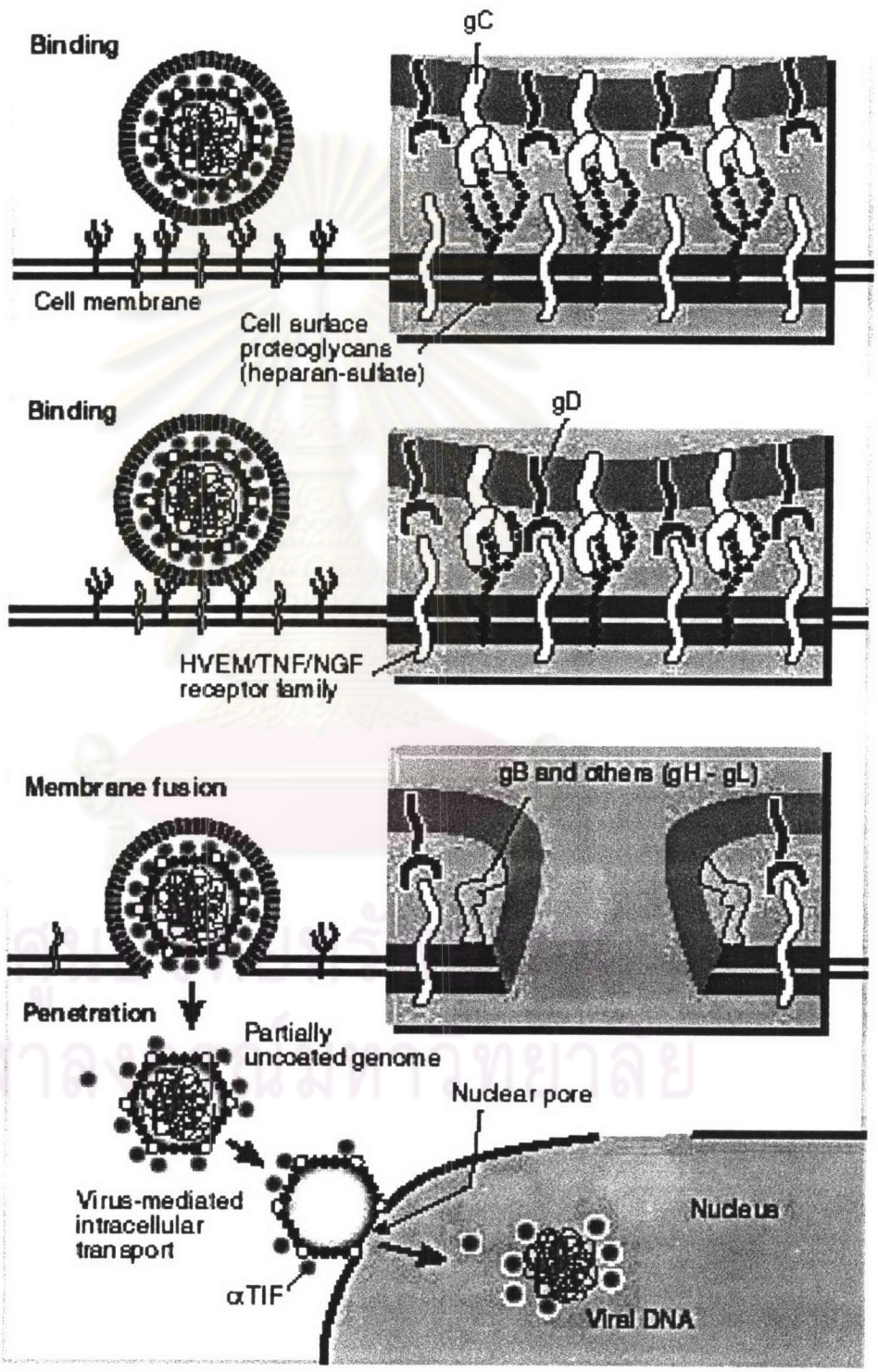

Figure 3: Schematic representation of the initial steps in HSV infection-HSV entry (95).

Gene expression

HSV transcription and protein synthesis are highly ordered. Although the absolute levels of viral protein synthesis may vary, different genes can be grouped on the basis of their requirements for synthesis. Hence, HSV genes have been subdivided into three distinct classes of mRNAs based on their time and requirements for expression (alpha, beta, and gamma) (63) (Table 2).

Table 2: Three distinct classes of mRNAs are made: (89).

Alpha - immediate early (IE) mRNAs	5 trans-acting regulators of virus transcription
Beta - (delayed) Early mRNAs	Further non-structural regulatory proteins & minor structural proteins
Gamma - Late mRNAs	Major structural proteins

Alpha genes

There are five alpha genes which have been identified and described as ICPs (infected cell proteins), these include ICP0, ICP4, ICP22, ICP27, and ICP47. The Alpha genes are by definition expressed in the absence of viral protein synthesis and contain the sequence GyATGnTAATGArATTCyTTGnGGG upstream of their coding regions. Their peak synthesis occurs two-four hours post infection, but they continue to accumulate until late in infection. All alpha genes appear to function as regulatory proteins with the possible exception of ICP47 (96).

Beta genes

These genes are not expressed in the absence of alpha proteins and their expression is enhanced in the presence of drugs which block DNA synthesis. They reach peak rates of synthesis five-seven hours post infection. The genes have been

subdivided into the Beta 1 and Beta 2 subclasses. Beta 1 genes appear early after infection, but require the presence of four protein for their synthesis. Examples of beta 1 genes include the large component of ribonucleotide reductase and the major DNA binding protein (ICP8). Beta 2 genes include viral thymidine kinase (TK) and the viral DNA polymerase. Beta gene synthesis immediately preceeds the onset of viral DNA synthesis and most viral genes involved in viral nucleic acid metabolism appear to be Beta genes (63,69).

Gamma genes

This class of genes is for convenience also separated into two groups. Gamma 1 genes are expressed early in infection and are only minimally affected by inhibitors of DNA synthesis (example, major capsid protein). In contrast, Gamma 2 genes are expressed late in infection and are not expressed in the presence of inhibitors of viral DNA synthesis.

The location of the gene classes within the HSV genome is of interest. Alpha genes map at the termini of the long and short components and tend to cluster together. In particular, alpha genes surround the HSV origin of replication in the short region (oriS). Each Alpha gene has its own promoter-regulatory region and transcription initiation and termination sites. Beta and gamma genes are scattered in both the long and short components. Interestingly, the Beta genes specifying the DNA polymerase and the DNA binding protein flank the origin of replication in the long region (oriL). There is little gene overlap and few instances of gene splicing for any of the HSV gene classes (63).

Both IE and E proteins are required for genome replication. A virus-encoded DNA-dependent DNA polymerase and DNA-binding protein are involved in replication, together with a number of enzymes (e.g. TK) which alter cellular biochemistry. In addition, cellular proteins are required for genome replication, therefore HSV replication occurs in the nucleus. Viral DNA replication is the target for a number of successful anti-Herpesvirus drugs (e.g. acyclovir, gancyclovir, etc). The pattern of replication is complex, involving at least three potential origins of replication, and resulting in the

formation of high molecular weight DNA concatemers. Virus particles (core plus capsid) assemble in the nucleus - genomic concatemers are cleaved and packaged into pre-assembled capsids. The envelope is acquired from the inner lamella of the nuclear membrane and particles accumulate in the space within the inner and outer lamellae. How these particles are transported to the cell surface is not clear and may or may not involve the golgi apparatus. Mutations in certain envelope glycoproteins interfere with cytoplasmic transport. Any remaining virus particles are released when the cell lyses (~24 hours after infection) (89).

Latency

The ability of HSV to establish a lifelong latent infection in the human host is the most intellectually challenging aspect of HSV biology. The virus enters nerve endings and is transported retrograde to the nucleus of sensory nerves innervating mucosal epithelium. In latently infected neurons, viral genomes acquire the characteristics of endless or circular DNA (97,98) and no replicating virus can be detected in the sensory ganglia. In a latent infection the viral genome is maintained intact in specific sensory neurons where it is genetically equivalent to that presents in a viral particle, but the highly regulated productive cycle cascade of gene expression, so characteristic of HSV infections, does not occur. In a fraction of neurons harboring latent HSV, the virus is periodically, reactivated. During latent infection, the expression of most viral genes is absent during latent infection, but a number of latently infected neurons express a single transcript-the latency associated transcript or LAT, which is encoded in the repeat regions of the genome. Then LAT is the major viral genes products expressed in this stage (99).

LAT expression facilitates reactivation, but its mechanism of action is unclear at this time. It does not appear to directly involve the expression of a protein. A two kb intron is spliced from the primary transcript. This intron is stable in the nucleus of the latently infected neuron and persists as a circular "lariat" form, but this stable intron is not required for the facilitation of reactivation mediated by LAT. The spliced, polyadenylated LAT can be detected with difficulty in some latently infected neurons; a region of about

350 bases in the extreme 5' end of this transcript is required for LAT-facilitated reactivation (100).

Reactivation occurs following physiological stress to the animal. During this event some productive phase transcripts and proteins can be detected in the neuron, and infectious virus appears at the periphery (the site where the virus originally entered). This virus can initiate a general infection at the peripheral site where limited virus replication takes place until the host defense systems respond and suppress it (99, 100).

Pathogenesis and diseases of HSV infection

HSV infection is a viral infection of the skin and/or mucous membranes manifested by painful, blister-like lesion on a red base most often on the mouth or face (oral herpes) or in the genital area (genital herpes). Though HSV-1 is statistically more often a cause of oral herpes and HSV-2 is more often a cause of genital herpes, either type can cause HSV infection in either the oral or genital area (10).

HSV is passed from one person to another directly from skin to skin to mucous membrane contact with infected lesions as in touching, mouth to genital exposure (possible to pick up genital herpes from your partner's herpetic cold sores). Some individuals may shed the herpes virus, that is, lose particles of herpes virus from the skin surface, without realizing they have active herpes lesions (called asymptomatic viral shedding). Self-innoculation of HSV from one area of the body to another is possible for example, the lips to the eyes or from the genital area to the eyes. Thus, HSV causes the various diseases (Table 3) (5,10).

The pathogenesis of human disease is dependent on intimate, personal contact of a susceptible individual with someone excreting HSV. Virus must come in contact with mucosal surfaces or abraded skin for infection to be initiated. With viral replication at the site of infection, either an intact virion or, more simply, the nucleocapsid is transported by neurons to the dorsal root ganglia, where latency is established. After latency is established, a proper stimulus will cause reactivation to occur; virus becomes evident at mucocutaneous sites, appearing as skin vesicles or mucosal ulcers. Primary infection

can spread beyond the dorsal root ganglia, thereby becoming systemic: however, this event is unusual (6).

Due to infection with HSV-1 generally is limited to oropharynx and is transmitted by direct contact of a susceptible individual with infected secretion (such as virus contained in vasicular fluid). Thus, initial replication of virus will occur in the oropharyngeal mucosa. The trigeminal ganglion becomes colonized and herbors latent virus (5,6). Acquisition of HSV-2 infection is usually the consequence of transmission by genital contact. Virus replicates in genital, perigenital or anal skin sites with seeding of the sacral ganglia. After the establishment of latency, a recurrence of HSV is known as reactivated infection or recurrent infection. This form of infection leads to recurrent vesicular lesions of the skin such as HSV labialis or recurrent HSV genitalis. Reinfection with a different strain of HSV can occur, albeit extremely uncommon in the normal host and is called exogenous reinfection (6).

Table 3: HSV infection causes the various of diseases which are different lesions (5).

Type of HSV	Lesions & Characteristic of infection	Diseases
HSV-1	1. oral – facial infection	- acute gingivostomatitis - herpes labialis / fever blister - pharyngitis
	2. skin infection	- eczema herpeticum - traumatic herpes - herpetic whitlow
	3. eye infection	- herpetic keratoconjunctivitis - keratitis - iridocyclitis
	4. brain infection	- herpes encephalitis - aseptic meningitis - meningoencephalitis
HSV-2	1. genital infection	- genital herpes - cervicitis - vulvovaginitis
	2.neonatal infection	- neonatal herpes

Epidemiology

The HSV prevalence is increasing worldwide. HSV-1 infection is generally referred to as "fever blisters" or "cold sores." About 75% of HSV-1 lesions occur above the waistline. Between 40% and 50% of the US population is infected with HSV-1, and most infections spread during the initial, subclinical phase (101). Consequently, recurrent herpes labialis is a common problem, affecting 20% of the adult population in the United States (102). HSV-1 neonatal infection may be acquired from the mother's vaginal secretions during the birth process. Transfer can also occur during handling of the infant by family members or other people infected with the virus. Interestingly, the prevalence of the disease in children ranges from 10% to 100% in various populations, and it is seldom observed in babies less than one year of age (101).

While keratoconjunctivitis caused by HSV is the leading cause of infectious blindness in the United States. It initially presents with superficial corneal ulcers, which can be seen on slit-lamp examination. The disease may cause recurrent erosion of the conjunctiva and cornea that progresses to blindness. Finally, encephalitis is one of the most life-threatening complications of HSV infection. Without therapy, the mortality rate exceeds 70% and only about 9% of surviving patients return to normal health (101). The virus is recovered from the cerebrospinal fluid in 25% to 40% of patients. Herpes simplex encephalitis occurs in immunocompetent patients, but the risk of disseminated disease tends to be increased in immunocompromised patients (101,103).

HSV-2 infection is the primary cause of genital herpes. It is highly prevalent in human populations in many parts of the world, and is the most common cause of genital ulcer disease (GUD) worldwide. HSV-2 prevalence is increasing (104-106), In the developing countries, the major public health importance of HSV-2 lies in its potential role as a co-factor for HIV transmission. HSV-2 is highly prevalent in most regions experiencing severe HIV epidemics, with infection rates rising steeply with age to reach levels of 70% or more among adult women and men in some African countries (4).

The high prevalence of HSV-2 in many populations results from the fact that it is a lifelong infection, which is highly infectious and often transmitted in the absence of symptoms. HSV-2 prevalence varies widely, with generally higher rates in developing

than in developed countries and in urban than in rural areas. Prevalence is higher in the USA (22% in adults) (107) compared with Europe (generally less than 15%). However, substantially higher rates are seen in Sub-Saharan Africa and the Caribbean, with prevalence in adults of around 50% in many countries. Overall, prevalence is higher in women compared with men, especially among the young (108-110), and rates of up to 40% have been recorded among women aged 15-19 in Kisumu, Kenya (111).

However, genital herpes can also be due to HSV-1 infection and a study in Scotland found that 40% of genital herpes were due to HSV-1 in 1991 (112). Both HSV-1 and HSV-2 are able to infect and reactivate in the same anatomic area, although the natural history of these infections is markedly different, with HSV-2 recurring more frequently than HSV-1, so most clinical reactivations are likely to be due to HSV-2 (113).

Antiviral drug

Although HSV infections in the normal host are usually self-limited, patients with impaired immune systems may suffer chronic, debilitating and even fatal infections. In the early 1980's acyclovir (ACV) first became available for the treatment of HSV and has had a tremendous impact on the morbidity and motality of HSV infections, especially in immunocompromised hosts.

The development of effective antiviral therapies is an important biomedical scientific achievement of the late 20th century, and the various manifestations of HSV have been widely treated using antiviral therapies for more than 40 years. For research, antiviral chemotherapy [the nucleoside [analogues]; idoxuridine (1959), triflurothymidine (1964), and adenine arabinoside (1968)] was rudimentary prior to the discovery of acyclovir (ACV) in 1974. The first report detailing the selective antiviral activity of acyclovir against herpesviruses was published in 1978 (114), marking the start of an exciting chapter in clinical medicine.

Acyclovir

Acyclovir (acycloguanosine: ACV) is an analogue of the natural nucleoside guanosine (Figure 4) that must be transported into cell and phosphorylated to ACV - triphosphate to become the active antiviral compound. It has a high degree of activity against HSV. This specificity accounts for the low incidence of ACV toxicity in normal cells (115). Then ACV is today still the drug of choice for treatment of HSV infection.

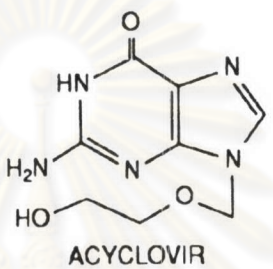

Figure 4: Structure of ACV that is an analogue of the natural nucleoside (5).

(I) Mode of action of ACV

ACV is selectively phosphorylated only within virus-infected cells by viral TK and further phosphorylation by cellular enzymes leads to the production of ACV-triphosphate, which competes with the natural nucleotide, dGTP, resulting in the selective inhibition of the analogue triphosphate into the growing DNA chain (116).

Viral TK is much more efficient in phosphorylating ACV when compared with cellular TK, which partially explains ACV's selective action. As a result of this step, the amount of ACV triphosphate present in HSV-infected cells may be 40 to 100 fold greater than in uninfected cells (117-121). ACV triphosphate is a more efficient inhibitor of HSV DNA polymerase in comparison with the cellular DNA polymerase. ACV that becomes incorporated into newly synthesized DNA acts as a chain terminator and also results in the inactivation of viral DNA polymerase by the formation of an irreversible complex (121-123). Interestingly, not only phosphorylation of ACV minimal in uninfected cells, but cellular DNA polymerases have must lower affinities for the antiviral triphosphates

compared with HSV DNA polymerases. The excellent clinical safety record of ACV, and this prodrug reflects the high selectivity for infected cells and this negligible activity in uninfected cells (124-125).

However, not long after the introduction of ACV into clinical practice, isolates of ACV-resistant HSV (ACV^r HSV) were described both in association with persistent lesions and in pretreatment isolates (44,57,127-130).

(II) Mechanism of resistance

In the laboratory, two kinds of resistant mutants of HSV can be generated. The viral TK and DNA polymerase are both intimately involved in mechanisms of resistance to ACV (39,40). Firstly, there are HSV mutants which lack the enzyme TK. This enzyme is required to activate the acyclovir molecule by adding on a high energy phosphate group, converting acyclovir to acyclovir monophosphate. Subsequent phosphorylation to the active precursor molecule, acyclovir triphosphate, which mimics the natural nucleoside, is carried out by cellular enzymes. Three distinct classes of ACV^r TK mutant have been identified: *TK*-negative (TK^N), *TK*-partial (TK^P), and *TK*-altered (TK^A) mutants. TK^N mutants lack TK activity, whereas TK^P mutants express reduced levels of TK activity. TK^A mutants are substrate specificity mutants, which phosphorylate thymidine but not ACV. Secondly, there are mutants of the DNA polymerase enzyme gene (required for the replication of the genome of the virus) which are resistant to ACV activity.

In patients, *TK* mutants are far more frequent than DNA polymerase mutants [approximately 95 to 96% of ACV^r HSV isolates are TK deficient (TK^N and TK^P), and the remaining isolates are usuallly TK^A] (131) and in immunosuppressed patients such as HIV infected persons, they may be responsible for resistance to ACV therapy. In immunocompetent persons, the occasional isolation of *TK* mutants is fortunately not associated with clinical nonresponsiveness to therapy. This applies even to persons on long term (up to a year or more) suppressive therapy for recurrent HSV infections.

(III) Susceptibility assay

A variety of phenotypic methods has been used to determine the susceptibility of HSV isolates to antiviral drugs, including dye uptake assays, viral DNA inhibition assays, enzyme immunoassays, and plaque reduction assay (PRA) (132). Efforts have been made to standardize these assays, since many variables can influence the final result (133-135). The PRA is gold standard method and is used most widely for routine susceptibility testing. This assay determines the inhibitory concentration (IC_{50}): the concentration of agent required to inhibit viral replication by 50 percent.

(IV) Surveillance in the immunocompetent population

The results from early work showed that ACV treatment in immunocompetent patients was not associated with the emergence of resistant virus. The historical prevalence of ACV^r HSV isolates from untreated, immunocompetent patients as detected by the PRA is 0.3% (136). Furthermore, there has been no detectable change over time in this prevalence based on data for isolates collected during clinical trials, from patients who had not responded well to ACV, and from population-based surveys (107,137-139). The prevalence of ACV^r HSV in these studies ranged from patients with genital herpes (107), untreated recurrent herpes labialis (137,138), or unspecified HSV infections (139).

Until recently, little attention had been paid to surveillance for resistant HSV isolates in patients with recurrent herpes labialis despite the widespread availability of antiviral treatments for this indication in some markets. Isolates from the two surveys of herpes labialis conducted in the United Kingdom and the United States were tested for susceptibility to penciclovir in addition to ACV. HSV isolates from 924 and 1,004 subjects, respectively, were tested in these surveys. One ACV^r isolate was identified in the 1998 United Kingdom survey which was cross-resistant to penciclovir; no other resistant were identified (138). Two isolates identified in the U.S. survey as ACV resistant had showed ACV IC_{50} of 2.4 and 3.2 μg/ml, respectively (137). Further analysis of these isolates has shown that they are sensitive to ACV (IC_{50} in the PRA in Vero cells were 1.24 and 0.72 μg/ml, respectively [M. Davis,personal communication, October 2001]).

Based on these two surveys, it appears that widespread availability of topical ACV in the United Kingdom (ACV became available without prescription in 1993) has had no measurable impact on the prevalence of resistant HSV to date. There is no evidence from these studies that isolates from patients who had received antiviral treatment were any less susceptible to ACV than isolates from untreated patients (80,107,137-139).

Fife *et al* concluded that six years of suppressive ACV therapy in immunocompetent patients with recurrent genital herpes did not lead to the selection of ACV^r virus (140). Sequential isolates from 13 patients showed no evidence of reduced antiviral susceptibility after cessation of suppressive therapy.

Although the ACV resistant mutants that exist naturally in any virus population probably account for the very low background prevalence of resistant isolates in the immunocompetent population, clinical resistance to ACV is exceptionally rare in this group. Isolated cases of clinical resistance have been reported in patients with genital herpes (34,36,141,142) or herpes keratitis (27,40).

In summary, clinical resistance to ACV is exceptionally rare in immunocompetent patients even though resistant HSV is detectable, albeit at a low frequency, in this population. This is because the normal immune response leads to the rapid resolution of the infection. Clinical resistance in an apparently immunocompetent individual should raise suspicion of some unappreciated immune deficit.

V. Surveillance in the immunocompromised population

Surveys in North America and Europe of HSV isolates from immunocompromised patients treated with ACV indicate that the prevalence of resistant HSV is generally between 4 and 7% (57,107,143,144). Results from a continuing survey of HIV-positive patients within the United States and Canada in 1998 to 2000 (Task Force on HSV resistance) are very similar. Thus, despite widespread and increasing use of antivirals to treat HSV infections, the frequency of resistant HSV even in high-risk, immunocompromised patients has remained stable for almost 20 years.

In patients with defective T-cell-mediated immunity, the virus is cleared very slowly from the lesions (18,145). Consequently, the lesions tend to be more prolonged

and more severe than in immunocompetent individuals (57). Extensive viral replication occurring in the setting of prolonged antiviral therapy and immuosuppression provides an ideal scenario for the selection of resistant virus, analogous to selection in cell culture. Moreover, multiple courses of treatment may be required to manage recurrent episodes (127,144). Consequently, patients with profound immunosuppression are more likely to carry ACV^r HSV than patients with moderate immunosuppression (57).

ACV^r HSV can emerge rapidly during the course of antiviral therapy in immunocompromised patients (40,44,127,146). For example, Crumpacker *et al*, described a child with a congenital immune deficiency, who received three courses of intravenous ACV for the treatment of recurrent mucocutanous HSV-1 infection (127). An isolate obtained at the start of the third course was sensitive to ACV, but within 9 days of starting the third course, an ACV^r HSV isolate was collected.

Recurrent HSV lesions developing after resolution of a clinically resistant lesion in immunocompromised patients often respond to ACV therapy. While reactivation of the latent virus leads to renewed replication of sensitive HSV within the lesion, despite the development of resistant virus at the periphery in a prior episode. However, in the same study, all eight second recurrences were resistant to ACV therapy (32), suggesting that resistant virus became latent and reactivated.

The immunocompromised host faces an increased risk of developing severe HSV infections and the emergence of acquired resistance compared with an individual with a fully functional immune system. Indeed, the probability of developing unresponsive lesions appears to be related to the severity of immunosuppression. Of 184 cases of clinical resistance reported between 1982 and 1994, 160 occurred in patients with AIDS and 24 occurred in patients who were otherwise immunocompromised, usually because of bone marrow transplantation (131). Typically, these patients present with chronic, nonhealing lesions that are unresponsive to high-dose ACV therapy.