CHAPTER III

REVIEW OF LITERATURE

History

More than 100 different herpesviruses have been described in various animal species. By the distinctive architecture of the virus virion, herpesviruses are identified. There are eight known human herpesviruses, these include: Herpes simplex virus type 1 (HSV-1), Herpes simplex virus type 2 (HSV-2), Varicella zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human herpesvirus 6 (HHV-6), Human herpesvirus 7 (HHV-7), and Human herpesvirus 8 (HHV-8) or Kaposi's sarcoma associated herpesvirus (Table 1).

Hepes simplex viruses (HSV) were the first of the human herpesviruses to be discovered since ancient Greektime (27). The word herpes is derived from the Greek word " herpein " which means creep or crawl which refers to the spreading nature of visualized skin lesion (1,3). HSV manifests itself as a disease with breakout on skin and mucous membrane (1-2). HSV was isolated in 1938 by Dodd and others from the mounts of children exhibiting acute symptoms (28). In 1962, Schneweis KE *et al.*, demonstrated that there were two serotypes of HSV, HSV-1 and HSV-2 (2), whose formal designations under International Conference for Taxonomy of Viruses (ICTV) are human herpesviruses 1 and 2 (29). In the same year, 1962, Nahmias AT and Dowdle WR, demonstrated that HSV-1 was more frequently associated with nongenital infection (infection above the waist), while HSV-2 has associated with genital disease (infection below the waist) (27). However, either type can occasionally be found in either area or at other sites (30). HSV is one of the most difficult viruses to control and has plagued mankind worldwide for thousands of years. Until now, there is no conclusive data revealing the exact extent of the virus's infiltration into human population but the number of sufferer's worldwide, in the year1999, is estimated to be at a approximately 86 millions (31). Infection with HSV can result in several diseases ranging from inapparent infections and self-limiting cutaneous lesions to fatal encephalitis (1).

Table 1. Properties Distinguishing the Herpesviruses (32)

Subfamily	Virus	Primary target cell	Site of latency	Means of spread
Alphaherpesvirinae				
Human herpesvirus 1	Herpes simplex type 1 (HSV-1)	Mucoepithelail	Neuron	Close contact
Human herpesvirus 2	Herpes simplex type 2 (HSV-2)	Mucoepithelail	Neuron	Close contact
Human herpesvirus 3	Varicella zoster virus (VZV)	Mucoepithelail	Neuron	Respiratory and close contact
Gammaherpesvirinae				
Human herpesvirus 4	Epstein-Barr virus (EBV)	B lymphocyte and epithelial cells	B lymphocyte	Close contact (kissing disease)
Human herpesvirus 8	Human herpesvirus 8 (HHV-8) or Kaposi's sarcoma-associated herpesvirus (KSHV)	Endothelial cells	Unknown	Exchange of body fluids
Betaherpesvirinae				
Human herpesvirus 5	Cytomegalovirus (CMV)	Monocyte,lymphocyte and epithelial cells	Monocyte, lymphocyte and others*	Close contact, transfusion, tissue transplant and congenital
Human herpesvirus 6	Herpes B lymphotropic virus (HBLV)	T lymphocytes and others*	T lymphocytes and others*	Respiratory and close contact
Human herpesvirus 7	Human herpesvirus 7 (HHV-7)	T lymphocytes and others*	T lymphocytes and others*	Unkonwn

* indicates that others cells may also be the primary target of site of latency

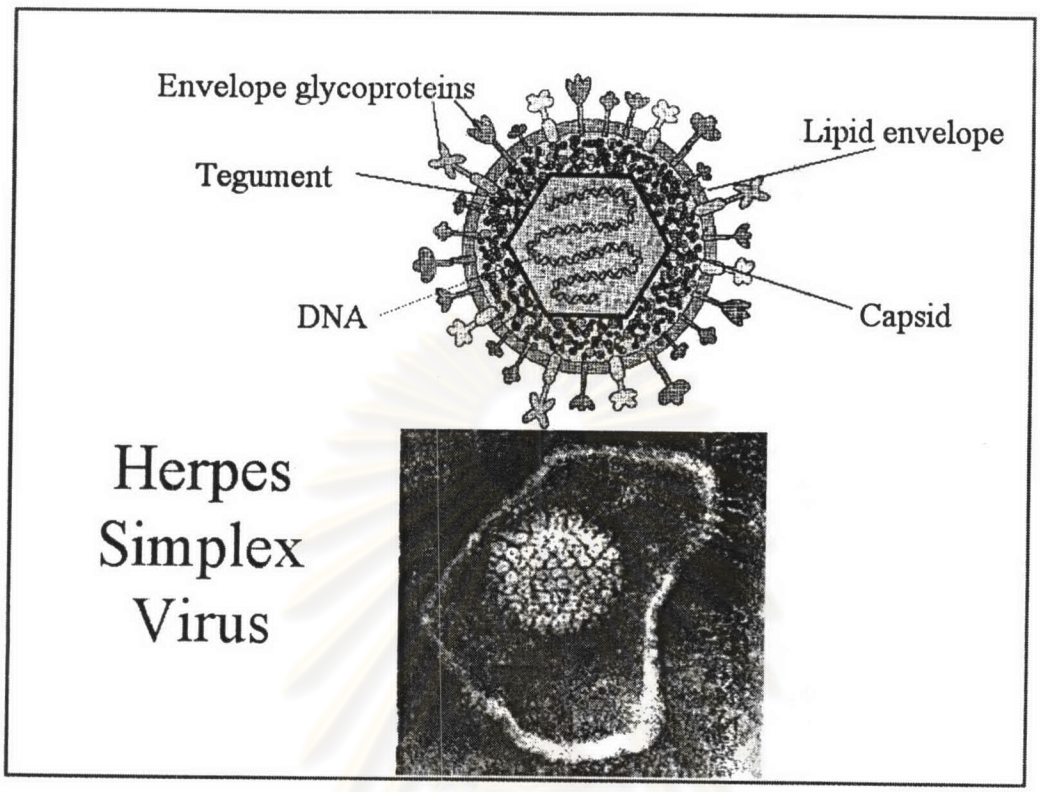
Virology

Herpes simplex viruses are the prototype members of the family Herpesviridae. They have been classified in subfamily alphaherpesvirinae, genus simplex virus. There are two antigenic types, designated HSV-1 and HSV-2, which share antigenic cross-reactivity but different neutralization patterns (33,34). The hybridization study has revealed that HSV-1 and HSV-2 had 50% sequence homology (35). The herpesviruses are widely separated in terms of genomic sequence and proteins, but all are similar in terms of virion structure and genome organization. As same as others, the structure of HSV particle is very complex [Figure 1(a)]. Complete virion is 200-300 nanometer in diameter and consists of four structural elements: a central electorn-opaque core, an icosahedral capsid containing the core, an electron-dense tegument surrounding the nucleocapsid, and an outer spiked membrane or envelope. The viral core appears as electron-dense toriod containing large DNA genome around a proteinaceous core. The DNA genome is a double-stranded linear molecule with a base composition of 68% C+G (HSV-1) or 69% (HSV-2) and molecular weight of 96 x 10^6 kilodaltons. The complete DNA sequence is now known for HSV-1. The DNA isolated from virions is characterized by random nicks and gap (36,37). HSV-1 and HSV-2 DNA consist of two covalently linked components designated L (long) and S (short) [Figure1 (b)] (38). The L component comprises 82% of the viral DNA and consists of a long unique sequence, UL, flanked by inverted repeat region called *ab* and *a'b'*. The S component is 18% of the viral DNA and also has a short unique sequence (Us) flanked by inverted repeats designated *a'c'* and *ca*. Because of this arrangement, the L and S regions can invert relative to each other, giving rise to four possible genome populations which differ only in the orientation of these regions. The DNAs of HSV type 1 and 2 share approximately 50% homology of their base pair, although they differ in many sites of cleavage by restection endonucleases (39,40).

Surrounding the core is an icosahedral nucleocapsid. The capsid, 125 nm in diameter, consists of 162 capsomers with pentons at the 12 vertices and 150 elongated hexons (9.5 x 12.5 nm) comprising the faces and edges (41). There are three capsid structures in infected cells: C capsids contain DNA and are derived from mature virions; A and B capsids lack DNA and are found in the nuclei of infected cells. B capsids are capable of packaging viral DNA during maturation of the virion whereas A capsids are dead-end structures that are unable to package

DNA. Outside the capsid is tegument, a protein-filled region, which appears amorphous in electron micrographs. It consists of virally encoded proteins and enzymes involved in the initiation of replication such as enzymes, which are needed to take control of the cell's chemical processes and subvert them to virion production, and some of which defend against the host cell's immediate responses. The outer layer of virion called envelope. This lipid envelope composes of host membrane, which has been modified by the insertion of herpes glycoproteins. These glycoproteins appear in electron micrographs as short spikes embedded in the envelope. Surface spikes vary in size, distribution, and composition; gB, gC, and gD are major components of three morphologically distinct spikes of 8 nm, 14 nm and 24 nm, respectively (42).

(a)



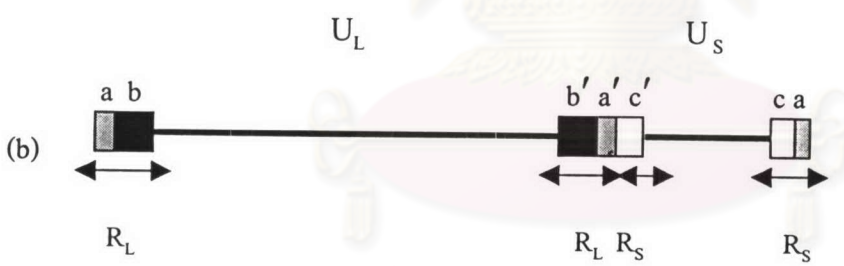


Figure 1. (a) Schematic representation of the architecture of the HSV virion and electron micrograph of HSV virion by the negative stain technique (43).

(b) Schematic representation of the HSV genome. The genome is divided into a U_L and U_S region. The unique sequences are separated by inverted repeats (shown as boxes and designated as R_L and R_S) (38)

HSV proteins

HSV-1 and HSV-2 encode at least 84 different polypeptides, about 5.3 x 10^6 daltons of proteins (44-46). The sum of molecular weight (MW) of the virion structural and non-structural proteins are 1.99 x 10^6 and 1.79 x 10^6 daltons and thus, account for 38% and 34 % respectively, of the potential coding capacity of the genome (44). HSV genes encode several different proteins and each can have various biological functions. By using the technique of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), approximately 50 virus specific infected cell proteins (ICP) could be detected during productive infection (12,47,48). There were at least 30 virion-specific proteins (VP) in both types of HSV virions (2,50). The HSV proteins have been identified and divided into three groups according to their temporal order of appearance and requirements for their expression, designated immediate early (IE) or α, Early (E) or β and Late (L) or γ protein. The first group of genes to be expressed are the IE genes, whose products called IE proteins induce the expression of the E, and L genes. This phenomenon is well known as cascade fashion (2,6,12,13). The IE genes were originally defined as genes which are expressed in the absent of *de novo* protein synthesis. The IE proteins represent the virus specific polypeptides which consisting of one minor structural and several structural proteins, are made immediately upon infection (13). Their peak synthesis occurs two to four hours post infection (h.p.i.) and continue to accumulate until late of infection. There are five IE proteins, ICP0, ICP4, ICP22, ICP 27 and ICP47. Four of the five IE proteins play critical role in the HSV gene expression, with the exception of ICP47 (50,51). ICP4 and ICP22 are essential regulatory proteins in all experimental systems (*in vitro* and *in vivo*). ICP0 and ICP22 are dispensable in at least some systems but the evidence indicates that each plays an important regulatory role in viral gene regulation (52-54). Recent studies have shown at least part of their interrelated role, but a complete understanding of how these proteins function in the cascade regulation of viral gene expression is not yet available.

The most extensively studied HSV protein is ICP4 (2). The 175 kilodaltons (kDa), ICP4 protein is encoded by the R_s1 gene, which is present in two copies in the viral genome. It was established so far that functional ICP4 is necessary for the activation of both early and late gene expressions (55). It is an essential regulatory protein, which binds DNA at specific sites and can both transactivate and repress the expression of viral genes including its own genes (2,56,57).

ICP 27, the 63 kDa phosphoprotein is the second IE proteins that is essential for lytic replication of HSV. As regards ICP4, multifunctions of this protein have been ascribed, including roles in both the transcriptional and post transcriptional regulation of viral gene expression (58,59). Thus, ICP27 has been involved in the switch from early to late gene expression during virus replication, polyadenylation site selection and 3 ;RNA processing. ICP27 is primarily required for the regulation of late gene expression and it appears to play a role in the shut-off host protein synthesis. This protein also affects early gene expression in the absence of ICP4, and it can affect the ability of ICP4 to bind to DNA. Although the mechanism of ICP27 regulates the expression of viral genes has become clearer, the extent of its role in regulating the switch from early to late gene expression is still questioned. Several studied have shown that ICP27 is necessary for normal expression levels of several early genes (60). Some of early genes that require ICP27 code for proteins necessary for viral DNA replication indicating that the normal viral DNA synthesis is required for ICP27.

Previous work has shown that ICP22 which approximately 18 kDa, encoded by the U_s1 gene, is involved in the control of viral gene expression, although the precise mechanism remains to be elucidated. This is partially due to the fact that ICP22 is not required for virus replication in many cell culture systems such as Vero and HEp-2 cell lines (38). However, in rodent or rabbit skin cell lines and in primary human fibroblasts, ICP22 is necessary for efficient virus replication (52-54) and for the expression of both ICP0 and a subset of viral late genes (54). Early in infection, ICP22 localizes in punctuated nuclear structures. At the time of viral DNA synthesis, ICP22 colocalizes in infected nuclei with ICP4, viral DNA, RNA polymerase II and a small cellular protein (EAP) which is named on the basis of its association with Epstein-Barr virus small nuclear RNAs. This aggregation will occur in the present of a functional protein kinase encoded by U_L13 gene and is necessary for optimal late gene expression (61,62). In other recent studies, ICP22 has been shown to require for the alternative splicing of the R_L2 gene (gene code for ICP0) and for accumulation of the viral host shut-off protein in infected cells (63,64). In addition, ICP22 was reported to interact with several cellular proteins such as a novel cellular protein, designated p60 that interacts with ICP22 and ICP0, in yeast two-hybrid system and in *in vitro* biochemical assays. However, the exact role of p60 in the life cycle of the virus is not known (51,65).

ICP0, a ring finger protein of 775 amino acid, encoded by IE gene 1 and the best described as a promiscous transactivator, a description of its ability in transient assays to transactivate a variety of unrelated genes either by itself or in concert with ICP4. The exact role of ICP0 protein in the regulation of viral gene expression was also difficult to establish. Although it is not absolutely required in tissue culture systems, virus deleted in ICP0 coding region is growth impaired, but only at low multiplicity of infection (MOI). Upon primary exposure, HSV-1 initiates a lytic infection in the epithelium and subsequently establishes a lifelong latent infection in sensory neurons (2), and ICP0 has been implicated in the regulation of both the lytic cycle and reactivation from latency. Several evidences indicate that ICP0 might play a specific role in the control of the balance between the latent and lytic states, such that in its presence the latter is favored (66-71). There is no evidence that ICP0 binds to DNA, either specifically or non-specifically, but ICP0 localizes to the nucleus soon after infection, where presumably its role in transactivation is manifested. Later in infection, ICP0 is translocated into the cytoplasm of primary cells (72), suggesting that its role in transactivation is more complex than originally thought or that the protein has multi-functional roles. Several findings reported in the last few years demonstrated that ICP0 interacted with multiple cellular proteins. Consistent with these data, ICP0 was found to bind the cellular ubiquitin-specific protease USP7, formerly called herpesvirus-associated ubiquitin-specific protease (HAUSP) (73-75), with high affinity and specificity. It has been found to interact with and stabilize cyclin D3 (72). Furthermore, ICP0 induces the proteasome-dependent degradation of a number of cellular proteins, suggesting that changes in the intranuclear environment may be involved in the function of ICP0 (76-78). Early in infection ICP0 was observed to localize in the nucleus at pre-existing nuclear sub-structure known as a Nuclear Domain 10 (ND10). Within a few hours this structure is disrupted, the ring finger domain of ICP0 is required for this disruption. The inhibition of this process was performed by proteasome inhibitor drugs treatment. From this observation pointed that ICP0 induces the degradation of specific cellular targets, and that this degradation is an integral component of the mechanism by which ICP0 activates viral gene expression during productive infection (77-79). Recently, it was reported that the expression of ICP0 in primary neurons in culture and in a differentiated neuronal cell line is very poor (80). This result suggests that the lack of ICP0 accumulation may favor establishment of the latent state in neurons by preventing the normal cascade of viral gene expression necessary for lytic infection. From the model, which purposed to explain the role of ICP0 and proteasome-mediated proteolysis in HSV infection

suggests that a cellular repressor of the viral lytic pathway is a target of ICP0-induced proteolysis. Thus, viruses lacking in ICP0 can replicate only when there are a large number of input viral genome. Further, in certain cells, which the expression of ICP0 is deficient such as neuron cells, latency would be more likely than productive infection (81). A specific receptor of HSV replication is not required in an alternative view of this process, but a combination of positive and negative forces acting to redirect critical cellular functions to the incoming extrachromosomal viral genome is prefer. These positive acting mechanisms would be completed by ICP0 mediated degradation of specific cellular proteins, a process which would be necessary before other critical cellular components could be re-targeted to the HSV template.

All IE genes appear to function as regulatory protein with the possible exception of ICP47. Several recently published reports, have shown that this IE protein plays an interesting role in immunological activity. HSV-1 ICP47 (ICP47-1) effectively blocks the major histocompatibility complex (MHC) class I antigen presentation pathway (50,82). The study of human fibroblasts demonstrated that ICP47-1 caused MHC class I proteins to be retained in the endoplasmic reticulum (ER) of cells and that antigen presentation to CD8+ T cells was inhibited after ICP47-1 was expressed (82,83). ICP47-1 binds with high affinity to the human transporter associated with antigen presentation (TAP) and blocks the binding of antigenic peptides. This process prevented peptide transport across the ER membrane by TAP (84,85), so that, without peptides, class I proteins were retained in the ER. By contrast, ICP47 did not be found to inhibit MHC class I antigen presentation in mouse cells (50) and could inhibit murine TAP poorly (84,85). HSV-2 ICP47 (ICP47-2) has only 42% amino acid sequence identity with ICP47-1, but it could block human TAP and bound to TAP with similar high affinities as same as ICP47-1. Moreover, at its high concentration, murine TAP was blocked poorly and ICP47-2 could not bind to murine TAP too.

The early protein group also contains minor structural and non-structural proteins, however, they are synthesized at the highest rate from five to seven h.p.i and decreasing thereafter (13). This group includes enzymes involved in viral DNA synthesis, such as DNA polymerase and thymidine kinase. Their synthesis requires the presence of functional IE proteins. Examples include ICP8 (the major DNA binding protein), RR2 (the small subunit of ribonucleotide reductase) and TK (thymidine kinase). Their synthesis requires a functional ICP4 protein. The

group of late protein contains major structural protein. They form a continual differing in their timing and dependence on viral DNA synthesis for expression. The proteins of this group are synthesized in the presence of functional IE and E proteins.

HSV encodes 11 known viral envelope glycoproteins : gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, gM and a 12th (gN) is predicted (46). Of these, only gB, gD, gH, and gL are essential to the process of infection in cell culture, while the other seven contribute to virus infectivity and spread in the host (86). Although, an additional glycoprotein, gK, has been shown to be absent from the virus envelope, it is required for the production of infectious virions (87,88). Infection involves virus attachment to the cell surface membrane followed by virus penetration and entry of the nucleocapsid into the cytoplasm (2,89). Current line of evidence indicates that virus attachment is a two-step process (90) involving different glycoproteins and several receptors. Both gC and gB are the major components of the viral envelope which involved in the initial attachment phase of virus to cell surface heparan sulphate. Several reports have implicated gD as an HSV receptor-binding protein. For example, UV-inactivated virions lacking gD, are able to block infection by HSV (91,92). HSV glycoproteins gB, gD, and gH are essential for HSV replication in tissue culture (93). However, virions lacking anyone of these glycoproteins absorb to cells (94,95) and Monoclonal antibodies (MAbs) to each fail to block attachment of wild-type virion to cells, although they do greatly reduce infectivity (96). Glycoprotein (g)L is essential for proper processing and insertion of gH into the virus envelope (97). Genetics studies of glycoprotein have demonstrated that virus penetration is a highly complex process involving the cooperative activities of multiple viral glycoproteins (91,94,98). Glycoprotein gE and gI could form a hetero-oligomer complex that functions as Fc receptor for IgG. Thus, HSV achieves the ability to evade the host immune response by blocking their specific Fc interactions. (99).

The human receptors for HSV entry

The initial step in HSV infection is binding to heparan sulphate proteoglycan molecules on the surface of cells (9). It is structurally and chemically similar to heparin, except that heparin is more highly sulphated (100). The conclusions that cell surface heparan sulphate serves as the receptor for HSV adsorption are based on several lines of evidence. First, enzymatic removal of cell-surface heparan sultate reduces binding and infectivity of subsequently added HSV, whereas digestion of other cell-surface GAGs has no effect (9). Agents that bind to heparan sulphate also block HSV adsorption (101). Experimental manipulations to reduce the levels of cell surface heparan sulphate lead to reduction in viral infection concurrent with the reduction in viral adsorption, indicating that the adsorption of virus to heparan sulphate is a required step in the pathway of infectious entry (11). Second, HSV virions directly bind to immobilized heparin, and soluble heparin can block the binding of virions to epithelial cell lines *in vitro* (9). These results provided the molecular basis for the early observations that heparin could inhibit HSV infection (102-104). Third, heparin-binding proteins, such as platelet factor 4 (9) and fibroblast growth factor (FGF) (105), can inhibit HSV adsorption and plaque formation, possibly by competing with virus for receptor sites associated with the cell surface heparan sulphate. The involvement of a cell-surface heparin-like molecule in HSV binding has been confirmed by several laboratories (106,107). Although attachment to cell-surface heparan sulphate mediates initial contact of HSV with the cell, subsequent interactions of the virus with other cell membrane components are required for the virus to penetrate the cellular plasma membrane (107).

In addition to the heparan sulphate proteoglycans, which mediate the attachment of virion to cells, HSV requires an entry receptor. Recently, several mediators of HSV-1 and/or HSV-2 entry to human cells have been identified (16,18,108-110). They can be divided into three structurally unrelated molecular families (15) which are (i) HveA (Herpes virus entry mediator A), (ii) members of the nectin family, (iii) 3-*O*-sulphated heparan sulphate. These molecules serve as receptors for HSV gD (15). HveA (HVEM or TR2) is a member of TNFR superfamily of proteins (15,17). HveB (PRR2α or nectin-2α), PRR2δ (nectin-2δ) and HveC (PRR1 or nectin-1δ) are related members of the Ig superfamily (111,112). A splice variant of HveC, called "HIgR," also mediates HSV entry through its interaction with gD (16). There are some reports demonstrated that, truncate, soluble forms of gD lacking transmembrane and cytoplasmic

domains, bind directly to soluble forms of each of these receptors (113-116). In addition, antibodies to HveA, HveB, and HveC block HSV infection in various cell lines (16-18). Thus, it is clear that, HSV can utilize several different and structurally unrelated cell surface proteins as receptors.

The first identified cellular mediator of HSV entry was the herpesvirus entry mediator (HVEM, renamed HveA, or TR2) which discovered in 1996 (17). HveA is a novel member of the TNF receptor family (17,19). It mediates HSV-1 entry when transfected into receptor-negative Chinese hamster ovary (CHO) cells (17). HveA is capable of binding gD (114). Expression of HveA appears to be most abundant in hematopoietic cells and lymphoid tissues such as the spleen and thymus (19,117,118). Lymphotoxin, inducible expression, complete with herpesvirus protein D for HVEM, a receptor expressed by T lymphocytes (LIGHT) and lymphotoxin alpha (Ltα) have been identified as the natural ligands for HveA (15,119). The structures of both ligands are related to TNF, exist as trimer (120,121) and presumable signal by inducing or altering receptor aggregation on the cell surface (108,122-125). In response to ligand binding, the cytoplasmic domain of HveA interacts with a subset of adapter proteins in the TNFR- associated factors (TRAFs) family leading to activation of the nuclear factor κB (NF-κB) and Jun-containing transcription factor AP-1 (JNK/AP-1) pathways (19,117,118). Monoclonal antibodies against the extracellular domain of HveA block several aspects of T cell activation, such as proliferation and cytokine production, suggesting that HveA is directly involved in this process (126). HveA has been originally identified as a receptor which function for many strains of HSV-1 and HSV-2 (17). However, HveA failed to mediate the entry of certain laboratory strains with mutations in gD such as rid1 and rid2 (15,17).

The key properties of the nectin1 receptors, HIgR and HveC as HSV receptors are as follows (15). First, they serve as receptors for all HSV-1 and HSV-2 strains tested. Second, they are broadly expressed in numerous human tissues including target tissues of HSV infection, e.g. CNS and ganglia. Third, they present on many human derived cell lines. Examples of cell lines expressing HIgR and HveC are epithelial (HEp-2, HeLa, 5637, T24), fibroblastic (MRC5.26), haematopoietic (TF1, U937), endothelial (ECV 304), neuroblastoma (Nalm6, IMR32, Lan5) (15,127,128). As mentioned above, they interact physically with gD and monoclonal antibody to nectin1 (mAb R1.302) has an ability to block virus infectivity (15).

The two of human nectin2 receptors, HveB and PRR2δ show about 30% identity at the amino acid level with nectin1 in the ectodomain region. There are three features different from HIgR and HveC. (i) The virus range differs greatly. Thus, HveB and PRR2δ do not serve as receptor for wild-type HSV-1 and are almost inactive towards wild-type HSV-2. They do serve as receptors for unrestricted HSV-1 mutants U10, U21, rid1, rid2 and ANG. (ii) HveB and PRR2δ expressed cells require exposure to high multiplicity of infection in order to become infected. (iii) Although the soluble form of human nectin2 interacts physically with mutant gD from the U21 virus, the binding is weaker than that of soluble human nectin1 to gD. Thus, HveB and PRR2δ act as low efficiency receptor for the unrestricted HSV mutants.

A novel role of 3-*O*-sulphated heparan sulphate in HSV entry has been reported by Shukla et al., (110). This new type of molecule can serve as HSV receptor when expressed in receptor negative CHO cells. It mediates the entry of HSV-1 but not of HSV-2. By coimmunoprecipitation and affinity coelectrophoresis assays have shown that *O*-sulphated heparan sulphate proteoglycan can bind gD *in vitro* whereas binding to gD could not be detected by EIA. Unmodified heparan sulphate does not bind gD. All together, heparan sulphate in some cells may act as both attachment and the entry receptors when modified by *O*-sulphation. Until now, it is not known what human cell lines and human tissue can be infected via this pathway of entry.

HSV replication cycle

HSV replication occurs in a wide variety of cell lines. Replication of the viruses has been examined in a number of cell systems, and the duration of successive steps in the replication cycle depends upon the type of cell, the virus strain, and the multiplicity of the infection (14). The general features of the replication cycle can be illustrated by type 1 infection of HEp-2 cells in which virus DNA synthesis begins about three hours after infection and new progeny virus is detectable three hours later. The duration of a complete cycle is about 12–18 hours and between 10^3 and 10^5 virus particles are produced per cell. It is an inefficient process, and results in the production of only one infectious virus particle for every 100 to 1000 are produced (14,41).

Attachment of HSV to cells occurs by binding of virions to the cell surface heparan sulphate progeoglycans. This attachment is mediated by two virion glycoproteins, gC and gB and this process followed by the interaction of gD with one of several cellular receptors such as HveA (89). HSV entry occurs by fusion of virion envelope with plasma membrane. The current model of HSV entry envisions that fusion of virion envelope with plasma membrane requires the intervention of four glycoproteins, gD, gB and the heterodimer gH-gL, in a manner yet to be clarified (129)(Figure 2). Once fusion is complete, the nucleocapsid is released, with a portion of tegument, into the cytoplasm. Once within the cell the capsid is transported to the nuclear membrane. The DNA is then released from the capsid into the cell nucleus where the viral DNA and RNA are synthesized. Several lines of evidence indicated that HSV-DNA is transcribed by a host transcriptase, designated RNA polymerase II (130,131).

A few hours of infection, host protein synthesis is inhibited and host polyribosomes disaggregate (132); synthesis of host RNA is also reduced (133,134). Fedwick and Walker (135) concluded that the suppression of host protein synthesis also of host DNA and RNA synthesis, is caused by a component of the infecting virus particles. The former is accompanied by cessation of glycosylation of host proteins early in infection (134,136,137) and glycosylated proteins which appear later on the modified membrane are virus-specified. Late changes include segregation of the nucleus and alteration of the nucleus and alteration of the membranous structure of infected cells (137).

After viral DNA is translocated from the cytoplasm into the nucleus; it is transcribed and approximately 50 ICPs are produced in the infected cells (12,44,138). The regulation of all viral proteins synthesis is co-ordinately regulated and sequentially ordered in a cascade fashion (13,139). By the immunofluorescent technique, specific antigen localization is first observed in the nuclei of infected cells after two h.p.i. stained with rabbit anti-HSV antiserum (140). This is followed by perinuclear fluorescence, ranging from a fine line to a broad band involving part or all of periphery of the nucleus (141,142). At four to six hours, the cytoplasm was stained slightly and the staining becomes very bright at nine hours (142). The cytoplasm is completely filled with diffuse intense fluorescence and a distinct layer of fluorescent material at the cell membrane can be seen. These antigens on the cytoplasmic membranes, resemble the components of the HSV envelope specific antigens during growth.

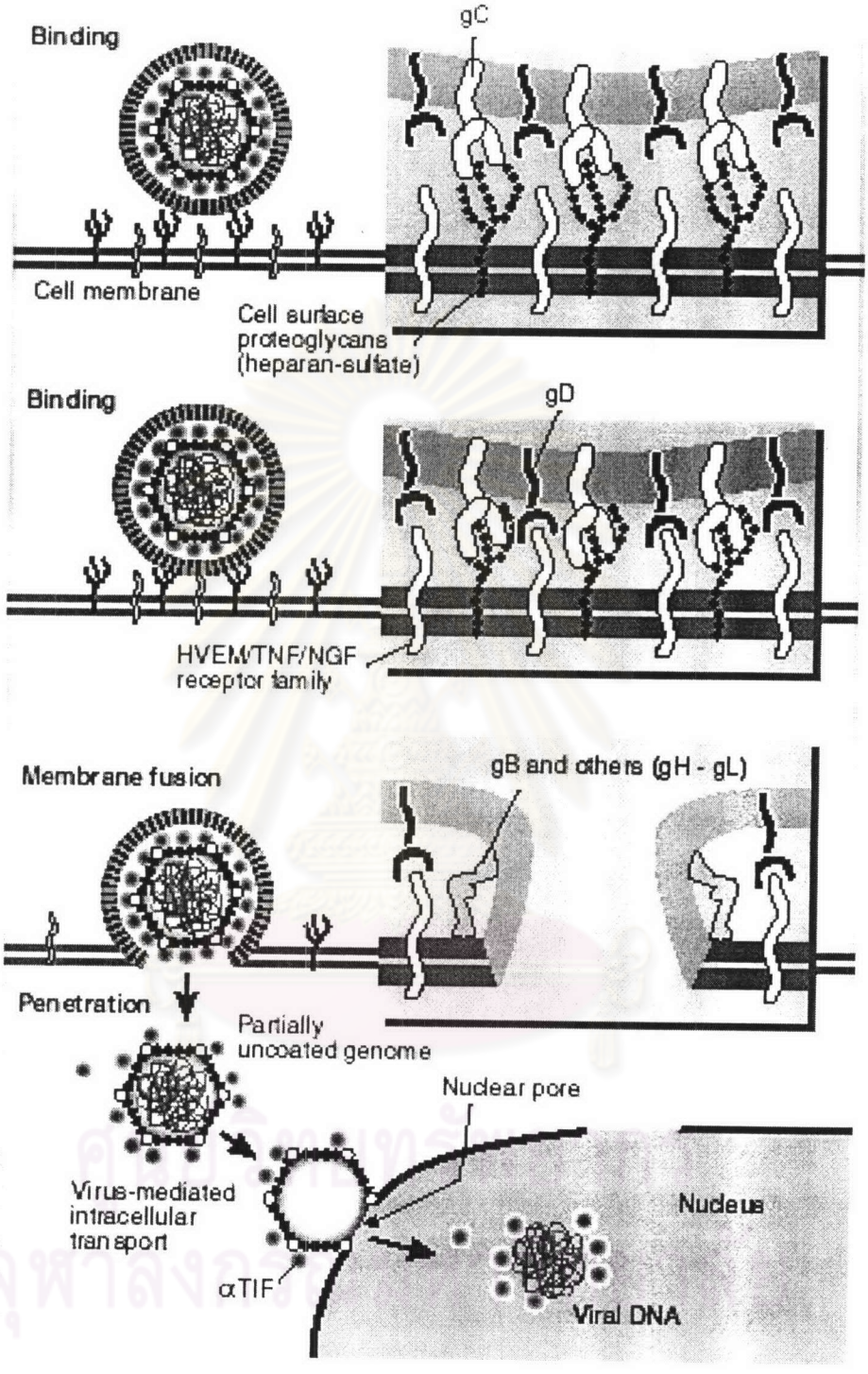


Figure 2. Schematic representation of the initial steps in HSV infection—HSV entry (129)

All of viral proteins synthesis (IE, E and L) was blocked by α–amanitin (a potent eukaryotic RNA polymerase II inhibitor) in cells sensitive to this drug, but not in cells containing an α–amanitin resistant RNA polymerase (143). Synthesis of IE proteins requires only the presence of viral DNA, since an accumulation of functional IE mRNAs in the cytoplasm is found in the presence of inhibitors of protein synthesis (13). If translation is blocked shortly after infection, IE mRNAs accumulate in the nucleus, but no other virus mRNAs are transcribed. Synthesis of E gene products turn off IE products and initiates genome replication. 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 such as thymidine kinase which alter cellular biochemistry. In addition, cellular proteins are required for genome replication, therefore HSV replication occurs in the nucleus. Some of the L structural proteins are produced independently of genome replication called γ-1, and γ-2 are only produced after replication.

HSV DNA replication can be detected as early as three h.p.i. and proceeds for and additional nine to 12 hours. Viral DNA replication is the target for a number of successful anti-herpesvirus drugs such as acyclovir and gancyclovir. The pattern of replication is complex and involved at least three potential origins of replication, and is resulting in the formation of high molecular weigh DNA concatemers. The initial step of HSV DNA replication is denaturation of the DNA at the replication origin with origin binding protein (U_L9). The helicase/primase ($U_L5/U_L8/U_L52$) and single stranded DNA binding protein (U_L29) associate to allow the DNA polymerase/U42 complex to begin DNA synthesis. Once new strand growth progresses, the circular replication structure is nicked to form a rolling circle intermediate. Long concatemeric strands of progeny DNA are encapsidated by the interaction of cleavage/packaging proteins with the specific packaging signals ("a" sequence) at the end of viral genomes. The procapsid proteins (U_L18, U_L19 and U_L38) assemble around scaffolding proteins (U_L26 and $U_L26.5$) that are then digested away. The empty capsid incorporates DNA by means of the action of cleavage/packaging proteins. The filled capsid associates with tegument proteins (aTIF,VHS) and migrates through the nuclear membrane to become enveloped. Viral glycoproteins are translated from HSV RNA on the rough endoplasmic reticulum then transported to the golgi body in vesicles to continue the glycosylation process. The glycoproteins are then transported in vesicles to the nuclear or plasma membrane. The viral capsid associates with the virus modified

membrane which can involve the interaction with virus-encoded matrix proteins. Final release of the enveloped virion from the cell involves the virus being incorpotated into exocytotic vesicles then released from the cell as free virus (Figure 3).

Following permissive infection, HSV-infected cells degenerate and show cytopathic changes. They form syncytial cells (polykaryotes) by the fusion of neighbouring cells. The infected cells may round up or become piled up and form clumps (134). The cytopathic effects will occur varying with the cell types. In some cells, virus can cause syncytial formation, in others it cannot. In addition to formation of polykaryotic cells, intranuclear inclusion bodies could be observed (14).

Replication of HSV in T lymphocyte

All members of the herpesvirus family, including HSV-1 and HSV-2, have the ability to establish latent infection in susceptible hosts. Although HSV-1 and HSV-2 establish latent infection in cells of neural origin, they are also able to infect and replicate in peripheral blood mononuclear cells, including B and T lymphocytes and monocytes (25).

The system of HSV replication in leukocyte culture was first described by Nahmias *et al.* (20). They found that viral replication did not occur in the leukocyte cultures in the absence of phytoheamagglutinin (PHA, a T cell mitogen) (21). The work of Kirchner *et al.,* (22) by using of neuraminidase treated sheep red blood cells to separate the human peripheral lymphocytes, indicated that population T cells was able to replicate HSV after pre-stimulation with T cell mitogen PHA and concanavalin A, while population B cell was not. However, the T cell cultures investigated at that time had not been highly purified and some investigators were claimed that B cells also replicate HSV after mitogen activation (20). Since 1983, the serial works of Braun and coworkers demonstrated that T cells are the cell type in which virus replication takes place (20,21). Moreover, they also showed that interleukin-2 stimulated T cells could be replicated by HSV. Many studies have focused on the infection of human T lymphocytes with HSV. Active CD4- and CD8- positive T cell subsets were equally capable of replicating HSV and MHC class II-expressing T cells actively replicated virus without prior mitogen stimulation (25,144). The effect of HSV-1 infection on human cytotoxic T-lymphocyte (CTL) lytic function was assessed

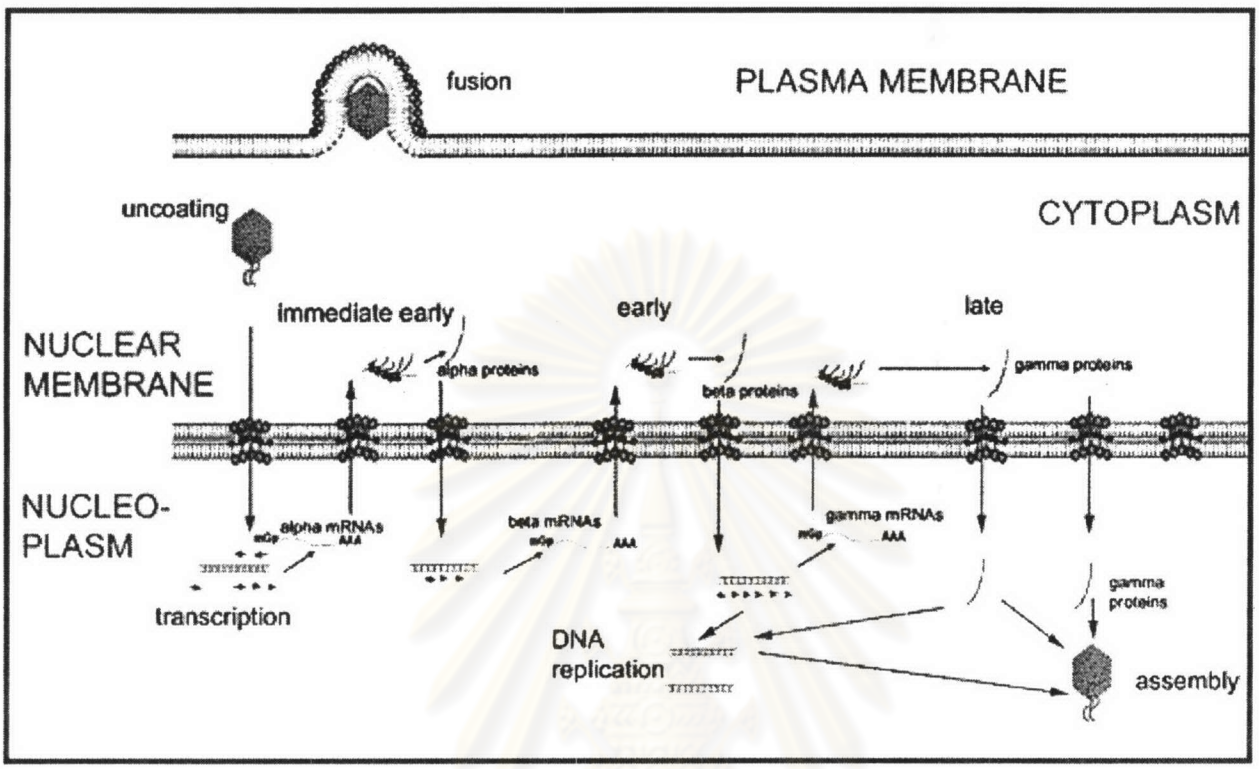


Figure 3. Schematic representation of an HSV replication cycle (145)

by Posavad *et al* (146). All HSV-infected CTL populations tested were significantly inhibited in lysing target cells. The inhibition of CTL lytic function by infection with HSV-1 was independent of T-cell receptor-mediated antigen recognition and did not involve virus-induced shutoff of host protein synthesis, the expression of HSV-1 transactivation protein, ICP4, or replicating virus. They demonstrated that ICP4 (α gene product), gB (γ1 gene product) and gC (γ2 gene product) were expressed in allo-CTL infected with 10 PFU of HSV-1 per cell at 4,7 and 11 hours post infection, respectively. Infectious virus production from HSV-infected allo-CTL peaked on day 2 postinfection and reached levels of $2x10^6$ PFU/ml, level within the ranges detected in mitogen-stimulated T cells (20,144,147,148). Therefore, CTL infected with HSV-1 not only express HSV-specific proteins but also produced infectious virus.

Pathogenesis and diseases of HSV infection

There are many routes of HSV infection. Transmission of the virus occurs as a result of direct contact with infectious secretion or rarely by laboratory accidents with infectious materials. Oral-facial infections are believed to acquire as a result of kissing and genital herpes simplex infections are acquired as a result of either genital-genital, oral-genital or anogenital contact. The HSV has multiple sites of infection related to the body and depending on the strain encountered. Typically, HSV-1 is correlated with oral and ocular infections while HSV-2 is correlated with genital infections. However, both HSV-1 and HSV-2 are known to cause genital-herpes, oral-facial infections (gingivostomatitis, labialis, pharyngitis), cutaneous infections, disseminated infection and erythema muliform. (149).

During the initial phases of HSV infection, the virus enters parabasal and intermediate epithelial cells where it multiplies, finally producing cell lysis. A local inflammatory response ensues and regional lymph nodes are involved. The viremia and visceral dissemination were rarely found, including in the immunocompromised individuals. After HSV replicates at the local port of entry, sensory or autonomic nerve endings are then infected. The viral nucleocapsid is transported intra-axonally to the nerve cell bodies in ganglia, usually the trigeminal and sacral ganglia, where further replication or latency can occur (150,151). In some cases the infiltrating virus can infect the spinal cord from the dorsal root ganglia (151).

In humans, the time interval between viral inoculation and approach to the ganglia is still unknown. Opposite to study in mice and guinea pigs, it demonstrated that HSV is recovered from ganglia by two days after vaginal or skin inoculation (152,153). During this early phase of infection, viral replication may occur in ganglia and continuous neural tissue with migration of infectious progeny virus back to mucosal or skin surfaces by way of the peripheral sensory nerves. This mode of viral spread helps to explain the large affected surface area and the high frequency of new lesion formation distant from the initial crop of vesicles in the patients with primary HSV infections. Recurrent HSV infections occur almost exclusively in individuals who has neutralizing antibody to the homologous virus. These recurrent episodes may result in asymptomatic HSV shedding, recurrent mucocutaneous and cutaneous lesions, pain including radiculopathy, and other illnesses (154). In general, recurrent HSV infections are milder than

primary infections. Although, it is believed that reactivation of latent, endogenous virus can cause recurrences, recent studies have demonstrated that exogenous reinfection with a different strain of HSV can occur (14). The frequency with which such reinfection occurs is unknown.

Latency

The hallmark of HSV infections is the establishment of the virus in a latent state following the primary disease episode. HSV establishes latent infection of sensory ganglion neurons, from which they reactivate at intervals causing asypmtomatic virus shedding or clinically disease. During this stage, it has been determined that the only detectable viral expression in infected cells are RNA called latency-associated transcriptions (LATs). Infectious virus does not present in latently infected ganglia. Evidence suggests that HSV replication during the maintenance stage of latency is blocked at the level of viral immediate early gene expression. Because of this, viral antigens are not detected during latency. Both HSV-1 and HSV-2 infected sensory ganglia have LATs which similar in overall structure and expression but they have limited in nucleotide sequences.

There are several available data suggest that regulation of latent infections likely involve four viral gene products: VP16, ICP4, ICP0 and LATs. ICP0 is well known for its function as a potent and global transactivator of viral gene expression (71,155). Recently, it was reported that the expression of ICP0 in primary neurons in culture and in a differentiated neuronal cell line is poor (80). This evidence suggests that the lack of ICP0 accumulation may support establishment of the latent state in neurons by preventing the normal cascade of viral gene expression necessary for lytic infection. Reactivation can be induced by physical, mechanical, hormonal, or chemical stimuli. Two events which necessary for reactivation are the termination of the latent viral state and the change of the neuron from nonpermissive to permissive for productive viral replication (6,37).

Immune response to HSV infection

The host's immune system is responsible for limiting the effect of primary HSV infections and holding the virus in its latent state as long as possible. The host response promotes host survival, helps infected cells, and establishes lifelong immunity to reinfection. Skin or mucosal infection of HSV-1 or HSV-2 is usually followed by transmission via sensory nerves to the spinal or trigeminal ganglia where lifelong latent infection establishes (14). The frequency of reactivation from ganglionic neurons leads to viral shedding into the oral cavity (HSV-1, trigeminal ganglia) or into the vagina, perianal, or penile skin (HSV-2, sacral ganglia). Type specific antibody accompanies infection and acts as a marker for subsequent episodes. Most initial and recurrent infections are asymptomatic or unrecognized (14).

The infection that occurs in the absence of detectable antibody directed to HSV known as primary infection (14,154). The initial immune response is complex and not completely understood but antibodies to HSV antigens can be detected within four to eight days of initial infection (14). This indicates the early or innate mechanisms of immunity, i.e, interferons, macrophage, and natural killer cells form the first line of defense against HSV while neutralizing antibody and specific T cells, which secrete antiviral cytokines and/or are activated to kill infected cells, are being produced. The first antibodies detected are IgM followed by IgG, which persists for longer period of time (14). IgM is the major class of antibody to be secreted into the blood in the early stages of a primary antibody response. IgG antibodies are the major class in the blood. It is produced in large quantities during secondary immune responses and binds to specific receptors on macrophages and neutrophils (154). At the site of epidermal infection, viral antigens are presented on dendritic cells and macrophages to CD4+ T-helper cells. These CD4+ T-helper cells initiate viral clearance by secreting cytokines such as gamma-interferon, stimulating recruitment and activation of macrophages and natural killer (NK) cells, mediating the immune response. The immune response effectively induces lysis of the infected cells expressing viral antigen, by integrated mechanisms involving CD4+ T-helper cells, CD8+ T-helper cells, natural killer (NK) cells, and the antibody-dependent cell-mediated cytotoxicity (ADCC) (156).

In recurrent infections, specific immune responses are activated much more quickly due to immunologic memory established during the onset of primary infection. In this phase of

infection, it is believed that HSV antigen is taken up by Langerhan's cells and presented on MHC class II effectively stimulating specific resident memory $CD4^+$ T-helper cells which are also stimulated by gamma-interferon (157). Langerhan cells, working in accordance with other sources of cytokines, coordinate a proper T-helper cell immune response. Even before restimulation of immune T cells, an initial cytokine, Tumor mecrotic factor (TNF)-alpha, was bound to macrophages and/or T cells emphasizing the beginning of the secondary immune response in the early stages of recurrent infection (157,158). Another early detectable cytokine represented in high titers is beta-interferon, which is released by fibroblasts, effectively inhibits viral replication (156,158). However, T cells along with the macrophages are the main mediators of the protective immune response against recurrent HSV. These are the $CD4^+$ and $CD8^+$ T cells, along with macrophages which are found in the mononuclear infiltrate surrounding infected epidermal cells. These T cells possess antiviral functions that include cytotoxicity, inhibition of viral growth, lymphokine secretion, and support of $CD8^+$ and humoral responses. The cytotoxicity response is mediated by early infiltrating $CD4^+$ T cells that are responsible for the secretion of gamma-interferon, similar to primary infection, associated with the T-helper cell immune response. The secretion of gamma-interferon may also help to overcome the down regulation of MHC Class I by TAP, which helps permit lysis by $CD8^+$ cytotoxic T cells. A study performed by Cunningham and Mikloska, 2001 found that $CD8^+$ T cell cytotoxicity could be detected only with gamma-interferon stimulated keratinocytes, underscoring the importance of this cytotoxic killer in an operational immune response (154). The role of the humoral immune response has also been examined that B cells are rarely found in these lesions, so the antibody response is not adequate. However, high levels of pre-existing neutralizing antibody may still play a role in preventing the spread of HSV, as in neonatal herpes and in the prevention of viremia (158). In addition, the size of the herpetic lesions and amount of viral spread are limited (157).

Epidemiology

HSV infection occurs worldwide without seasonal variation and naturally only infects human beings. It can be caused by either type of HSV. Susceptible individuals who are HSV seronegative develop primary infection after their first exposure to HSV-1 or HSV-2. Initial infection occurs when an individual who has antibodies to either HSV-1 or HSV-2 is infected with the other type of virus for the first time. Although HSV-1 and HSV-2 are usually transmitted by different routes and affect different area of the body, the sign and symptoms that they cause overlap (159). Most HSV-1 infections are acquired in childhood. There are many predisposing factors such as crowded living or day care situations. It was found that as many as 37% of children in day care acquire HSV-1 by age five, with peak seroconversion in children one to two years of age (160). HSV-1 seroprevalence steadily increases with age to about 90% by age 60 and is inversely proportional to family income (161) and level of education (162-164). It has been estimated that as many as 20% to 40% of some populations have recurrent oral-facial HSV infection (165,166). HSV-2 infections are usually sexually transmitted. Most genital HSV infections are caused by HSV-2; however, an increasing proportion is attributable to HSV-1. In the United States of America, the prevalence of HSV-2 infection has increased by approximately 30 % since the late 70's. So currently, it is believed that over 500,000 new cases of genital herpes annually in the United States and as many as 20% individuals may have recurrent genital herpes infections (159). The relative prevalence of HSV-2 infections varies significantly with socioeconomic and educational status (167). HSV antibody prevalence is approximately 60% in sexually active people in lower socioeconomic classes in the United States, whereas that in middle to high socioeconomic groups is averages 25%. Increased HSV-2 seroprevalence also has been reported in the United Kingdom, Israel, New Zealand, western Europe and Thailand (163,167). HSV infection is rarely fatal, except in the rare incidences of neonatal herpes infection and the rare incidences of HSV induced encephalitis, so HSV did not receive much attention for eradication, in the past. However, this view has been altered radically because of the increasing association of HIV transmission within HSV infected patients. Actually, the most important risk factor in the acquisition and transmission of HIV is genital ulcer disease, of which genital herpes is the most common cause within developing countries (168).