CHAPTER 2

Literature review

2.1 Viruses

Viruses are obligated pathogenic organism which have a nucleic acid genome of either DNA or RNA. The viral genomes are small comparing with a cellular genome. The genomes of different virus are ranging from in size 3000 to 1,200,000 bp. Viral genomes are associated with capsid protein that is a simplest form of the virus particle. In some viruses, this nucleoprotein is surrounded by a lipid bilayer (Cann, 2012; Dimmock *et al.*, 2007).

All viral genomes are surrounded by capsid proteins which are used for protection of viral nucleic acids from nuclease degradation. The capsid protein contains identification elements or attachment site that ensures a virus recognizes an appropriate target cell. Additionally, these surrounded proteins contain a genome-release system that ensures the virus genome is released from a particle only at the appropriate time and location. Moreover, some viral particle contains enzymes that are essential for the infectivity (Daheshia *et al*., 1998; Wagner *et al*., 2006).

Viruses can only reproduce in living cells. The outermost proteins of the virus particle allow the virus to recognize the correct host cell and entry into its cytoplasm (Fleming *et al*., 1997; Jassim and Naji, 2003).

2.2 The structure of virus

All viruses contain proteins and nucleic acids with at least 50%, and in some cases up to 90%, of their mass being proteins (Wagner *et al*., 2006). It would appear that there are many ways in which proteins can be arranged around the nucleic acids. However, viruses use only a limited number of proteins. The limitation on the range of structural proteins is due to restrictions that impose by considerations of efficiency and stability (Fatahzadeh and Scwartz, 2007). While proteins may have

regular secondary structure elements in the form of alpha helix and alpha structure, the tertiary structure of the protein is not symmetrical. This is a consequence of hydrogen bonding, disulfide bridges, and the intrusion of proline in the secondary structure. Although most virus particles have a regular morphology, nucleic acids may be covered by a single, large protein molecules which are irregular shape. However, viruses must contain more than a single protein can also be deduced solely from considerations of the coding potential of nucleic acid molecules (Daheshia *et al*., 1998; Wagner *et al*., 2006; Whitley *et al*., 1998).

2.3 Herpes simplex virus

2.3.1 Background and biological properties

Herpes simplex virus (HSV) infectious diseases occur worldwide and produce serious illnesses. The virus is responsible for a wide variety of common human infection, which is easily transmitted in both developed and developing countries. The first recognized of herpes occur in ancient Greek. The word herpes itself derived from "*herpein*" that means to creep or crawl and describes spreading and characteristic nature pattern of the skin lesion caused by herpes simplex virus. HSVs are classified into genus *Simplexvirus*, a member of subfamily *Alphaherpesvirinae,* family *Herpesviridae*. This classification is based on their biological properties, common structure and location in latent state (Albà *et al.,* 2001; Gupta *et al*., 2007; Pereira *et al*., 2012).

Herpesviridae is human pathogenic viruses and causes major illness of morbidity and mortality. HSVs use the host cell machinery and directly transcript viral genes. HSV infection has a short replication cycle and the virus has ability to destroy infected cells with a variety of diseases (Armaka *et al*., 1999; Taylor *et al*., 2002). Additionally, HSV infection produces clinical symptoms ranging from mild to serves disease. Primary infection causes vesicular lesions in the mucosal epithelia cells. Viruses are able to spread and establish latent infection in host cells within sensory neurons. Recurrent disease is usually present at or near the site of primary infection (Mandal *et al*., 2008; Tolo *et al*., 2006).

Herpesviruses compose of two serotypes, which are herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). HSV-1 and HSV-2 are similar in tissue tropism and

DNA sequences. Polymerase chain reaction (PCR) technique shows the sequence homology of both HSV-1 and HSV-2 approximately 85% similarity (Arama *et al*., 2010; Riley, 1998; Whitley and Roizman, 2001). Therefore, different remarkable between two viral subtypes are also classified by their location in the latent state, epidemiology, DNA homology, antigenic determinants, and symptom of disease (Brugha *et al*., 1997; Lucotte *et al*., 1995; Taylor *et al*., 2002). Therefore, HSV-1 and HSV-2 are closely related in clinically indistinguishable and share many common epitope and characteristics, which are resulting in cross-reactive responses in serological assays (Whitley *et al*., 1998).

Both types of HSV infections cause chronic disease and affect populations worldwide in both rural and urban. It is life-long infections with intermittent clinical and subclinical viral reactivation and shedding from mucosal surfaces. The reactivation is depended upon the type involved (Lückemeyer *et al*., 2012; Madhavan *et al*., 1999; Sierra *et al*., 2011).

2.3.2 Structure properties of herpes simplex virus

HSV is a diverse group of a large double-stranded linear DNA molecule and the diameter of virion particle is approximately 150-200 nm in size. HSV particle consists of nucleotide with 32-75% G+C, depending on the virus species (Spear and Roizman, 1972). DNA core is packed in an icosahedral capsid that coated with a layer of proteins called tegument and enclosed with a glycoprotein-containing envelope. The envelope protects viral genome from various conditions in extracellular environment. Moreover, several glycoproteins in envelope are essential for viral particle fusion and entry into infected cells (Spear, 2004).

There are four structural features of HSV particle are as follows.

2.3.2.1 Viral genome

Structure of viral particle consists of a central opaque dense core containing viral genome in form of torus structure. The genome encodes about 100-200 genes that is packaged tightly in an icosahedral capsid. Inner core is surrounded by an envelope, which is derived from cellular membranes (Figure 2.1) (Furlong *et al.,* 1972; Karasneh and Shukla, 2011; Spear, 2004).

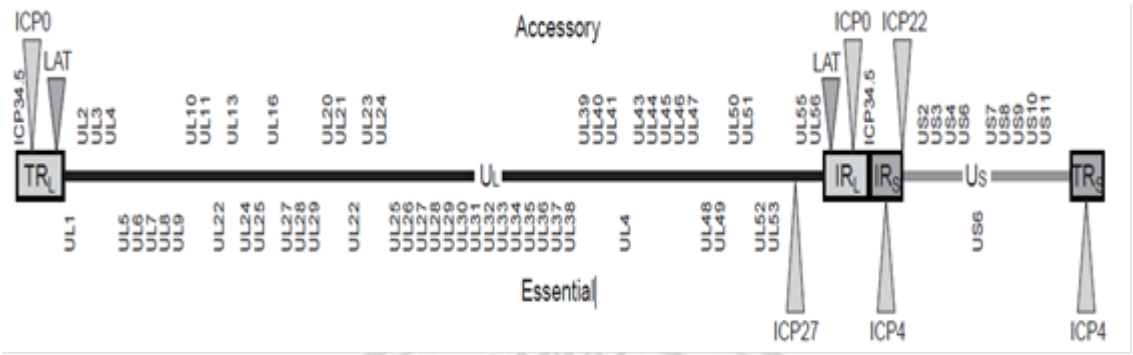


Figure 2.1 HSV genome (Simonato *et al*., 2000)

The HSV genome is a single large double-stranded linear DNA molecule. It consists of approximately 150,000 base pairs and encodes at least approximately 80 proteins. However, only half of these proteins are necessary for directly control of viral replication and facilitate interaction between virus and different host cells. Other proteins have function on interaction with host cell or immune response (Taylor *et al*., 2002).

Viral genome and their protein products are generally composed of two covalently linked segments, called long (L) or short (S) regions, which are based upon their relative length. Each segment contains unique sequence; U_L (unique long) and U_S (unique short) region that are flanked by large inverted repeats (Figure 2.2) (Taylor *et al*., 2002).

Viral genome is released into the nucleus for viral gene expression. HSV genome encodes several numbers of essential enzymes, which are DNA-dependent DNA polymerase and other enzyme such as thymidine kinase, ribonucleotide reductase, serine-protease and protease. Thus, these enzymes and DNA polymerase are suitable targets for antiviral treatment (Figure 2.2) (Simonato *et al*., 2000; Taylor *et al*., 2002).

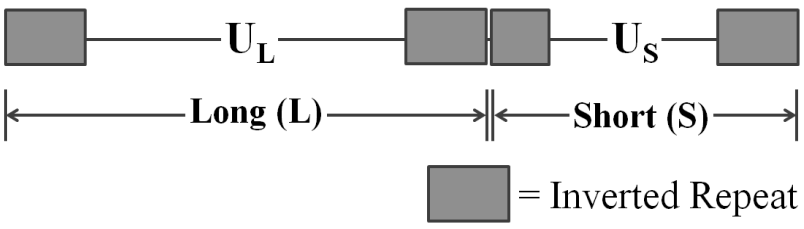


Figure 2.2 HSV genome organizations (modified from Taylor *et al*., 2002)

2.3.2.2 An icosahedral capsid

HSV genome is packed within an icosahedral capsid and surrounded by a membrane that is derived from host nuclear membrane. Major capsid shells are 125 nm in diameter thick and icosahedral structure. Capsid protein displays 162 capsomers, which composes of 12 pentons and 150 hexons (Davison, 2010; Spear, 2004). The pentons and hexons are connected together by 320 triplexes and triplex lies at local three-fold position created by a group of three capsomers (Baines, 2011). In addition, HSV composes of 3 capsid types, which are A, B and C-capsids. All of them have same structure but only C-capsid contains DNA and it is able to mature into infectious virus (Figure 2.3) (Brown and Newcomb, 2011; Spencer *et al*., 1997).

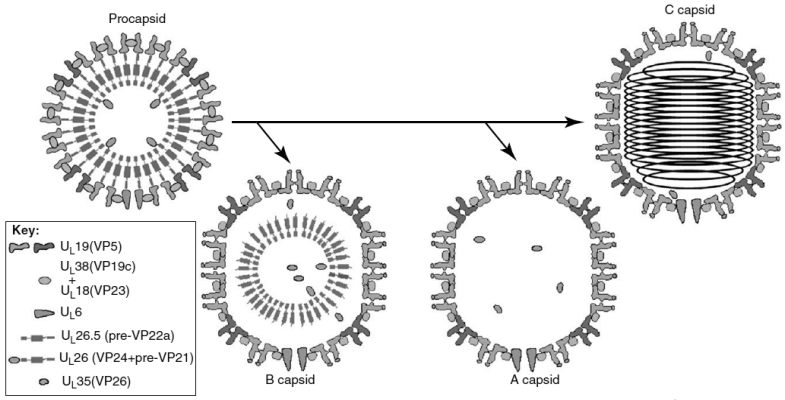


Figure 2.3 Structure of HSV-1 maturation (Baines, 2011)

2.3.2.3 A tegument

Tegument is an amorphous layer of proteins surrounded the capsid. The space of tegument is between envelope and capsid. This component which contains viral proteins and enzymes that are essential for replication. (Figure 2.4) (Taylor *et al*., 2002).

2.3.2.4 Envelope

Envelope or outer membrane of mature HSV is derived from portions of inner lamella nuclear membrane and cisternae of endoplasmic reticulum of infected cell. Viral particles move from nucleus into cytoplasm. Virus exits cytoplasmic membranes of the cell by budding into vesicles derived from Golgi complex and contains viral glycoprotein spikes. Therefore, virus that has fragile membrane and damage envelope is not infectious. The infectious virus can be transmitted by direct contact with infected mucosal membrane or infections secretions from infected person (Spear and Roizman, 1972).

Herpesviruses have several viral-encoded glycoproteins embedded on their surface envelope. Major function of HSV envelope and envelope glycoproteins are important for interactions with host cell receptors to promote entry and fusion to the cells, and escape viral immune mechanisms by binding complement or a portion of the antibody (Riley, 1998; Sarmiento and Spear, 1979). Normally, enveloped viruses are sensitive to acid, detergent, dry, organic solvent and also potential target for antiviral agents (Figure 2.4).

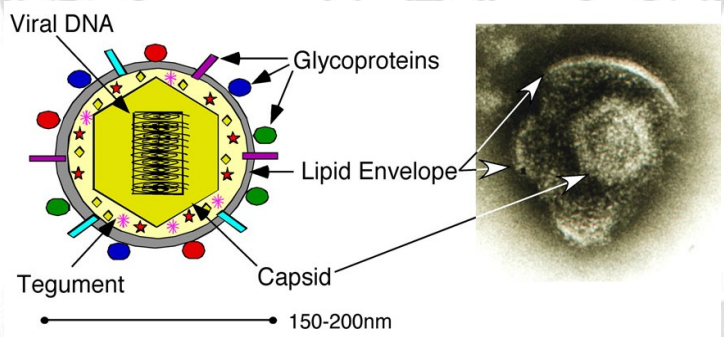


Figure 2.4 HSV structure composes of viral DNA, icosahedral capsid, tegument and envelope (Taylor *et al*., 2002)

2.4 Herpes simplex virus infection

Initial of infection

Primary infection occurs when individual is infected with either HSV-1 or HSV-2. This initial infection begins with HSV entry to the body through epithelial or mucus membrane by attachment with specific cell-surface receptor of host cell.

Then, the virus fuses its envelope with cellular membrane leading to viral invasion of epithelial cells. After that, virus transports capsid through nuclear pore and follows by releasing viral DNA into nucleus to begin intracellular cytolytic replication (Whitley and Roizman, 2001). These infection leads to damage the infected cells because HSV induces epithelial and dermal cells detachment, cell fusion and intranuclear inclusions. The lesions of fluid-filled blisters containing cellular debris, inflammatory cells, and progeny virions are found (Gupta *et al*., 2007).

Therefore, primary symptoms of HSV infections cause "flu-like" syndrome. The patients are present fever, headache, malaise, diffuse myalgia follow by local symptoms consisting of itching, tenderness, dysuria lesions, painful and ulceration. Viral shedding occurs during primary infection or during subsequent recurrences leading possible transmission. Moreover, people with asymptomatic infections also shed the virus (Khan *et al*., 2005).

Latent infection

After primary infection, viral genomes in form of circular extra-chromosomal DNA are transported through periaxonal sheath of sensory nerves to nuclei of neuronal cell bodies of host nervous system that connect the point of entry body. The viral genome is maintained to establish life-long in sensory neurons of the peripheral nervous system in the state of latency. The virus can be reactivated episodically. Major reservoir of latent infections is sensory neurons in ganglion tissue, either trigeminal ganglion for HSV-1 or sacral lumbosacral ganglia for HSV-2 to persist in a dormant state for life. Thus, virus remains hidden in the cell by avoiding immune surveillance (Fatahzadeh *et al*., 2007; Piret and Boivin, 2011; Spear and Roizman, 1972).

The characteristic of this latent infection demonstrates that the viral genome remains in tissue without production of infectious viral particle to damages tissue. The presence of virus can be detected by immunofluorescence microscopy using antibodies against HSV immediately early protein (Figure 2.5) (Lachmann, 2003; Steiner and Kennedy, 1995). During latent period, viral DNA and RNA transcripts can be detectable but viral-encoded proteins are not produced. However, RNA transcripts that are produced during latency are important in reactivation of HSV from the latent state (Riley, 1998; Taylor *et al.,* 2002).

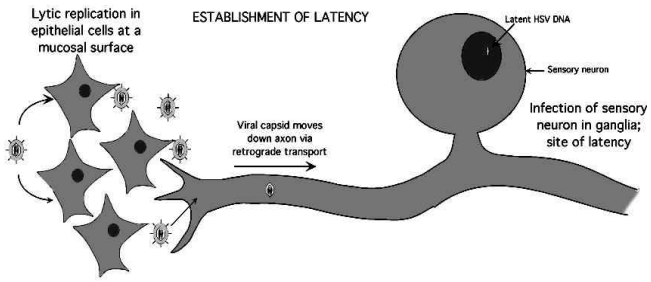


Figure 2.5 Stages of HSV initial infection (Taylor *et al*., 2002)

Reactivation and recurrent infection

HSV reactivation from latency is an essential element of herpesvirus life cycle. This process maintains the potential to reactivate episodes of replication and symptoms that occurs at the same site of primary infection by promoting viral replication in the nerve and leads to spread the viral infection to new host. However, reactivation of latent virus depends on an intact anterior nerve route and peripheral nerve pathways. Moreover, subclinical HSV infection depends on site, time and stage of immune system (Figure 2.6).

The sporadic reactivation of latent viral gene expression from establishment latency is generally triggered after response to variety of internal factors; physical properties and psychological stress such as fatigue, febrile illnesses, tissue damage, menstruation, sexual intercourse, temperature, ultraviolet irradiation, corticosteroid administration, laser surgery and nerve damage (Piret and Boivin, 2011; Taylor *et al*., 2002; Whitley and Roizman, 2001). Theses stimuli can reactivate the virus from neuron to move along the nerve from sensory ganglia to give renewal of proliferation of activated virus. The recurrent infections lead to clinical disease on the skin that involve mucosal membrane or squamous epithelial areas to cause lesions of vesicles cluster in the vicinity of the initial site of infection (Fatahzadeh and Schwartz, 2007; Itzhaki *et al*., 1997; Lachmann, 2003).

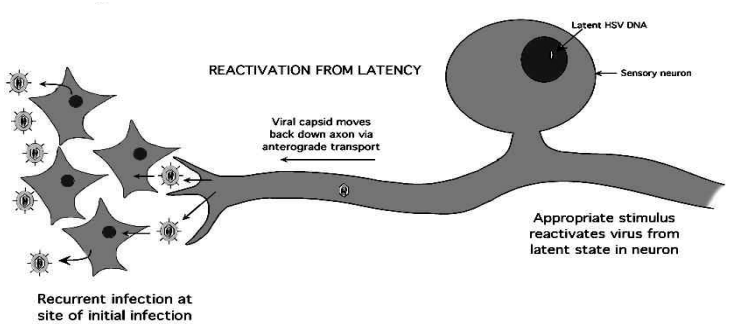


Figure 2.6 Stages of HSV reactivation (Taylor *et al*., 2002)

Viral reactivation can produce recurrent symptomatic and asymptomatic infection. HSV can be reactivated causing frequent recurrent infections in some patients while most people experience few recurrences (Spear, 2004).

Therefore, the diseases of HSV reactivation from trigeminal ganglion generally occur on oral and nasal mucosa, which are the border of the lip to produce infectious vesicles while lesion around genital area is caused by reactivation of HSV from sacral nerve ganglia. Moreover, clinical presentation of recurrent infection is generally less stringent, more localized, and shorter time of symptom than primary infection because of the immune responses is expressed (Figure 2.7) (Doherty *et al.,* 2010; Spear and Roizman, 1972).

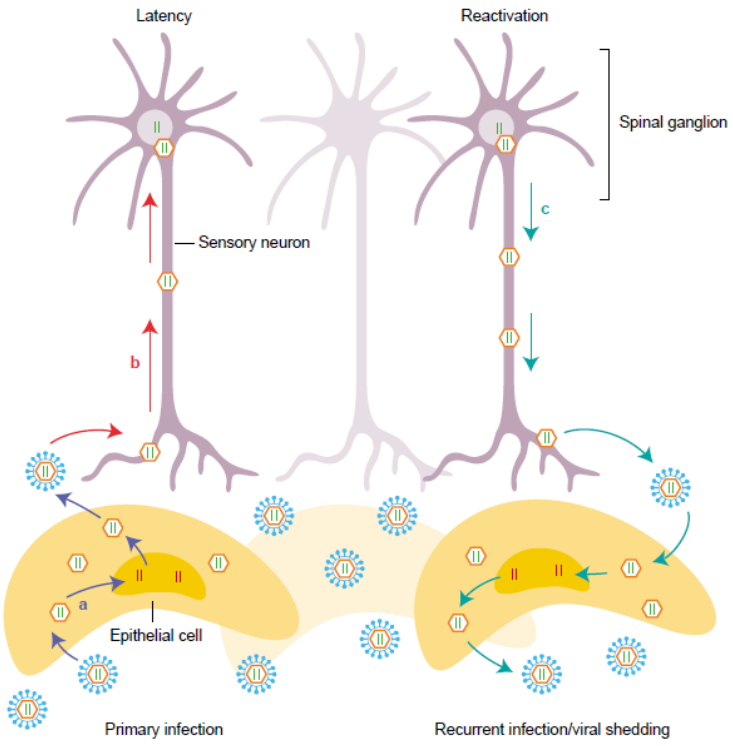


Figure 2.7 Primary and recurrent of HSV infection (Lachmann, 2003)

2.5 Herpes simplex virus attachment and entry

HSV infections are composed of attachment and entry to infected cell. Then, replication and expression of viral DNA are occurred after viral uncoating in nucleus. After that, nucleocapsids are assembled and exited from epithelial cells to cause a primary infection. Besides, some of viruses enter sensory neuron and travel retrograde to the nucleus where the viruses establish latency depending on type of HSV. Moreover, reactivation results from anterograde transport of viral particles by viral shedding from the neuron, and re-infection of epithelial cells at initial site of infection (Lachmann, 2003).

Replication of HSV occurs when viral enter into the susceptible host cell. Herpes simplex virus is able to replicate in many cell types and leads to infected cells changes from necrosis, intranuclear inclusion body, swelling with condense chromatin, nuclear degeneration and formation of multinucleated giant cells together with inflammatory response (Taylor *et al.*, 2002; Whitley and Roizman, 2001).

Entry of HSV into host cells acquires interaction between multiple HSV glycoproteins (g) components on surface of viral envelope with multiple specific cell surface receptor molecules on host cell surface. The important target receptors for HSV infection are epithelial cell, mucosa, neurons and leucocytes.

HSV encodes surface glycoproteins more than 11 known viral glycoproteins (B-M) including gB, gC, gD, gE, gF, gH, gI, gK, gM and gN. gB, gC, gD and gH are required for attachment to cell surface. Virus initially attaches to host cell by interaction process between virion gC and herparan sulfate (HS) proteoglycan on the host cell surface. HS is a ubiquitous glycosaminoglycan (GAG) found on cell membrane and basement membrane of many cell types (Hung *et al.,* 2002). However, the viral attachment to only HS does not enable entry to host cell. Then, gD protein interacts with one or more cellular co-receptor molecules on cell surface such as herpes virus entry (Hve) protein and leads to the stability of virion attachment. After that, gD conformation is changed and interaction with gH/gL herterodimer on viral surface triggers the fusion step of viral envelope and plasma membrane, and HSV nucleocapsid

penetrates to host cell (Figure 2.8) (Frampton *et al.,* 2005; Hung *et al.,* 2002; Spear, 2004).

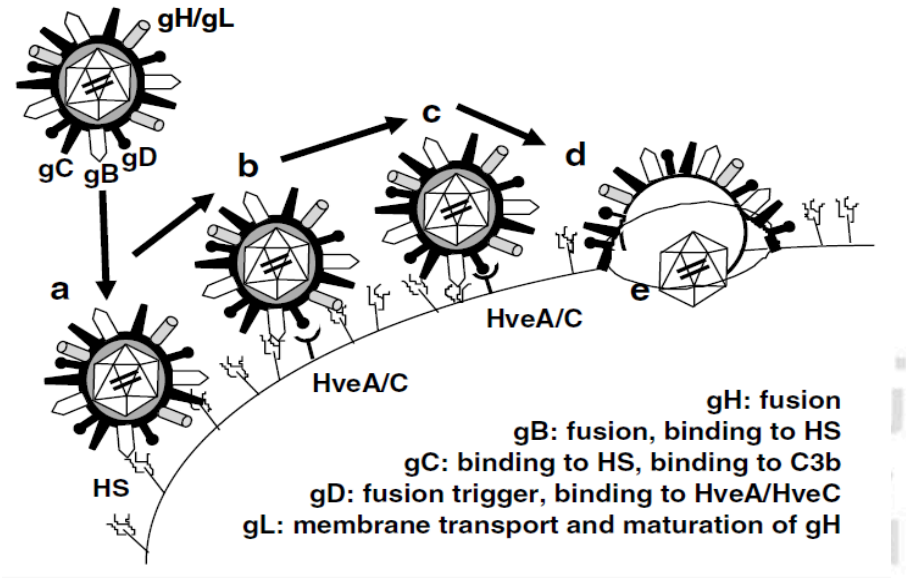


Figure 2.8 HSV-1 entries into host cell (a) Interaction binding between gC and gB with HS on host cell surface (b) Diffusion of cell surface to HveA/HveC receptor (c) gD binding with HveA/HveC receptor (d) Fusion viral envelope and cell membrane (e) Capsid protein is released into cytoplasm (Frampton *et al.,* 2005)

HSV co-receptors are divided into three structural families. First, herpesvirus entry mediator (HVEM) or HveA is a member of the tumor necrosis factor (TNF) receptor family (Spear and Roizman, 1972). Second, members of the poliovirus receptor related (PRR) immunoglobulin superfamily include HveB, HveC (nectin-1α), and HIgR (nectin-2α). Finally, specific site in heparan sulphate is 3-O-sulfated heparan sulfate (3-O-HS) (Figure 2.9) (Banfield *et al*., 1995; Mardberg *et al*., 2002; Montgomery *et al*., 1996). Additionally, it also binds to the C3b component of complement system and can block complement-mediated neutralization of virus. gB also interacts with HS or chondroitin sulfate (CS) proteoglycans (Bender *et al*., 2005; Spear, 2004).

Furthermore, gE and gI are structural proteins and immune escape proteins. gE is required for basolateral transmission of virus in polarized cells and efficient expression of late genes (Whitley *et al*., 1998). The other glycoproteins as well as several nonglycosylated membrane associated proteins have function in several important roles such as virus entry via fusion of viral envelope with cellular membrane, intracellular virion morphogenesis, egress and cell-to-cell spread (Melancon *et al*., 2005).

HSV-1 and HSV-2 have the different in some receptors. Both HVEM and nectin-1 are entry receptors for both serotypes while nectin-2 is virtually inactive for HSV-1 entry and weak entry for HSV-2. Moreover, glycoproteins gB, gH and gL are structurally conserved among all herpesviruses and probably have essential roles in viral entry. On the other hand, glycoproteins gC and gD are conserved among most of the neurotropic alphaherpesviruses but do not have recognizable structural homologues in members of the other two genus of the herpesvirus family (Spear, 2004).

Therefore, HSV can also entry through host cell by multiple steps that results from fusion viral envelope with host cell by pH independent fusion with plasma membrane and further fuses into acidic, or neutral endosomes, or macropinocytosis pathway (Figure 2.10). In addition, HSV glycoprotein can fuse with host membrane at neutral and low pH. However, specific pathway of HSV entry into host cell differs and depends on upon various cells type-specific (Campadelli-Fiume *et al*., 2012; Karasneh and Shukla, 2011).

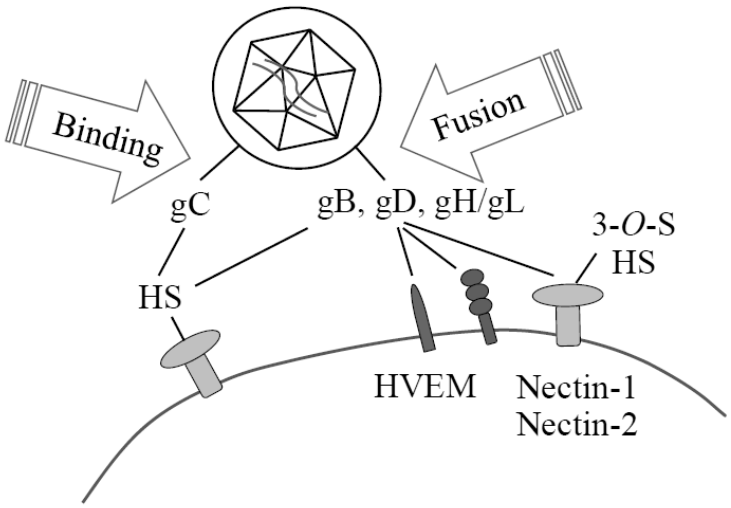


Figure 2.9 Cell surface receptors and viral glycoprotein requirement for HSV attachment and entry (modified from Spear, 2004)

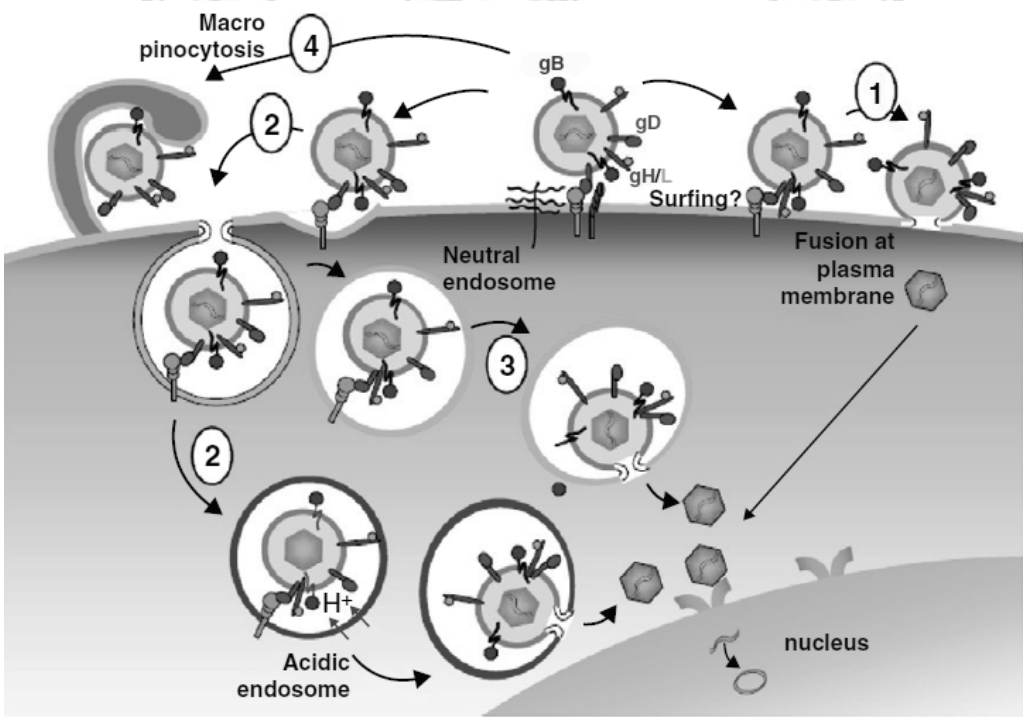


Figure 2.10 Herpes simplex virus entry pathway by Fusion at cell membrane (1) Endocytosis into acidic or (2) Neutral endosome (3) Macropinocytosis (4) (Campadelli-Fiume *et al*., 2012)

After HSV successful entries and penetrates into host cell by fusion of viral envelope with cellular membrane of host cell. Then, viral nucleocapsid and some tegument proteins (VP16, VP1-2) are transported through cellular microtubules network via nuclear pore into the nucleus following by viral DNA, which is released into nucleus whereas other tegument proteins such as the host shutoff protein (vhs, U_s11) remains in cytoplasm. This process causes disaggregation of polyribosomes and degradation of cellular DNA. Therefore, viral gene expression and replication occur in nucleus and viral assembly occurs in cytoplasm of the host cell (Whitley *et al*., 1998).

2.6 Replication and expression of herpes simplex viral DNA

Herpesvirus replication is occurred in neuronal nucleus. HSV DNA genome enters nucleus through nuclear pore via microtubules. After DNA replication, circular concatameric forms of HSV genome are made by a rolling circle mechanism.

Transcription of viral genome and viral protein synthesis occur in three sequential phases; immediate early (α), early (β) and late (γ) genes (Figure 2.11) (Preston *et al*., 1988). VP16 stimulates transcription of immediate early genes (IE) or alpha genes with transcription about 2-4 hours after infection. The alpha proteins consist of DNA-binding proteins, transcription factors and enzymes that are assigned to lytic infection by regulation of early and late gene expression. Moreover, IE genes also stimulate transcription of the early gene (β) synthesis. The early genes (E) or beta genes are expressed at 4-8 hours to 15 hours after infection. The early gene products compose of DNA polymerase for initial replication of viral DNA and enzymes that degrade cellular mRNAs and proteins. Moreover, other early gene products are required for DNA replication and act as transcription factors. Circular concatemers are made and then switch to linear chains of individual molecule that are cleaved into monomers by a rolling circle mechanism.

Finally, viral DNA replication stimulates the expression of late genes (L) or gamma genes, which encode structural proteins after 12 hour of viral infection. This product is mainly structural and additional proteins of virion. The proteins assemble to form the capsid and tegument, and they are also incorporated into nuclear membranes to form envelope of virions (Figure 2.11). The replication cycle is approximately 8-16 hours (Whitley *et al.,* 1998). Thus, transcription of viral DNA takes place in nucleus,

while all viral proteins are synthesized in cytoplasm of the infected cell (Taylor *et al*., 2002).

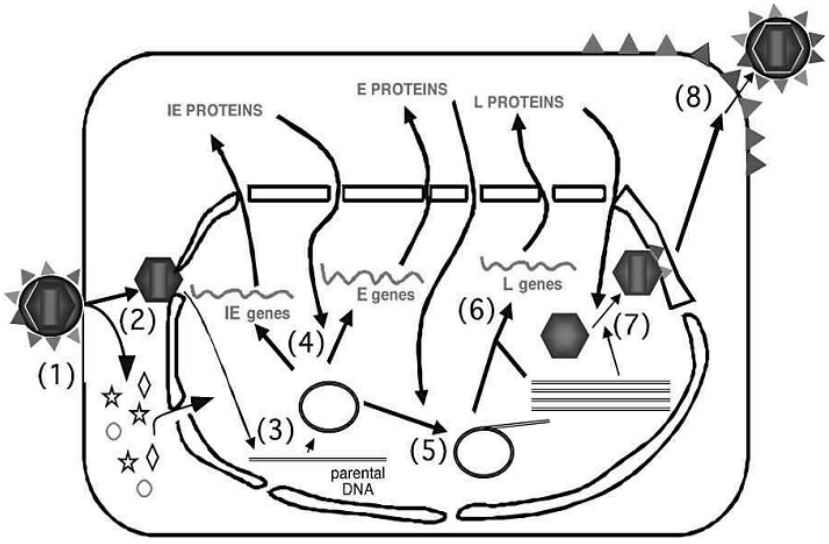


Figure 2.11 Replication of HSV cycle. The cycle of HSV infection composes of HSV particle binds to receptor on cell surface membrane and fusion (1) Viral nucleocapsid and tegument are released into cytoplasm of host cell and transported their nucleocapsid through nuclear pore (2) Viral DNA is released into nucleus (3) Transcription and translation of immediate early and early genes (4) Viral DNA synthesis (5) Transcription and translation of late genes (6) Encapsidation (7) Release of viral particle (8) (Taylor *et al*., 2002).

After infection, the virus uses the intracellular components of host cell to express viral proteins for replication and other process. Two types of proteins; nonstructural and structural proteins are expressed (Taylor *et al*., 2002). More than 80 HSV proteins are expressed in a highly regulated cascade fashion in a number of coordinately expressed groups of gene products that play roles in regulation of viral gene expression. HSV-1 and HSV-2 encode at least 84 different polypeptides and 45 genes are necessary for functions such as viral attachment, DNA repair and immune response for viral infection (Whitley *et al*., 1998).

Furthermore, at least six viral proteins have important roles in expression of viral genes and efficiently mobilize cellular proteins for synthesis of viral DNA and

proteins. Anti-tumor necrosis factor (αTNF) is a transactivator and essential structural protein of virion. Moreover, the infected cell protein 4 (ICP4) is the large complex component and it can directly bind to both high and low affinity sites at transcription initiation site. Therefore, ICP4 activates early and late genes during viral lytic cycle while IE genes are repressed (Compel and Deluca, 2003). ICP0 is promiscuous transactivators of HSV genes that is expressed in both nucleus and cytoplasm and has important functions such as stabilization of cell cycle and also regulation of protein synthesis and proteolysis (Hancock *et al*., 2009). Another, α protein, ICP22 is a repressor of cellular cycle and promotes viral gene expression. Regulatory proteins ICP0, ICP4, and ICP22 are phosphorylated by cellular kinases (cell cyclin kinase, cdc2) and viral protein kinases (Us3 and U_L13) (Guo *et al*., 2010; Whitley and Roizman, 2001).

ICP27 is a transporter from nucleus to cytoplasm and regulates the expression of late proteins. In addition, ICP27 is able to control the posttranslational processing of RNA (Figure 2.12). ICP47 is another multifunctional protein which blocks RNA splicing in early infection (Figure 2.12). Tegument protein is activated in HSV replication. Vhs is the product of gene U_L41, which activates RNA activity for degradation of all mRNA (Amen and Griffiths, 2011). Therefore, two viral proteins, vhs and protein kinase are important for infection of viral disease.

In addition, RNA polymerase II of host cell is responsible for synthesis of all viral mRNAs. Viral proteins are necessary for initiation and enhancement of transcription of certain genes. Therefore, viral genome also contains signals for processing of newly synthesized genomes for packaging into pre-formed capsids**.** Transcription of viral genome is controlled by cellular DNA-dependent RNA polymerases and the transcription is regulated by viral-encoded and cellular nuclear factors. Lytic, persistent or latent infection is controlled under these factors. However, cells that promote latent infection restrict transcription to specific genes without genome replication (Taylor *et al*., 2002).

Latency-associated transcripts (LATs) are produced by specific region of genome during a latent infection, and LATs are detectable only while viral replication does not proceed. Early protein such as DNA-dependent polymerases is required to promote replication. Moreover, other early proteins inhibit production and initiate

degradation of cellular messenger RNA and DNA. Thus, expression of early and late genes leads to cell death (Block and Hill, 1997; Kent *et al*., 2003).

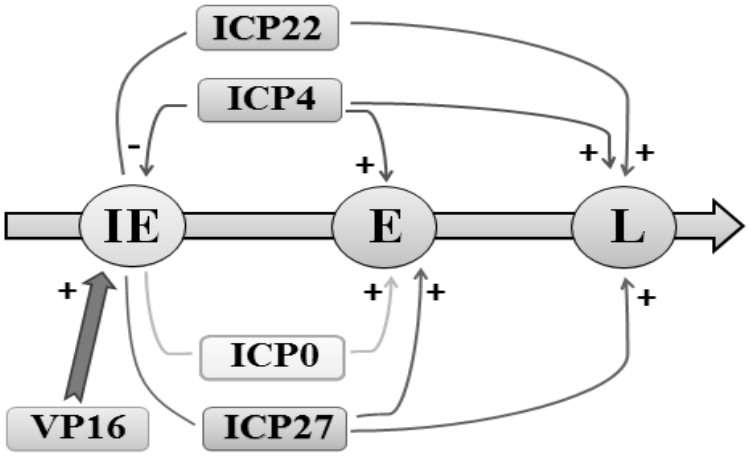


Figure 2.12 HSV gene expressions (modified from Simonato *et al*., 2000)

2.7 Virion assembly and release

Transcriptions of several early genes are needed to produce mature virus and their proteins are produced following DNA replication. Capsids are produced in nucleus and traverse at inner nuclear membrane, outer nuclear membrane and cytoplasmic membrane to release from the infected cell. Procapsids are precursors of mature capsid and formed from scaffolding that are necessary for initial DNA cleavage. They do not contain viral DNA, unstable and porous. Furthermore, procapsid had angular in shape that is lower than other type of capsid. C-capsids are found in mature infectious virions, which compose of packaged viral genomes. The scaffolding protein consists of a protease that can cleave substrate during capsid assembly. B-capsids do not contain viral DNA as well as A-capsid but they contain cleaved scaffolding protein. Assembly of viral capsid is occurred in nucleus, which requires synthesis of late proteins. However, VP5, VP23 and VP26 are capsid proteins, which lack nuclear localization sequences (NLS). Thus, these proteins form complex with NLS-containing protein in cytoplasm in order to transport into nucleus.

Encapsidation occurs in nucleus after newly viral DNA is synthesized by cleavage of HSV DNA concatemers into unit-length monomers molecules, which requires several viral gene including the U_L6, U_L15, U_L25, U_L28, U_L32, U_L33, U_L36 and U_L37. Then, emply capsid shells are loaded and packaged with viral DNA by a process that simultaneously resolves concatemers into the virions (Taylor *et al*., 2002; Whitley *et al.,* 1998). In addition, polyamines are facilitated to the encapsidation process but histone is not required (Roizman *et al*., 2007).

After encapsidation of full-length viral genomic DNA molecules, nucleocapsids attach to the nuclear surface of inner nuclear membrane and rapidly enveloped by budding and released into the space between the inner and outer nuclear membranes that contains the viral glycoproteins. All glycoproteins are synthesized and then receive the high-mannose sugar chains at the endoplasmic reticulum and diffuse to the nuclear membrane (Whitley *et al*., 1998). Interactions between capsid and tegument proteins and between tegument proteins and viral glycoproteins in the inner nuclear membrane promote this budding process. This early process takes place in nucleus and capsid is surrounded by the primary tegument protein. After egress start, disruption of nuclear lamina involving HSV proteins U_L43 and U_L34 phosphorylated membrane proteins has been inserted. Capsids are coated with tegument proteins and bind with inner nuclear membrane containing U_L31/U_L34 and HSV membrane glycoproteins gB, gD, gH/gL and gM, and are bud into perinuclear space (Whitley *et al*., 1998).

At perinuclear, the primary enveloped capsid buds after glycoproteins are processed via outer nuclear membrane where the primary envelope is de-enveloped, the nucleocapsids are delivered into the cytoplasm by transferring in a vesicle to the Golgi apparatus via areas where viral proteins are concentrated and released into cytoplasm (Farnsworth and Johnson, 2006). Here, virions become encased within transport vesicles and are transported through vesicles that are formed by fragmentation and dispersion of Golgi stacks to the extracellular space. These delivers enveloped virions into cytoplasmic vesicles and subsequently move into the cell surface. Secondary envelopment occurs as herpesvirus tegument-coated capsids bind onto viral glycoprotein-enriched regions of the Golgi apparatus, trans-Golgi network (TGN), or endosomes. The basolateral cell surfaces promote virus spread to other cells (Whitley and Roizman, 2001; Whitley *et al*., 1998). Then, enveloped virions within membrane

vesicles are transported to cell surfaces, where there are fusion between vesicle and plasma membrane. The virus is released by budding pathway and virus is able to spread from cell to cell that is protected from host response (Figure 2.13) (Johnson *et al*., 2011). This process takes about 18 hours (Whitley *et al.,* 1998).

Therefore, the virus that is released from one cell can infect another cell by transportation across neighboring cells. Rapid cell-to-cell spread within the epithelium and HSV directly spread from one cell to another cell through the extracellular space, which involves a set of viral glycoproteins; gE and gI. Both gE and gI mediate HSV transfer across cell junctions by interacting with cell junction components (Taylor *et al.,* 2002; Turner *et al*., 1998). However, virus induces cell fusion at approximately 5 hours post infection at 37ºC. At that time infectious virions are produced and released out of infected cells (Melancon *et al*., 2005).

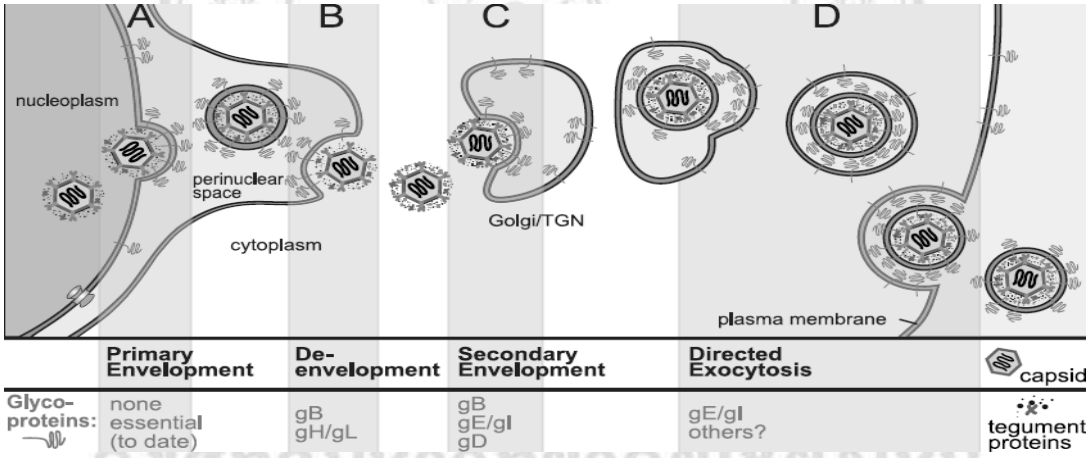


Figure 2.13 HSV release from infected cell (Johnson *et al*., 2011)

2.8 Pathogenesis and Immunity

HSV infections are a major morbidity due to virus is neurotropic and establishes latency in neurons of dorsal root ganglia and autonomic nervous system in primary sensory neurons (Lachmann, 2003; Whitley and Roizman, 2001).

Pathogenesis of HSV disease depends on types of HSV. Both types of HSV can infect human and other animals but only human are present the symptom of disease. Clinical manifestation of HSV infectious disease exhibits different severity, which

depends on the port of entry, immune status, and also depends on primary or recurrent infection in hosts.

HSV enters susceptible individual by personal contact between excreting HSV through mucosal skin surfaces. Viral replication is at the site of infection and an intact virion is transported retrograde by neurons to the dorsal root ganglia. Thus, latent infection is established in the innervating neurons and hid from immune response. After viral infection, parabasal and intermediate cells of epithelium swell with condensed chromatin in nucleus. Then nuclear degeneration and cell membrane breakage are detected in the cell. Therefore, at least 48-72 hours after viral infection, cytopathogenic effect (CPE) are produced in infected cells (Whitley and Roizman, 2001).

Generally, HSV-1 infection is mainly associated in predominates cause of orofacial infections. The virus can infect and produce skin lesions around face, mouth, lips, which is commonly called cold sores or fever blisters. HSV-1 replicates in mucosal epithelia cells in primary infection and results in either symptomatic disease at the site of viral entry or asymptomatic infection. During infection, the virus becomes latent in their local sensory ganglion, trigeminal ganglia and reactivation of virus causes the lesions at or near site of viral entry into the body. HSV-2 usually associates with infection of genital areas and can be latent in lumbosacral ganglia and spread through sexual contract (Riley, 1998).

Periodically reactivating of latent virus causes symptomatic lesions or undergo asymptomatic. Viral shedding may be systemic symptoms. Frequency of HSV-2 recurrence is greater than HSV-1 when infection involves the genital area. HSV-1 can cause serious diseases such as cornea keratitis and encephalitis. Neuoinvasiveness of HSV infection is invading of the brain and causing neurotoxicity from cytoskeletal disruption, and senescence of cells. Therefore, HSV can cause lytic infection of most cells, persistent infection of lymphocytes and macrophage, and latent infection in neurons.

These pathogenesis and symptoms of HSV-1 and HSV-2 infection are similar but the differences between two types of viruses including growth characteristics, antigenicity and their diseases. Generally, HSV-1 is usually associated with infections above the waist such as lips, mouth and throat while HSV-2 occurs at genital, cervix,

vulva, vagina, penis and may occur on another area such as legs and buttocks. Both of oral and genital herpatic may be associated by red, itchy, painful, fever, headache and muscle ache.

First episode of disease tends to be more severe than recurrences. Initial lesion appears as the clear vesicle containing infectious virus that are formed on erythematous base and progresses to pustule lesions, ulcers, and crusted lesions. Therefore, reddened area is present and gives rise to a macula, which crusts to form a papula and fluid in the blister is fully of virus. In addition, the symptomatic oropharyngeal disease is characterized by lesions of the buccal and gingival mucosa, and fever.

Intraoral ulceration mostly occurs during primary infections whereas lesions on lip suggest recurrent infection. The orolabial lesions are preceded by pain, burning, tingling, or the vermillion border of the lip. Vesicles usually occur and persist for 48 hours then pustules or ulcers and crusts are formed within 72-96 hours. The lesions are completely healed after 8-10 days. Primary genital herpes also appears as macules and papules. The infection follows by vesicles, pustules, and ulcers. The symptoms of an infection include burning or tingling sensations, following by multiple painful vesicles at the site of infection (Whitley *et al*., 1998). The complications after HSV infection include paraesthesias and dysaesthesias of the legs and perineum. Dysuria, localized inguinal adenopathy, and malaise are also formed (Whitley and Roizman, 2001). Therefore, cytolysis occurs from induction of multinucleated giant cells with condensed chromatin in the nuclei of the cells. Degeneration of the cellular nuclei and macromolecule within the epithelium are followed. Vesicular fluid contains large amount of virus, cellular debris, inflammatory cells and multinucleated giant cells. The vesicular fluid becomes pustular with the recruitment of inflammatory cells and subsequent formation of scabs. Thus, vesicles are replaced by shallow ulcers. Scarring is uncommon when mucous membranes are involved. In addition, present of virus indicates by formation of syncytia or Cowdry type A inclusion bodies in nucleus. Cowdry type A acidophilic intranuclear inclusion bodies are produced after changes in the nuclear structure and followed by glomerate of chromatin. However, presence of clinical symptoms is variable since tissue damage and inflammatory response are caused by a combination of viral pathology and host immune response (Fatahzadeh and Schwartz, 2007).

Primary episode of HSV-2 expresses multiple painful vesicles in clusters on a surface and may be associated with puritis, dysuria, vaginal discharge, and tender regional adenopathy. Moreover, many women have experience on fever, malaise, and myalgia 1 to 2 days before the appearance of lesions and the lesions may last 4-5 days before crusting but skin may not re-epithelialize for almost 10 days. Whereas, non-genital lesions may arise on mouth, buttock, legs, fingers, and eyes. On the other hand, symptomatic recurrent of HSV episodes are characterized by a prodromal followed by painful, vesicular lesions and fever. Moreover, difference in recurrence rate depends on HSV types, which recurrence of HSV-1 is less frequent than HSV-2.

Cell characteristics of primary and recurrent infection of both types of HSV are associated with viral-mediated cell death and inflammatory response. The first line of cellular defense of HSV infection is innate immune system, which composes of main function to limit further infection and initiate cellular and humoral-mediated immune system (Chan *et al*., 2011). After infection, the reactivity of lymphocyte blastogenesis develops within 4-6 weeks and sometimes as early as 2 weeks. Host defenses against HSV infections involve multiple immune cells such as macrophages, natural killer cells (NK cell), different T-cell sub-populations, and antibodies that mediate neutralization and/or antibody-dependent cell mediated cytotoxicity and cytokine response (Marques and Straus, 2000). The humoral immune response is important to reduce the viral titers during primary infection but does not prevent either recurrences or exogenous reinfection. Cellular immune response is the main factor in determining both the severity and the rate of primary infection as well as recurrence of HSV. However, the severity of HSV infection is related to the level of cellular immune response of host cell (Marques and Straus, 2000; Whitley *et al*., 1998).

Cell mediated immunity is important to control HSV infection. During primary infection, interferon (IFN), cytotoxic T cells and macrophages are essential for controlling HSV infections. They are able to kill infected cells by IFN, which is important in limiting the initial infection. Moreover, NK cell are acted to limit and resolve the progression of the infection. Macrophages are activated by delayed-type hypersensitivity and cytotoxic killer T-cell responses. However, the immunopathology are caused by cell-medicated and inflammatory response of the

disease symptoms. If functional of cell mediated immunity inactive, HSV infection may spread to the necessary organs and the brain (Whitley and Roizman, 2001).

During primary infection, immunoglobulin M (IgM) antibodies appear transiently followed by IgG and IgA, which persist for long periods. The humoral immune responses limit viral spread by neutralization of surface glycoprotein of extracellular virus and direct coating itself with IgG via Fc receptors and complement receptors. However, virus can avoid neutralization of antibody by latent infection in neuron. Moreover, virion and virus infected cell may express antibody and complement receptor that can bind to antibody and complement and weaken these humoral defenses. Virus entry to axonal rapidly before NK cell or the IFN response occurs. Therefore, reactivation and reinfection may occur even in the presence of antibodies (Whitley and Roizman, 2001).

Programmed cell death is also the host defense to response viral protein or host immune system. HSV can block programmed cell death by its own or by host proteins. At least 3 proteins, U_S3, gD, and gJ can block programmed cell death by specific cell injury. HSV can make complementary RNA that can anneal and activate host protein kinase R (Whitley and Roizman, 2001).

Once virus enters trigeminal ganglion (TG), neurons are destroyed and are probably responsible for stimulation an inflammatory response. LAT expression is measured in neurons infected with latent virus. Therefore, prolonged inflammatory reaction in the TG is observed. This inflammation persists well beyond the time when viral replication or viral antigens are detectable and the prominent cell is the cluster of differentiation 8 (CD8)+ T lymphocyte. In addition, CD8+ T cell can block HSV-1 reactivation from latency in sensory neurons. Several cytokines and chemokine with IFN γ and TNF are produced from the inflammatory cells in the TG (Khanna *et al*., 2004). However, the virus can be reactivated despite the presence of antibody (Figure 2.14).

However, HSVs have ability to evade the host defense by interaction of viral protein with cellular proteins. The interactions of viral and cellular proteins are involved in blocking host cell response. HSV α protein (ICP47) can block the presentations of antigenic peptides by binding to the transporter protein to transporter associated with antigen processing 1 (TAP 1) or TAP2 and block transporting peptides

to the endoplasmic reticulum. Moreover, HSV has a system for blocking host responses against infection because the cells degrade some of the newly synthesized viral proteins (Whitley and Roizman, 2001).

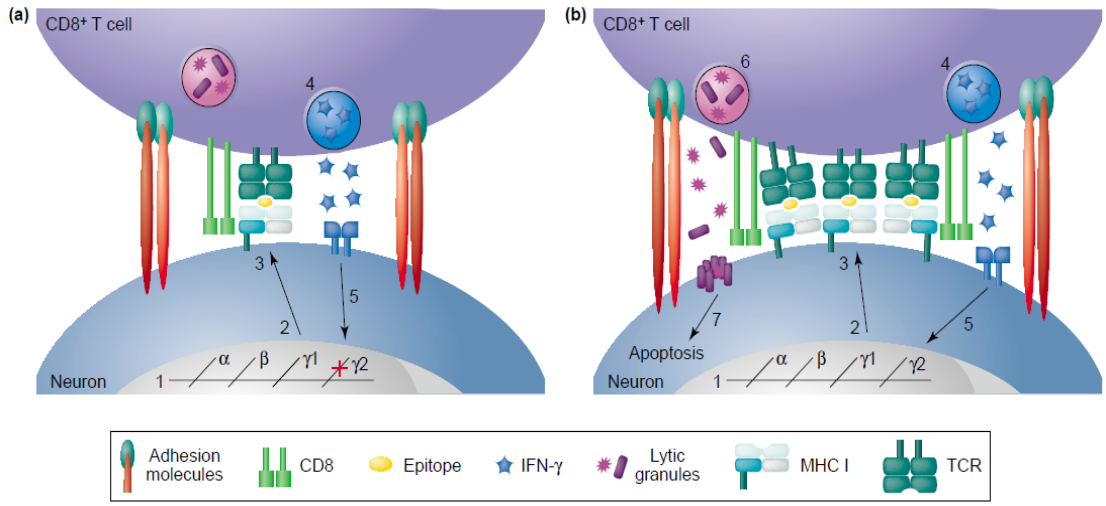


Figure 2.14 Immunology models of HSV-1 latency and reactivation (Khanna *et al*., 2004)

2.9 Transmission and disease of HSV

HSV infections are distributed worldwide and human is reservoir for transmission to susceptible individuals during close personal contact while animal vectors of human HSV infection have not been found. HSV-1 and HSV-2 have different routes and involve different areas of the body and the two types of virus overlap in sign, symptom, epidemiology and clinical manifestations (Spear, 2004; Whitley *et al*., 1998). HSV-1 is more likely to reactivate frequently from oral sites and HSV-2 is more likely to reactivate from genital sites (Spear, 2004). Therefore, recurrent infection is varies between men and women by 2.7 and 1.9 times per 100 days, respectively. In addition, women with initial genital herpes can shed the infection without symptoms which occur by 12% of primary HSV-1, 18% of primary HSV-2 and 23% of non-primary HSV-2 infection (Whitley and Roizman, 2001).

HSV-1 prevalence varies with age, race, geographic location, and socioeconomic status worldwide. The higher rate of seropositivity has been reported from less industrialized countries. Therefore, HSV-1 is the large proportion of population as a result of poor hygiene in underdeveloped countries that HSV-1

antibodies are found in more than 90% of children. The lowest rates of infection are found in children while the highest rates are in prostitutes and among them 80% are infected with HSV-2. Therefore, HSV-1 is primarily associated with oral, pharyngeal, facial, ocular, and central nervous system infections, which are transmitted by oral secretions and non-genital contract. Moreover, it can remain viable on the skin, clothing or plastic for short time and accommodates transmission through close nonsexual contact such as kissing on the cheeks or sharing common wares (Whitley and Roizman, 2001).

Genital herpes is very common and most affects adult people. It is important sexually transmitted disease (STD) caused by HSV-2 infection and the exception of a minority of cases caused by HSV-1 (Kriebs, 2008). Therefore, older age, female gender, race, poor socioeconomic status, low level of education, sexually transmitted disease, early age at first intercourse and a higher number of lifetime sexual partners lead to the risk of genital herpes infection in human (Fatahzadeh and Schwartz, 2007). Furthermore, HSV-2 is seroepidemiologically associated with human cervical cancer, as a cofactor with human papillomavirus (Brugha *et al*., 1997; Gupta *et al*., 2007).

Vertical transmission of neonate from an infected mother to her baby usually occurs during vaginal delivery pregnancy. Maternal genital infection during the time of delivery, the quantity and quality of maternal antibodies, duration of ruptured membranes in the presence of active infection and the use of fetal scalp monitor during delivery may affect neonate infection (Anzivino *et al*., 2009; Brugha *et al*., 1997; Piret and Boivin, 2011; Sacks *et al*., 2004).

Oral herpes

Oral herpes is the most common manifestation, which is caused by HSV-1 infection. The lesions are generally occurred near oral mucosa on lips and facial area and it spreads to all parts of the mouth.

The symptom of oral herpes infection appears as a small group of clear vesicles, which is commonly called fever blisters or cold sores. Itching, pain, enlarged submandibular lymph node, sore throat, malaise and rapidly ulcerative lesions distribute

throughout the mouth involving the hard palate, gums, chest, pharynx, gingivae, buccal mucosa, lip and the top of tongue. In addition, it is also possible that HSV can be reactivated from the trigeminal ganglia and results in the recurrent HSV infected lesions at the same location inside the mouth. However, the symptoms of recurrent episode are less severe and more localized than primary episode (Lachmann, 2003).

Herpetic gingivostomatitis

Gingivostomatitis is common symptom of primary HSV-1 and HSV-2 infection in children. Therefore, children are almost infected by HSV-1, whereas young adults may be infected with HSV-1 or HSV-2. The symptoms of herpetic gingivostomatitis include painful vesicular lesions, fever and submandibular lymphadenopathy. The ulcerative lesions affect skin and mucous membranes. However, majority of infections are subclinical (Marques and Straus, 2000).

Herpes pharyngitis

Clinical symptoms of herpes pharyngitis are ranged from mild to severe pharyngitis. Herpes pharyngitis occurs with sore throats. The patients with HSV pharyngitis exhibit ulcerative lesions, vesicles at posterior pharynx with exudative lesions following the illness with the fever, malaise, and myalgia (Marques and Straus, 2000).

Herpes stomatitis

Herpes stomatitis is infection of oral cavity that commonly causes blisters, fever, itching, trauma, ulcerative lesions and inflammation. Severe HSV stomatitis is similar to a primary gingivostomatis symptom and may occur in immunosuppressed patients (Pithon and Andrade, 2010).

Herpetic keratitis and HSV keratoconjunctivitis

Herpetic keratitis caused by HSV-1 infection occurs in cornea of eye and this infection almost limits to one eye. The symptoms are an acute onset of pain, watery discharge, itching, blurred vision, lid swelling, and conjunctiva of eye (Spear, 2004).

Thus, primary infection causes an acute corneal damage, and recurrence or chronic epithelial keratitis leads to involvement of deeper layer of cornea, which causes severe dendritic ulcers, permanent corneal scarring, corneal damage and major cause of corneal blindness (Lachmann, 2003).

HSV keratoconjunctivitis is ocular infection with HSV-1 infection of eye and associated with unilateral or bilateral conjunctivitis. For primary infection the bilateral keratoconjunctivitis manifests with a pain, watery discharge, itching, blurred vision, lid swelling, photophobia, tearing, and eyelid edema. While, recurrent disease is caused by viral shedding from reactivation of virus in trigeminal ganglia, this is unilateral and causes dendritic ulcers leading to corneal scarring. Therefore, the superficial ocular infections involve the eyelids, conjunctiva or corneal surface and lead to serious disease causing vision loss. In addition, retinal necrosis is also associated with HSV-1 infection (Marques and Straus, 2000; Whitley *et al*., 1998).

Herpetic whitlow

Herpetic whitlow disease is infection of finger by contact with HSV from body secretions. The virus enters the body and establishes infection through abrasions or through small wounds on the hands or wrists skin. HSV-2 whitlow is caused by contact of HSV-2 from genitals to hands. Lesions are present at fingertip and can be pustule and very painful. In addition, fever and local lymphadenopathy are commonly seen (Marques and Straus, 2000; Spear, 2004).

Eczema herpeticum

Eczema herpeticum or Kaposi varicelliform shows clinical skin symptom that is present as active eczema, preexisting atopic dermatitis and small blisters. Infection can be spreaded along skin at the site of eczema lesions. It also has potential to affect adrenal glands, liver, adrenals and other organs. This infection is rarely found but it is severe disease (Gupta *et al*., 2002).

Genital herpes

Genital herpes is life-long and commonly results from HSV-2 infection but about 10% of cases are from HSV-1 infection. This disease is public health concern due to its serious psychosocial morbidity and frequency recurrent painful genital lesions are occurred several times in a year (Marques and Straus, 2000). Genital herpes are transmitted by direct contact of recipient's mucous membranes or skin infected with sexual partner (Brugha *et al*., 1997; Spear, 2004).

The clinical apparence of genital herpes infection may be symptomatic or asymptomatic depending on multiple factors such as the site of infection and immune statue of host. Therefore, the diseases are often occurred more than asymptomatic infection and the diseases show as macules, papules and pustules with ulcers. In men, lesions have been found on glans or shaft of penis and sometimes in urethra whereas lesion may be seen on vulva, vagina, cervix, perianal region, or inner thigh in female patients. These painful lesions may be synchronous with a variety of systemic signs and symptoms including fever, malaise, headache, myalgia, inguinal adenitis, dysuria, itching, vaginal and urethral discharge, tender inguinal lymphadenopathy and glandular inflammation of groin area. Secondary episodes of genital herpes result from viral reactivation from sacral ganglion. The infections are frequently less severe than first episode (Anzivino *et al*., 2009; Brugha *et al*., 1997; Marques and Straus, 2000).

Moreover, HSV proctitis can be found. It is inflammation lesion in lower rectum and anus area with pus, blood discharge, painful and irritation around infection area. Primary episode of this infection is longer in duration and more severe than recurrent genital HSV infection. Moreover, recurrent infection may be asymptomatic but the virus can be spreaded to other people (Whitley *et al*., 1998).

HSV meningitis and encephalitis

Neurovirulence of HSV infection is neuroinvasiveness of virus from peripheral sites and replication of virus in neuronal cells. Therefore, the virus can invade and replication in central nervous system (CNS) and also can be established latent infection (Whitley *et al*., 1998).

HSV meningitis is often a complication of primary genital HSV-2 infection. The patients with HSV meningitis have headache, fever, stiff neck and mild photophobia (Marques and Straus, 2000).

HSV encephalitis is usually associated with HSV-1 infection in brain. The disease generally shows acute onset of focal neurologic symptoms and fever (Lachmann, 2003; Marques and Straus, 2000). Pathology of this infection causes damage to temporal lobes of the brain and focal necrosis. The infection also gives rise to erythrocytes in the cerebrospinal fluid, seizures, focal neurological abnormalities, and other characteristics of viral encephalitis. However, the lesion is limited in one of temporal lobes (Sauerbrei *et al*., 2000). The most severe sporadic encephalitis causes high morbidity and mortality from CNS damage (Spear and Roizman, 1972). Herpes encephalitis can be demonstrated from the primary infection or secondary infection (Lachmann, 2003).

HSV infection in the neonate and newborn

HSV infection of neonate has a very high mortality, which results from infection with either HSV-1 or HSV 2. The infection is frequency occurred during passage of infant through genital birth canal of mother. The direct inoculation with infectious genital secretions is found in mother with genital ulcers (Lachmann, 2003).

Neonatal herpes may occur in the absence of skin lesions. The infection of HSV in neonates during and after birth is localized around skin, eye and mouth. However, neonate has an underdeveloped immune system and virus can spread rapidly into many peripheral organs (Kimberlin, 2004).

2.10 Laboratory diagnosis

Clinical laboratory diagnoses of viral infections are detected and confirmed by several methods. Therefore, specific and sensitivities of the test depend upon the type of test and quality of specimen obtained. However, detection of diseases in asymptomatic individuals is still limited. Diagnosis procedures include viral antigen, viral DNA, infectious virus and viral antibodies detection.

Clinical specimens are taken from lesions by collecting the lesion fluid or cotton swab to the skin vesicles, eyes, mouth, cerebrospinal fluid (CSF), rectum, blood, stool, urine, throat, nasopharynx, and corneal lesion. All of clinical specimens are transferred into media and sent to virology laboratory (Whitley *et al*., 1998). Diagnosis HSV infection is also performed from biopsy, viral culture, antigen detection by enzyme immunoassay, immunofluorescence, detection of nucleic acid with PCR, microarray technology, and loop mediated isothermal amplication (LAMP) (Navaneethan *et al.,* 2010).

2.10.1 Cytology technique

This methodology is standard method for diagnosis HSV infectious specimen, which shows positive result in cell culture within 2-3 days after inoculation. HSV determination in cultured cells is obtained from scraping of skin vesicles called Tzank smear, which approximately 60% sensitive. Papanicolaou (PaP) smear or biopsy specimen can also be used to demonstrate cytologic changes of tissue culture cells.

Detection of HSV infection in cell culture was observed by characteristic of cytopathological effects (CPEs), which are present of enlarged foci, syncytia "ballooning" cytoplasm and multinucleated giant cells formation. Moreover, Cowdry type A intranuclear inclusion bodies are also shown. However, cytology technique is less sensitive than detection by immunological methods (Brugha *et al*., 1997).

2.10.2 Serological assays

Detection of HSV-1 and 2 infections by serological assays are widely used for diagnostic a primary HSV infection and epidemiological studies. However, serological diagnostic of HSV infection can only determine the past exposure because the rise of antibody titers is not found in recurrent disease (Marques and Straus, 2000; Riley, 1998).

Most laboratories analyze subtype of viral isolates using highly sensitive and type-specific serological assays. Monoclonal anti-HSV antibodies are used in

immunodot enzyme assays (IEA), fluorescent antibody assay, immunoperoxidase method, enzyme-linked immunoabsorbant assay (ELISA) and western immunoblot analysis (WBA) assays (Brugha *et al*., 1997; Riley, 1998; Wald *et al*., 2001).

2.10.3 Molecular biology method

Determination of HSV type is detected by molecular method using restriction endonuclease cleavage of HSV DNA and DNA hybridization using HSV type-specific DNA probe to detect different types of HSV and PCR technique. HSV DNA is amplified by using a common appropriate type-specific forward primer and reverse primers for HSV-1 and HSV-2 (Clark *et al*., 2011). This technique shows specific, sensitive biological technique and rapid detection more than viral culture. Therefore, PCR is recognized as the reference standard method to detect herpes simplex encephalitis caused by HSV infection (Madhavan *et al*., 1999; Riley, 1998; Whitley *et al*., 1998).

2.11 Prevention and treatment HSV infection

Direct contact with herpes blister on mucocutaneous lesion from patients should be avoided to reduce the risk of infection because HSV can spread by close contact and during sexual intercourse. However, symptoms of some HSV infected patient are unapparent so virus can transmit without prevention. Additionally, people who work in hospital must be careful when handling potentially infected tissue or fluids and should wear the gloves to prevent infection of the fingers and clean by washing soap (Riley, 1998).

HSV is an enveloped virus so it is easily inactivated by dryness and detergents (Brugha *et al*., 1997; Danaher *et al*., 2011). Nowadays, HSV infections are generally managed by potential antiviral drugs as specific inhibitors to virus at various stages in HSV replication cycle by inhibition of virus nucleic acid or protein synthesis. Furthermore, new anti-herpes drugs, which have alternative pathways and different inhibitors on HSV activity, are also searched for potentially valuable target against HSV infections disease (Greco *et al*., 2007). Thus, the effective antiviral drug therapy for treatment HSV infection must selectively target to specific virus particle or viral

replication cycle inside host cells without affecting on host cells metabolism. These effective drugs will reduce health care costs, shorten herpes disease episodes, shorten time to heal of HSV disease, and reduce recurrences of herpes and transmission. Additionally, antiviral agents that used in primary infection should be able to reduce probability of patient suffering recurrent episodes (Lachmann, 2003; Jassim and Naji, 2003).

There are three different classes of anti-HSV drugs have been used. First, nucleoside analogs that acts as inhibitor of thymidine kinase (TK) activity in infected cell. Second, DNA polymerase inhibitors that acts as inhibitor of DNA polymerase activity of viral DNA polymerase. Third, helicase-primese inhibitors those are able to inhibit helicase and primase activity and also viral DNA synthesis. Although, several effective anti-HSV drugs are available for treatment HSV infection but the disease is not completely cured because latency establishment from virus since viral genomes in ganglia are not affected by drugs and most patients have recurrent HSV infection (Dobson *et al*., 1998; Lachmann, 2003; Whitley and Roizman, 2001). However, anti-HSV agents have been used to reduce clinical and subclinical rate of infection and long-term therapy with these agents may result in emergence of drug resistant virus (Lückemeyer *et al*., 2012).

Anti-herpes simplex virus agents that have been approved for treatment of HSV infection are as follows

2.11.1 Acyclovir

Acyclovir (ACV) (9-[2-hydroxyethoxymethyl] guanine), is synthetic acyclic purine-nucleoside analogue. It has been known as effective and safety antiviral drug, and used for long-term prophylaxis with low side effects. It reduces clinical establishment of acute or latent infection severity in variety of clinical situations by reduction of the duration of pain, complications and viral reactivation (Dobson *et al*., 1998; Marques and Straus, 2000).

Therefore, ACV is prototype of antiviral agent that can be converted to its active form after phosphorylation by a viral encoded TK enzyme, which has 100-fold selective affinity greater than cellular polymerase. Then, ACV-monophosphate is subsequently phosphorylated by host cell enzymes to ACV-triphosphate. Thus, ACV-triphosphate becomes active only in infected cells. These incorporated ACV-triphosphate prevents chain elongation and terminates viral DNA synthesis lead to inhibit viral DNA polymerase and viral replication because of lacking a hydroxyl group in the 3′ position (Figure 2.15) (Lachmann; 2003; Marques and Straus, 2000; Piret and Boivin, 2011).

However, resistance strains may be occurred in those people that had long-term ACV prophylaxis. Predominantly of ACV resistance strains (95 %) result from generation at least one of base mutation. Thus, defective HSV mutants or deficient in TK are unable to phosphorylate acyclovir (Lachmann, 2003; Marques and Straus, 2000).

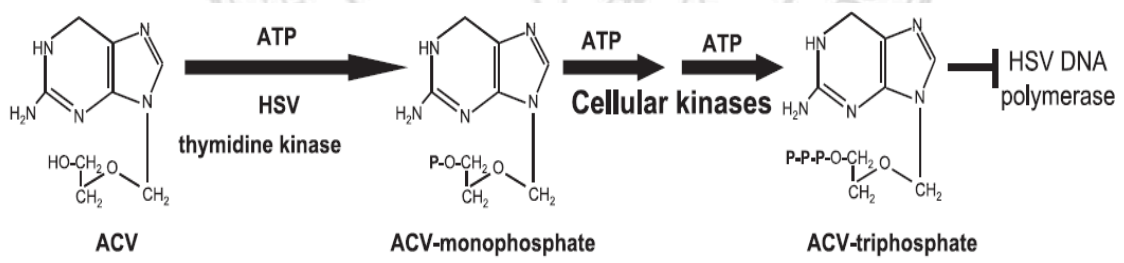


Figure 2.15 Mechanism of ACV inhibitor in HSV treatment (Griffiths, 2011)

2.11.2 Famciclovir

Famciclovir is a diacetyl ester prodrug analog compound. It is well absorbed in oral cavity. It is another guanosine analogue and able to convert rapidly by phosphorylation to its active antiviral metabolite, penciclovir triphosphate (Faro, 1998; Saltzman *et al*., 1994).

2.11.3 Valacyclovir

Valacyclovir is the L-valyl ester oral prodrug of ACV that shows similar viral inhibitory activity as ACV. The drug is completely absorbed in oral cavity, rapidly hydrolyzed and converted to acyclovir in intestinal wall and liver. Valacyclovir has effective affinity to viral TK enzyme. It can reduce subclinical and HSV shedding (Marques and Straus, 2000; Riley, 1998; Whitley *et al*., 1998).

2.11.4 Penciclovir

Penciclovir (9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine) is an acyclic guanine nucleoside analogue. A spectrum of activity and mode of action mechanism is similar to ACV. Penciclovir is phosphorylated and converted into penciclovir triphosphate. Viral DNA synthesis and replication are inhibited and chain elongation is limited (Piret and Boivin, 2011).

2.11.5 Cidofovir

Cidofovir (1 - [(S) - 3 - hydroxy – 2 - (phosphonomethoxy) propyl] cytosine dehydrate) is acyclic nucleotide phosphonate analog with broad-spectrum against HSV infection activity. Cidofovir is phosphorylated by the cellular kinases to cidofovirtriphosphate to obtain active molecule and acts as competitive inhibitor of viral DNA polymerase (Lachmann, 2003; Marques and Straus, 2000).

2.11.6 Foscarnet

Foscarnet (trisodium phosphonoformate) is an organic analogue, which acts as viral DNA synthesis inhibitor. ACV-resistant infections strain can be treated by forcarnet, which is prevent the cleavage of pyrophosphate from deoxynucleotide triphosphates and the action is not requite phosphorylation. However, foscarnet is more toxic than ACV (Marques and Straus, 2000). Mechanism and pathways of several antiherpes drugs are summarized in Figure 2.16.

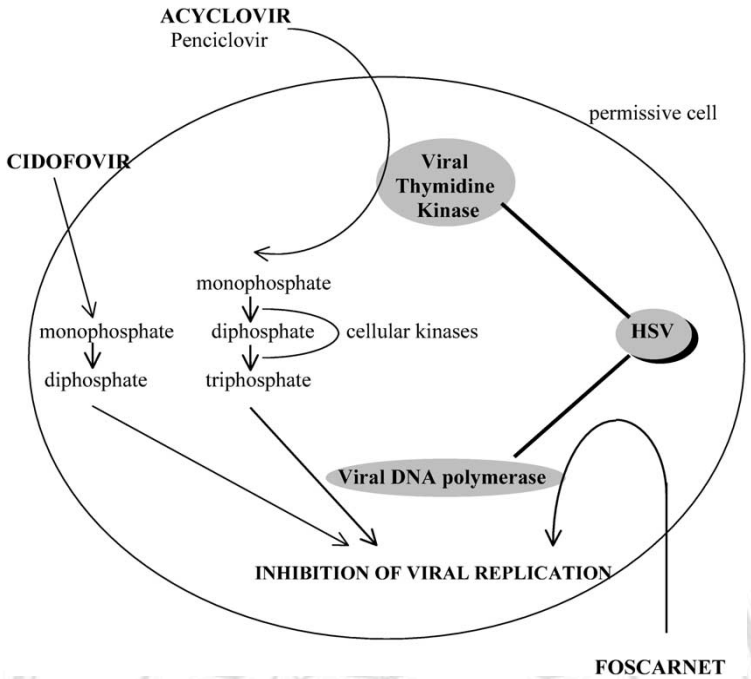


Figure 2.16 Mechanism of antiviral against herpes simplex virus infection (Morfin and Thouvenot, 2003)

2.12 Algae

Algae are the organisms that are commonly like plants which ranging in size from microscopic single cells to gigantic seaweed. They are in aquatic ecosystems and provide an important function, and essential for human existence as the main producer of oxygen in the aquatic ecosystem. Moreover, the algae played role to produce the organic material from sun light, carbon dioxide, and water (John *et al*., 2002).

In addition, algae have been used in animal and human diets since the ancient time. Generally, algae are mostly found living in fresh water, marine, brackish, rivers, lakes or ponds. However, they can also be found in almost every other environment such as growing in the snow that living in lichen associations, in desert soils and hot springs (Jones *et al*., 2014). They can be segregated using living habitats into 2 groups; floating algae or phytoplankton, and benthic algae, which are attached to substrates (John *et al*., 2002). Algae are usually found in most bodies of water. There are two different types of algae, which called “Macroalgae” and “Microalgae”.

The macroscopic algae or macroalgae can be found by the naked eye in the field. Macroalgae are commonly known as “seaweed” and considered as ‘macrophytes’ since they often form floating masses that can be easily harvested (Wellman *et al*., 2003). They can grow as a slime colony, in mats and sheets, or filaments. However, the morphology of some macroalgae was also developed as a higher plant (Sheath and Cole, 1992). Most freshwater macroalgae live in running water. There are usually found the abundance of macroalgae that followed by their attachment behavior to stream and rivers (Entwisle, 1989).

On the other hand, the microscopic algae or microalgae were well-known to develop in many commercial applications. Algal products can be used to enhance the nutritional value of food and animal feed owing to their chemical composition and they play a crucial role in aquaculture (Hoek *et al.*, 2010).

In Thailand, there are few studies of many freshwater macroalgae which are available for recovery many advantages under various conditions (Powthongsook, 2000). Moreover, in the northern and north-eastern areas, the freshwater macroalgae were the most important natural resources for consumption by local people. Hence, the numerous freshwater macroalgae could grow fully covering the bottom of the pond. (Peerapornpisal *et al.*, 2006). Especially, four freshwater macroalgae, which common names are “Hed Lab”, “Lon”, “Tao” and “Kai”, are the Thai famous edible macroalgae (Peerapornpisal *et al.*, 2005; Thiamdao *et al.*, 2011).

2.12.1 *Spirogyra*

Spirogyra is a member of the Algae. The algae are simple plants ranging from single-celled organisms (*Chlamydomonas, Euglena*) to complex seaweeds. They contain chlorophyll and make their food by photosynthesis. *Spirogyra* is a filamentous algae. Its cells form long, thin strands that, in vast numbers, contribute to the familiar green, slimy ‘blanket weed’ in ponds. Seen under the microscope, each filament consists of an extensive chain of identical cells (Lee, 2008). Since the genus *Spirogyra* was established by Link (1820), more than 400 species have been identified and described worldwide (Usha and Panikkar, 1994). This genus is one of the largest genera among Zygnemataceae. In addition, the thalli are unbranched uniseriate

filaments. Cells morphology are cylindrical, 10 to >200 μm in diameter, most are 20 to 60 μm, and up to several times as long; cell walls are two-layered with inner cellulose, and an outer mucilage layer that makes filaments very slimy to the touch. The end walls are plane. The basal cells are infrequently identified with rhizoidal holdfasts. 75% of species were found the flat or plane end walls, but 25% of species were reported the cup-like form. Cells contain uninucleates, chloroplasts from 1-15 per cell, the plastids are ribbon-like and pressed against the cell-wall within the parietal layer of the cytoplasm (Figure 2.17). The edges of chloroplasts are ruffled. In the cells, many disc-shaped pyrenoids are laid along ribbon-like chloroplasts. The cytoplasmic strands support the centrally located nucleus within the central vacuole (Kudlubowska, 1994; Hoshaw and McCourt, 1988; Usha and Panikkar, 1994).

Spirogyra is at large genus which found in fresh water such as pools, ponds, lakes, ditches. In addition, they are also in slow running water of rivers and streams. In general, they occur free floating bright green masses in the water, but only few species such as *spirogyra dubia, spirogyra affinis, spirogyra rhijoides* develop specialized structure 'hapteron' with the help of which they can get attached with substratum area. In particular, the strands of *Spirogyra* and other *Zygnematales* filaments are slippery in nature which a fine mucilage layer coats the filament, commonly feel "soapy" to the touch, known as "Pond Silk" or "Water silk".

The Systematic positions of *Spirogyra* are as follows: (Lee, 2008)

Kingdom: Plantae

Phylum : Chlorophyta

Class : *Zygnematophyceae*

Order : *Zygnematales*

Family : *Zygnemataceae*

Genus : *Spirogyra*

The class *Zygnematophyceae* was recognized the motile cells of advanced members of the class are similar to the flagellated male gametes of the bryophytes. This motile cells are asymmetrical and have two laterally or subapically inserted flagella (Belcher and Swale, 1978; Lee, 2008). The microtubular root system contains a multilayered structure that is associated with a broad microtubular root and a second,

smaller, microtubular root. The rhizoplasts and eyespots are not present. The mitotic spindle is persistent during cytokinesis, and cell division occurs. In particular, sexual reproduction results in the formation of a dormant zygote. Then, meiosis occurs when the zygote germinates The algae in the class are predominantly freshwater algae (Belcher and Swale, 1978; Hoek *et al*., 2010; Lee, 2008).

The order *Zygnematales* are a closely related group of freshwater algae that are unique among the Chlorophyta in having sexual reproduction by isogamous conjugation (McCourt *et al*., 2000). The gametes of this order are non-flagellated (Gontcharov *et al*., 2002). The union of the two gametes can be through a conjugation tube formed by the parent cells, or the gametes can move from their parent cells into the medium and fuse.

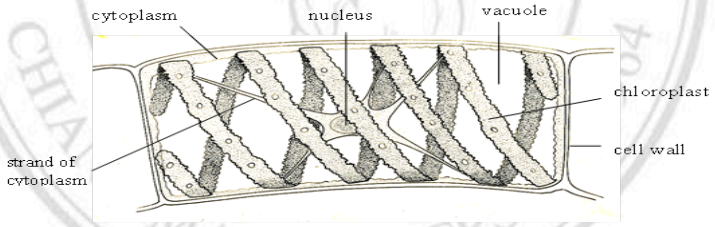
Within the order *Zygnematales*, *Spirogyra* is within family *Zygnemataceae.* The Family *Zygnemataceae* is cylindrical cell which are permanently unbranched filaments, and the cell walls lack pores. Union of the two aplanogametes is usually by the establishment of a conjugation tube between two cells. Members of the *Zygnemataceae* are among the most common filamentous freshwater algae, favoring small stagnant bodies of water and usually flowing water (Berry and Lembi, 2000).

Zygnemataceae are especially abundant in the spring months, generally occurring as bright green free-floating masses. Planktonic species of *Spirogyra* or *Mougeotia* often have twisted or spirally coiled threads (Lee, R.E. 2008; Lynn and Brock, 1969). *Spirogyra* occurs primarily in the spring time because it tolerates high light intensities in cool water (Graham *et al.*, 1995). *Spirogyra* has ribbon shaped chloroplasts with a number of pyrenoids along the length of the chloroplast (Figure 2.17). In those cells, actin microfilaments attached to the chloroplasts are directly responsible for movement of the chloroplast with a phytochrome system directing actin functioning (Mineyuki *et al*., 1995).

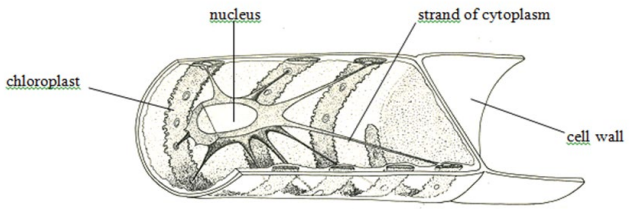
The body of *spirogyra* is a gametophytic thallus which is elongated cylindrical, unbranced silky thread like structure known as filament. The cells are separated by a partition wall known as Septum. Cell wall is the outermost protective double layer structure. The inner layer of which is made of cellulose and the outer layer contains pectose. The outer most lining of pectose turns into Pectin and get dissolved in water to

form Mucilage which surrounds the filament and forms mucilagenous sheath. The protoplast contains plasma membrane, cytoplasm, single large centrally located vacuole and chloroplast containing pyrenoids (Mineyuki *et al*., 1995). Cell sap occupies the central part of the cell. Spirally coiled ribbon shaped chloroplast containing pyrenoids are present in position (Figure 2.17). The rounded proteineous structures known as pyrenoids lie equidistant from each other inside the chloroplast. They contain central core of starch surrounded by protein plates and help in formation and storage of starch (Lee, R.E. 2008; Mineyuki *et al.*, 1995).

A nucleus of *Spirogyra* is suspended in the center of the cell. Every cell in the filament except the basal one is capable of cell division. Moreover, the reproduction of *Spirogyra* is asexual reproduction that occurs by fragmentation of the filaments, whereas sexual reproduction occurs by conjugation. Particularly, the conjugation is initiated by two filaments coming to lie next to one another, and being bound together in a layer of mucilage (Figure 2.18).



(A)



(B)

Figure 2.17 The single cell of *Spirogyra*; cell contains a helical chloroplast, a nucleus, cytoplasm and a vacuole enclosed in a cellulose cell wall (A), The 3D presentation of cell structure. (B). (Mackean, D.G.; http://www.biology-resources.com/amoeba.html)

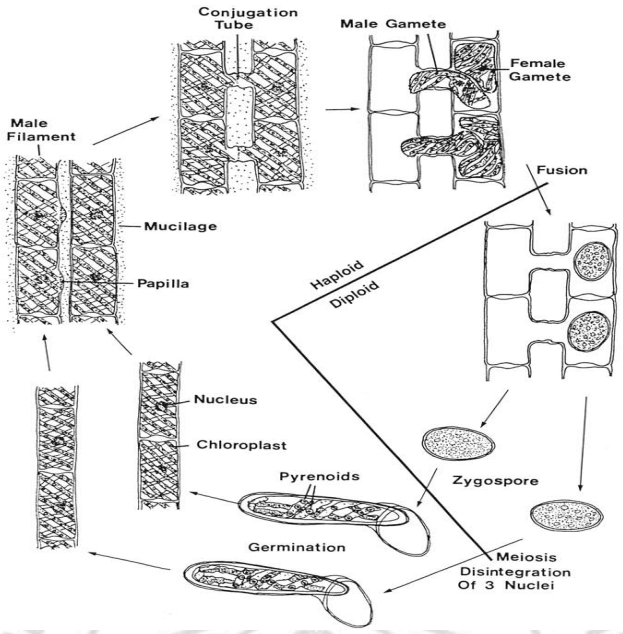


Figure 2.18 The life cycle and conjugation of *Spirogyra* (Lee, 2008)

The two types of conjugation of *Spirogyra*, which is called scalariform conjugation, occurs between two separate filaments. Another type of conjugation is lateral conjugation, which occurs between cells of the same filament. The conjugation tube is formed between adjacent cells (Lee, R.E. 2008; Peerapornpisal *et al.*, 2006). Therefore after the completion of scalariform conjugation the cells of one filament appear empty and those of the another filament contain zygospores, after completion of lateral conjugation empty and zygospore containing cells alternate in the filament (Lee, R.E. 2008). Zygospore represents the only diploid stage in the lifecycle of *spirogyra* and it is yellowish brown ellipsoidal structure having diploid nucleus and covered by three layered wall. Before germination, the diploid nucleus divides meiotically and form four haploid nuclei. Out of which three degenerate and one remains at functional nucleus.

From the investigation in Thailand, Tiamdao (2011) reported the new records of the species of *Spirogyra*, such as *Spirogyra inflata* (Vaucher) Dumortier, *Spirogyra chunkingensis* Jao, *Spirogyra grossii* Schmidle, *Spirogyra submaxima* Transeau, *Spirogyra Africana* (Fritsch) Czurda, *Spirogyra crassoidea* Transeau and *Spirogyra dictyospora* Jao. The characters of Tao or *Spirogyra* were rather solid and large size of cell dimension was seen (Berry and Lembi, 2000, Kim *et al*., 2004; Hainza *et al*., 2009).

Additionally, diversity of *Spirogyra* including *S. grossii, S. chungkingensis, S. gracilis* and *S. submaxima* were observed at Na Ku Ha Village, Phrae province. At this village, Tao or *Spirogyra* can be cultivated all year. Especially, *S. ellipsospora* was reported up to 100% at Na Ku Ha Village (Tiamdao *et al.,* 2011).

"Tao or Tao Num" was assigned to generic name *Spirogyra*. Tao has been widely found in the ponds, streams and rivers of north and northeast of Thailand. Tao has nutritional value, which is similar to vegetable. "Yum Tao" is the most famous local dishes of the North and Northeast of Thailand. Especially in Na Ku Ha Village, Tambon Suan Khuan, Muang District, Phrae Province, where the local people can cultivate Tao or *Spirogyra* in the small ponds within the project of One Tambon One Product (OTOP) (Tiamdao *et al.,* 2011). Nowadays, Tao is used as an ingredient in cosmetic, food supplement and pharmaceutical products due to its high antioxidant activities (Panyoyai, 2008; Malaiwan, 2005). The study of the nutritional values revealed high contents of protein (18.6 g/100g DW), magnesium (241.1 g/100g DW) and manganese (35.80 g/100g DW) (Peerapornpisal *et al*., 2005).

2.12.2 Usefulness of algal studies

Currently, evidence of phycochemical and pharmacological studies on algae is available. Several studies have been carried out over the past years with the aim to discover new antibiotic or cytotoxic metabolites of algae **(**Piccordi *et al.,* 2000; Ördög *et al.,* 2004). Interestingly, blue-green algae improves metabolism by lowering cholesterol in animals and humans. The level of the total cholesterol in rat serum was reduced when a high cholesterol diet was supplemented with blue-green algae (Iwata *et al.,* 1990). Cyanobacteria have unique food storage compounds, myxophycean starch and cyanophycin. In addition, the diglycosyl diacylglycerols substances showed antitumor activity (Tokuda *et al.,* 1996).

The evaluation of the benefit of algal extracts of fresh water and marine algae were interested in cytotoxic and antiviral activities. The methanolic extract of *Spirulina* exerts antiviral effect on HSV-1 with IC_{50} value of 25.1 μg/ml (Chirasuwan *et al*., 2009). They indicated that the compound called sulphoquinovosyl diacylglycerol (SQDG**)**, contained palmitic acid and linoleic acid groups that showed antiviral

activities. It was reported that SQDG of some cyanobacteria (*Phormidium* and *Lyngbya*) possessed both anti-human immunodeficiency virus type 1 (HIV-1) and antitumor activity (Loya *et al.,*1998). In addition, the report of antiviral activity against HSV-2 was exhibited (Corona *et al.,* 2002). It was found that the methanolic extract of *Spirulina maxima* had anti-HSV-2 activity with EC_{50} 6.9 mg/ml, and IC_{50} 0.13 mg/ml. They suggested that the antiviral activity could be due to highly polar compounds present in methanol extract. On the other hand, hot water extract of *Spirulina maxima* inhibited the infection for adenovirus type 3 with a percentage less than 20%, with an IC_{50} 5.2 mg/ml. They also suggested that the antiviral activity of algal water extract might be attributed to the presence of sulfated polysaccharide **(**Singh *et al.,* 2011**;** Sayda *et al.,* 2010). It was reported that Spirulan (Sulphated polysaccharide composed of O-rhamnosyl-acofriose and O-hexuronosylrhamnose) revealed the activity by inhibition of HIV-1 and HIV-2 reverse transcriptase, HSV and influenza virus (Singh *et al.,* 2011). Scytovirin was firstly isolated from the aqueous extract of *Scytonema varium* could bind to the envelope glycoprotein of HIV and inactivate the viral particles (Bokesch *et al.,* 2003). Furthermore, the studies of ethanolic extract of *Spirogyra gratiana* indicated this fresh-water algae extract demonstrated the highest antioxidant activity by DPPH radical scavenging activity test (Kartal *et al.,* 2009).

The worldwide researches of marine algal extracts were shown. Polysaccharides were extracted from red seaweed; *Gracilaria corticata* that revealed high molecular weight of galactan sulfate. It could exhibit antiviral activity against HSV-1 and HSV-2 by inhibition of viral attachment to the host cell (Mazumder *et al.,* 2002). The studies from sulfated polysaccharide from hot water extraction of brown seaweed, *Sargassum patens* showed antiviral activity against HSV-1, HSV-2 and HSV-1 acyclovir resistant strains (Zhu *et al*., 2003). Additionally, sulfated polysaccharide fraction isolated from the hot water extract of the green seaweed, *Caulerpa racemosa* showed the high inhibition of the reference strains and thymidine kinase resistant strains of HSV-1, acyclovir-resistant strains of HSV-1 and HSV-2 (Ghosh *et al*., 2004). The sulfated fucoidan from brown seaweed, *Stoechospermum marginatum* extract illustrated no cytotoxicity on Vero cell and inhibited virus adsorption (Adhikari *et al.,* 2006).

2.13 Phytochemicals

Phytochemicals are naturally large group of plant derivative compounds. There are a variety of phytochemicals that have been found in algae. Phytochemicals have different biological activity properties (Allahverdiyev *et al*., 2004; Kumar *et al*., 2009; Raja and Sama, 2012). The phytochemicals found in algae have complementary, overlapping mechanisms of action against variety of disease (Jassim and Naji, 2003). Phytochemicals can be grouped as follows.

2.13.1 Flavonoids

Flavonoid is the large group of phytochemicals, which are polyphenolic compounds. It have been recognized that phytochemicals have variety potential biological functions and can inhibit human disease with anti-bacteria, anti-virus, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and anti-oxidant activities (James *et al*., 2009; Rispail *et al.,* 2005; Xu *et al*., 2007).

2.13.2 Alkaloids

Alkaloids generally contain nitrogen-bearing molecule as pharmacologically active compounds. There are different types of alkaloids such as tropane alkaloids, sanguinarine, berberine and reserpine. Moreover, pharmaceutical of alkaloids have been reported such as anti-spasmodic and anti-bacterial activities (Yadav and Agarwala, 2011).

2.13.3 Tannin

Tannins are water-soluble phenolic compounds produced by all plants. It is a health-promoting compound and uses as anti-carcinogenic, anti-mutagenic, anti-microbial, anti-oxidant and anti-radiation activities (Karamać *et al*., 2007).

2.13.4 Anthraquinones

Anthraquinones are class of natural compounds found in many plant families, which are composed of various chemical compounds. These compounds show

pharmaceutical bioactivity properties such as anti-microbial, anti-cancer and anti-oxidant activities (Dave and Ledwani, 2012; Locatelli *et al*., 2009; Sakulpanich and Gritsanapan, 2009).

2.13.5 Cardiac glycosides

Cardiac glycosides contain digitoxin, digoxin and ditoxin. It has been recognized for treatment cardiac, congestive heart failure by helping to support its strength and rate of heart contraction.

2.13.6 Phenolic compounds

Phenolic compounds are one of the large and diverse groups of molecules that found in many different families of plant. It can be classified into non-soluble compounds such as condensed tannins, ligans and cell-wall bound hydrocinnamic acids. Soluble compounds are phenolic acids, phenylpropanoids, flavonoids and quinoes (Rispail *et al*., 2005). These compounds are impact on human health, which show pharmaceutical properties such as anti-apoptosis, anti-aging, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activity (Yadav and Agarwala, 2011).

2.13.7 Coumarins

Coumarins are classified into benzopyrone family. It is a large class of compounds in plant. Various bioactive activities are shown on anti-microbial, anti-tumor, anti-malarial, anti-cancer, anti-inflammatory, anti-coagulant, anti-oxidant, anti-coagulant and anti-proliferative activities (Al-Haiza *et al*., 2005; Jain and Joshi, 2012; Mirunalini and Krishnaveni, 2011; Sahoo *et al*., 2012).