# CHAPTER I

## INTRODUCTION

Herpes simplex virus or HSV is one of herpesviruses in family Herpesviridae which has been classified into subfamily alphaherpesvirinae (1-3). They are divided into two types; Herpes simplex virus type 1 (HSV-1) and Herpes simplex virus type 2 (HSV-2). HSV is a linear double stranded (ds) DNA virus and has icosahedral capsid surrounded with envelope. HSV envelope contains 11 glycoproteins (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM) (4,5). It is a fast growing virus, which can form cytopathic effect. HSV is a neurotropic virus because after primary infection, the virus escapes the immunity and establishes latent infection in dorsal root ganglia (6). Therefore, HSV can cause recurrent infection. Transmission is by direct contact to the infectious lesions such as oral, genital fluid or secretion (7). Tissue tropism is skin and mucous membrane. Moreover, HSV can replicate in multiple cell types in epidermis and dermis such as germinal epithelium and fibroblast (1,8). HSV usually causes localized infection however it can cause systemic infection in newborn, infant, AIDS patient and immunosuppressive person.

Infection of susceptible cells is initiated by the attachment of virion via gC and/or gB to cell surface heparan sulphate proteoglycans. This step is followed by the interaction of gD with one of several cellular receptors, then gB, gD and gH-gL complex induces cell-cell fusion (4,9-11). Next step, the capsids are transported toward the nucleus and the DNA passes into the nucleus where the transcription and replication occur. The transcription of HSV has a specific characteristic called cascade regulation (2,6,12,13). First, immediate early (IE) genes are transcribed and translated to IE proteins in two to four hours after infection. The protein products from these genes are transported back into the nucleus, where they induce transcription of the early (E) genes. E genes encode about 15 proteins involved in viral DNA replication. This step occurred in five to seven hours after infection. After that, viral DNA replication then occurs. Late (L) genes encode structural proteins, formed after DNA replication is complete. This will occur nine to 11 hours after infection. Then, the assembly occurs in the nucleus, a capsid is formed and the DNA enters the capsid. The capsid buds through localized areas of the inner nuclear membrane. In some undefined way, virions are released to the environment (2,14).

Recently, several mediators of HSV-1 and/or HSV-2 entry into human cells have been identified. These molecules act as receptors for HSV gD and can be divided in three types according to their structural molecules. There are HveA, HveB, HveC, PRR2δ and 3-*O*-sulphated heparan sulphate (15). HveA or herpesvirus entry mediator (HVEM) is a member of the tumor necrosis factor receptor (TNFR) superfamily of proteins. HveB (PRR2α and nectin2α), PRR2δ (nectin2δ) and HveC (PRR1δ and nectin1δ) are related members of the immunoglobulin (Ig) superfamily. A splice variant of HveC, called "HIgR," also mediates HSV entry through its interaction with gD. Truncated, soluble forms of gD, lacking the transmembrane and cytoplasmic domains, bind directly to truncated, soluble forms of each of these receptors. In addition, antibodies to HveA, HveB, and HveC block HSV infection in various cell lines so it confirms that HSV can utilize several different and structurally unrelated cell surface proteins as receptors (15-18). However, different cells have different kinds of HSV receptors. Peripheral T cell and T cell line expressed HveA (17,19).

It is well-known that HSV usually replicates in epithelial cells and fibroblasts, especially in epithelial cells which are commonly used as a model to demonstrate HSV replication cycle (8,14). Until 1964, HSV has been shown to replicate in lymphocytes (20). Nahmias *et al*, have first reported the replication of HSV in human peripheral leukocytes after activation with phytohemagglutinin (PHA) documenting that HSV, in addition to its well known neurotropism, might also have lymphotropic properties (21). This idea is supported by the work of Kirchner *et al.* They found that HSV was able to replicate in population of T cells after prestimulating with PHA and concanavalin A (22). Beside this, there are several reports referring that HSV could infect lymphocytes. Kirchner and Schroder have reported the replication of HSV in EBV-stimulate B cell (23). Daniel *et al,* have shown the ability of HSV replication in macrophage when culture for several days (24) and Rudiger *et al,* have reported that HSV is able to replicate in a variety of different blood cells (25). In addition, Naraqi *et al,* have reported the successful isolation of HSV from the leukocytes of acutely infected patients (26) suggesting the possibility of spread viral infection mediated by infected lymphocytes. Moreover, replication of virus in leukocytes may represent a very important *in vivo* mechanisms of virus in the body. It is therefore of interest to study about HSV replication in T lymphocyte compare to epithelial cell which until now, the mechanism of HSV growth in T lymphocyte has not been clearly defined.

In this study, experiments were performed to (i) compare the HSV growth rates in HEp-2, Jurkat cell and Jurkat cell that was activated with PHA (ii) compare viral growth rates between HSV-1 and HSV-2, and (iii) explain which mechanisms cause different viral growth in each cell type. To achieve these goals, HSV both type 1 and 2 were grown in Vero cell, HEp-2 cell, Jurkat cell and activated Jurkat cell by varying the multiplicity of infection (MOI) and viral production was determined by plaque titration at various time after infection. The kinetics of viral antigen expression were demonstrated by Flow cytometry and indirect immunofluorescence assay (IFA). The antibodies used in this experiment were either polyclonal antibody to HSV-1 or HSV-2 and the specific antibody to three types of IE proteins, ICPO, ICP22 and ICP47. One proposed hypothesis is increasing of HveA receptor might be a mechanism to support viral growth in T cells. Therefore, detection of HveA receptors by RT-PCR was done.