INTRODUCTION

1. Human Herpesviruses

Herpesviruses are highly disseminated in nature. Of nearly 100 herpesviruses species found in the world, 8 have been isolated so far from humans. These are herpes simplex virus 1 (HHV-1), herpes simplex virus 2 (HHV-2), varicella-zoster virus (HHV-3), Epstein- Bar Virus (HHV-4). cytomegalovirus (HHV-5), human herpesvirus 6 (HHV-6) (Roizman, 1996), human herpesvirus 7 (HHV-7) (Black & Pellett, 1993) and human herpesvirus 8 (Levy, 1995).

A typical herpes virion consists of a core containing a linear double-stranded DNA of about 120 to 230 kilobasepairs (kbp), an icosadeltahedral capsid with 162 capsomeres, an amorphous asymmetric tegument surrounds the capsid and an envelope containing viral glycoprotein spikes on its surfaces (Roizman, 1996).

Although herpesviruses are difficult to differentiate from each other through their morphology, members in this group can be distinguished by the variable features on DNA molecular weight, base composition and sequence arrangement, biological properties, diseases association and clinical manifestations.

1.1. EPSTEIN-BARR VIRUS (EBV)

1.1.1. Discovery and Isolation of Epstein-Barr Virus

Epstein-Barr virus or human herpesvirus 4 is a gamma herpes virus in the Herpesviridae family. EBV was first discovered in continuous cultures of tumor cells from an African Burkitt's lymphoma (Epstein *et al.*, 1964). The virus particles were visualized on electron microscopic examination and later found to be biologically and antigenically distinct from other members of the then known human herpesviruses (Epstein *et al.*, 1965; Henle & Henle, 1966). Subsequent cell culture experiments showed that the virus could be rescued from circulating lymphocytes (Nilsson *et al.*, 1971) and also from throat washings of seroconverted individuals (Gerber *et al.*, 1972). Natural primary EBV infection takes place in children as young as 6 months which results in permanent seroconversion and harbouring of the virus throughout life.

1.1.2. Ultrastructure of EBV

An EBV particle is about 180 nm in diameter and consists of an outer membrane envelope, an icosahedral nucleocapsid with 162 capsomer and a core of protein and DNA (Kieff *et al.*, 1982). The viral membrane has spikelike projections on its outer surface which are composed of one or two species of high-molecular-weight glycoproteins (Edson & Thorley-Lawson, 1981). EBV genome is a linear double-stranded DNA molecule which is 172 kb in length and encoding approximately 100 genes.

The EBV genome consists of unique and tandemly repeated DNA elements. There are multiple terminal repeat (TR) sequences approximately 500 basepairs (bp) long at each end of the DNA. The number of repeats varies among different EBV isolates.

There are four direct tandem internal repeat sequences (IR) which separate the EBV genome into five unique regions (Dambaugh & Kieff, 1982).

1.1.3. Infection of EBV

In vivo, epithelial and B-lymphocytes are hosts of EBV. *In vitro* however, only B-lymphocytes are readily infected by infectious EBV. It is suggested that the oropharynx is the site of initial infection with EBV (Sixbey *et al.*, 1983) and the oropharyngeal epithelium is the primary site of viral replication. EBV infection becomes subsequently generalized with the appearance of virally infected B cells in the peripheral blood (Robinson *et al.*, 1981).

The route of infection by EBV is generally thought to be through mouth to mouth contact. Healthy carriers shed EBV intermittently into their saliva, indicating presence of productive viral infection in the epithelial, oropharynx and salivary glands (Wolf *et al.*, 1984). EBV is than transmitted via the saliva to non-infected individuals.

1.1.4. EBV Cycle in Human Cell

Entry of EBV into B-cells is initiated by an interaction between the major viral envelope glycoprotein gp350 and the B-lineage-associated C3d complement receptor CR2 designated as CD21 (Jondal *et al.*, 1976). Infection of B-lymphocytes with EBV results in persistent latent infection and immortalization of the cells to perpetual proliferation (Yao *et al.*, 1989). In the transformation process, EBV drives B-cell to become activated and than the infected B-cell remains at the lymphoblastoid stage and

committed to indefinite proliferation. In contrast, EBV infection in mucosal epithelium cell was predominantly lytic infection (Sixbey *et al.*, 1984).

In vitro activation of the latent EBV or induction of EBV lytic cycles in some lymphoblastoid cell line can be done by addition of phorbol esters, sodium butyrate, nucleoside analogues, or antibody to IgM to the culture medium (Kieff *et al.*, 1982). These agents induce the expression of ZEBRA protein which will transactivate a number of genes associated with the EBV lytic cycle. Then, a cascade of events occurs in viral replication leading to sequential expression of early and late gene products. The late gene products such as viral capsid antigen (VCA) and glycoproteins are expressed for packaging of the viral DNA and forming the structural components of the virion. These EBV virion structural proteins have been studied in detail because of their possible role as target of neutralizing antibodies.

1.1.5. Primary EBV Infection and Seroepidemiology of the Virus

Seroepidemiological studies indicated that EBV was widespread in all human populations with the great majority of adults having antibodies to the virus (Henle & Henle, 1979). The age at which primary EBV infection occurs varies among populations. Infection in the first few years of life is common among lower socioeconomic groups. Appearance of antibodies directed to the viral capsid antigen (VCA) often reaching close to 100% by age 10 years (Tan & Henle, 1972; Henle & Henle, 1979; Deveraj *et al.*, 1987). Clinical manifestations of this early childhood infection are usually mild. In contrast, primary EBV infection in adolescents and adults always develop more severe

syndromes and up to 50% present with clinical manifestations of infectious mononucleosis (Sawyer *et al.*, 1971).

1.1.6. Acute Infectious Mononucleosis (IM)

Clinical infectious mononucleosis occurs most commonly in areas where exposure to and infection with EBV are delayed until adolescence and young adult life. These places include Australia, Canada, England, European countries, New Zealand and the United States (Evans & Neiderman, 1982). In Malaysia, similar to other developing countries, IM is rare, since children have seroconverted by age 10 (Deveraj *et al.*, 1987). Acute infectious mononucleosis is characterized by malaise, sore throat, lymphadenopathy, lymphocytosis and the appearance of atypical cells in the circulation. EBV serology in infectious mononucleosis patients revealed the presence of IgM antibody to VCA and elevated titres of IgG antibodies to VCA and early antigen (EA) (Horwitz *et al.*, 1985).

1.1.7. African Burkitt's Lymphoma

There are three recognized form of Burkitt's lymphoma (BL) namely, endemic, sporadic and acquired immunodeficiency syndrome (AIDS)-associated Burkitt's lymphoma. All three forms of BL bear one of three reciprocal translocations between chromosome 8, near the site of the c-myc locus at 8q24, with either the Ig heavy-chain locus on chromosome 14 or one of the light-chain loci on chromosome 2 or 22.

EBV is associated with endemic BL. This form of BL accounts for approximately half of all childhood lymphomas occurring in equatorial Africa and Papua New Guinea

with an incidence of 8 to 10 cases per 100,000 people per year. Burkitt was the first to postulate an infectious agent being involved in the tumor's etiology (Burkitt, 1962). EBV genome is present in the majority of BL from areas where the disease is endemic (Neri *et al.*, 1991). In the early work (Henle *et al.*, 1969), titers of anti-VCA and anti-EA(R) antibodies in the serum of endemic BL patients were found considerably higher than in appropriate control groups. Moreover, a prospective seroepidemiological survey involving 42,000 Ugandan children from an endemic area showed that individuals who subsequently developed BL had significantly raised anti-VCA titers months or years before the clinical onset of BL (Geser *et al.*, 1982).

Although endemic BL shows the strongest and most consistent association with EBV, the role that the virus plays in the etiology and pathogenesis of the tumor is controversial. Various models for the role of EBV in the pathogenesis of BL have been proposed (Lenoir & Bornkamm, 1987) and it is conceivable that endemic BL develops in three stages. The first stage involves primary EBV infection, resulting in virus-induced immortalization of B lymphocytes. Second stage involves risk factors, most probably holoendemic malaria which act as potent stimulators of B-cell hyperplasia or mediators of T-cell suppression (Lam *et al.*, 1991). The third and final step involves the translocations of the distal part of chromosome 8 to chromosome 14, 2 or 22 leading to the constitutive deregulation of the c-myc oncogene and subsequent monoclonal B-lymphocyte proliferation, resulting in BL.

1.1.8. Nasopharyngeal Carcinoma (NPC)

NPC is a tumor of epithelial origin and incidences of NPC vary markedly between different population groups. It is rare in Europe and America, but common in Southeast Asia, particularly among the Southern Chinese (Armstrong *et al.*, 1979) and intermediate rates are seen in North and East African and Eskimos. An association between EBV and NPC was firstly described by Old *et al.* in 1966, based on the observation that IgA antibodies directed against EBV early antigen (EA) and VCA were elevated in patients with NPC (Henle & Henle, 1979) and EBV genome is found in every NPC specimen (Nonoyama & Pagano, 1973).

Other than EBV, genetic or environmental factors may contribute to the development of NPC. Early evidence for a genetic determinant among Chinese was a human leukocyte antigen (HLA)-associated risk for NPC (Simon *et al.*, 1974). For example, the presence of HLA-A2 and HLA-B sin 2 major histocompatibility complex alleles in Chinese individuals was shown to be associated with an increased risk of NPC.

Among the environmental or cultural factors, it has been suggested that constant ingestion of Cantonese-style salted fish, especially during childhood, correlates with increased risk for NPC (Armstrong *et al.*, 1983; Yu *et al.*, 1986; 1989).

Thus, the etiology of NPC is multifactorial, including virological, genetic and environmental factors.

1.1.9. EBV Seroepidemiological Studies in Malaysia

Tan & Henle (1972) showed that EBV seroconversion occurred at very early ages among children in West Malaysia with 83% of the samples in the ages of 1-2 years being

positive for IgG against EBV-VCA. EBV seroconversion rate was 90-100% for those aged 5 and above. Deveraj *et al.* (1987) obtained similar results among 216 children aged 0-11 years from East Malaysia. Yadav *et al.* (1990) found 100% EBV seroconversion in 95 Kadazans aged 0-79 years from Sabah in East Malaysia.

1.2 HUMAN HERPESVIRUS 6 (HHV-6)

1.2.1. Discovery and Isolation of HHV-6

HHV-6 was firstly discovered by Salahuddin *et al.*, (1986) while investigating the potential role of new human retroviruses in various lymphoproliferative disorders. This novel human herpesvirus was isolated from the peripheral lymphocytes of six adult patients with various lymphoproliferative disorders including one patient with AIDS-related lymphoma.

HHV-6 was shown to propagate best in freshly isolated primary lymphocytes and caused the formation of large, refractile and short-lived cells (Salahuddin *et al.*, 1986). When the infected cultures were examined under the electron microscope, herpesvirus-like particles were found. Monoclonal antibodies, well characterized human antibodies and high-titred polyclonal monospecific antibodies directed against other human herpesviruses or primate herpesviruses, all failed to react with this new virus by immunofluorescence assay (IFA). In addition, DNA hybridization studies by Josephs *et al.* (1986) indicated that this newly isolated virus was distinct from other human herpesviruses. HHV-6 was initially named human B-lymphotropic virus (HBLV) because the early study (Salahuddin *et al.*, 1986) indicated a B cell tropism of this virus.

Within a short time after the discovery of HBLV by Salahuddin *et al.* (1986), similar viruses were isolated from AIDS patients in Uganda (*Downing et al.*, 1987), United Kingdom (Tedder *et al.*, 1987) and France (Agut *et al.*, 1988). Later, this virus was successfully isolated from the peripheral blood lymphocytes of children with exanthem subitum (Yamanishi *et al.*, 1988) and from saliva of healthy adults (Pietroboni *et al.*, 1988a; Levy *et al.*, 1990 and Mukai *et al.*, 1994).

Although this newly isolated virus showed tropism to B cells (Salahuddin *et al.*, 1986), Downing *et al.*, (1987) and Tedder *et al.*, (1987) were able to demonstrate *in vitro* tropism of the virus for T cells, monocytes, macrophages, glial cells and fibroblast. Soon thereafter, Ablashi *et al.* (1987) and Lusso *et al.* (1987) found additional cell lines that could be infected *in vitro* by HBLV. Accordingly, Ablashi *et al.* (1987) suggested HBLV should be classified as human herpesvirus type 6 (HHV-6).

1.2.2. Ultrastructure of HHV-6

Since the discovery of HHV- 6, the ultrastructure forms of mature and immature virus particle were studied through electron microscopy of thin sections of cells infected with HHV-6.

Mature HHV- 6 virions are 170-200nm in diameter. The viral particles composed of four major elements: an electron-dense core containing linear, double-stranded DNA; a nucleocapsid composed of 162 capsomers arranged in an icosadeltahedron structure; a tegument surrounding the capsid and a membrane envelope contains numerous glycoprotein spikes. This enveloped mature form of virus can be observed in cytoplasmic vacuoles, on the surface membrane as well as outside the infected cell (Yoshida *et al.*,

1989a). The nucleocapsid had a diameter of approximately 90-110nm and surrounded by a tegument of uniform 20-40nm thickness.

Immature virions are found in the nucleus and cytoplasm of infected cells. The naked viral nucleocapsids are formed in the nucleus. They usually appear as single, ringed particles with electron dense center representing the core. Another immature form is the nucleocapsid coated with a tegument and located in the cytoplasm. These viral particles without envelope are approximately 140-180nm in diameter (Yoshida *et al.*, 1989a).

1.2.3. Morphogenesis of HHV- 6

Morphogenesis of HHV - 6 was proposed by Roffman *et al.* (1990). After the replication of viral DNA in the nucleus of infected cell, the viral DNA was inserted into preassembled capsids. These naked nucleocapsids were then budded into tegusomes to acquire the tegument. Tegusome is a spherical, double membrane intranuclear inclusion which formed through cytoplasmic invagination. Thereafter, tegumented nucleocapsids fuse with nuclear membrane and result in the release of tegumented nucleocapsid into the cytoplasm vacuoles. Later, fusion of the vacuole membrane with the cell membrane releases the mature viral particles into the extracellular space.

1.2.4. Physicochemical Characteristics of HHV-6 Virus Replication

HHV-6 is readily propagated in freshly isolated human cord blood mononuclear cells. Cocultivation of stimulated cord blood lymphocytes with infected cells resulted in widespread infection of the cells by HHV-6, with T cells being the primary target of

infection (Lopez *et al.*, 1988). T-cell activation is required for efficient propagation of HHV-6 (Frenkel *et al.*, 1990). The virus does not replicate effectively in quiescent lymphocytes but replicates efficiently in cells exposed to the polyclonal mitogen phytohemagglutinin (PHA). In addition, virus replication requires exogenous interleukin-2 (IL-2) in the medium. However, IL-2 at concentration exceeding 10 U/ml strongly inhibited virus replication *in vitro* (Roffman and Frenkel, 1990). Virus infectivity is stable below 37^0 C and at neutral or slightly alkaline pH from 7.0 to 8.5. (Shiraki *et al.*, 1991). HHV-6 infectivity is unstable when the temperature goes above 42^0 C and pH below 6.5.

The membrane antigen(s) was proposed to be involved in virus penetration and replication. Although HHV-6 has tropism for $CD4^+$ T cells (Takahashi *et al.*, 1989), infection of HHV-6 is not inhibited by the presence of anti-CD4 antibodies (Lusso *et al.*, 1989b). This indicates that CD4 is not the membrane receptor for HHV-6 and HHV-6 infection occurs via an CD4-independent mechanism. Other studies showed antibodies against CD3 to enhance the HHV-6 replication in lymphocytes (Kikuta *et al.*, 1990b; Roffman and Frenkel, 1991). However the mechanism of enhancement of virus replication by these antibodies to CD3 remains obscure. Another study demonstrated that monoclonal antibodies to HHV-6 glycoprotein gp100 inhibit the penetration of virus into susceptible cells (Foa-Tomasi *et al.*, 1991). The precise role of glycoprotein for infection and replication of the virus has not been determined.

1.2.5. Effects of HHV-6 Infection on Host Cell

Most cells infected by HHV-6 become larger, refractile and balloon-like as early as 2 days after infection (Salahuddin *et al.*, 1986), but some cell lines do not produce this cytopathic effect (CPE) although they are infected and expressing viral proteins (Ablashi *et al.*, 1988). HHV-6 induces a strong shut-off of host cells DNA replication, which is almost complete by 3 days post-infection. (Di Luca *et al.*, 1990).

In *in vitro* studies, HHV-6 infection induces production of cytokines in peripheral blood mononuclear cells. Interferon alpha (IFN α) is released rapidly and the activity reached the maximum level after 2 days of exposure to HHV-6 (Kikuta *et al.*, 1990a). Human cell population responsible for IFN α production after HHV-6 infection comprised non-T cells and monocytes. HHV-6 is also a potent inducer of interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) production (Flamand *et al.*, 1991), transcription of IL-1β and TNF-α being maximum at 12 and 6 hours after HHV-6 infection, respectively. Peak levels of IL-1β and TNF-α were reached at 24 hours post-infection before gradually decreasing with time. These strong immunomodulatory effects exerted by HHV-6 infection may be an important part of host response to HHV-6.

Horvat *et al.* (1993) showed that HHV-6 infection inhibited the proliferative response of human peripheral blood mononuclear cells *in vitro*. HHV-6 infection also cause *in vitro* suppression of bone marrow progenitor cells differentiation (Knox and Carrigen, 1992). The mechanism of suppression may be due to either HHV-6 encoded or induced soluble mediator(s) that can interfere with the response of bone marrow to growth factor and block the normal differentiation of marrow precursors (Burd *et al.*,

1993). Strong evidence of marrow suppressive role for HHV-6 *in vivo* was shown by Drobyski *et al.* (1993) in marrow transplant recipients.

1.2.6. HHV-6 Genome

The HHV-6 genome is a linear, double stranded DNA molecule (Salahuddin *et al.*, 1986) of 160-170kbp (Josephs *et al.* 1988). It contains a central segment of a largely unique sequence of approximately 140kbp and having a sequence of approximately 10kbp duplicated in the same orientation at both left and right genomic termini (Lindquester and Pellett, 1991). The density of the genomic DNA is approximately 1.702g/cm^3 and has a mean G+C content of 43% (Lindquester and Pellett, 1991).

The complete DNA sequence of HHV-6 strain U1102 (Gompels *et al.*, 1995) showed the viral genome to be 159,321bp in size, has a base composition of 43% G+C and contains 119 open reading frames. The overall genome structure of this virus strain is 143 kbp bounded by 8 kbp of direct repeats. This genome contains 102 separate genes likely to encode protein. The genes are arranged colinearly with those in the genome of the previously sequenced betaherpesvirus such as human cytomegalovirus and has a distinct arrangement compared to the sequenced gammaherpesviruses and alphaherpesviruses.

Other sequencing data demonstrated close homology of genetic organization in long unique segments of HHV-6 and human cytomegalovirus DNA (Lawrence *et al.*, 1990; Littler *et al.*, 1990). The homologous genes are envelope glycoproteins gB and gH, DNA polymerase, major capsid protein, phosphotransferase and putative large tegument protein genes (Josephs *et al.*, 1991). The similarity of genetic organization in large parts

of HHV-6 and human cytomegalovirus genomes has resulted in HHV-6 being classified in the betaherpesviruses group.

1.2.7. Variants of HHV-6

HHV-6 can be divided into two distinct groups. There are HHV-6 variant A and variant B. The variants can be differentiated from each other by *in vitro* cell tropism (Ablashi *et al.*, 1991; Dewhurst *et al.*, 1992; Yasukawa *et al.*, 1993), expression of cell markers and virus glycoproteins (Foa-Tomasi *et al.*, 1992; Furukawa *et al.*, 1994), reactivity with panels of monoclonal antibodies (Ablashi *et al.*, 1991; Dewhurst *et al.*, 1992; Campadelli-Fiume *et al.*, 1993; Pellett *et al.*, 1993), restriction endonuclease profile (Ablashi *et al.*, 1991), nucleotide sequences (Aubin *et al.*, 1991; Dewhurst *et al.*, 1994; Yamamoto *et al.*, 1994), seroepidemiology (Yadav *et al.*, 1991) and disease associations (Ablashi *et al.*, 1991; Akashi *et al.*, 1993; Drobyski *et al.*, 1993; Yalcin *et al.*, 1994).

Although the two variants can be distinguished by the above characteristics, they are genetically very closely related. Some genomic segments have nucleotide sequences that differ by as much as 25% between the 2 groups (Chou and Marousek, 1994). In contrast, some genomic segments only have as little as 4 % (Aubin *et al.*, 1993) and 0.2% to 3% difference (Gompels *et al.*, 1993).

1.2.8. Interaction Between HHV-6 and Other Viruses

Initial genomic analysis (Joseph *et al.*, 1986) and serological finding (Morris *et al.*, 1988; Irving *et al.*, 1990b) showed HHV-6 to be distinct from other herpesviruses.

Later, Efstathiou *et al.* (1988) identified a region of HHV-6 genome similar to human cytomegalovirus. Soon after, Lawrence *et al.* (1990) showed HHV-6 to be closely related to human cytomegalovirus (CMV) and Osman *et al.* (1997) detected cross-reactive responses to CMV in renal allograft recipients.

Primary infection by EBV or CMV were shown to increase the HHV-6 IgG titre and induce appearance of HHV-6 IgM (Enders *et al.*, 1990; Linde *et al.*, 1990). These reaction or induction may be due to polyclonal B-cell stimulation by related antigens or reactivation of HHV-6.

The role of HHV-6 in EBV reactivation in EBV-genome positive human lymphoid cell lines was first demonstrated by Flamand *et al.* (1993). HHV-6 can upregulate the expression of the immediate-early antigen BZLF1 and early antigens in both EBV producer and nonproducer cell lines. Furthermore, HHV-6 infection in these cells also increased the expression of late EBV gene products such as viral capsid antigen and viral membrane glycoprotein gp 350. These upregulation and activation of EBV genome was mainly caused by HHV-6 variant A infection (Cuomo *et al.*, 1998).

Since HHV-6 is frequently isolated from AIDS patients, the relationship between HHV-6 and human immunodeficiency virus (HIV) is of interest. Brown *et al.* (1988a) showed that the prevalence of anti-HHV-6 antibodies was not greater among HIV-1 seropositive individuals. Although both viruses have tropism for $CD4^+$ lymphocytes, no correlation was found between HHV-6 and the course of HIV infection (Spira *et al.*, 1990). However, one report showed co-infection of HHV-6 and HIV can inhibit HIV replication *in vitro* (Pietroboni *et al.*, 1988b). In other reports, HHV-6 infection is active and disseminated in patients with AIDS (Lusso and Gallo, 1994). HHV-6 infected cells

can be detected in lung, lymph-node, spleen, liver and kidney tissues from AIDS patients (Knox & Carrigen, 1994). HHV-6 transactivates the HIV-1 long terminal repeat (LTR) and might contribute directly or indirectly to pathogenesis of HIV (Horvat *et al.*, 1989, Lusso *et al.*, 1989a; Lusso & Gallo, 1994). It was also shown that, as asymptomatic HIV infection progressed to AIDS, concurrent replication of HHV-6 variant A was increased and HHV-6 IgG antibodies elevated (Ablashi *et al.*, 1998).

HHV-6 is capable of infecting human cervical epithelial cells *in vitro* without CPE (Chen *et al.*, 1994) and altering expression of human papillomavirus (HPV) genes. HHV-6 infection enhances expression of HPV RNAs encoding the viral oncoprotein E6 and E7.

1.2.9. Primary HHV-6 Infection

Primary HHV-6 infection occurs most commonly in children (Okuno *et al.*, 1989). Most of them become seroconverted by the ages of 1 to 3 years old (Briggs *et al.*, 1988; Brows *et al.*, 1988b; Ward *et al.*, 1993; Black *et al.*, 1996; Chua *et al.*, 1996). Primary infection in newborns is rare because transplacental maternal IgG to HHV-6 protects the newborns from HHV-6 infection (Yoshikawa *et al.*, 1989; Huang *et al.*, 1992). HHV-6 seroconversion usually happens following the waning of maternal antibodies 3-6 months after birth (Balachandra *et al.*, 1989; Farr *et al.*, 1990). The seroconversion is shown by the appearance of IgM and IgG among infants from 6 to 12 months (Saxinger *et al.*, 1988). IgM antibodies appear 5 to 7 days after infection, reaching maximum titres at 2 to 3 weeks and declining to undetectable level after 2 months (Suga *et al.*, 1992). IgG response develops at 7 to 10 days postinfection and is

present at detectable levels thereafter. The most common childhood disease associated with HHV-6 is exanthem subitum, also known as roseola infantum.

1.2.10. Transmission of HHV-6

Although HHV-6 infection is ubiquitous and seroconversion happens early in life, the route of transmission is not known. Horizontal transmission is probable since infectious cell-free HHV-6 has been reported to be present in the saliva of healthy adults and HIV-infected patients (Pietroboni *et al.*, 1988a). HHV-6 DNA and antigen have also been detected in saliva (Aberle *et al.*, 1996), salivary gland (Fox *et al.*, 1990) and bronchial gland epithelium (Krueger *et al.*, 1990). These results suggested that HHV-6 transmission is most probably via oral secretion and primary infection occurs via the oropharynx (Levy *et al.*, 1990). The possibility of horizontal infection was also shown by Okuno *et al.* (1991) in an outbreak of HHV-6 primary infection in an orphanage. Mukai *et al.* (1994) gave a more convincing result from their study which showed that the viruses isolated from siblings and mothers of children with primary HHV-6 infection have nearly identical restriction endonuclease profile. However, the site and target cell of primary infection within the oropharynx is not known yet. Ultimately, lymphocytes with $CD4^+$ cells become infected.

Breast milk has been demonstrated not to be a route of HHV-6 transmission (Dunne & Jevon, 1993) and it is not a significant source of HHV-6 infection in infancy (Kusuhara *et al.*, 1997).

The possibility of congenital transmission of HHV-6 was demonstrated by a few research groups. HHV-6 specific sequences were detected in organ tissues of fetus

(Aubin *et al.*, 1992) and in cord blood (Adams *et al.*, 1998). IgM antibodies against HHV-6 were also found, although only rarely (0.28%) in cord blood (Dunne & Demmler, 1992). This showed that congenital HHV-6 infection may account for a small proportion of HHV-6 seropositivity.

Although HHV-6 DNA is shed in the genital track of some women and there is a possibility that infectious virus is transmissible through sexual contact and to newborn infants by perinatal spread (Leach *et al.*, 1994), the presence of HHV-6 DNA within the genital tract of pregnant woman rarely result in infection of the neonates (Maeda *et al.*, 1997).

1.2.11. Persistence and Latent Infection of HHV-6

Frequent detection of HHV-6 DNA in human cells demonstrated the persistence and latent infection of HHV-6. HHV-6 DNA can be detected in peripheral blood mononuclear cells (PBMC) from healthy adults by the polymerase chain reaction (PCR) (Cone *et al.*, 1993; Cuende *et al.*, 1994; Hall *et al.*, 1994; Di Luca *et al.*, 1994; Aberle *et al.*, 1996). HHV-6 DNA was demonstrated in up to 98% of PBMC specimens from children and adults (Hall *et al.*, 1998). HHV-6 DNA has also been found in many other tissues and cells such as salivary glands (Fox *et al.*, 1990), lymph node tissue (Sumiyoshi *et al.*, 1993), bronchial gland (Krueger *et al.*, 1990), central nervous system (Caserta *et al.*, 1994) and vaginal secretions (Leach *et al.*, 1994; Maeda *et al.*, 1997).

In vitro HHV-6 was shown to infect and establish latent infection in adherent monocytes (Kondo *et al.*, 1991). The latent virus can be reactivated by treatment with phorbol ester. *In vivo* the latent virus may be reactivated, leading to the expression of

HHV-6 antigens, as seen in some benign and malignant lymphoproliferative diseases (Luppi *et al.*, 1998).

1.2.12. Exanthem Subitum (ES)

HHV-6 was firstly identified as the major causative agent in exanthem subitum by Yamanishi *et al.* (1988). This finding was support by later reports on isolation of HHV-6 from ES patients (Yoshida *et al.*, 1989a, Asano *et al.*, 1991 and Pruksananonda *et al.*, 1992). HHV-6 associated exanthem subitum is characterized by high fever (>39^0 C) lasting for 3-5 days, this period of acute febrile illness is followed by the appearance of an erythematous and macular or macropapular rash (most frequently involving the face and trunk) of 1-3 days duration. Cell-associated viremia can be detected from peripheral blood mononuclear cells as early as the first day of fever (Asano *et al.*, 1989a; Asano *et al.*, 1991). Cell-free virus can be detected in the plasma soon after the release of virus from the infected cell. The viremia is reduced during the rash phase and is usually gone by the time the disease resolves. The disappearance of the virus from blood corresponds to the induction of specific immunity to the virus. Antibody activity to the virus can be detected as soon as 3 days after the onset of fever (Asano *et al.*, 1989a). Immunological response starts with IgM production followed by elevation of IgG antibodies a week later (Ueda *et al.*, 1989; Yoshida *et al.*, 1989b; Irving *et al.*, 1990a). Most exanthem subitum is caused by infection of HHV-6 variant B and very rare by variant A (Dewhurst *et al.*, 1993). The prevalence of ES caused by HHV-6 is higher in Japan (Kusuhara *et al.*, 1992; Okada *et al.*, 1993) compared to Europe (Segondy *et al.*,

1992; Portolani *et al.*, 1993) and North America (Pruksananonda *et al.*, 1992). The distribution of HHV-6 variants may explain the geographical distribution of ES.

Besides the classical syndrome of ES in primary HHV-6 infection, various related syndromes have been described in conjunction with primary HHV-6 infection. The most common syndrome is febrile illness without skin eruption (Suga *et al.*, 1989; Segondy *et al.*, 1992; Portolani *et al.*, 1993; Ward & Gray, 1994). In contrast, two reports were published on HHV-6 infection with exanthem subitum-like rash without fever (Asano *et al.*, 1989b; Okada *et al.*, 1993).

1.2.13. Clinical Manifestation and Complications of HHV-6 Infection in Children

Clinical manifestation and complications of HHV-6 primary infection involve many body systems. Complications reported by Asano *et al.* (1991) including diarrhoea, bulging fontanelle and bronchopneumonia. Idiopathic thrombocytopenic purpura was reported by Yoshikawa *et al.* (1993) as complications of primary HHV-6 infection. Manifestations such as encephalitis and gross hepatosplenomegaly were reported by Irving *et al.* (1990a). Liver dysfunction, anemia and granulocytopenia associated with manifestations of HHV-6 infection were recorded by Takikawa *et al.* (1992). More common complications of HHV-6 infection in children involve central nervous system (CNS). CNS complications including meningoencephalitis (Ishiguro *et al.*, 1990; Yoshikawa *et al.*, 1992), meningitis (Huang *et al.*, 1991), encephalitis/encephalopathy (Suga *et al.*, 1993), febrile seizures (Segondy *et al.*, 1992; Hukin *et al.*, 1998) and recurrence febrile convulsions (Kondo *et al.*, 1993). These neurological complications

were caused by invasion of HHV-6 in CNS. A case of hepatitis associated with HHV-6 infection was also reported by Tajiri *et al.* (1990).

The most serious manifestation of HHV-6 infection is in causing death. The fatal outcome associated with HHV-6 infection including fatal fulminant hepatitis (Asano *et al.*, 1990), fatal haemophagocytic syndrome (Huang *et al.*, 1990), fatal encephalitis/encephalopathy (Asano *et al.*, 1992), fatal disseminated infection with HHV-6 (Prezioso *et al.*, 1992) and fatal Griscelli's Syndrome (Wagner *et al.*, 1997).

1.2.14. HHV-6 Infection in Adults

A high rate of HHV-6 seroconversion in early life makes infection in adults rare. Infections in adults appear to have more severe consequences than the childhood acquisition. The common complications of HHV-6 infection in adults are prolonged lymphadenopathy or mononucleosis-like illness and hepatitis. Mononucleosis-like illness was reported in Niederman *et al.* (1988), Steeper *et al.* (1990) and Akashi *et al.* (1993) reports. These cases were demonstrated by serological studies to be non-Epstein-Barr virus, non cytomegalovirus heterophile-negative mononucleosis-like illness. Serological evidence of HHV-6 infection was shown by detection of specific IgM and high titre of IgG against HHV-6. Hepatitis in adults infected by HHV-6 was reported by Steeper *et al.* (1990) and Sobue *et al.* (1991).

1.2.15. HHV-6 Seroepidemiological Studies in Malaysia

A few seroepidemiological studies on HHV-6 in Malaysia have been carried out. Yadav and colleagues in 1990 reported low HHV-6 IgG seroprevalence of 34% among

95 Kadazans in Sabah. In 1991, the same group reported HHV-6 (GS strain) IgG seroprevalence of 80% in 50 Malays, 64% in 50 Chinese, 64% in 50 Indians and 58% in 84 indigenous tribes in Malaysia. Levine *et al.* (1992) reported that the presence of IgG to HHV-6 was 41.5% of 41 samples from Malaysian Chinese and 54.2% of 48 samples from Malaysian Indians (both groups aged 21-40 years). Chua *et al.* (1996) screened 600 samples aged 1 to 83 years in his study and found HHV-6 seroprevalence to be 83.7%, with no significant difference between the sexes and among the various ethnic groups (Malays, Chinese and Indians).