CHAPTER 1

INTRODUCTION
The history of Herpes viruses dates to the ancient Greek times where physicians like Hippocrates described the cutaneous spreading of herpes simplex lesions. The word “herpes,” which means “to creep or crawl,” was used in reference to the spreading nature of the herpetic skin lesions. However, the origin of herpes virus in human history remains unknown and the virus has been prevalent ever since anyone could diagnose fever blisters. Herpes viruses are common inhabitants of animal kingdom and nearly 100 distinct herpesviruses have been isolated from a variety of species which include non-human primates, cat, dog, snakes, rabbit, mouse, rat, frog, and birds (1). To date, nine herpes virus types are known to infect man frequently and constitute the Human Herpesvirus family. Virus family members were initially named after the clinical symptoms, for instance, herpes simplex virus and herpes Zoster virus, or after their discoverers, such as Epstein Barr virus or based on their pathology namely, cytomegalovirus. However, according to the International Committee on Taxonomy of Viruses (ICTV) endorsed nomenclature, the Human herpesvirus family was subdivided into 3 subfamilies designated as alpha, beta, and gamma. Individual members were clubbed into these sub-families based on their biological properties (Table 1) (2).

<table>
<thead>
<tr>
<th>Sub-family</th>
<th>Members</th>
<th>Common name</th>
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<tbody>
<tr>
<td>Alpha</td>
<td>Human herpesvirus-1</td>
<td>Herpes Simplex Virus type-1 (HSV-1)</td>
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<tr>
<td></td>
<td>Human herpesvirus-2</td>
<td>Herpes Simplex Virus type-2 (HSV-2)</td>
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<td></td>
<td>Human herpesvirus-3</td>
<td>Varicella-zoster Virus (VZV)</td>
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<tr>
<td>Beta</td>
<td>Human herpesvirus-5</td>
<td>Cytomegalovirus (HCMV/CMV)</td>
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<td></td>
<td>Human herpesvirus-6A</td>
<td>(HHV-6A)</td>
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<td></td>
<td>Human herpesvirus-6B</td>
<td>(HHV-6B)</td>
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<td></td>
<td>Human herpesvirus-7</td>
<td>(HHV-7)</td>
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<tr>
<td>Gamma</td>
<td>Human herpesvirus-4</td>
<td>Epstein-Barr Virus (EBV)</td>
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<td></td>
<td>Human herpesvirus-8</td>
<td>Kaposi’s Sarcoma associated Virus (KHSV)</td>
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Table 1: The Human Herpesvirus family members
Disease associations of human herpesviruses

In addition to causing acute diseases, they are capable of establishing a latent infection and reactivating under a variety of stimuli, a feature common to all herpesviruses. HSV-1 & HSV-2 are widespread in distributions, that cause fever blisters and genital sores respectively, and occasionally central nervous infections. They show a worldwide distribution and exhibit unique biological properties such as neurovirulence, establishment of latency in the nerve ganglia and the potential to reactivate equally in both immunocompetent as well as immunocompromised individuals, though the reactivations are more common and severe in the latter cases. HSV-1 is transmitted chiefly through saliva, whereas HSV-2 is transmitted either sexually or from mother to child via the genital tract during birth (3). Primary infection with VZV causes chickenpox in children, and this virus is the most infectious of the herpesviruses that can potentially spread via inhalation of an aerosol of nasopharyngeal secretions from a patient. Once the clinical symptoms resolve, VZV latently persists lifelong in the nervous system of the infected person, with reported 10-20% of cases where it reactivates producing herpesvirus zoster (Shingles), a manifestation generally detected in elderly immunocompromised individuals (4). EBV was discovered almost half century ago from Burkitt's lymphoma derived cells. It is highly prevalent in developing countries, particularly in equatorial Africa and infects most of the children in early years of life generating an asymptomatic primary infection followed by lifelong dormant infection. However, if the infection is delayed until adolescence, it usually presents as infectious mononucleosis (5). A very rare event in a few carriers of this virus is the emergence of Burkitt's lymphoma and nasopharyngeal carcinoma. EBV infection is also implicated in development of autoimmune diseases and post transplant lymphoproliferative disorders. Human B lymphocytes once infected with EBV can be
propagated indefinitely in culture. This property of EBV has been extensively exploited for generating biological material for functional and molecular studies (6). CMV infection was initially documented more than a century ago, originally described as presence of typical cytomegalic cells in parotid glands of infants. However, technological limitations held the successful isolation of the virus for almost 50 years till 1957 (7). Like other herpesviruses, it is widespread in the human population and about 50% of adults are carriers of CMV. CMV infection has been implicated as a major cause of hearing loss and mental retardation in cases of congenital infection and can cause life-threatening complications in infants & in immunocompromised individuals. The viral reactivation is a major concern for transplant recipients and AIDS patients (8). Although CMV is not yet recognized as an oncogenic virus, some evidences suggest a possible role of CMV infection in malignant diseases from different cancer entities (9). HHV-7 is another ubiquitous virus that latently infects over 90% of the human population by the age of 3. HHV-7 infections have been associated with exanthem subitum, hepatitis, multiple sclerosis (MS), and transplant complications (10). HHV-8 is commonly known as the Kaposi's sarcoma-associated herpesvirus as it was initially detected in Kaposi’s sarcoma (KS) tissues from an AIDS patient. It is an oncogenic virus that is distinguishable from other herpesviruses as it exhibits a limited and an uneven distribution in the human population. It is also implicated in pathogenesis of other malignancies such as multicentric Castleman’s disease and primary effusion lymphoma. Nearly, 30% of the viral genes encode novel proteins not found in other human herpesviruses; most of these proteins are responsible in KS pathogenesis (11).
**HHV-6**

*Clinical associations*

The natural history of HHV-6 infection can be represented by 3 stages. The first being primary infection that takes place between 6 and 12 months of age and is known to cause acute febrile illness (12). The species B is implicated in most of the primary infections and has been recognized as the etiologic agent of Roseola infantum (also termed as *exanthem subitum* or the sixth disease), a common childhood disease with skin eruptions that is often benign, self-limiting and is observed in subset of patients following primary infection (13). In certain cases, it leads to complications such as encephalitis, febrile seizures, gastrointestinal symptoms, and respiratory distress. However, most of the primary infections are characterized by fever with a self-resolving course (14). Primary infections in adults are rare, and can particularly have fatal consequences in case of immunocompromised cases (14, 15). The second stage of infection can be observed in healthy individuals, where the virus remains latent in the lymphocytes, monocytes, undergoing low level of replication mostly in the salivary glands and is secreted in the saliva. At this stage, although the virus is pathologically quiescent, but frequent shedding in the saliva acts as the source for transmission to uninfected individuals (16). This stage can last for the lifetime of an individual. The third stage is observed relatively infrequently and is represented by complications arising in immunocompromised individuals mostly due to endogenous latent virus reactivation or superinfection in a previously infected individual (14). Moreover, pediatric transplant recipients who are under the age of two are vulnerable to primary infection from an HHV-6 positive donor and have been reported to have fatal consequences (14). The scenario of virus reactivation is of profound clinical significance in transplant recipients where the virus is presumed to induce immunomodulation resulting in a myriad of clinical syndromes such
as drug induced hypersensitivity syndrome, allograft dysfunction, acute cellular rejection, an increased risk of opportunistic infections (17, 18). Moreover, prospective studies have demonstrated that HHV-6 seroconversion was associated with enhanced episodes of CMV reactivation and disease in transplant settings (14).

HHV-6 has also been implicated with the pathogenesis of multiple sclerosis (MS). It has also been observed that an anti HHV-6 IgG titer is positively associated with MS relapse in a dose dependent manner, indicating that either HHV-6 infection or an immune response to HHV-6 antigens may have an effect on the clinical course of MS (14, 19).

Apart from this, HHV-6 infections have been associated with encephalitis in immunocompromised as well as immunocompetent conditions, although, the latter is observed relatively rare (20). A number of studies have suggested a possible role of HHV-6B in the pathophysiology of Mesial Temporal Lobe Epilepsy (MTLE), as evidenced by the presence of viral DNA in brain resections from MTLE patients and also the viral tendency to aggregate in the temporal lobe (21). As HHV-6 was initially isolated from patients with lymphoproliferative disorders, the virus has been extensively investigated for its possible role in malignancy. Several studies have reported a link between HHV-6 and nodular sclerosis subtype of Hodgkin’s lymphoma (HL) (22). The viral DNA is frequently detected in T-cell non-Hodgkin’s lymphoma (NHL). Previous studies from our lab have also reported activation of HHV-6 in HL and NHL (23). HHV-6 has also been postulated to play a role in the development of cervical cancer as well as adult pediatric gliomas (24, 25). Though few reports suggest its role in malignant transforming activities, overall there is still lack of conclusive data linking HHV-6 to human malignancies (14, 26). HHV-6 has been reported to exhibit molecular interactions with viruses such as Human ImmunodeficiencyVirus-1 (HIV-1), EBV and Human Papilloma Virus (HPV) (14). There seems to be a synergistic relationship between HHV-
and EBV, considering the facts that while HHV-6 infection activates EBV replication and enhances its transforming potential, on the other hand EBV infection makes the B-cells more susceptible to HHV-6 infection. Few HHV-6 gene products have been shown (in vitro) transactivation of HPV transforming genes. However, there is lack of evidence demonstrating a direct association of HHV-6 in the development of cervical cancer.

**Immune response**

Both HHV-6 species can infect several types of immune cells with their primary target being CD4+ T lymphocytes that play a major role in generating immune responses. The virus exhibits molecular mimicry as it harbors a number of host acquired genes, whose products, such as virally encoded chemokine, G-protein coupled receptors, can interfere with the normal host defense responses (27). It also modulates the cellular immune response to viruses by up-regulating the production of IFN-α in mononuclear cells, inhibiting the production of IF-γ in Peripheral Blood Mononuclear Cells (PBMC), regulating the expression of a panel of interleukins (IL) including IL-1β, IL-6, IL-8, IL-10, IL-12 & IL-18 (14). Other mechanisms of immunomodulation include alteration in the expression of cell-surface molecules for instance, up-regulating the expression of Tumor Necrosis Factor-α family of receptors & IL-2 receptors (14). Interestingly, the expression of the cellular receptor CD46, used for virus entry inside the cells, is down-regulated post infection (27). It up-regulates CD4 levels in continuous T-cell lines and down regulates CD3 expression (14). Additionally, HHV-6 infection exerts an immunosuppressive effect by inhibiting proliferative response of PBMC to mitogens and down regulating IL-2 expression (14).

**Transactivation of heterologous genes**

HHV-6 also harbors a number of genes that exhibit transactivating potential and this feature can be harnessed to develop an indicator cell line for HHV-6 viral titer assay, as
reporter for other herpesviruses. Some of the reported targets include heterologous promoters for instance, HIV-1 Long Terminal Repeat (LTR, that harbors the viral promoter region) and CD4 (28, 29). The viral genes reported to transactivate HIV-1 LTR include some genes encoded by the Immediate Early locus-A (IE-A) as well as IE-B (IE locus B) loci (14). The region within LTR that bears recognition sequence for transactivation by HHV-6 is located between -103 to -48 base pairs (bp) from the LTR cap site. Extensive studies on the LTR and HHV-6 interaction revealed that this transactivation takes place predominantly through nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) binding sites in the LTR (30).

Antivirals for HHV-6

The therapy includes drugs used to treat its most closely associated member HCMV, as till date no particular drug is specifically approved for the treatment of acute HHV-6 infections. Commonly used drugs include those targeting the viral DNA replication, for instance Foscarnet (pyrophosphate analog), Ganciclovir and Cidofovir (nucleoside analogs) (31). Potential new anti-HHV-6 agents are currently in various stages of development and include drugs targeting all double stranded DNA viruses and even those targeting the malaria parasite (32). Most of them are experimentally available although none have commercial approval.

Background of the present studies

Previous studies from this laboratory reported HHV-6 status in specific immunocompromised conditions. The virus was frequently found activated both in HL and NHL (23). The status was also evaluated in HIV infected mothers and their newborns, and it was found that perinatally co-transmitted HHV-6 was always activated in the neonates born with HIV infection (33). Additionally, while evaluating the cellular distribution of the virus in PBMC samples obtained from immunocompetent healthy
individuals, one individual presented with unusually high amount of the viral DNA showing the presence of single copy of the virus per cell in preliminary observations. Subsequently, an HHV-6 positive B cell line, designated as PJH6, was derived from the PBMC of the said individual by EBV transformation. The present work was aimed at a comprehensive characterization of this new HHV-6 isolate along with development of a simple assay for HHV-6 titration.

Aims and Objectives

- Ultra structure of the isolate and virus infectivity study.
- Chromosomal integration profile of the isolate.
- Role of viral IE gene products on activation of select cellular genes.
- Harnessing the isolate to develop a basic gene transfer vector.
- Development of a single step relative viral titer assay.