Human herpesvirus 6: molecular biology and clinical features

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Human herpesvirus 6 (HHV-6) exists as distinct variants HHV-6A and HHV-6B. The complete genomes of HHV-6A and HHV-6B have been sequenced. HHV-6B contains 97 unique genes. CD46 is the cell receptor for HHV-6, explaining its broad tissue tropism but its restricted host-species range. HHV-6 utilizes a number of strategies to down-regulate the host immune response, including molecular mimicry by production of a functional chemokine and chemokine receptors.

Immunosuppression is enhanced by depletion of CD4 T lymphocytes via direct infection of intra-thymic progenitors and by apoptosis induction. Infection is widespread in infants between 6 months and 2 years of age. A minority of infants develop roseola infantum, but undifferentiated febrile illness is more common. Reactivation from latency occurs in immunocompromised hosts. Organ-specific clinical syndromes occasionally result, but indirect effects including interactions with other viruses such as human immunodeficiency virus type 1 and human cytomegalovirus or graft dysfunction in transplant recipients may be more significant complications in this population. Recent advances in quantitative PCR are providing additional insights into the natural history of infection in paediatric populations and immunocompromised hosts.

Introduction

Human herpesviruses 6 (HHV-6) is a human pathogen of emerging clinical significance (Hall, 1997; Levy, 1997). Salahuddin et al. (1986) were the first to isolate HHV-6, using peripheral blood lymphocytes (PBL) obtained from patients with lymphoproliferative disorders. Initially termed human B-lymphotropic virus (HBLV), cell tropism is greatest for T lymphocytes (Lusso et al., 1988). Two genetically distinct variants of the virus exist, HHV-6A and -6B (Schirmer et al., 1991). HHV-6 is a β-herpesvirus related to HHV-7 and, to a lesser extent, human cytomegalovirus (HCMV). Yaminishi et al. (1998) identified HHV-6 as a causative agent of exanthem subitum (roseola), a common childhood exanthem. HHV-6 infection is common in the first 2 years of life. Although overt clinical disease is infrequent in adults, HHV-6 reactivates with immunosuppression. HHV-6 has been linked with a variety of human diseases including multiple sclerosis (MS), although the significance of these associations is unclear (Challoner et al., 1999). Recent advances in the molecular biology of HHV-6 include the detection of the cell-surface receptor for HHV-6 and the determination of the genetic sequences of HHV-6A and -6B (Clark, 2000).

Genome

Herpesviruses are enveloped double-stranded DNA viruses with an icosahedral capsid. HHV-6 and HHV-7 form the genus Roseolavirus of the β-herpesviruses (Berneman et al., 1992). HHV-6 is separated into HHV-6A and -6B variants on the basis of distinct genetic, immunological and biological characteristics (Schirmer et al., 1991; Ablashi et al., 1995). The HHV-6 genome contains 160–162 kb, with a 143–144 kb central unique region (U) containing open reading frames (ORFs) U1–100 and flanking 8–9 kb terminal direct repeats (DR) at either end (Gompels et al., 1995; Dominguez et al., 1999; Isegawa et al., 1999). The terminal and junctional segments of the DRs contain human telomere-like repeats of undetermined function (Gompels et al., 1995). The genome contains seven regions of genes conserved amongst all herpesviruses, a group of genes found only in β-herpesviruses and several genes specific to the genus Roseolavirus (Clark, 2000). HHV-6A (U1102 strain) (Gompels et al., 1995) has been compared with HHV-6B (Z29 and HST strains) (Dominguez et al., 1999; Isegawa et al., 1999). HHV-6B contains 119 ORFs and 97 unique genes in comparison with 110 ORFs for HHV-6A. The nucleotide sequence similarity is 90%, but the DR and the region encoded between U86 and U100 (respectively 85 and 72% nucleotide sequence similarity) are more divergent (Dominguez et al., 1999). Screening of 14 different strains illustrates that differences are conserved throughout viral evolution as variant-specific features but are greater for HHV-6A strains (Isegawa et al., 1999). In addition to genetic differences, variations in reactivity with monoclonal antibodies, cell tropism and disease manifestations support the view that HHV-6A and -6B are distinct β-herpesviruses.
Little is known regarding the transcriptional regulation of IE genes: however, analysis of U95 has revealed that the R3 region, one of three major regulatory regions adjacent to IE-A, contains both NF-kB and AP-2 binding sites and enhances the U95 promoter activity (Takemoto et al., 2001). The U89 gene product, IE1, of HHV-6A is capable of transactivating heterologous promoters (Martin et al., 1991). Recently, the IE1 protein of HHV-6B has been cloned (Gravel et al., 2002). IE1 is phosphorylated on serine/threonine residues, is conjugated by the small ubiquitin-like modifier (SUMO-1) peptide and localizes to the nucleus within 4 h post-infection. IE1 from HHV-6B has only 62% amino acid identity to the equivalent protein in HHV-6A. IE1 may play a role in latency-associated gene transcription (Kondo et al., 1998). The U16/17 gene products result from differential splicing; some are IE proteins and others are late proteins (Flebbe-Rehwaldt et al., 2000). The U17/16 splice product demonstrates positional homology to HCMV IE UL36, but some of the U16–17 gene transcripts are unique to HHV-6. In general, the U17/16 transcripts show lower levels of transcription than U89. U16 transactivates the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR). U3 also has the capacity to transactivate genes (Mori et al., 1998).

HHV-6 genes involved in replication include U27, which encodes the polymerase processivity factor (Zhou et al., 1997). U27 RNA transcripts are polyadenylated and polyadenylylated and polyadenylated. The 41-kDa early protein encoded by U27 localizes to the nucleus and co-immunoprecipitates with a 100-kDa DNA polymerase (Lin & Ricciardi, 1998). This interaction enhances DNA transcription by the DNA polymerase. U41 encodes a single-stranded DNA-binding protein, U43/UL74, and U77 encode elements of the helicase/primase complex and U73 encodes the origin-binding protein (Clark, 2000). Viral replication in vitro is enhanced by the presence of multiple copies of the origin of lytic replication (oriLyt). Homologues of the consensus herpesvirus cleavage-packaging motifs pac-1 and pac-2 are found in DR4 and DR5 and come into close contact at the junction between concatameric viral genomes to form a pac-2–pac1 functional element during HHV-6 DNA replication (Deng & Dewhurst, 1998). This sequence provides the substrate for packaging and cleavage of unit-length HHV-6 genomes required for the formation of mature virions. The sequence enhances the replication of oriLyt-containing plasmids in infected cells and possesses a novel S1 nuclease-sensitive conformation. U94 encodes a homologue of the human adenovirus-associated virus type 2 (AAV-2) rep gene (Thomson et al., 1991). Interestingly, this gene is one of only two HHV-6 genes without a homologue in HHV-7. The gene product, RepH6, can restore function when expressed in Rep-defective AAV-2 mutants. It is transcribed under IE conditions and plays a role in viral DNA replication and gene regulation and inhibits both cell transformation and transcription from the HIV-1 LTR. RepH6 is transcribed in latently infected lymphocytes and contributes to the maintenance of latency: stable expression of RepH6 results in susceptibility to infection but low-level viral replication and gene transcription in the absence of cytotoxic effects (Rotola et al., 1998). RepH6 localizes to the nucleus and binds human TATA-binding protein, a transcription factor (Mori et al., 2000). Genes involved in nucleic acid metabolism include U28, which encodes the R1 subunit of the ribonucleotide reductase, U45, a dUTPase, U69, a phosphotransferase, and U81, a uracil-DNA glycosylase (Clark, 2000).

The genes U39 and U48 encode the conserved surface glycoproteins gB and gH, which contribute to virus-cell fusion. HHV-6 gB has 39% amino acid sequence identity to HCMV gB, the amino-terminal portion of which contains epitopes recognized by neutralizing antibodies (Chou & Marousek, 1992). This suggests potential immunological cross-reactivity between HCMV and HHV-6 gB. HHV-6 gH forms complexes with glycoprotein gl (encoded by U82), resulting in the formation of a gp100 complex (Liu et al., 1993). HCMV gH and gl can substitute for the comparable HHV-6 glycoproteins and heterologous complexes result (Anderson et al., 1996). A heterologous complex of HCMV gl with HHV-6 gH is particularly stable. gH/gl complexes play a role in cell infection and fusion of infected cells and, during cellular co-infection, might contribute to β-herpesvirus interactions in vivo. gp100 plays a role in the transport and processing of gH (Isegawa et al., 1999). U72 encodes gM and U100 the glycoprotein gp82–105 that is unique to the genus Roseolovirus of human herpesviruses. The U100 gene is subject to differential splicing, and a number of envelope-expressed polypeptides result. In contrast to the other glycoprotein-encoding genes, U100 of HHV-6A and -6B demonstrate only 72.1% sequence identity (Isegawa et al., 1999). This glycoprotein may therefore have a role in the differential effects of HHV-6A and -6B infections. Along with gB and gH, gp82–105 contains epitopes recognized by neutralizing antibodies and therefore represents a target for variant-specific neutralizing antibodies (Pfeiffer et al., 1993). U11 encodes the major structural antigen p100, a phosphoprotein, which differs between HHV-6A and -6B. In contrast to HHV-6A and -6B infections. Along with gB and gH, gp82–105 contains epitopes recognized by neutralizing antibodies and therefore represents a target for variant-specific neutralizing antibodies (Pfeiffer et al., 1993). U11 encodes the major structural antigen p100, a phosphoprotein, which differs between HHV-6A and -6B, with only 80.1% sequence identity (Isegawa et al., 1999).

An unusual feature of HHV-6 in comparison to other herpesviruses is the lack of viral glycoproteins in the plasma membrane. Viral glycoproteins are stored in newly formed annulate lamellae, in which they may undergo O-glycosylation (Cardinall et al., 1998). It is proposed that, during viral
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morphogenesis, nucleocapsids released from the nucleus have a primary envelope that lacks glycoproteins but, in the cytoplasm, this is removed and replaced by a secondary envelope containing glycoproteins acquired from the annulate lamellae (Torrisi et al., 1999). Further modification of glycoproteins by glycosylation during transit through the Golgi apparatus occurs before mature virions are released.

Capsid maturation, DNA packaging, glycoprotein modification and the formation of mature virus all require the U53-encoded protease. The protease undergoes auto-processing at two sites, which could represent targets for novel antivirals against HHV-6 (Tigue & Kay, 1998). Another target for antivirals is the protein kinase encoded by U69, a homologue of HCMV UL97 (Ansari & Emery, 1999). Expression of this gene using recombinant baculoviruses confers ganciclovir resistance against HHV-6 (Tigue & Kay, 1998). Another target for antivirals is the protein kinase encoded by U69, a homologue of HCMV UL97 (Ansari & Emery, 1999). Expression of this gene using recombinant baculoviruses confers ganciclovir sensitivity.

Herpesviruses provide examples of viral piracy of host genes. U83 encodes a functional chemokine (Zou et al., 1999). Although the gene has relatively little sequence similarity to human chemokine genes, the protein expressed has the typical cysteine residues of a chemokine, transduces signals that involve calcium fluxes and induces chemotactic activation. The U83 gene is regulated by novel splicing, with an alternative cysteine residues of a chemokine, transduces signals that involve calcium fluxes and induces chemotactic activation. The U83 gene is regulated by novel splicing, with an incomplete transcript being produced in the absence of HHV-6 protein production, as occurs with IE genes, and the full transcript being produced as a late gene (French et al., 1999). The chemokine may correspond to members of the CC or CXC chemokines that recruit lymphocytes and monocytes. U12 encodes a functional β-chemokine receptor (Isegawa et al., 1998). U51 also encodes a β-chemokine receptor whose intracellular trafficking is regulated in cell-specific fashion such that it is transported to the cell surface only in activated T lymphocytes and not in cell lines of non-lymphoid origin (Menotti et al., 1999). The U51-encoded chemokine receptor binds chemokines and also down-regulates the chemokine that binds to CCR5, RANTES (regulated upon activation, normal T-cell expressed and secreted), suggesting a novel immunomodulatory mechanism for HHV-6 (Milne et al., 2000).

DR7 encodes a protein with malignant transforming capability that binds the tumour-suppressor protein p53 and transactivates the HIV-1 LTR (Kashanchi et al., 1997). Table 1 summarizes some of these genes.

Cell tropism

HHV-6 replicates in activated CD4 T lymphocytes in vivo (Lusso et al., 1991; Takahashi et al., 1989). HHV-6A and -6B variants differ in the respective ability with which they can replicate in specific transformed T-lymphocyte cell lines. In addition, CD8 T lymphocytes, γδ T lymphocytes and natural killer (NK) cells support HHV-6 replication in association with surface expression of CD4 (Lusso et al., 1991, 1993, 1995). CD4 T lymphocytes can be dually infected with HHV-6 and HIV-1 (Lusso et al., 1989). HHV-6 can infect macrophages, dendritic cells, fibroblasts, epithelial cells and bone-marrow progenitors (Kempf et al., 1997; Asada et al., 1999; Robert et al., 1996; Simmons et al., 1992; Luppi et al., 1999). HHV-6 blocks the differentiation of macrophages from bone-marrow progenitors (Burd et al., 1993) and decreases in-vitro colony formation from both granulocyte/macrophage and erythroid progenitors (Isoyama et al., 1997). Both HHV-6 variants infect human fetal astrocytes in vitro (He et al., 1996), but HHV-6A may have greater neurotropism in vivo (Hall et al., 1998).

HHV-6 is characterized by a broad tropism for human cell types but a narrow range of host species. Santoro et al. (1999) identified the cellular receptor for HHV-6, CD46. CD46 is a ubiquitous type-1 glycoprotein that is a member of a family of regulators of complement activation (Santoro et al., 1999). Some T-cell lines, despite expressing CD46, are unable to support replication of HHV-6A, suggesting that co-receptors may exist. In peripheral blood mononuclear cells, activation stimuli can reactivate HHV-7 from latency but not HHV-6B (Katsafanas et al., 1996). Reactivation of HHV-6B from latently infected cells following activation only occurs after infection with HHV-7. This suggests that, in CD4 T lymphocytes, HHV-6B reactivation may be preceded by HHV-7 replication that is subsequently down-regulated by HHV-6B replication.

Immunology

In children with primary infection, anti-HHV-6 antibody is detectable from 3–7 days (Dockrell et al., 1999a). IgM production peaks in the second week and is detectable for 2 months after infection. IgG antibodies rise by 2 weeks post-infection and are detectable for life in 90% or more of adults (Yamanishi et al., 1988; Dockrell et al., 1999a; Robinson, 1994). Neutralizing antibodies recognize linear and conformational epitopes on gB, gH and gp82–105 (Pfeiffer et al., 1993). Reactivation of infection induces secondary antibody rises. Despite glycoprotein homology between β-herpesviruses, dual antibody increases to HCMV and HHV-6 are usually associated with specific antibodies against each virus, not cross-reactive antibodies (Irving et al., 1990). Cell-mediated immunity against HHV-6 is a critical element of the host defence. Analysis of proliferative responses and IFN-γ production by T-lymphocyte clones in response to β-herpesvirus confirms that the majority of reacting clones respond to specific HHV-6 antigens and not common β-herpesvirus or variant-specific antigens (Yasukawa et al., 1993). Individuals with defects in NK cell function are susceptible to herpesvirus infections. HHV-6 infection of NK cells results in increased production of IL-15 that both increases NK-mediated killing of HHV-6 and stimulates IFN-γ production from CD4 T lymphocytes and NK cells (Flamand et al., 1996; Gosselin et al., 1999).

One of the most intriguing features of HHV-6 is its ability to evade host immune defences. HHV-6 induces CD4 T-lymphocyte depletion. Although the narrow host range of HHV-6 has limited the utility of animal models, severe combined immunodeficiency (SCID) mice implanted with human fetal thymus and liver (huSCID Thy/Liv mice) have
provided a useful model (Gobbi et al., 1999). In this model, thymocyte depletion is induced in directly infected cells in HHV-6A- or -6B-infected mice. Although all subpopulations decline, intra-thymic T progenitor cells are particularly susceptible. Progressive destruction of the thymus could induce T-lymphocyte depletion by affecting T-lymphocyte development. A further mechanism of T-lymphocyte depletion is the induction of apoptosis (Inoue et al., 1997). Both HHV-6A and -6B induce apoptosis in the CD4⁺ T-cell line JHAN. The majority of apoptotic cells are uninfected cells and apoptosis induction is independent of viral replication, as evidenced by apoptosis induction by UV light-irradiated and ultracentrifuged HHV-6 cell-culture supernatant. Furthermore, TNF-α and Fas cross-linking enhance the observed apoptosis. In contrast, the induction of apoptosis in CD4⁺ cord-blood lymphocytes occurs in

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<tr>
<th>Gene</th>
<th>Gene product</th>
<th>Comments</th>
<th>Reference(s)</th>
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<tr>
<td>IE proteins</td>
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<tr>
<td>U89 IE protein (IE1)</td>
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<td></td>
<td>Martin et al. (1991); Gravel et al. (2002)</td>
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<tr>
<td>U95 IE protein</td>
<td>Homologue of MCMV IE2</td>
<td></td>
<td>Takemoto et al. (2001)</td>
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<tr>
<td>U3 IE protein</td>
<td>Member of β-herpesvirus US22 gene family; homologue of HCMV UL24</td>
<td></td>
<td>Mori et al. (1998)</td>
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<tr>
<td>Genes required for DNA synthesis and viral replication</td>
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<tr>
<td>U27 Polymerase processivity factor</td>
<td>41-kDa nuclear protein; binds DNA polymerase</td>
<td>Zhou et al. (1997)</td>
<td></td>
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<tr>
<td>U38 DNA polymerase</td>
<td>100-kDa protein</td>
<td></td>
<td>Lin &amp; Ricciardi (1998)</td>
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<tr>
<td>U43/7477 Helicase/primer complex</td>
<td></td>
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<td>Clark (2000)</td>
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<td>U73 Origin-binding protein</td>
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<td>Clark (2000)</td>
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<tr>
<td>U94 RepH6</td>
<td>Homologue of AAV-2 rep gene, unique to HHV-6. Binds human TATA-binding protein (transcription factor). Involved in maintenance of latency by down-regulating gene transcription. Also inhibits cell transformation and inhibits transactivation of HIV-1 LTR.</td>
<td>Thomson et al. (1991); Rotała et al. (1998); Mori et al. (2000)</td>
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<td>Nucleic acid metabolism</td>
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<tr>
<td>U28 R1 subunit of ribonucleotide reductase</td>
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<td>Clark (2000)</td>
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<tr>
<td>U45 dUTPase</td>
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<td>U69 Phosphotransferase</td>
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<td>U81 Uracil-DNA glycosylase</td>
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<td>Glycoproteins and structural antigens</td>
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<tr>
<td>U11 p100 major structural antigen</td>
<td>Phosphoprotein that varies between HHV-6A and -6B</td>
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<td>Isegawa et al. (1999)</td>
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<td>U39 gH</td>
<td>Conserved glycoprotein with role in virus–cell fusion</td>
<td></td>
<td>Chou &amp; Marousek (1992)</td>
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<td>U48 gI</td>
<td>Forms complexes with gL. Role in virus–cell fusion</td>
<td></td>
<td>Liu et al. (1993); Anderson et al. (1996)</td>
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<tr>
<td>U72 gM</td>
<td>Conserved glycoprotein</td>
<td></td>
<td>Liu et al. (1993); Anderson et al. (1996)</td>
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<tr>
<td>U82 gL</td>
<td>Glycoprotein with role in transport and processing of gH. Complexes with gH</td>
<td></td>
<td>Pfeiffer et al. (1993)</td>
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<td>U100 gp82–105</td>
<td>Glycoprotein with marked variation between HHV-6A and -6B</td>
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<td>Miscellaneous</td>
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<tr>
<td>U33 Pro tease</td>
<td>Required for mature virion production</td>
<td></td>
<td>Tigue &amp; Kay (1998)</td>
</tr>
<tr>
<td>U83 Viral chemokine</td>
<td>Novel splicing results in different transcripts</td>
<td></td>
<td>Zou et al. (1999); French et al. (1999)</td>
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<tr>
<td>U12 Chemokine receptor</td>
<td>β-Chemokine receptor</td>
<td></td>
<td>Isegawa et al. (1998)</td>
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<tr>
<td>U51 Chemokine receptor</td>
<td>β-Chemokine receptor. Signalling can result in down-regulation in RANTES</td>
<td></td>
<td>Menotti et al. (1999); Milne et al. (2000)</td>
</tr>
<tr>
<td>U69 Protein kinase</td>
<td>Confers ganciclovir sensitivity</td>
<td></td>
<td>Ansari &amp; Emery (1999)</td>
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<tr>
<td>DR7 p53-binding protein</td>
<td>Transforming activity and transactivation of HIV-1 LTR</td>
<td></td>
<td>Kashanchi et al. (1997)</td>
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directly infected cells and does not appear to involve TNF-α or Fas (Ichimi et al., 1999). In a further study using cord-blood mononuclear cells, p53 played a role in apoptosis induction (Kim et al., 1997). HHV-6-induced apoptosis of CD4 T lymphocytes has also been demonstrated ex vivo in PBL derived from patients with acute HHV-6 infection (Yasukawa et al., 1998). HHV-6A but not -6B can also deplete CD4 T-lymphocyte numbers by inducing CD46-mediated cell fusion, which is dependent on gB and gH (Mori et al., 2002). HHV-6 also decreases PBL proliferation via transcriptional down-regulation of IL-2 in mitogen-stimulated purified T-lymphocyte populations in vitro (Flamand et al., 1995). In addition, HHV-6 down-regulates CDS transcription (Lusso et al., 1991). HHV-6 infection of monocytes in vitro results in decreased generation of reactive oxygen intermediates after certain stimuli (Burd & Carrigan, 1993).

HHV-6 adds to the net state of immunosuppression in HIV-1-co-infected individuals (Lusso & Gallo, 1995). However, the exact relationship between HHV-6 and HIV-1 has been controversial. U86 and U89 transactivate the CD4 promoter (Flamand et al., 1998). By up-regulating CD4 expression on CD4 T lymphocytes and extending the range of cells expressing CD4 to include CD8 T lymphocytes, HHV-6 can potentially increase the range of cells susceptible to HIV-1 infection (Lusso et al., 1991, 1995). HHV-6 transactivates the HIV-1 LTR via U17/16 and U16-gene products (Flebbe-Rehwaldt et al., 2000; Lusso et al., 1989). HHV-6 also up-regulates cytokines, including IL-1β and TNF-α, that could increase HIV-1 replication (Flamand et al., 1991). Nevertheless, some reports suggest that HHV-6A or -6B down-regulates HIV-1 replication in vitro (Levy et al., 1990a; Carrigan et al., 1990). There is also disagreement as to the effect of HIV-1 on HHV-6 replication (Levy et al., 1990a; Sieczkowski et al., 1995). In HIV-seropositive individuals with CD4 T-lymphocyte counts < 400 cells ml⁻¹, HHV-6 DNA is detected less frequently and is present at lower copy number in peripheral blood mononuclear cells than in individuals with counts > 400 cells ml⁻¹ (Fairfax et al., 1994). It is unclear, however, whether this reflects differential HHV-6 replication or death of subpopulations of CD4 T lymphocytes that support HHV-6 replication in individuals with more advanced HIV-1 infection.

In vivo HIV-1 proviral DNA levels are higher in tissues co-infected with HIV-6 (Emery et al., 1999) while, in patients with AIDS, HHV-6 is found in a higher proportion of tissue specimens than in HIV-seronegative individuals (Corbellino et al., 1993). Furthermore, in children with vertically acquired HIV infection, HHV-6 infection in the first year of life is associated with a more rapid progression of HIV infection (Kositanont et al., 1999). Despite these observations, the SCID-hu Thy/Liv mouse, when infected with HHV-6A or -6B and CXCR4-tropic HIV-1 (the intra-thymic T-progenitor cell is susceptible to infection with R4 virus), provides no evidence for up-regulation of either virus or of enhanced cytotoxicity, despite replication of both viruses in the thymic tissue (Gobbi et al., 2000). An explanation that potentially accounts for this experimental variation has been provided recently (Grivel et al., 2001). Using human tonsil cultured ex vivo and infected with HHV-6A and either a CXCR4 (T-tropic) or CCR5 (M-tropic) strain of HIV-1, it has been demonstrated that HHV-6 selectively suppresses the CCR5-tropic virus by up-regulating RANTES. RANTES is known to enhance replication of CXCR4-tropic virus; therefore, HHV-6A replication could contribute to the shift from CCR5-tropic to CXCR4-tropic virus in late-stage infection.

HHV-6 also modulates the inflammatory response to infection. Using a nylon-based immunomicroarray containing over 1000 immune response-related genes, it has been found that HHV-6A or -6B down-regulates the chemokine receptor CXCR4 in directly infected CD4 T lymphocytes by decreasing the expression of the CXCR4 gene repressor, YY1, and c-Myc, altering the chemotactic response of directly infected cells (Yasukawa et al., 1999; Hasegawa et al., 2001).

**Epidemiology**

Infection with HHV-6 is ubiquitous in the first 2 years of life. The incidence of infection peaks at 6–9 months (Casey et al., 2001). This is earlier than the peak for HHV-7 infection. Seroprevalence studies suggest that more than 90 % of adults are seropositive for infection (Levy et al., 1990b; Saxinger et al., 1988). Infection occurs worldwide, without geographical restriction (Okuno et al., 1989; Krueger et al., 1998; Knowles & Gardner, 1988). Seropositivity may fall with advanced age, giving the false impression of primary infection in older age groups (Brown et al., 1988). Seroprevalence studies provide little data on variant-specific infections. However, it is believed that the majority of clinical infections in immunocompetent hosts are HHV-6B infections and that HHV-6A contributes to infections in immunocompromised hosts and some neurological manifestations (Hall et al., 1998). The mode of transmission is unclear, but HHV-6 is present in saliva and replicates in epitelial cells, suggesting that oral secretions contribute to transmission, especially of HHV-6B (Clark, 2000; Simmons et al., 1992; Levy et al., 1990b). Sequence analysis of HHV-6 isolated from mother/infant pairs suggests that mother-to-infant transmission can occur (van Loon et al., 1995). There is no convincing evidence of sexual spread. Siblings and low parental income are risk factors for early HHV-6 infection (Lanphear et al., 1998).

**Clinical features in immunocompetent hosts**

A subset of infected children develop the childhood exanthem roseola infantum (exanthem subitum) with high fever and the development of a rash after resolution of fever
(Yamanishi et al., 1988). Roseola may also be caused by HHV-7. However, only 17% of children with acute infection develop roseola, and the majority develop an undifferentiated febrile illness (Hall et al., 1994; Pruksanarondna et al., 1992). In a large prospective study involving emergency room visits for acute infections in children under the age of 3 years, primary HHV-6 accounted for 10% of febrile illness overall and 21% in the children aged between 6 and 12 months (Hall et al., 1994). Of children with primary HHV-6, 13% had febrile seizures, and these accounted for one-third of all febrile seizures in children less than 2 years of age. Rare complications included hepatitis, arthritis, encephalopathy and haemophagocytosis syndrome (Hall et al., 1994; Take gi et al., 1996). Uvulo-palatoglossal junctional ulcers provide a useful early clinical sign of roseola (Chua et al., 2000). In contrast to western populations, a study of paediatric disease in Zambia has found that HHV-6A is a common cause of infection (Kasolo et al., 1997).

Primary infection in older age groups is rare. The most common features are an undifferentiated febrile illness or infectious mononucleosis-like illness (Niederman et al., 1988; Akashi et al., 1993; Irving & Cunningham, 1990). Skin rash, hepatitis and atypical lymphocytosis may occur. HHV-6 may reactivate in immunocompetent individuals during pregnancy or periods of critical illness requiring admission to intensive-care units (Dahl et al., 1999; Razonable et al., 2002). In these studies, there is no evidence of HCMV or HHV-7 reactivation and reactivation has been asymptomatic, so the significance is unclear. In the study involving intensive-care units, almost all episodes were due to HHV-6A (Razonable et al., 2002).

There are also numerous reports of other possible disease associations involving HHV-6. The difficulty with these studies is that, as HHV-6 infection is ubiquitous and the tissue tropism widespread, the detection of HHV-6 DNA in a pathological condition may be a consequence of viral reactivation by a pathological condition rather than the aetiological cause. A biological explanation of how HHV-6 might contribute to the pathogenesis of the condition is usually lacking.

The association of HHV-6 with MS is one example (Challoner et al., 1995). HHV-6B is detected in brains of both nont-transplanted and transplant (BMT) recipients and 32% (range 0–82%) for solid organ transplant recipients have been reported (Dockrell & Paya, 2001). Prospective studies employing DNA detection by PCR or viral culture in sequential blood samples post-transplantation provide the strongest evidence for HHV-6 infection. Studies that diagnose HHV-6 infection by culture or DNA detection in urine or saliva result in detection of viral shedding during latent infection, a phenomenon common to other herpesviruses including HCMV and one that leads to an overestimate of the incidence of active infection. The majority of HHV-6 infections post-transplantation are due to reactivation of HHV-6B, and the peak incidence of infection is 2–4 weeks post-transplantation (Singh & Carrigan, 1996; Griffiths et al., 2000). However, cases of primary infection due to transmis sion of the virus in donor tissue have been described, and superinfection with a donor strain distinct from the strain latent in the recipient may occur (Lau et al., 1998). Anti- lymphocyte globulin treatment is a risk factor for HHV-6 infection post-transplantation (Jacobs et al., 1994).

Case reports describe disease associations, but prospective studies suggest these are rare. The strongest clinical associations are with undifferentiated febrile illness, encephalitis and bone marrow suppression. In a cohort of orthotopic liver transplant (OLT) recipients, HHV-6 infection accounted for
11% of febrile episodes (Chang et al., 1998), although the exact contribution will vary depending on the HCMV-preventive strategy employed. Fever may be high (>41°C) and associated with leukopenia (Jacobs et al., 1994). A non-specific rash may occur in association with HHV-6 infection post-transplantation (Yoshikawa et al., 2002). Interstitial pneumonitis is most frequent in BMT recipients (Cone et al., 1993, 1994; Carrigan et al., 1991). Dual infections with both HHV-6B and -6A may occur. In contrast to other aetiological agents of interstitial pneumonitis, the outcome may be more favourable with HHV-6 (Cone et al., 1993, 1994, 1996; Carrigan et al., 1991). Hepatitis is associated in particular with OLTT recipients and presents a mixed pattern of liver function abnormalities (Ward et al., 1989). HHV-6 viraemia modifies the natural history of hepatitis C virus (HCV) infection post-transplantation (Singh et al., 2002). The frequency of HCV recurrence in OLTT recipients is unaltered, but higher fibrosis scores result when HCV recurrences are associated with HHV-6 viraemia.

Clinical features of encephalitis include headache, seizures, confusion, abnormal movements and disturbance in higher mental function (Singh & Paterson, 2000). Cerebrospinal fluid (CSF) analysis may be normal or, less frequently, reveals a mononuclear cell infiltrate of lymphocytes and sometimes monocytes with elevated protein levels. The magnetic resonance imaging (MRI) is often normal. Symmetrical non-enhancing white matter lesions or grey matter lesions occur occasionally. These MRI appearances must be distinguished from the non-enhancing white matter lesions associated with immunosuppression-induced encephalopathy. Both HHV-6A and -6B can be detected in pathological specimens, and ante-mortem diagnosis can be aided by detecting HHV-6 DNA in the CSF. HHV-6 DNA detection in CSF correlates with the presence of central nervous system symptoms (Wang et al., 1999). The risk of encephalitis in BMT recipients may be increased by the use of anti-CD3 monoclonal antibody for acute graft-versus-host disease prophylaxis (Zerr et al., 2001). Encephalitis mortality can exceed 50% (Singh & Paterson, 2000).

Idiopathic bone marrow suppression is more common in BMT or stem-cell transplant recipients, but can also occur following solid organ transplantation (Carrigan & Knox, 1994; Singh et al., 1997; Wang et al., 1996; Drobyski et al., 1993). In those cases without other aetiological agents identified, HHV-6 can be cultured in the bone marrow (Drobyski et al., 1993). All cell lineages can be involved, but leukocytes and platelets are most often suppressed (Knox & Carrigan, 1992). Potential causes of marrow suppression include indirect effects mediated by cytokines and HHV-6 soluble products or direct infection of bone-marrow progenitors (Luppi et al., 1999; Isomura et al., 1997; Knox & Carrigan, 1992). HHV-6A may induce a more severe form of bone marrow suppression that occurs later in the post-transplant period than that associated with HHV-6B (Carrigan & Knox, 1994; Rosenfeld et al., 1995). Risk factors for bone marrow suppression include HHV-6 infection prior to engraftment and high levels of HHV-6 DNA in PBL (Imbert-Marcille et al., 2000; Ljungman et al., 2000).

The indirect clinical sequelae of HHV-6 post-transplantation include altering the net state of immunosuppression with modification of the natural history of HCMV infection. HHV-6 infection is associated with severe clinical symptoms in solid organ transplant recipients if concomitant HCMV infection occurs (Herbein et al., 1996). Serological evidence of HHV-6 infection in OLTT or renal transplant recipients is an independent risk factor for the development of HCMV disease and, in particular, disease with organ involvement (Dockrell et al., 1997; Desjardin et al., 1998, 2001). As serology is an insensitive method of detecting infection in this population, these studies suggest that serological evidence of HHV-6 infection is a marker of HCMV disease. Potential explanations include direct viral interaction in vivo or relate to the degree of immunosuppression required for reactivation of both viruses. Using quantitative PCR to determine levels of HHV-6 and HCMV DNA post-transplantation, the level of HCMV DNA and the presence of HCMV disease are related to the level of HHV-6 DNA and HHV-7 DNA (Mendez et al., 2001). In another study using quantitative PCR, a higher level of HCMV DNA was associated with the presence of HHV-6 DNA in peripheral blood cells (Humar et al., 2000). In a further study, HHV-7 DNA with or without HHV-6 DNA was associated with the development of HCMV disease (Osman et al., 1996). Other prospective studies have, however, failed to demonstrate an association between HCMV disease and HHV-6 DNA detection, although they have suggested an association with HHV-7 DNA (Kidd et al., 2000; Tong et al., 2000). These studies, when related to in vitro data, suggest an association but do not prove a causal relationship between HHV-6 and/or HHV-7 reactivation and HCMV disease. The picture is clearly complex and the ability to detect the exact timing of reactivation and to distinguish reactivation from latent infection is critical. Of note, Griffiths et al. (1999) have related time of detection of HHV-6 DNA (median time 20 days) to that of HHV-7 (median time 26 days) and HCMV (median time 36 days). In addition to an association with HCMV disease, HHV-6 infection post-transplantation is associated with fungal infection (Dockrell et al., 1999b; Rogers et al., 2000) and, in association with HCMV, has been linked to graft dysfunction, biopsy-proven graft rejection or episodes of rejection 30 days or more post-transplantation (Griffiths et al., 1999; Lautenschläger et al., 1998; Humar et al., 2002). Although HHV-6 up-regulates Epstein–Barr virus replication in vitro (Flamand et al., 1995), the relationship between these viruses in vivo requires clarification (Lin et al., 1999).

Diagnosis

HHV-6 can be cultured from clinical specimens by co-culture with lymphoblasts prepared by IL-2 and PHA treatment of PBL (Yamamishi et al., 1988). PBL from the patient may be used directly to produce lymphoblasts. Cord blood cells may also be used to culture HHV-6. Evidence of
viral replication is provided by demonstration of cytopathic effect and HHV-6 antigen using monoclonal antibodies (Singh & Carrigan, 1996). Culture is time-consuming but time to diagnosis is decreased using a shell vial technique similar to that used for HCMV. Serological diagnosis most often employs immunofluorescence assay (IFA), using test slides with treatment of the sample with IgG absorbent, or an enzyme immunoassay (EIA) (Chiu et al., 1998; Chou & Scott, 1990). Appropriate controls should be used to exclude cross-reactions. The presence of anti-HHV-6 IgM or a fourfold rise in anti-HHV-6 IgG supports a clinical diagnosis in an immunocompetent individual likely to be seroconverting, such as a small child. Serological tests provide epidemiological data but provide only retrospective diagnosis. IgM responses may be absent or may result in cases other than primary infection, serological cross-reactions with other β-herpesviruses may occur and serology is insensitive in immunocompromised populations. Biopsy specimens aid in the diagnosis of organ-specific syndromes, particularly in immunocompromised individuals by demonstrating cytopathic effects and HHV-6 antigens, although it should be noted that owl’s eye inclusions characteristic of HCMV infection do not occur with HHV-6 infection (Mattes et al., 2000).

Increasingly, diagnosis of HHV-6 is by PCR-based methods. These techniques are rapid, utilize samples obtained by noninvasive techniques and are sensitive in immunocompromised populations. Qualitative PCR may be insensitive at distinguishing latent from active infection; however, modifications that raise the threshold above that encountered in peripheral blood mononuclear cells during latent infection or that utilize acellular samples have been described (Secchiero et al., 1995a; Carrigan, 1995). A multiplex PCR allows for simultaneous detection of HHV-6A, -6B and -7 (Kidd et al., 1998). Quantitative PCR assays using internal standards and known quantities of cloned virus have been developed (Secchiero et al., 1995b). RT-PCR using blood cells or biological fluids facilitates detection of active infection (van den Bosch et al., 2001). However, early quantitative PCR assays were labour-intensive and real-time quantitative PCR using the TaqMan reaction combines single-step amplification with computer-based analysis (Locatelli et al., 2000; Gautheret-Dejean et al., 2002). These assays have sensitivity equivalent to nested PCR protocols, a wide dynamic range and are reproducible. Detection of HHV-6 DNA is an accurate way of diagnosing primary infection in paediatric populations (Chiu et al., 1998). Although HHV-6 DNA detection in whole blood has only a 57% positive predictive value for primary infection, the presence of HHV-6 DNA in whole blood in the absence of specific IgG antibody, HHV-6 DNA in plasma or a high HHV-6 viral load are predictive of primary infection (Chiu et al., 1998). In a further study, a high HHV-6 viral load in peripheral blood mononuclear cells or the combination of HHV-6 DNA in peripheral blood mononuclear cells in the absence of HHV-6 DNA in saliva was suggestive of primary infection (Clark et al., 1997). Real-time PCR is a sensitive way of detecting HHV-6 DNA in transplant populations, although one study has suggested that, in this population, strain-specific differences may occur: HHV-6A and -6B are detected in plasma but only HHV-6B DNA in PBL (Nitsche et al., 2001). An alternative approach to virus quantification is provided by antigenaemia assays, as are used for the detection of HCMV, and these methods have also been applied to HHV-6 diagnosis post-transplantation (Lautenschlager et al., 2000).

**Therapy**

HHV-6 infection in immunocompetent children is usually self-limited and does not require antiviral therapy. In immunocompromised individuals, rare cases with organ-specific syndromes, such as encephalitis, necessitate specific therapy. Future studies need to address whether pre-emptive therapy initiated on the basis of a rising HHV-6 viral load is a valid strategy to prevent indirect effects such as HCMV reactivation or graft rejection in transplant recipients. Ganciclovir, foscarnet and cidofovir are active against HHV-6 in vitro, but acyclovir is not (Singh & Carrigan, 1996; Yoshida et al., 1998). Cidofovir has the best inhibitory activity in vitro (Yoshida et al., 1998). Ganciclovir is active against both HHV-6A and -6B, although the 50% effective inhibitory concentration is higher for HHV-6B. Foscarnet is also effective in vitro against both HHV-6A and -6B. Case reports and small series demonstrate the efficacy of ganciclovir and foscarnet in immunocompromised hosts with organ-specific syndromes (Singh & Paterson, 2000; Wang et al., 1999). The decrease in HHV-6 DNA level parallels that of HCMV during ganciclovir and/or foscarnet infusion post-transplantation (Mendez et al., 2001; Zerr et al., 2002). A single isolate of HHV-6A has remained detectable in an immunocompromised patient despite both ganciclovir and foscarnet therapy, suggesting that in vitro studies may not always predict in vivo response (Zerr et al., 2002). Despite a lack of efficacy in vitro, high-dose acyclovir prophylaxis has decreased HHV-6 DNA detection in BMT patients post-transplantation (Wang et al., 1996). The HHV-6 homologue of HCMV UL97 is U69 and, like its HCMV homologue, point mutations in U69 are associated with decreased susceptibility to ganciclovir in vitro and with prolonged exposure to ganciclovir in vivo (Manichanh et al., 2001).

**Conclusions**

Infection with HHV-6A and -6B is ubiquitous. Although clinical diseases are rarely recognized in association with these viruses in immunocompetent adults, they still have significant potential as human pathogens. The indirect effects of reactivation in immunocompromised populations are still poorly understood. Future studies need to improve our understanding of areas such as the differences between the two HHV-6 variants, the maintenance of latency, the basis of HHV-6-associated immunosuppression and the extent of disease associations. The recent availability of the complete sequences of HHV-6A and -6B can facilitate these investigations.
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References


Human herpesvirus 6


