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., 1995). 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 (ZZ9 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.
HHV-6 gene transcription follows the co-ordinated pattern that characterizes herpesviruses, with immediate-early (IE), early and late proteins expressed (Mirandola et al., 1998). IE proteins are synthesized within minutes to hours of infection, are independent of de novo protein synthesis and often require virion-associated proteins for expression. Variant-specific differences in the temporal regulation and splicing patterns of IE protein-encoding genes have been noted. Transcriptional transactivators encoded include the gene products of U86–89 (encoded in gene block IE-A) and U16–19 (encoded in gene block IE-B) (Gompels et al., 1995; Schiewe et al., 1994). Homologues of the HCMV US22 family of IE genes include U3 and U95 (Takemoto et al., 2001).

Little is known regarding the transcriptional regulation of IE genes: however, analysis of U95 has revealed that the R3 region, one of three major repetitive 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) 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., 2002). The U16/17 gene products result from differential splicing; some are IE proteins and others are late proteins (Fiebbe-Rehwaldt et al., 2000). The U17/16 splice product demonstrates positional homology to HCMV IE UL56, 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 (HHV-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 poly-ribosome-associated, confirming a role in protein synthesis. 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/UL77 encodes 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-I and pac-2 are found in DR4 and DR6 and come into close contact at the junction between concatameric viral genomes to form a pac2–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 adeno-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 HHV-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 cytopathic 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. gl 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 Roaovirus 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 (Pfleiffer 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 (Cardinale et al., 1998). It is proposed that, during viral
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 when recombining 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 expression that involves 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 CCR5 ligand-receptor class, which mediate chemokine activation.

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% of 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 JJHAN in vitro. 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene product</th>
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<tr>
<td>IE proteins</td>
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<td>U89</td>
<td>IE protein (IE1)</td>
<td>Transcriptional transactivator. Nuclear phosphoprotein</td>
<td>Martin et al. (1991); Gravel et al. (2002)</td>
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<td>IE protein</td>
<td>Homologue of MCMV IE2</td>
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<td>U3</td>
<td>IE protein</td>
<td>Member of β-herpesvirus US22 gene family; homologue of HCMV UL24</td>
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<td>Genes required for DNA synthesis and viral replication</td>
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<td>U27</td>
<td>Polymerase processivity factor</td>
<td>41-kDa nuclear protein; binds DNA polymerase</td>
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<td>U38</td>
<td>DNA polymerase</td>
<td>100-kDa protein</td>
<td>Lin &amp; Ricciardi (1998)</td>
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<td>U41/74/77</td>
<td>Helicase/primase complex</td>
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<td>U73</td>
<td>Origin-binding protein</td>
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<td>Nucleic acid metabolism</td>
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<td>U28</td>
<td>R1 subunit of ribonucleotide reductase</td>
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<td>dUTPase</td>
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<td>gH</td>
<td>Conserved glycoprotein with role in virus–cell fusion</td>
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<td>gI</td>
<td>Forms complexes with gL. Role in virus–cell fusion</td>
<td>Liu et al. (1993); Anderson et al. (1996)</td>
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<td>Tigue &amp; Kay (1998)</td>
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<td>Viral chemokine</td>
<td>Novel splicing results in different transcripts</td>
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<td>β-Chemokine receptor</td>
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<td>Confers ganciclovir sensitivity</td>
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<td>p53-binding protein</td>
<td>Transforming activity and transactivation of HIV-1 LTR</td>
<td>Kashanchi et al. (1997)</td>
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Human herpesvirus 6

HHV-6 is ubiquitous in the first 2 years of life. 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 epithelial 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 (Lanphier 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. HHV-6 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 CD3 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 HIV-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 HHV-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 (Kostianont 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 induces a type-1 response in T lymphocytes (Mayne et al., 2001). Among the genes up-regulated were IL-18, the IL-2 receptor and members of the TNF receptor superfamily. HHV-6 also up-regulates the transcription of the chemotactant IL-8 in a hepatoma cell line (Inagi et al., 1996) and adhesion molecules in liver allografts (Lautenschläger et al., 1999). These effects may contribute to organ-specific inflammation during HHV-6 infection. HHV-6 down-regulates the chemokine receptor CXCR4 in directly infected CD4 T lymphocytes by decreasing the association 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 epithelial 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 (Lanphier et al., 1998).
Primary infection in older age groups is rare. The most common feature is 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 vertical and horizontal acq

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(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; Pruksanarundta 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; Takagi et al., 1996). Uvulo-palatoglossal junctional ulcers provide a useful early clinical sign of roseola (Chua et al., 2000). In contrast to these recent reports, a study of paediatric disease in Zambia has found that HHV-6A is a common cause of infection (Kasolo et al., 1997).

Other suggested but unproven disease associations for HHV-6 include progressive multifocal leukoencephalopathy, Bell’s palsy, influenza encephalopathy, drug-induced hypersensitivity syndrome, pityriasis rosea, fetal hydrops, Kikuchi’s necrotizing lymphadenitis, lymphoproliferative disorders (including non-Hodgkin’s lymphomas, Hodgkin’s lymphoma, acute lymphoblastic leukaemia), oral carcinoma, cervical carcinomas and chronic fatigue syndrome (Levy, 1997; Caserta et al., 2001; Reeves et al., 2000; Pitkaranta et al., 2000; Sugaya et al., 2002; Suzuki et al., 1998; Kosuge et al., 2000; Ashshi et al., 2000; Daibata et al., 1999; Bandobashi et al., 1997; Pellett et al., 1992). Of relevance to the discussion of associations with cancer is the observation that HHV-6 can integrate into chromosomes and be passed on to an index case’s offspring (Daibata et al., 1999).

**Disease associations in immunocompromised hosts**

HHV-6 reactivates in HIV-seropositive individuals, but specific clinical syndromes associated with reactivation are rare. Cases of pneumonia and encephalitis are described most frequently (Caserta et al., 2001). With the exception of vertically acquired HIV infection, evidence of indirect effects on HHV disease progression have not been proven (Kostantin et al., 1999). There is greater evidence of disease association in organ transplant recipients (Singh & Carrigan, 1996; Griffiths et al., 2000; Singh, 2000; Emery, 2001; Dockrell & Paya, 2001).

The reported incidence of HHV-6 infection post-transplantation varies in accordance with diagnostic methodology; median incidences of 48% (range 28–75%) for bone marrow 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 transmission 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...
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; Drobskyi et al., 1993). In those cases without other aetiological agents identified, HHV-6 can be cultured in the bone marrow (Drobskyi 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 does 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 OLT 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 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., 1999; 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; Lautenschlager et al., 1998; Humar et al., 2002). Although HHV-6 up-regulates Epstein–Barr virus replication in vitro (Flamand et al., 1993), the relationship between these viruses in vivo requires clarification (Lin et al., 1999).

Diagnosis

HHV-6 can be cultured from clinical specimens by coculture 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. Bipsysm 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 non-invasive 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


Human herpesvirus 6


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