Review

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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.*, 1993). 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.

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- κ B 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.*, 2002). The U16/17 gene products result from differential splicing; some are IE proteins and others are late proteins (Flebbe-Rehwaltdt *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 polyribosome-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/U74/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 DR_L and DR_R and come into close contact at the junction between concatameric viral genomes to form a *pac2*–*pac1* junctional 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 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 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 *Roseolavirus* 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, 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 (Cardinali *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 (Torrissi *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 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 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 CX₃C 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, $\gamma\delta$ 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 (Isomura *et al.*, 1997). Both HHV-6 variants infect primary 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

Table 1. Selected HHV-6 genes and gene products

Gene	Gene product	Comments	Reference(s)
IE proteins			
U86	IE protein	U86–89 form IE-A gene block. Homologue of HCMV IE86	Schiewe <i>et al.</i> (1994)
U89	IE protein (IE1)	Transcriptional transactivator. Nuclear phosphoprotein	Martin <i>et al.</i> (1991); Gravel <i>et al.</i> (2002)
U95	IE protein	Homologue of MCMV IE2	Takemoto <i>et al.</i> (2001)
U16–19	IE protein(s)	U16–19 form IE-B gene block. U16 transactivates HIV-1 LTR	Flebbe-Rehwaltdt <i>et al.</i> (2000)
U3	IE protein	Member of β -herpesvirus US22 gene family; homologue of HCMV UL24	Mori <i>et al.</i> (1998)
Genes required for DNA synthesis and viral replication			
U27	Polymerase processivity factor	41-kDa nuclear protein; binds DNA polymerase	Zhou <i>et al.</i> (1997)
U38	DNA polymerase	100-kDa protein	Lin & Ricciardi (1998)
U41	Single-stranded DNA-binding protein		Clark (2000)
U43/74/77	Helicase/primase complex		Clark (2000)
U73	Origin-binding protein		Clark (2000)
U94	RepH6	Homologue of AAV-2 <i>rep</i> 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	Thomson <i>et al.</i> (1991); Rotola <i>et al.</i> (1998); Mori <i>et al.</i> (2000)
Nucleic acid metabolism			
U28	R1 subunit of ribonucleotide reductase		Clark (2000)
U45	dUTPase		
U69	Phosphotransferase		
U81	Uracil-DNA glycosylase		
Glycoproteins and structural antigens			
U11	p100 major structural antigen	Phosphoprotein that varies between HHV-6A and -6B	Isegawa <i>et al.</i> (1999)
U39	gB	Conserved glycoprotein with role in virus–cell fusion	Chou & Marousek (1992)
U48	gH	Forms complexes with gL. Role in virus–cell fusion	Liu <i>et al.</i> (1993); Anderson <i>et al.</i> (1996)
U72	gM	Conserved glycoprotein	
U82	gL	Glycoprotein with role in transport and processing of gH. Complexes with gH	Liu <i>et al.</i> (1993); Anderson <i>et al.</i> (1996)
U100	gp82–105	Glycoprotein with marked variation between HHV-6A and -6B	Pfeiffer <i>et al.</i> (1993)
Miscellaneous			
U53	Protease	Required for mature virion production	Tigue & Kay (1998)
U83	Viral chemokine	Novel splicing results in different transcripts	Zou <i>et al.</i> (1999); French <i>et al.</i> (1999)
U12	Chemokine receptor	β -Chemokine receptor	Isegawa <i>et al.</i> (1998)
U51	Chemokine receptor	β -Chemokine receptor. Signalling can result in RANTES down-regulation	Menotti <i>et al.</i> (1999); Milne <i>et al.</i> (2000)
U69	Protein kinase	Confers ganciclovir sensitivity	Ansari & Emery (1999)
DR7	p53-binding protein	Transforming activity and transactivation of HIV-1 LTR	Kashanchi <i>et al.</i> (1997)

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

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 (Kirn *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 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, $\gamma\delta$ T lymphocytes and NK cells, HHV-6 can potentially increase the range of cells susceptible to HIV-1 infection (Lusso *et al.*, 1991, 1993, 1995). HHV-6 transactivates the HIV-1 LTR via U17/16 and U16+ gene products (Flebbe-Rehwaltdt *et al.*, 2000; Lusso *et al.*, 1989). HHV-6 also up-regulates cytokines, including IL-1 β 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; Siczekowski *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 (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 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 chemoattractant IL-8 in a hepatoma cell line (Inagi *et al.*, 1996) and adhesion molecules in liver allografts (Lautenschlager *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 (Case-rta *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 (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; Pruksananonda *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 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 control patients and those with MS. HHV-6 antigens are detected in the nuclei of oligodendrocytes from patients with MS but not control samples. Furthermore, oligodendrocytes expressing HHV-6 are more frequent adjacent to plaques of demyelination. Other groups have either supported these findings (Knox *et al.*, 2000) or been unable to replicate them (Coates & Bell, 1998). Increased levels of anti-HHV-6 IgM in patients with relapsing remitting MS (Soldan *et al.*, 1997), increased rates of detection of HHV-6A DNA in cell-free specimens from MS patients (Akhyani *et al.*, 2000) or increased serum levels of soluble CD46 in MS patients compared with controls have also been reported (Soldan *et al.*, 2001). Therefore, whether HHV-6 contributes to MS pathogenesis is unclear.

Other suggested but unproven disease associations for HHV-6 include progressive multifocal leukoencephalopathy, Bells 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 pneumonitis and encephalitis are described most frequently (Caserta *et al.*, 2001). With the exception of vertically acquired HIV infection, evidence of indirect effects on HIV disease progression have not been proven (Kositantont *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

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 ($> 41^{\circ}\text{C}$) 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 OLT 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 OLT 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 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.*, 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; 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 co-culture with lymphoblasts prepared by IL-2 and PHA treatment of PBL (Yamanishi *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 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.

Acknowledgements

I would like to thank Pauline Whitaker for secretarial assistance.

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