

# Highlights from 5th International Conference on HHV-6 and -7

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## KEY WORDS

■ HUMAN HERPESVIRUS-6 ■ HUMAN HERPESVIRUS-7 ■ CENTRAL NERVOUS SYSTEM DISEASE ■ TYPE-SPECIFIC ASSAYS ■ CELLULAR RECEPTORS ■ TRANSPLANT RECIPIENTS ■ ANTIVIRAL THERAPY

## SUMMARY

This article reports on key presentations at the 5th International Conference on Human Herpesvirus (HHV)-6 and -7, organized by the HHV-6 Foundation. New assays for HHV-6 and -7 promise to be more accurate and better able to distinguish between HHV-6A and B or differentiate active from latent infection. Nevertheless, more research is needed to enhance the sensitivity and specificity of these assays. Cellular receptors for both HHV-6 and -7 have been identified. Both viruses have *in vitro* tropism for neurons and dendritic cells of the central nervous system (CNS), and their role in producing CNS disease in the immunocompromised (particularly transplant recipients and the HIV-infected) is well established. HHV-6 may enhance the progression of simian immunodeficiency virus in monkeys, as suggested by *in vivo* data. In immunocompetent children and adults, HHV-6 and/or -7 may play a role in triggering and perpetuating several diseases of the nervous system, namely encephalitis, multiple sclerosis, chronic fatigue syndrome and epilepsy.

THE 5TH INTERNATIONAL Conference on Human Herpesvirus-6 and -7 was held in Barcelona, Spain, on May 1–3 2006. The conference included overview presentations by Robert C Gallo, Koichi Yamanishi, Edward S Mocarski, Niza Frenkel, Dharam Ablashi, Steven Jacobson, Ursula A Gompels, Henri Agut, Philip E Pellett, Stephen Dewhurst, Louis Flamand, Carolyn B Hall, Per Ljungman, Gerhard Krueger, Paolo Lusso, Lieve Naesens, Erik De Clercq, William D Gaillard, Schlomo Shinnar and Anthony L Komaroff. Here, we summarize the conclusions drawn from new research presented at the conference.

## Cell and Molecular Biology

Human cytomegalovirus (CMV) may activate HHV-6 replication in human fibroblast culture. HHV-6 infection of astrocytes dysregulates the expression of glutamate transporters. The virus is capable of a complete replication cycle in both a T-cell and an astrocyte line. HHV-6 was found to induce cell-cycle arrest, the accumulation of p53 and the binding of p53 to DNA. The A variant of HHV-6 (HHV-6A) was shown to induce a greater cytopathic effect, and more persistent infection, in an oligodendrocyte cell line than the B variant (HHV-6B). In HIV-infected patients, HHV-6B was more often involved in encephalitis, whereas HHV-6A was more commonly encountered in other neurological disorders.

The protein encoded by the U94/rep (latent) gene, a gene unique to HHV-6, inhibited replication of HHV-6, HHV-7 and CMV. Antibodies to the U94 protein were found to be increased in patients with multiple sclerosis (MS). The DR7B oncoprotein encoded by HHV-6B was

frequently found in Reed–Sternberg cells infected with the virus. One team has found chromosomal integration of both HHV-6A and -6B in 1–3% of immunocompetent subjects. This team also demonstrated integration of the complete virus, expression of viral genes and passage in the germ line, and noted correspondingly high levels of viral DNA in blood, cerebrospinal fluid and hair follicles. The clinical relevance of such integration is unknown and may be unique to HHV-6.

Several presenters, including Gallo, argued that HHV-6A and -6B should be considered as separate herpesviruses and be reclassified, given the emerging evidence of differences in their biology.

## Assays

The absence of reliable, sensitive and specific assays for active HHV-6 infection has hampered research. At the conference, several promising new molecular and serological assays were described. Monitoring a real-time polymerase chain reaction (PCR), using a control synthetic DNA molecule, led to improved accuracy, repeatability and reproducibility in detecting HHV-6 in biological fluids. Another PCR assay, tested on 39 samples, was sensitive and specific, and distinguished infection between HHV-6A and -6B. A loop-mediated isothermal amplification technique for detecting the virus, tested in 300 samples, was compared with viral isolation: the assay had a sensitivity of 95% and a specificity of 95%. An antibody avidity assay has been used to help identify recent infection and an electrochemiluminescence assay, developed by the National Institute of Neurological Disorders and Stroke, can differentiate between HHV-6A and -6B infection, as well as active versus latent infection. Detection of HHV-6 DNA in the blood is usually interpreted as indicating primary infection, reactivation or reinfection. However, chromosomal integration of HHV-6 DNA also leads to high levels of viral DNA, whether or not the infection is active.

## Epidemiology

Most children in developed nations are infected by HHV-6B by 3 years of age. HHV-6 DNA can be detected in saliva of 2–3% of children aged 3–5 years, and in up to 25% of adults; it has also been detected in cervical samples from 20% of US women. HHV-7, but not HHV-6, DNA can be detected in breast milk. The prevalence of HHV-6A is thus far unknown, because previously no serological assay has been able to differentiate antibodies to the A and B variants.

## Immunology

Both HHV-6A and -6B productively infect CD4+ cells, although the A strains are capable of broader tropism,

including natural-killer cells,  $\gamma\delta$  cells and CD8+ cells. HHV-6 infection enhances production of inflammatory cytokines and chemokines, including interferon [IFN]- $\alpha$ , interleukin [IL]-1 $\beta$ , IL-6, IL-10, IL-15, IL-21 and RANTES, while downregulating production. The HHV-6A receptor is CD46, a molecule that is critically important in immunologic synapse, IL-2 and IFN- $\gamma$  formation; the receptor for HHV-6B is uncertain. HHV-6 and HHV-7 infections divert Class I MHC molecules from the cell surface into a lysosomal compartment, which possibly aids the viruses to escape immune detection.

## Transplantation

In transplant patients, HHV-6 causes encephalitis (with a 40% mortality rate and lasting cognitive deficits in many people). Typically, these patients have magnetic resonance imaging (MRI) abnormalities in the medial temporal lobes, and many have delirium and seizures; 60% of children with HHV-6 encephalitis have lasting cognitive impairment. HHV-6-associated bronchiolitis obliterans occurs in lung transplant recipients. HHV-6 infection complicates haematopoietic stem cell transplantation in 40–50% of cases, causing hepatitis, pneumonitis, CMV reactivation, bone marrow suppression and encephalitis. The virus is also associated with high levels of IL-6 and tumour necrosis factor (TNF)- $\alpha$ . In pediatric renal or bone marrow transplantation, HHV-6 reactivation is strongly associated with acute rejection.

## Encephalitis

A large, prospective, 5-year study of encephalitis in over 1000 immunocompetent subjects found relatively few cases of HHV-6 infection (identified by PCR). However, the assay was not very sensitive, and serological studies (antibodies to early antigens) of cerebrospinal fluid (CSF) suggested a higher incidence of HHV-6 infection. A prospective study in immunocompetent children with encephalitis revealed infection with HHV-6 and/or HHV-7 in 17%; few had pleocytosis. Diffusion-weighted MRI revealed a transient decrease in white-matter diffusivity, consistent with glial or neuronal axonal involvement, in one documented case of HHV-6 encephalopathy. An individual with severe depression, cognitive deterioration and hyperintense signal in the white matter on MRI, who was admitted to psychiatric hospital several times, had high levels of HHV-6 DNA in the serum and CSF; with foscarnet treatment, the patient returned to normal health.

## Epilepsy

Between 35% and 40% of children with febrile seizures have acute HHV-6 or HHV-7 encephalitis. In mesial temporal lobe epilepsy, analysis of brain tissue removed during surgery (primarily from the hippocampus and temporal lobes) revealed HHV-6B DNA. Viral DNA was also demonstrated in primary cultured astrocytes.

## Chronic Fatigue Syndrome

Evidence of active HHV-6 infection in blood is found more often in patients with chronic fatigue syndrome (CFS) than in age- and gender-matched controls. A new study, comparing CFS cases with healthy controls, has found HHV-6 antigenaemia in 31% of CFS cases versus 7% of controls, and viral DNA by PCR in 22% of cases versus 0% of controls. In an uncontrolled study, 12

subjects with severe CFS and evidence of both HHV-6 and Epstein–Barr virus (EBV) infection received valganciclovir for 6 months; nine of the patients had a dramatic improvement in symptoms, along with reductions in HHV-6 titres and EBV antibodies. Controlled studies are planned.

## Multiple Sclerosis

Prospective studies of relapsing–remitting MS and secondary-progressive MS detect HHV-6 DNA in serum by PCR on at least one occasion, often during relapse.

Cell-free HHV-6 DNA has been found (by PCR) in 20–25% of plasma and CSF specimens taken from MS patients, with significantly higher viral loads in those with active disease. Because CD46 is the receptor for HHV-6 and is important in complement regulation, neural stem cells were infected with HHV-6A and then exposed to human complement; massive deposition of opsonic C3 fragments resulted, with widespread cell death.

Human herpesvirus-6 infects glial precursor cells, the ancestors of myelin-producing oligodendrocytes, leading to cell cycle arrest: findings which suggest that defective remyelination might play a role in MS. Higher levels of anti-HHV-6-immunoglobulin G in CSF were found in MS patients receiving the immunosuppressive drug, natalizumab; immunohistochemical studies have also confirmed the presence of HHV-6 in progressive multifocal leucoencephalopathy (PML) lesions, suggesting that HHV-6 may be able to increase replication of JC virus, the cause of PML.

A study in the common marmoset *Callithrix jacchus* demonstrated a relapsing neurological illness with demyelination following infection with HHV-6A (GS strain). Autopsy revealed plaques in subcortical white matter, optic neuritis and demyelination in the spinal cord. No such manifestation occurred in animals inoculated with HHV-6B (Z-29 strain).

## Other Disease Associations

In >80% of patients with acute liver failure not due to infection with the hepatitis A–E viruses, infection with HHV-6 was the likely cause; such patients were at considerable risk of HHV-6 infection through transplanted donor livers. This virus was associated with both interstitial pneumonitis and myocarditis in HIV-infected patients. In patients with drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome, reactivations of HHV-6 and EBV, but not CMV, in peripheral blood mononuclear cells are often noted. HHV-6 genomes are frequently detected in the coronary arteries of patients with Kawasaki disease.

## Antivirals

A compound named CMV423 is more potent against HHV-6 *in vitro* than foscarnet, ganciclovir and cidofovir. An orally-available compound, cyclopropavir, has high activity against HHV-6A. Ether lipid esters of cidofovir are more potent than cidofovir against both HHV-6A and -6B. Cidofovir and foscarnet have activity against HHV-6-infected astrocytes.

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