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Human Herpes Virus 8 (HHV8)

Human Herpesvirus-8 (HHV-8) is one of the few currently recognised cancer-causing viruses (oncoviruses) in humans. It is still often referred to as Kaposi's sarcoma associated herpesvirus (KSHV) since it was originally identified in Kaposi's Sarcoma (KS) lesions from AIDS patients[1].

Structure and genome

HHV-8 is a virus belonging to the Herpesviridae family, of the Gammaherpesvirinae subfamily and of the Rhadinovirus genus. The 9 herpesviruses infecting humans are classified into 3 subfamilies based on their biological properties and tropism. The alpha subfamily are neurotropic viruses and include herpes simplex viruses (HSV) 1 and 2 (also referred to as HHV-1 and -2), and Varicella zoster virus (VZV or HHV-3). Viruses belonging to the beta family may infect a wider range of tissues and cell types and include human cytomegalovirus (CMV or HHV-5), HHV-6A, HHV-6B and HHV-7. HHV-8 and the Epstein Barr Virus (EBV or HHV-4) belong to the gamma subfamily of herpesviruses, based on their tropism for lymphocytes.

Like other herpesviruses, HHV-8 has a highly organized icosahedral-shape nucleocapsid containing the linear, double-stranded DNA genome. The nucleocapsid is enclosed in a protein layer (the tegument), itself surrounded by a lipid bilayer containing the glycoproteins necessary for viral attachment and entry into cells. The virion size is 120 nm in diameter, with a genome size of approximately 165kb, encoding about 90 genes.

In addition to the conserved herpsevirus genes necessary for its infection and replication functions, HHV-8 is unique amongst herpesviruses in that it possesses a variety of additional genes "pirated" from host cells, such as a DNA polymerase, interleukin-6 (IL-6), BCL-2, a G-protein coupled receptor, complement-binding proteins, thymidine kinase.

Pathophysiology

HHV-8 has been shown to target mainly human B cells[2] as well as monocytes[3], endothelial/spindle[4] cells and keratinocytes[5]. Following infection, HHV-8 is endocytosed by macropinocytosis and may remain in a latent state[6] where only the

viral latency-associated nuclear antigen (LANA) is expressed. LANA suppresses the viral genes required for viral production and assembly, thereby sustaining latency. In this state, the virus remains as a naked piece of circular DNA (episome) within the infected cells. LANA has also been shown to interact with various cellular proteins.

Of particular interest is its ability to bind to and inhibit two key cellular tumour-suppressing proteins, p53[7, 8] and Rb[9]. Infected cells thereby become protected from apoptosis and may more readily undergo uncontrolled proliferation under certain conditions (e.g. immunodeficiency and immunosuppression).

Epidemiology

HHV-8 infection and seroprevalence are limited in the general population (from 0-5% in Northern Europe to up to 80% in Central and Eastern Africa). Since most HHV-8infected individuals are asymptomatic, the mechanisms of transmission still remain poorly understood. HHV-8 has been found in saliva, nasal secretion and seminal fluid[10, 11]. The main routes of transmission identified to date are from mother to foetus (through saliva) in endemic population (Africa), and through sexual contacts within the homosexual population in the USA and Europe.

Infection (or reactivation) is of particular concern to the immunosuppressed and immunocompromised. Cancer patients receiving chemotherapy, AIDS patients and organ transplant patients are therefore all at a high risk of showing signs of infection.

HHV-8 in disease

HHV-8 is now accepted as the causative agent in:

- all forms of Kaposi's sarcoma (KS), either AIDS-associated or non AIDSassociated[12]. KS is a multicentric angioproliferative disorder of endothelial origin which varies in terms of types of lesions, clinical aggressiveness, site of presentation and treatment. HHV-8 is detected in 100% of KS lesions and in 50-70% of the peripheral lymphocytes of the same patients.
- body cavity lymphoma or primary effusion lymphoma (PEL)[13, 14, 15, 16]. PEL is a non-Hodgkin B-cell lymphoma affecting various body cavities (pleural space, pericardium, peritoneum) and is almost always associated with HIV. HHV-8 genome is always found in the effusion fluid, frequently in association with EBV (70%).
- some forms of Castleman's disease (CD)[16]. CD is a rare lymphoproliferative (B-cell) disorder resulting from hypersecretion of IL-6 either of endogenous or viral (HHV-8) origin. CD may affect one (unicentric) or multiple (multicentric) lymph node(s). In unicentric CD, there are usually little or no symptoms and removal of the affected lymph node is usually curative. 50 % of the cases of multicentric CD (MCD) are caused by HHV-8, and where MCD is associated with HIV, HHV-8 is found in 100% of the cases. The other non-HIV-related MCD are of unknown origin. The main complication resulting from MCD is the development of non-

Hodgkin's lymphoma and autoimmune haemolytic anaemia as a result of the proliferating B-cells.

In addition, HHV-8 has also been detected but not causally linked to Bowen's disease, a malignant squamous cell carcinoma, in HIV-negative patients[17]. In addition, HHV-8 has also been shown to be able to invade and persist in the central nervous system, where it may be linked to some forms of encephalitis, meningitis[18], and primary CNS lymphoma[19]. Although the role of HHV-8 in neuropathology has not been ascertained, early detection of HHV-8 in the CSF might prove helpful for clinical differential diagnosis.

Treatment

There is no treatment of HHV-8 infection since primary infection is usually asymptomatic. Once KS has been diagnosed, a local treatment may be applied such as surgical removal of the lesion, radiotherapy or local chemotherapy. Although treatment of HIV-infected individuals with highly active antiretroviral therapy (HAART) has significantly reduced the incidence of KS amongst AIDS patients, efficient and tolerable therapies for MCD and PEL are still lacking.

Clinical diagnostics

Since HHV-8 cannot be readily cultivated from infected material, primary diagnosis of HHV-8-associated diseases is usually based on cytological examination of biopsies: skin, lung of intestine lesions for KS, effusion fluids for PEL or lymph nodes for CD. This may be associated with immnunofluorescence staining for LANA-1, which will confirm the latent infection by HHV-8. Detection of anti-HHV-8 IgGs have also been described as a means of confirming infection[20].

HHV-8 viral DNA may also be detected by PCR either from biopsies or from peripheral blood lymphocytes. HHV-8 being predominantly intracellular, it is however rarely detected in plasma, except in immunosuppressed HIV individuals. Various studies have shown that 0-10% of HHV-8-infected individuals without KS and 0-52% of HHV-8-infected individuals with KS will present a viraemia at any given time[21, 22]. In other studies the presence of HHV-8 in the blood predicted the risks of KS development[23] and the amount of HHV-8 in PBMCs, not plasma, correlated with clinical staging of KS[24]. Quantitation of HHV-8 viral load in the blood is therefore a useful mean of assessing response to therapy or survival[25]. HHV-8 detection may also be of clinical relevance in organ donor screening and monitoring transplant patients with KS resulting either from HHV-8 reactivation or primary infection from the donor[26].

At Micropathology Ltd, we offer detection and quantitation of HHV-8 using probe-based qPCR, targeting the ORF-26 (minor capsid protein) gene of HHV-8. This is a new assay with updated qPCR methodology. Quantitation standards used in our assay are calibrated by digital PCR as no international standard is currently available for

HHV-8. We perform a routine surveillance of public databases to ensure optimal assay performance against newly published and relevant variants.
Validated sample types are plasma, serum, EDTA whole blood and CSF. HHV-8 has also been successfully detected in other sample types such as pleural fluids, ascites, pericardial fluid and skin biopsies but due to the paucity of positive specimens of these types, validation of these sample types has not been performed to date. These sample types can however be processed at Micropathology and the result will be sent with a caveat stating “Please note, X is not a validated sample type for this assay””.
For additional information regarding the HHV8 assay, please contact the laboratory.

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