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HHV-8, an Always Intriguing, Sometimes Relevant Herpesvirus

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Abstract

The virus associated with Kaposi's sarcoma (KSHV) is also known as the human herpesvirus type 8 (HHV-8), due to its biological and genetic homology to other human herpesviruses. Since its identification in 1994, a growing body of evidence supports its role in the pathogenesis of Kaposi's sarcoma. Moreover, other lymphoproliferative disorders, such as Multicentric Castleman's disease and primary effusion lymphomas, have been also linked to HHV-8 infection. However, many questions still remain unanswered. HHV-8 seems a necessary but not sufficient factor in the oncogenic process. The exact pathways leading to tumoural development are not well understood. This review summarises the epidemiological, clinical and biological studies on which current understanding of HHV-8 and its pathogenic role are based. The relationship between HHV-8 and HIV infection is also discussed.

Key words

HHV-8. Kaposi's sarcoma. Primary effusive lymphomas.

Introduction

Kaposi's sarcoma (KS), the most common HIV-associated tumour, was suspected for a long time to be caused by an infectious agent, based on a series of epidemiological observations[1]. Chang *et al.*[2], using representational differential analysis, identified for the first time in 1994 DNA sequences unique to KS tissue. All four clinical subtypes of the disease (classic, endemic, iatrogenic, and epidemic) were then screened for the DNA fragment with a sensitive PCR, and were detected in most of the samples whereas all control samples were negative[3-12]. This specificity suggested a causal role for the putative new virus in KS, prompting Chang *et al.* to name it 'Kaposi's sarcoma-associated herpesvirus' (KSHV). Given its homology to other human herpesviruses, it was named human herpesvirus type 8 (HHV-8).

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The virus

The entire HHV-8 genome was sequenced in 1996[13,14]. It consists of a 140.5-Kb long unique coding region flanked by terminal repeat units (TR). Sequence analysis has confirmed that the virus is phylogenetically most closely related to herpesvirus saimiri (family, herpesvirus; subfamily, γ-herpesvirus; genus, rhadinovirus). Like other γ-herpesviruses, HHV-8 is lymphotropic[15,16].

The unique coding region encodes at least 81 open reading frames (ORF). Like other members of the herpesvirus family, the HHV-8 genome is divided into seven 'block regions' (numbered 1-7), with 'intervening blocks' of DNA (designated as 'interblock regions' a-h) (see Fig. 1). Conserved block regions encode putative viral structural proteins and enzymes required for viral replication, whereas the intervening blocks encode proteins unique to the subfamily and genus[17]. In other known herpesviruses, interblock regions contain genes involved in the maintenance of viral latency and viral transforming properties[18].

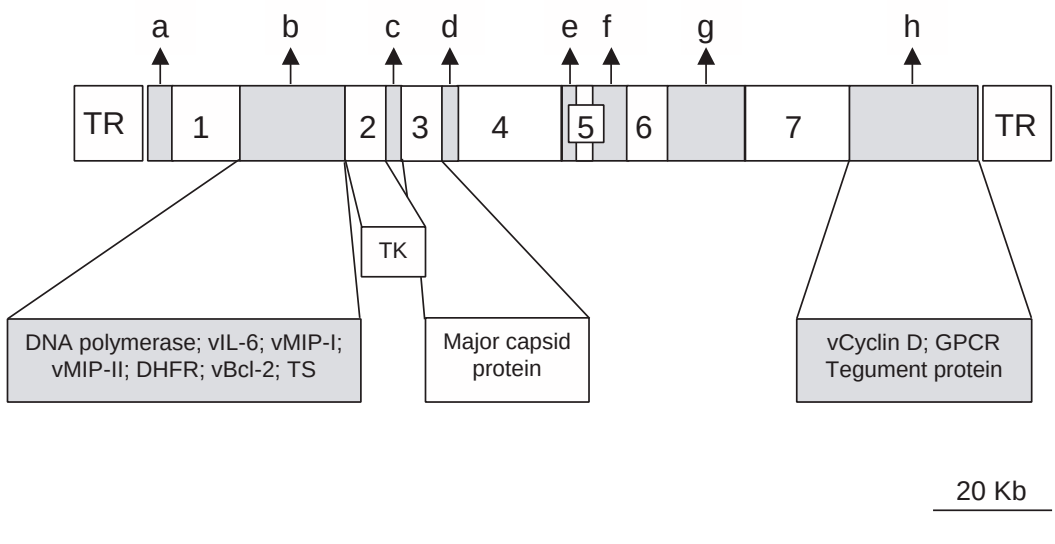

Fig. 1. *The HHV-8 genome. vIL-6: Viral interleukin-6; vMIP-I and II: viral macrophage inflammatory protein I and II; DHFR: dihydrofolate reductase; vBcl-2: viral Bcl-2; TS: thymidylate synthase; TK: thymidine kinase; vCyclin D: viral cyclin D; GPCR: G-protein coupled receptor.*

Variability in DNA sequences encoding capsid proteins make it possible to categorise HHV-8 into distinct viral subtypes (see Table 1). Types A and C are phylogenetically closer to each other, and far from subtypes B and D. HHV-8 subtype B has been found almost exclusively in patients with KS born in Africa, while subtype D has been identified only in individuals originally from the Pacific Islands. In Europe and the USA, subtypes A and C predominate. The latest has also been detected in the Middle East and Asia. It has been suggested that HHV-8 is an ancestral virus whose genomic diversity is the result of migratory currents combined with a process of biological evolutionary selection. Studies performed so far have not been able to correlate certain HHV-8 subtypes with more aggressive KS forms[19,20]. Although occasionally more than one genotype has been detected in the same specimens, the development of HHV-8-associated tumours in a given patient is generally linked to a single genotype[21].

Table 1. *HHV-8 subtypes based on the variability of DNA ORF K1 sequences.*

HHV-8 Subtypes	Geographic area
A	Europe and USA
B	Africa
C	Europe, USA, Middle East and Asia
D	Pacific Islands

Sequencing of the HHV-8 genome has revealed homology between some of its coding regions and human genes involved in signal transduction and regulation of cell cycle progression. HHV-8 genes encode for functional proteins in both the latent and lytic phase of HHV-8 infection. It is believed that certain viral proteins mimic the action of activators and regulatory cellular proteins[17] and this could account for the transforming properties of the virus, as will be discussed later. These include homologues to Bcl-2 (ORF16), cyclin-D (ORF72), G-protein-coupled receptor, interleukin 6 (IL-6), some macrophage inflammatory proteins, and interferon regulatory factor[22-24]. Some of the specific latent and lytic genes of HHV-8, which are under investigation, are listed in Tables 2 and 3.

Epidemiology

Table 2. *Latent HHV-8 genes and gene products.*

HHV-8 gene	HHV-8 protein	Function
ORF 73	LANA	Episome maintenance
		Plasmid stabilization
ORF 72	v-cyclin	Growth control
ORF 71	v-FLIP	Block apoptosis
ORF K12	Kaposins	Unknown

Table 3. *Lytic HHV-8 genes and gene products.*

HHV-8 gene	HHV-8 protein	Function
ORF 74	KSHV-GPCR	Chemokine homologue
		Constitutive signalling trigger
ORF K6	VMIP-II	Triggers angiogenesis
ORF K1	Unknown	Transmembrane signalling
ORF K9	Unknown	Blocks IFN-induced gene expression

Although there is no total agreement between the different HHV-8 serologic tests, all have shown the same epidemiological trends: HHV-8 seroprevalence is higher among homosexual men and subjects born in certain regions (Africa and Mediterranean countries)[25-27].

In the general population, the highest seroprevalence has been found in Sub-Saharan African countries. For instance, in Uganda, where endemic KS is one of the most common tumours, anti-HHV-8 antibodies were detected by ELISA or immunofluorescence assays in up to 31-35% of the population[28,29]. In Mediterranean countries the prevalence is approximately 10%, whereas in northern European, South-East Asian and Caribbean countries, seroprevalence is in the 2-4% range. In the United States, a 'mixing bowl country', HHV-8 seroprevalence is in the range of 5-20%[30]. Moreover, in a study performed in South Africa, black individuals were more often positive for anti-HHV-8 antibodies compared to white subjects[31]. The reasons behind these racial and geographical trends are unknown. However, no sex-related differences have been observed in HHV-8 seroprevalence, despite KS occurrence in Africa among men being about 8 to 10 times as common as among women[32].

In subjects with KS of any subtype or other HHV-8-related diseases like Primary Effusive Lymphomas (PEL) and Multicentric Castleman's Disease (MCD), the prevalence of HHV-8 antibodies is over 80%. For instance, in a study performed in the United Kingdom, Greece, Uganda and USA[29] 82% of patients with AIDS-related KS and 94% of individuals with classic KS had anti-HHV-8 antibodies.

In patients with HIV-1 infection and no KS, the HHV-8 seroprevalence is higher (20-50%) than in the general population, except in South-East Asia and the Caribbean basin, where no AIDS-associated KS has been recognised[30]. Among HIV-infected patients, homosexual men have anti-HHV-8 antibodies more often (31-35%) than haemophiliacs, i.v. drug users or transfusion recipients (1-5%). On the other hand, HIV-negative homosexuals present higher seroprevalence for HHV-8 (8-13%) than persons a deemed 'low risk' for KS, including blood donors (1-3%)[28,29,33-35].

HHV-8 serologic studies are limited due to the lack of highly sensitive and specific assays. Different techniques have been used in the studies mentioned above (ELISA, immunofluorescence, and immunoblot). These are designed to detect antibodies against latent nuclear antigens or capsid recombinant proteins[29,35-36]. Of note is the fact that there is no clear correlation among the different tests when they are used in populations at low risk for HHV-8 infection. Therefore, although current serologic assays may be appropriate for epidemiological purposes, the results cannot be interpreted as markers of infection in a particular subject. Recently introduced assays combining several HHV-8 antigens, which are more sensitive and specific, might be more useful in clinical settings[32]. Moreover, the profile of anti-HHV-8 antibodies responding to latent or lytic antigens is not homogeneous[37]. A deeper insight into the serological responses developed against HHV-8 might improve the diagnostic and prognostic implications of the available assays.

PCR techniques have been used during the last few years to detect HHV-8 DNA in several compartments and body tissues[38-50]. Some studies performed on HIV-positive subjects have reported prevalences of HHV-8 infection similar to those obtained using serological assays[50]. However, others have observed a poor correlation between PCR and serology, suggesting that both techniques might be complementary tools in the diagnosis of HHV-8 infection[51].

The available evidence strongly suggests sexual and mother-to-child infection as the most probable routes for HHV-8 transmission[20,26]. Sexual transmission is linked to homosexual practices, whereas the evidence for heterosexual acquisition of HHV-8 is much less convincing[26,27]. Semen and urogenital tract biopsy specimens in healthy adults have been examined by PCR for the presence of HHV-8, obtaining a wide range of positivities (0-91%)[44,52-56]. Results have also been highly variable in HIV-infected individuals[44,56]; for instance, PCR performed in semen was positive for HHV-8 in 25-64% of homosexuals and 14-25% of patients with KS.

The correlation found between the risk for HHV-8 infection in children and the status of their mothers suggests a mother-to-child transmission of the virus[57]. However, the timing and route of such transmission are unknown, and the possibility that other family members could be the source of infection has not been ruled out. In that regard, data from a study performed in prepuberal Ugandan children suggest a pattern of horizontal transmission similar to that of the Epstein-Barr virus, the human herpesvirus most closely related to HHV-8[32].

Tumoural HHV-8-associated diseases

Up to now, three diseases have been linked to HHV-8 infection: KS, PEL and MCD. Both KS and PEL occur almost exclusively among HHV-8-infected patients (with or without concurrent HIV infection), unlike MCD which develops quite often without HHV-8 infection. The role of HHV-8 in the pathogenesis of these entities is not well understood. It has been proposed that the virus could be: (i) the causative agent; (ii) a cofactor; or (iii) a bystander, whose growth and maintenance might be enhanced by the tumour. Available data suggest that HHV-8 is an essential cofactor for the development of KS and PEL.

Differences exist in cellular tropism and viral gene expression among the three diseases[58]. The diverse clinical manifestations of HHV-8 infection should be the result of variable expression of the viral genome in different types of infected cells. Both B and T lymphocytes are infected in MCD. A few cells in the lesions seem to contain full HHV-8 DNA, but all of them express lytic phase genes. KS and PEL show a similar pattern of genomic expression opposed to MCD: Tumoural cells are massively infected by HHV-8 but only a few express lytic phase genes. The type of cells differs in both tumours: Spindle and endothelial cells are mainly infected in KS, whereas B cells predominate in PEL[58].

There is a notable heterogeneity in the levels of viral IL-6 expression, being much higher in PEL and

MCD compared to KS[59]. The biological activity of viral IL-6 is not well known. Recent *in vitro* studies have proved that it is a multifunctional cytokine, promoting polyclonal hypergammaglobulinemia, angiogenesis, plasmacytosis in spleen and lymph nodes, and hematopoiesis in the myeloid, erythroid and megakaryocytic lineages[60].

Viral IL-6 expression in tumoural specimens correlates with other markers of the lytic viral cycle[59], seeming to play an important role in the process of cellular proliferation and tumorigenesis. Thus, ORF74 encodes the G-protein-coupled receptor, expressed both in KS and in PEL cells. Signalling by this receptor leads to cellular transformation and tumourigenicity, and induces a switch to an angiogenic phenotype mediated by the 'vascular endothelial growth factor' and the Kaposi's-spindle-cell growth factor[61].

HHV-8 and KS

Evidence suggesting an involvement of HHV-8 in the pathogenesis of KS can be grouped into six categories: (i) high seroprevalence of anti-HHV-8 antibodies in patients with KS[29]; (ii) temporal association of HHV-8 infection and KS development[38,39,62,63]; (iii) detection of HHV-8 DNA in tumoural cells, specially lytic cycle genes[58,64]; (iv) encoding of transforming proteins, antiapoptotic factors and inflammatory cytokines by HHV-8 genes, which have certain homology with human genes[17,22-24]; (v) HHV-8 stimulating the proliferation of microvascular endothelial cells[65]; and (vi) the impact of certain antiviral drugs on the incidence of KS[66]. However, the timing and relationship between HHV-8 infection and KS oncogenesis are not well understood[32,67,68].

Cross-sectional studies, both serologic and genetic show a good correlation between HHV-8 infection and KS occurrence. Moreover, longitudinal studies have proved the presence of HHV-8 prior to the appearance of KS lesions in HIV-positive patients[38]. The detection of anti-HHV-8 antibodies[69] and of HHV-8 DNA in PBMC predicts the development of KS in HIV-positive individuals[43,49,70]. Among HIV-infected subjects with positive PCR for HHV-8 in PBMC, up to 55% developed KS over a median period of 3.5 years. In contrast, only 9% of those negative for HHV-8 PCR in PBMC subsequently developed KS[39,41].

It is not clear if an active and disseminated HHV-8 infection, with positive viremia, is necessary to occur at the time of KS development. Several authors have highlighted the fact that the infection might have been present a long time before the tumoural lesions were first recognised, indicating that the incubation period could be very long[11,15,21,63]. Moreover, the detection of HHV-8 in lymph nodes from healthy subjects suggests that, during the course of HHV-8 infection, a silent infection could have been reactivated[71]. However, the detection of HHV-8 in PBMC from patients with KS most probably reflects infection of B cells, likewise other herpesviruses[39,70], and could act as an important reservoir *in vivo*.

The clinical implication of HHV-8 viremia in KS has not yet been established. The proportion of HIV-infected patients with positive PCR for HHV-8 in PBMC is in the range of 25-90%[11,13,15,16,19,72]. HHV-8 viremia has been correlated with tumoural activity and stage in classic KS[50,73]. Although it has been suggested that HHV-8 viremia predicts KS progression[73], according to other authors, it does not seem to be a useful marker of tumoural activity or response to treatment[72].

Within KS lesions, HHV-8 infects Kaposi's spindle cells and endothelial cells and monocytes[42,64,74,75]. The first two express the CD34 antigen, suggesting that spindle cells derive from endothelial cells. *In vitro* studies have shown that HHV-8 infection of endothelial cells induces cellular proliferation and increases survival[65].

HHV-8 is detected in the majority (if not all) of tumoural cells in KS lesions. However, transcripts expressed during the lytic phase, indicating active infection, are expressed in only a small proportion of cells[58]. Several homologues of cellular cytokines, chemokines and antiapoptotic factors encoded by the HHV-8 genome are expressed during the viral lytic cycle in cell lines from PEL or in KS samples[76]. Cyclin-D and kaposin are readily detected in spindle cells[64,77], unlike viral IL-6, which is generally not expressed at detectable levels in KS specimens[23]. However, the recognition of IL-6 in a subset of KS samples suggests that IL-6 expression may be important for KS pathogenesis in some settings[59].

Cellular products released during the lytic phase of HHV-8 infection exert regulatory effects on the cellular cycle and on apoptosis, and could play a key role in the tumourigenesis process. Thus, the activation of lytic cycle gene expression seems to be crucial in the oncogenesis of KS and PEL. ZEBRA and Rta proteins mediate the activation of lytic cycle genes in the Epstein-Barr virus[78,79]. A homologous of the Rta has been recently identified for HHV-8, which corresponds to the ORF50[80].

HHV-8 and lymphoproliferative processes: PEL and MCD

PEL is a distinct subtype of non-Hodgkin's lymphoma involving the pleural, pericardial and/or peritoneal cavities, which grow as lymphomatous effusions, in the absence of clinically identifiable tumour masses[81]. Cancer cells derive from B cells, likewise other HIV-associated lymphomas. They have indeterminate immunophenotype, but harbour B-cell markers with clonal rearrangements of the immunoglobulin genes. PEL develops mainly, although not exclusively, in HIV-infected subjects, and HHV-8 is invariably present[82-85]. In fact, cellular lines obtained from this lymphoma have allowed a better characterisation of the virus[15,86]. In some cases, tumoural cells are dually infected with Epstein-Barr virus and HHV-8, but the role that this interaction may have in the oncogenic process is unknown[82,87]. All tumoural cells in PEL are infected by HHV-8, but only a minority seems to present active infection[58].

Castleman's disease is an atypical, non-malignant, lymphoproliferative disorder. It may be localised or multicentric with multisystemic involvement and generalised adenopathies. From a histological standpoint, a hialine vascular type, a plasmacytic cell type, or combination of both have been described. The last two forms are the ones occurring in HIV-positive individuals, and HHV-8 DNA has often been detected in the lesions[88-90]. In MCD, only a small percentage of tumoural cells are infected with HHV-8, but all of them express lytic phase genes and contain relatively high levels of transcripts, suggesting an active infection. They are located mainly within the follicular mantle of small lymphocytes surrounding the germinal centres. Although HHV-8 infects mainly B cells, it can also be detected in T cells from peripheral blood and lymph nodes[58,91].

IL-6 is a B-cell growth and differentiating factor. The high expression of the viral homologous of this interleukin in MCD, both in tumoural cells and in lymph nodes, suggests that HHV-8 plays an important role in this pathogenesis. Thus, IL-6 would act as an autocrine or paracrine factor in the lymphoproliferative process[58,59]. On the other hand, the massive transcription of other genes related to the lytic phase, like Bcl-2 (ORF16), cyclin-D (ORF72), and G-protein-coupled receptor, along with IL-6 seems to be characteristic of cells belonging to aggressive forms of MCD and PEL. These proteins, involved in the control of cellular proliferation and apoptosis, may be also expressed to a lesser extent in non-malignant lymphoproliferative processes, suggesting that the distinct expression of some genes defines the nature of the different lymphoproliferative entities[92].

HHV-8 and HIV

HHV-8 is non-pathogenic in the majority of healthy individuals, but can be highly oncogenic in the context of HIV-1 infection and iatrogenic immune suppressive conditions. This fact suggests that a defect in the cell-mediated immunity favours the development of KS and other HHV-8-related diseases. Conversely, an intact immune system is often capable of controlling the tumoural process. For instance, 39% of patients who develop KS after transplantation experience clinical remission of the disease after immunosuppressive therapy withdrawal[93]. Moreover, in patients with a history of KS, the tumour may recur within several months after introducing immunosuppressive medications[94]. An immune defect could imply a lack of control of HHV-8 infection, allowing the virus to act as an oncogenic factor. However, KS appears in individuals with no apparent immune defects in Mediterranean countries and endemic areas in Africa, indicating that there must be other factors involved in the pathogenesis of the tumour.

The frequent association of KS with HIV infection suggests an additive or synergistic effect between HIV and HHV-8 in the tumoural pathogenesis. An HIV regulatory protein, tat, stimulates the growth of KS cells in culture[95,96]. Thus, HIV may be permissive for KS, independently of the immune deficiency caused by the infection of CD4+ lymphocytes. However, HIV and HHV-8 seem to have separate effects on the pathogenetic process of KS. Although HIV stimulates HHV-8 replication *in vitro*[97], there are no data comparing HIV and HHV-8 viral loads *in vivo*. Moreover, serological studies have shown that anti-HHV-8 titers do not correlate with the presence or absence of HIV infection[98]. Likewise, recent studies have not found a correlation between HIV RNA in plasma and the presence of HHV-8 in PBMC detected by PCR, suggesting that the two viruses do not act at the same time point and through the same ways to induce KS development[72]. This and other studies[38] have failed to show a correlation between HHV-8 viremia and the degree of immunosuppression.

Therapeutic implications

Retrospective studies have shown that HIV-infected patients receiving ganciclovir or foscarnet for the treatment of CMV infection had a lower risk of KS[99,100], suggesting a protective role against HHV-8. In a recent study, the risk of developing KS in HIV-infected patients with CMV retinitis was reduced by 75% in those treated with oral ganciclovir, and by 93% in those treated with intravenous ganciclovir, as compared with patients given placebo. According to the authors, the reduction in the incidence of KS was not explained by the use of HAART, since it was comparable for the three treatment groups[66]. *In vitro* studies have shown that HHV-8 is very sensitive to cidofovir, moderately sensitive to ganciclovir and foscarnet, and slightly sensitive to acyclovir[101,102].

The clearance of HHV-8 viremia in the majority of patients with HIV and KS treated with liposomal doxorubicin, which was not explained by changes in the CD4 or total lymphocyte counts, has led to the suggestion that this chemotherapeutic agent might have an antiviral effect against HHV-8[72]. In one study[98], HHV-8 could not be detected in patients experiencing complete remission after treatment of KS with liposomal doxorubicin.

A relevant point for the use of antiviral drugs as prophylaxis for HHV-8-associated proliferative diseases is the timing of such treatment. Cidofovir and ganciclovir do not inhibit DNA synthesis during the latent cycle, suggesting that viral DNA replicates by DNA polymerases from the host. Therefore, a clinical benefit is not expected for PEL and KS, since latent cycle cells predominate in both tumours[101]. Assuming that HHV-8 replication occurs prior to the onset of KS and that reactivation of the virus from latent phase is crucial for the pathogenesis of KS, a protective role of the antiviral treatment in those patients infected by HHV-8 without KS might theoretically be expected[101]. On the other hand, *in vitro* studies have shown that α-interferon inhibits both the reactivation of HHV-8 in PEL cells and the latent infection in PBMC from immunosuppressed patients with or without KS[103].

Lastly, studies evaluating agents aimed at blocking the Rta protein or the transcription of the ORF50 in the

HHV-8 genome, which are key pieces in the activation of the lytic cycle, are currently in development.

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