

## REVIEWS

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### HUMAN HERPESVIRUS 8 (HHV-8) AND THE ETIOPATHOGENESIS OF KAPOSI'S SARCOMA

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**OBJECTIVE:** To review the current literature on human herpesvirus 8 with particular attention to the aspects related to the etiopathogenesis of Kaposi's sarcoma.

**MATERIALS AND METHODS:** The authors searched original research and review articles on specific aspects of human herpesvirus 8 infection, including virology, epidemiology, transmission, diagnosis, natural history, therapy, and Kaposi's sarcoma etiopathogenesis. The relevant material was evaluated and reviewed.

**RESULTS:** Human herpesvirus 8 is a recently discovered DNA virus that is present throughout the world but with major geographic variation. In the Western world, the virus, transmitted mainly by means of sexual contact, is strongly associated with Kaposi's sarcoma and body cavity-based lymphoma and more controversially with multiple myeloma and other non-proliferative disorders. There is no specific effective treatment, but HIV protease inhibitors may play an indirect role in the clearance of human herpesvirus 8 DNA from peripheral blood mononuclear cells of HIV-infected patients. Human herpesvirus 8 DNA is present in saliva, but there are as yet no documented cases of nosocomial transmission to health care workers. The prevalence of human herpesvirus 8 among health care workers is probably similar to that in the general population.

**CONCLUSION:** Human herpesvirus 8 appears to be, at least in Western Europe and United States, restricted to a population at risk of developing Kaposi's sarcoma. Human herpesvirus 8 certainly has the means to overcome cellular control and immune responses and thus predispose carriers to malignancy, particularly Kaposi's sarcoma. The wide diffusion of Human herpesvirus 8 in classic Kaposi's sarcoma areas appears to represent an important factor in the high incidence of the disease. However, additional co-factors are likely to play a role in the development of Kaposi's sarcoma.

**DESCRIPTORS:** Human herpesvirus 8. KSHV. Kaposi's sarcoma. AIDS. HIV.

#### INTRODUCTION

Kaposi's sarcoma (KS) is the most common oral malignancy of HIV disease. Epidemiological studies had previously suggested that KS may have an infective etiology, and in 1994 a virus termed Kaposi's sarcoma-associated herpes virus (KSHV) or human herpes virus-8 (HHV-8) was confirmed to be the cause. Since the first description of HHV-8<sup>1</sup>, more of its virology, pathogenic mechanisms, and clinical

consequences have been elucidated. There is strong evidence that HHV-8 not only is the causative agent of KS<sup>2</sup> but also of primary effusion lymphoma (PEL) (also termed body cavity-based lymphomas (BCBL)<sup>3</sup> and multicentric Castleman disease (MCD)<sup>4</sup>. Human herpesvirus 8 has also

been tenuously etiologically linked with multiple myeloma<sup>5</sup> and non-neoplastic disorders such as sarcoidosis<sup>6</sup>.

#### EPIDEMIOLOGY OF HHV-8 - ASPECTS OF TRANSMISSION

Human herpesvirus 8 is principally acquired sexually. Anal intercourse may be particularly associated with transmission<sup>7</sup>. In a large study of US

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males, sex with other men was associated with a greater risk activity for HHV-8 acquisition than was a history of blood or blood products receipt or injecting needle usage<sup>8</sup>. Likewise, in a cohort of Danish homosexual men, HHV-8 seropositivity correlated positively with the frequency of receptive anal intercourse<sup>9</sup>. Females infected with HIV by their bisexual male partners were found to be at greater risk of developing KS than those who became HIV-infected as a consequence of vaginal intercourse with male hemophiliacs, transfusion recipients, or injecting drug users<sup>7,10</sup>.

HHV-8 is present in prostate tissue and in semen, but the frequency of carriage varies considerably between groups. Hybridization *in situ* revealed HHV-8 in the prostate glandular epithelium of an elderly HIV-seronegative male<sup>11</sup>. In addition, in a cohort of Italian patients of unknown HIV status, who had no apparent clinical disease other than occasional varicoceles, HHV-8 DNA was detected by polymerase chain reaction (PCR) in 12% of tissue specimens from the urogenital tract, and in 44% of prostate tissues<sup>12</sup>. In addition, 81% of ejaculates from non-HIV-infected Italian individuals were HHV-8 positive<sup>12</sup>. HHV-8 was detected in prostate tissue of men with KS by *in situ* hybridization; between 1% to 5% of cells expressed viral transcripts associated with HHV-8 replication, while more than 90% expressed gene products associated with viral latency, findings suggesting that the prostate may be a site for viral replication and subsequent shedding into semen<sup>13</sup>.

HHV-8 DNA was detected by PCR in semen samples of 2/15 HIV-negative and 8/15 HIV-positive men living in Central Africa<sup>14</sup>. In contrast, HHV-8 was not detected in the semen of 4 HIV-infected American males with KS<sup>15</sup>, and only 2 out of another cohort of 14 HIV-infected American homo-

sexual men with KS were found to be HHV-8 DNA positive<sup>16</sup>. Accordingly, not all HIV-infected patients from the US had evidence of HHV-8 in semen<sup>17</sup>.

Transmission of HHV-8 in feces was suggested by the findings that oro-anal contact was associated with a higher likelihood of KS in homosexual males<sup>7</sup>. Nevertheless, supporting evidence is equivocal since HHV-8 DNA was not detectable in feces from one cohort of HIV-infected patients<sup>18</sup> but was found in 24 out of 51 duodenal and rectal biopsies from another cohort<sup>19</sup>.

Sexual transmission is unlikely to be the only route of transmission of HHV-8, particularly in geographic areas where HHV-8 infection is endemic, and where prior to the AIDS era, KS was a common tumor not subsequently found to be associated with HIV infection (e.g. in Central and East Africa)<sup>20</sup>, such as in Uganda<sup>21</sup>. A recent study found HHV-8 infection in early childhood in Uganda unassociated with antibodies to hepatitis A or C virus or with the quality of water supply, which points to a horizontal mode of transmission different to that of developed countries<sup>22</sup>.

HHV-8 has also been found in saliva of HIV-infected patients. In one study, HHV-8 DNA was found in unstimulated whole saliva from 25 of 76 HIV-infected individuals *without* oral KS<sup>23</sup>, and another study showed HHV-8 in the saliva of individuals having no detectable viral sequences in their peripheral blood mononuclear cells<sup>24</sup>. In addition, HHV-8 DNA was detected in the saliva of 5 of 29 (17%) symptomatic HIV-infected patients (only 1 had oral KS at the time of sampling), but in none of 15 healthy controls<sup>25</sup> or in any of 39 HIV-negative patients<sup>26</sup>.

Parenteral transmission is another potential route of HHV-8 infection. The virus has been detected in CD19+ cells of a healthy blood donor<sup>27</sup>. But in a separate study, 13 of 14 patients

were found to remain seronegative for HHV-8 despite receiving transfused blood cells from HHV-8 seropositive donors<sup>28</sup>.

Data concerning a vertical route of transmission of HHV-8 is contradictory. In one study, all 9 children born from HHV-8 IgG positive Haitian and American mothers were HHV-8 seronegative<sup>29</sup>. However, in another study, 8 children of 19 who had HHV-8 IgG seropositive mothers were also HHV-8 seropositive<sup>30</sup>. Apart from vertical transmission, intrafamilial person-to-person spread of the virus has also been suggested, since the HHV-8 seroprevalence of spouses, children, and siblings in families of KS patients is higher than in HHV-8 non-family controls<sup>31</sup>.

#### EPIDEMIOLOGY OF HHV-8 INFECTION - SEROLOGICAL DATA

Epidemiological data based upon the detection of IgG antibodies to HHV-8 is conflicting, owing to methodological differences and, perhaps more importantly, geographical differences in HHV-8 infection. Using an indirect immunofluorescence assay (IFA) based on nuclei of BCBL-1 cells (to exclude the detection of cytoplasmic antigens)<sup>32</sup>, a seroprevalence of 1% was found in a group of HIV-negative blood donors and 8% in HIV-negative patients with syphilis. A study with indirect immunofluorescence using an uninduced cell preparation with a high cut-off to avoid non-specific background<sup>33</sup> failed to detect HHV-8 antibodies in a large cohort of US blood donors. In contrast, an IFA based upon induced cell preparations optimized for the expression of both nuclear and cytoplasmic antigens<sup>34</sup> found 25% of a cohort of healthy American adults and 2% to 8% of children to have IgG antibodies to HHV-8.

An IFA that detected antibodies to a latency-associated nuclear antigen (LANA) found none of 195 exclusively heterosexual men from San Francisco to have antibodies to HHV-8, while 12.5% of men who reported mostly homosexual activity, and 39.6% who were exclusively homosexual were HHV-8 positive<sup>8</sup>. More recently, using uninduced and TPA-induced BCBL-1 cells<sup>35</sup>, IFA antibody titers to HHV-8 were found to be much higher in HIV-infected Los Angeles patients with KS than in those HIV-infected without KS; furthermore, antibody positivity rate was 8% in healthy individuals, 56% in HIV-positive homosexual men, and 12% in age-matched HIV-negative controls<sup>36</sup>.

Additionally, 28% of Italian blood donors were found to be HHV-8 seropositive by IFA in a recent published study<sup>37</sup>. This is similar to another study where 23% of the control group comprised of healthy volunteers and patients with various dermatological diseases from central and southern regions of Italy had HHV-8 DNA in their PBMCs<sup>38</sup>.

Using IFA, HHV-8 antibodies were found in 16/16 French KS patients but in only 3/83 patients with non-KS dermatologic diseases and in 2/100 healthy controls<sup>39</sup>. Likewise, only 5/169 (3%) of HIV negative patients from Honduras were found to be HHV-8 positive when using a cutoff point of 1:40<sup>40</sup>.

Studies with enzyme-linked immunosorbent assay (ELISA) utilizing recombinant capsid-related proteins of HHV-8 open reading frame (ORF) 65 (which has little sequence similarity to EBV-BFRF3)<sup>41</sup> found seroprevalence rates of 1.7% in UK, 5% in North American, 12% in Mediterranean blood donors, and up to 47% in Ugandan HIV-negative individuals. Investigations using an ELISA that utilizes 2 regions of the ORF 26 differing substantially from EBV-BDLF1, found 6 of 30 (20%) US

healthy blood donors to be HHV-8 seropositive<sup>42</sup>. Another study, using small viral capsid antigen (sVCA) found 3 of 28 (11%) US blood donors to be seropositive for HHV-8, although none of 25 hemophilic patients or 22 children (10 HIV-negative and 12 HIV-positive) were HHV-8 seropositive<sup>43</sup>. In another study, none of the 52 patients tested who were negative for HIV and hepatitis B and C virus had antibodies for HHV-8<sup>44</sup>. In addition, using a latency-associated nuclear antigen (LANA) IFA and an ELISA with plates coated with capsid-related protein encoded by ORF 65, 24.1% of 779 Italian blood donors from different regions had antibodies to at least 1 antigen<sup>45</sup>.

An ELISA for detection of the ORF 65.2 antigen showed positivity in 24/26 (92.3%) of Swiss HIV-positive patients with KS, in 21/87 (24.1%) of HIV-positive patients without KS, in 11/54 (20.4%) of HIV-negative homosexual men, and in 9/178 (5.1%) blood donors<sup>46</sup>. Another ELISA assay with the whole virus lysate as the antigen was used, and a seroprevalence of 11% (10/91) was found in US blood donors. In this study, the average titer of different groups was also measured: blood donors had an average titer of 118, classic KS patients had an average titer of 14,111, and HIV-associated KS patients had 4000<sup>47</sup>.

In order to standardize HHV-8 antibody assays, a blinded comparison was recently performed between 5 laboratories using 4 different IFAs and 3 ELISAs. It was found that while the HHV-8 antibody tests were adequate for epidemiological investigations, the poor specificity and sensitivity in detecting asymptomatic HHV-8 infection required further confirmatory testing using nucleic acid detection methods<sup>48</sup>.

Most serological studies (Table 1) suggest a global HHV-8 seroprevalence of 2% to 10%. Assuming a rate of 5% HHV-8 in the US and a 1970 baseline incidence of KS in men in the

US (about 0.3 cases per 100,000 men), the HHV-8 rate would be only 1 case of KS for every 17,000 HHV-8 infections<sup>50</sup>.

## DISEASE ASSOCIATIONS OF HUMAN HERPESVIRUS 8

### Kaposi's sarcoma

Two small fragments of the HHV-8 genome, KS<sub>330</sub>Bam and KS<sub>631</sub>Bam, were originally isolated from KS tissues using representational difference analysis<sup>1</sup>. Since then, other research groups, using PCR primers derived from KS<sub>330</sub>Bam sequence, have detected HHV-8 DNA in lesional tissues of all 3 epidemiological forms of KS: HIV-related<sup>15,51-67</sup>, endemic<sup>51,52,56,58,65,67,68-70</sup>, and immunosuppressed related<sup>65,67,71-73</sup>.

Human herpesvirus 8 DNA can be amplified from KS tissue in different clinical stages of the disease<sup>74</sup>. Furthermore, semiquantitative analysis has established that the HHV-8 DNA load is higher in patients with multicentric and visceral involvement compared with those with localized disease, and that the nodular stage also has a higher viral load than do the patch and plaque stages, thereby showing a correlation between viral load and disease severity<sup>75</sup>.

The presence of HHV-8 DNA in PBMCs of HIV-infected individuals is predictive of the subsequent appearance of KS lesions<sup>76,77</sup>. In addition, antibodies to HHV-8 are associated with having KS or being at increased risk of developing KS<sup>78,79</sup>.

### Body cavity-based lymphoma

Body cavity based-lymphoma (BCBL) (also termed primary effusion lymphoma (PEL)) is a rare, rapidly fatal malignancy, first described in AIDS patients.<sup>3</sup> The disease has a distinctive presentation, with malignant perito-

**Table 1** - HHV-8 seroprevalence in general populations.

Assay	Antigen	Country	Seroprevalence (%)	Reference
Indirect Immunofluorescence Assay	Latency Associated Nuclear Antigen	Us	1-8	32
Western Blot	Lna	Us	0	33
Indirect Immunofluorescence Assay	Latency Associated Nuclear Antigen	Us	25	34
Elisa	Orf 65	Uk, Us, Uganda	1.7, 5, 47	41
Elisa	Orf 26	Germany	11	149
Elisa	Orf 26	Us	20	42
Western Blot	Svca	Us	11	43
Indirect Immunofluorescence Assay	Orf 26/26	Us	0	44
Indirect Immunofluorescence Assay	Latency Associated Nuclear Antigen	Us	0	8
Indirect Immunofluorescence Assay/ Western Blot	Latency Associated Nuclear Antigen	Us	8	35
Elisa/Indirect Immunofluorescence Assay/Western Blot	Latency Associated Nuclear Antigen/Orf 65	Italy	24	45
Indirect Immunofluorescence Assay	Latency Associated Nuclear Antigen	France	2-3.6	39
Indirect Immunofluorescence Assay	Latent/Lytic	Us	12	79
Indirect Immunofluorescence Assay	Latency Associated Nuclear Antigen	France	2	94
Elisa	Orf 65.2	Switzerland	5	46
Elisa	Whole Virus	Us	11	47
Indirect Immunofluorescence Assay	Lytic/Latent	Italy	28/2	37
Indirect Immunofluorescence Assay	Lytic	Honduras	3-11	40

neal, pericardial, or pleural effusions in the absence of an identifiable tumor mass or nodal involvement. Carriage by BCBL cells of both EBV and HHV-8 infection has been observed, but association with HHV-8 alone has also been reported<sup>180-83</sup>. Notably, most cases of EBV-negative BCBL have occurred in patients who are also HIV negative, whereas BCBL in HIV positive patients are usually EBV-positive.

#### **Multicentric Castleman's disease**

Multicentric Castleman's Disease (MCD) is an atypical lymphoproliferative disorder mostly found in patients with HIV disease. HHV-8 has been found only infrequently in MCD of HIV negative patients<sup>4</sup>, making the association less strong in comparison with that of HHV-8 with KS or BCBL/primary effusion lymphoma.

#### **Other possible disease associations of HHV-8**

##### *Lymphoproliferative disease*

HHV-8 sequences have been detected in some angioimmunoblastic lymphadenopathies in non-HIV-infected patients, in particular in a distinct, benign lymphadenopathy histologically characterized by a predominantly follicular lesion with a giant hyperplastic germinal center and increased vascularity<sup>84</sup>.

HHV-8 DNA has also been detected rarely in other lymphoproliferative disorders, including non-Hodgkin's lymphoma, Hodgkin's disease, reactive lymphadenopathies<sup>85</sup>, and cutaneous lymphoma in AIDS<sup>59</sup>. The viral load is significantly higher in lymphoid tissue from HIV-infected persons as compared to HIV-seronega-

tive individuals<sup>85</sup>, although it is still lower than in splenic tissue or PBMCs from the same patients. This suggests that the HHV-8 detected in these lesions may reflect HHV-8 carriage by non-neoplastic B cells<sup>59</sup>. Detection of HHV-8 in mature T-cell lymphoproliferative disorders is equivocal<sup>3,86-88</sup>.

##### *Multiple Myeloma*

HHV-8 has been associated with multiple myeloma (MM) in several US studies. HHV-8 DNA was found in bone marrow dendritic cells of 25% of a cohort of patients with monoclonal gammopathy, a condition that may progress to MM<sup>5</sup>. HHV-8 DNA was also found by PCR in 6 out of 7 fresh biopsy samples, and by in situ hybridization in bone marrow dendritic cells in 17 out of 20 patients with MM<sup>89</sup>. In addition, 20 of 27 (81%) patients with

MM were HHV-8 seropositive using an ELISA assay, while only 22% of patients with other malignancies and 6% of blood donors were found to be HHV-8 seropositive<sup>90</sup>.

In contrast, studies in other countries have not detected HHV-8 associated with MM. Using a nested PCR and a serological assay to detect HHV-8 LANA and ORF 65 (lytic) protein, only 1 out of 20 Italian patients with MM was found to be HHV-8 seropositive<sup>91</sup>. None of 10 Swedish patients with MM were found to be HHV-8 positive by PCR or serological assay<sup>92</sup>. Furthermore, no statistically significant differences between MM patients and blood donors were observed in the frequency of HHV-8 seropositivity using an IFA assay<sup>76</sup>. Similar results have also been found by others<sup>93-97</sup>. Any significance of HHV-8 in the etiology of MM thus remains unclear.

#### *Other conditions*

HHV-8 DNA can be detected in normal skin of HIV-infected patients with KS<sup>62,64,98</sup>, and in normal skin from patients with endemic KS<sup>12,15,65,99,100</sup>, or in iatrogenic KS<sup>101</sup>. It is also found in lesional tissue of patients with squamous cell or basal cell carcinomas<sup>66</sup>. The HHV-8 infection load appears to be lower in normal skin and in cutaneous lesions other than KS than in lesional KS tissue<sup>64,66</sup>.

Sequences of HHV-8 DNA have been detected in immunosuppressed patients with glomerulonephritis<sup>54</sup>, with pemphigus vulgaris,<sup>102</sup> and with mycosis fungoides.<sup>103</sup> HHV-8 sequences have also been detected in angiosarcomas<sup>104,105</sup> and in angiolymphoid hyperplasia with eosinophilia<sup>104</sup>. However, HHV-8 DNA was not detected in immunosuppression-associated dermatofibromas, despite these sharing many histologic similarities with HIV-related KS<sup>106</sup>.

HHV-8 has been associated with sarcoidosis in Italian patients<sup>6</sup>, but not

in French patients<sup>107</sup>. Furthermore, a serological assay (ELISA) detected antibodies in only 3 out of 15 (20%) of Swiss patients<sup>108</sup>.

At present it is unclear if immunosuppression or granulomatous inflammation activate latent HHV-8 or whether the virus is indeed an etiological agent.

#### *Virological aspects of HHV-8*

Cell lines derived from BCBL frequently contain HHV-8 genomes. Their study has thus provided significant insights into the biological properties of HHV-8. The BC-1 line harbors HHV-8 but not EBV-DNA, while line BC-2 harbors both viruses<sup>3</sup>. Treatment of BC-1 with phorbol esters rapidly induces lytic growth of HHV-8, and progeny virus are then shed into the supporting medium<sup>109</sup>. The induced B cells can be observed to contain 110 nm intranuclear herpesvirus-like nucleocapsids and complete cytoplasmic virions<sup>81</sup>. The length of genome of the virus is estimated to be similar (e.g. 160-170 kb) to other gammaherpesviruses. The HHV-8 genome is, like that of EBV, maintained in latently infected B cells as extrachromosomal, episomal, monomeric circles, with induction from latency leading to the selective accumulation of linear genomic forms<sup>109</sup>. In contrast, only covalently closed, circular episomes of HHV-8 are identified in KS tissue, while linear forms, arising from viral replication, are additionally found in PBCs of KS patients<sup>110</sup>. While uninduced BCBL lines have not yet been shown to permit propagation of HHV-8, the virus can be cultured from skin lesions of patients with AIDS-associated KS using the human embryonal-kidney epithelioid line 293<sup>106</sup>, thus providing evidence that the virus is able of replicating vegetatively in vitro.

Sequencing of a 12.3-kb HHV-8 clone obtained from a genomic library derived from BC-1 revealed homology between HHV-8 with parts of the EBV

genome. The sequences of some ORFs of HHV-8 are homologous to EBV-membrane antigen p140, herpesvirus saimiri (HVS) p160, cellular type D cyclins, and HVS and cellular G protein-coupled receptors<sup>80</sup>. Furthermore, transcription of these 4 ORFs can be demonstrated in BC-1<sup>2</sup>. A novel abundant 1.2-kb RNA, polyadenylated nuclear RNA (called PAN RNA), has also been identified from the BC-1 line; it appears speckled in the nuclei by immunofluorescence and may be a early lytic cycle viral transcript<sup>111,112</sup>.

The BC-1 cell line was used to create a cosmid and phage genomic library, allowing full characterization of the HHV-8 nucleotide sequence, except for a 3 kb region at the right end of the genome<sup>113</sup>. The BC-1 HHV-8 genome is 140.5-kb long, with a unique coding region flanked by multiple 801-bp terminal repeat sequences. A genomic duplication that apparently arose in the parental tumor is present in this cell culture-derived strain. At least 81 ORFs and 5 internal repeat regions are present in the long unique region. In addition to viral structural and metabolic proteins, the virus encodes homologues as complement-binding proteins, 3 cytokines (2 macrophage inflammatory proteins (MIP), MIP-1a, MIP-1b, and interleukin-6, IL-6), dihydrofolate reductase, *bcl-2*, interferon regulatory factors, interleukin 8 receptor, neural cell adhesion molecule-like adhesin, and a D-type cyclin<sup>113</sup>. A subsequent study in which a 17-kb segment of HHV-8 between ORFs 11 and 17 was sequenced confirmed that the viral genome contains a single 13-kb divergent locus wherein are 9 ORFs that are homologous with, or related to, cellular proteins<sup>114</sup>. A fourth potential cytokine gene, BCK, was also identified, in addition to a viral thymidylate synthetase gene, the T1.1 abundant lytic cycle nuclear RNA gene, and 2 genes (1E1-A and 1E1-B) related to the immediate-early protein

of the gamma-2 class herpesvirus bovine herpesvirus type 4<sup>114</sup>.

The herpes viral-like particles produced from BC-1 cells can further infect PBMC-derived CD19+ B cells. This suggests that HHV-8 is transmissible and B-lymphotropic<sup>115</sup>.

More recently, other BCBL-derived cell lines have been established and characterized: BC-3, BCP-1, and CRO-AP/3 do not contain EBV DNA, while CRO-AP/5 and HBL-6 contains both EBV and HHV-8<sup>98,116-118</sup>. The structure of the HHV-8 gene in these cell lines has yet to be reported.

#### *HHV-8 homology to other viral and cellular proteins*

Analysis of the putative translation products of HHV-8 has revealed homology with a number of known human cell receptors, cell cycle enzymes, and chemokines important in a variety of cellular and immunological as well as homeostatic and angiogenic mechanisms. HHV-8 may thus possess a range of mechanisms capable of causing cell changes.

HHV-8 possesses several potential oncogenes. The open reading frame (ORF) K1, for instance, encodes a class I transmembrane glycoprotein with transforming properties in rodent fibroblasts<sup>119</sup>. The second HHV-8 potential oncogene is a G protein-coupled receptor (GCR), which is a putative translation product of ORF 74. These receptors are important in cellular growth and differentiation, with some GCRs being implicated in malignant transformation<sup>116</sup>. Expression of HHV-8 GCRs stimulates proliferation and causes transformation of rodent fibroblasts. Hence, the HHV-8 GCR homologue may be a mediator of KS tumorigenesis<sup>120</sup>. The closest cellular homologues to the putative HHV-8 GCR are the interleukin-8 (IL-8) receptors A and B, and the closest viral homologue is the herpesvirus

samiri (HVS) *ECRF3* gene, which encodes a functional IL-8 receptor<sup>121</sup>.

HHV-8 also contains a protein homologous with cell cycle controllers, such as a cyclin D-type v-cyclin that displays 53% homology to cyclin D2<sup>122</sup>. Cyclins are required for cellular division and are involved in the control of the G<sub>1</sub>S check point in the cell cycle<sup>123</sup>. HHV-8 cyclin protein is reported to possess kinase activity, which enables it to phosphorylate and thus inactivate the retinoblastoma (Rb) tumor suppressor protein. Hence HHV-8 has the potential to overcome cell-cycle control, thereby increasing the likelihood of tumor development<sup>124,125</sup>.

HHV-8 ORF 16 (vBcl-2) shares 15% to 20% amino acid identity with the Bcl-2 family<sup>113,126,127</sup> — a group of genes known to prevent apoptosis.

Other ORFs of HHV-8 have been found to encode proteins similar to macrophage inflammatory protein (MIP) chemokines (vMIP-1 from ORF K6 and vMIP-2 from ORF K4), interleukin-6 (from ORF K2), and interferon regulatory factor (vIRF) (from ORF K9)<sup>2</sup>. The HHV-8 ORF K6 protein (vMIP-1) inhibits HIV entry via the

CCR5 chemokine receptor, suggesting that vMIP-1 is functional in binding CCR5 and thus contributes to interactions between HHV-8 and HIV.

Potentially, HHV-8 may exert some inhibitory action in HIV infection, which might underlie the suggestion that patients with AIDS and KS have a better prognosis than patients with AIDS but not KS<sup>128</sup>. This may not be the case, however, since a recent epidemiological study found that the presence of KS appears to accelerate the clinical course of HIV infection resulting in shorter survival times<sup>129</sup>. Furthermore, vMIP-1 is expressed only by a small subpopulation of HHV-8 infected cells (productive cells), and therefore the impact of vMIP-1 on KS angiogenesis may be limited.<sup>130</sup> The virally-derived IL-6 (vIL-6) has been found to be expressed in some HHV-8 infected cells<sup>131</sup>.

HHV-8 may thus have the means of overcoming typical cellular strategies against viral infection including cell cycle control, apoptosis, and cell-mediated immunity (Table 2 summarizes HHV-8 genes possibly implicated in the pathogenesis of KS).

**Table 2 - HHV-8 genes implicated in tumorigenesis<sup>150</sup>.**

Host cell homologue	HHV-8 encoded protein	Possible function
D-Type cyclin	v-Cyc	Inactivation of pRB Promotes G1 to S phase transition
IL-8 GPCR	V-GPCR	Cellular growth signal
Interferon regulatory factor	v-IRF	Inhibits p21 and MHC class I expression
CC chemokines	v-MIP-I, vMIP-II, vMIP-1B	Chemoattraction, angiogenesis
IL-6	v-IL-6	Growth factor for KS cells
Bcl-2 family protein	v-Bcl-2	Inhibition of apoptosis
FLICE inhibitory protein	v-FLIP	Inhibition of CD95L and TNF-induced apoptosis
N-CAM family protein	v-Ox-2	Cellular adhesion molecule
CD21/CR2 complement binding protein	ORF 4	Escape from host immune response

### Antiviral therapy

HHV-8 replication in vitro can be inhibited by ganciclovir, foscarnet, and cidofovir, but not acyclovir<sup>133</sup>. In addition, lytic replication of HHV-8 can be inhibited by a low dose of cidofovir<sup>134,135</sup>.

Highly active antiretroviral therapy (HAART), however, is very effective in inhibiting HIV replication, increasing CD4 + cell counts and delaying, lessening, or resolving AIDS-associated opportunistic infections<sup>136,137</sup>. Preliminary data from case reports suggest that protease inhibitors may help induce remission of HIV-re-

lated KS<sup>125,138-143</sup> and clearance of HHV-8 DNA from PBMCs<sup>138,139,141,143,145</sup>. Moreover, HAART in 2 HIV-positive patients with BCBL resulted in HHV-8 DNA being undetected in their pleural effusions<sup>146</sup>. In contrast, PrI treatment produced no effect on the clinical and virological progression of an HIV-positive patient with MCD<sup>147</sup>.

### Oral health considerations of HHV-8 infection

HHV-8 is not found in saliva of healthy controls but is found in the saliva of up to 33% of patients with AIDS<sup>23</sup> and up to 75% of HIV infected

patients with KS<sup>24</sup>. Similarly, HHV-8 DNA was found only in symptomatic HIV-1-infected patients (5 [17%] of 29)<sup>25</sup>. More recently, 11 out of 24 saliva samples (45.8%) of patients with AIDS-KS were HHV-8 DNA positive, but in the control group, none of the 20 saliva specimens were positive for HHV-8 DNA<sup>38</sup>. HHV-8 has been identified in oral KS, other oral lesions (e.g. non-specific ulcers), and in normal oral mucosae in HIV disease<sup>148</sup>. Nevertheless, there are no data yet to suggest that dental health care workers are at notable risk of HHV-8 acquisition through occupational routes.

## RESUMO

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**OBJETIVO:** O objetivo do presente artigo foi revisar a literatura recente em relação ao herpesvírus humano tipo 8, com ênfase especial aos aspectos relacionados à etiopatogênese do sarcoma de Kaposi.

**MÉTODOS:** Os autores pesquisaram artigos de pesquisa original e revisões de literatura nos aspectos específicos da infecção pelo herpesvírus humano tipo 8, incluindo, virologia, epidemiologia, transmissão, diagnóstico, história natural e terapia. O material considerado relevante foi avaliado e revisado.

**RESULTADOS:** O sarcoma de Kaposi é considerado ainda a malignidade mais comumente observada em

pacientes infectados pelo HIV. Estudos epidemiológicos, assim como os baseados em técnicas de biologia molecular indicam que um agente sexualmente transmissível, independente do HIV, deve estar envolvido na etiologia do sarcoma de Kaposi, possivelmente como resultado da ação das *cell signaling proteins* superando os aspectos da resposta imune. O herpesvírus humano tipo 8 tem sido ainda sugerido como agente causal na patogênese de outras desordens, incluindo mieloma múltiplo, *multicentric Castleman's disease*, *body cavity-based lymphoma*, além de outras condições não-proliferativas como sarcoidose e pênfigo vulgar, embora grande parte dos estudos sorológicos apontem para uma soroprevalência em torno de 2 a 10%. O herpesvírus humano tipo 8 parece então, ser um vírus restrito a pessoas sob risco de desenvolver o sarcoma de Kaposi, associado à imunossupressão. O

tratamento para o sarcoma de Kaposi é normalmente paliativo, e inclui a aplicação de vimblastina intra-lesional, crio-cirurgia, interferon-alpha e outras formas de terapia. Mais recentemente, os inibidores da protease, foram também sugeridos como possíveis agentes implicados na remissão do sarcoma de Kaposi associado ao HIV e no desaparecimento do herpesvírus humano tipo 8 das células mononucleares do sangue periférico.

**CONCLUSÃO:** O herpesvírus humano tipo 8 está fortemente associado a todas as formas de sarcoma de Kaposi, *multicentric Castleman's disease* e *body cavity-based lymphoma*. Ainda, não existe tratamento definitivo para o sarcoma de Kaposi.

**DESCRITORES:** Herpesvírus humano tipo 8. KSHV. Sarcoma de Kaposi. AIDS. HIV.

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