Update on Kaposi's sarcoma and other HHV8 associated diseases. Part 2: pathogenesis, Castleman's disease, and pleural effusion lymphoma

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The pathogenesis of Kaposi's sarcoma (KS) is better understood since the identification of the novel human herpesvirus 8 (HHV8), which can be found in all forms of KS. Viral oncogenesis and cytokine-induced growth, as well as some states of immunocompromise, contribute to its development. Several virally encoded genes—eg, *bcl*-2, interleukin 6, cyclin D, G-protein-coupled receptor, and interferon regulatory factor—provide key functions on cellular proliferation and survival. Growth promotion of KS is further stimulated by various proinflammatory cytokines and growth factors such as tumour necrosis factor α, interleukin 6, basic fibroblast growth factor, and vascular endothelial growth factor, resulting in a hyperplastic polyclonal lesion with predominant spindle cells derived from lymphoid endothelia. HHV8 has recently been discovered to escape HLA-class-I-restricted antigen presentation to cytotoxic T lymphocytes by increasing endocytosis of MHC class I chains from the cell surface, thus enabling latent infection and immune escape in primary and chronic infection. Multicentric Castleman's disease is a rare lymphoproliferative disorder of the plasma cell type, which has been reported in both HIV-seropositive and HIV-seronegative patients, and which frequently contains HHV8 DNA. Pleural effusion lymphoma, or body-cavity-based lymphoma, belongs to the group of non-Hodgkin B-cell lymphomas characterised by pleural, pericardial, or peritoneal lymphomatous effusions in the absence of a solid tumour mass. Pleural effusion lymphoma has an intermediate immunophenotype lacking B or T lymphocyte antigens and also belongs to the diseases associated with HHV8.

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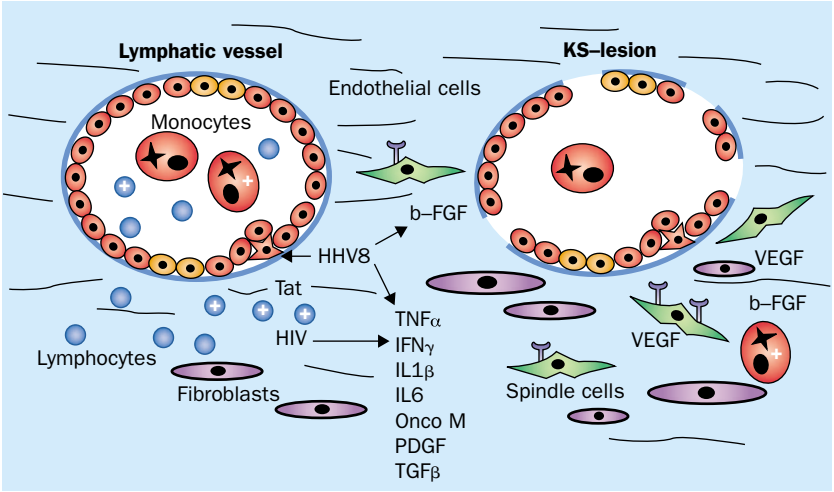

Figure 1. Schematic of KS pathogenesis. Some HHV8–infected endothelial cells together with HIV–infected monocytes and lymphocytes (marked with a "+") will eventually lead to atypical endothelial cells that may be transformed when exposed to a particular microenvironment resulting in a polyclonal cell population with spindle–shaped morphology. These spindle cells bearing a number of growth factor receptors will contribute to the formation of slit–like vessels with a discontinuous endothelial lining. During the course of the disease a few (or a single) predominant clones may grow out leading to an oligo–(mono)clonal cell population in advanced KS stages.

Part 1 of this update on Kaposi's sarcoma (KS) and other human herpesvirus 8 (HHV8)-associated diseases was published in the May issue of *The Lancet Infectious Diseases* (pp281–92). Part 2 summarises recent insights in the pathogenesis of KS and discusses other HHV8-related diseases such as Castleman's disease and pleural effusion lymphoma.

Pathogenesis

Any model of pathogenesis should explain several unique characteristics of KS. These characteristics include the high male-to-female ratio, the association with sexually transmitted HIV infection,[1] its declining incidence in the

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homosexual male population in the USA and Europe, its apparent exacerbation with opportunistic infection, and its occurrence in individuals at risk for but without evidence of HIV infection. The interplay of HHV8, HIV, and KS cells may help to explain the multicentric nature of KS and the effect of opportunistic infections and other inflammatory lesions to exacerbate KS cell growth.

Whether KS is a true malignancy or a polyclonal hyperplastic response to inflammatory signals released by viral infection has been debated.[2,3] KS has a proliferative component of spindle cells and endothelial cells, and has inflammatory and angiogenic properties (figure 1). Although HIV infection is clearly involved in the pathogenesis of AIDS-KS, KS is also seen in the absence of HIV in transplant recipients (transplantation KS) and in elderly men (classic KS). Furthermore, the fact that AIDS-KS may occur at normal CD4-cell counts suggests that HIV does contribute more than just immunodeficiency. HIV-infected cells, HHV8-infected cells, and KS cells each produce cytokines and growth factors that promote the development of clinical KS (figure 1).

HHV8

HHV8 or Kaposi's sarcoma herpesvirus (KSHV) was first identified by Chang et al[4] in 1994 on the basis of DNA sequences detected in tissues from patients with AIDS-KS. HHV8 has also been identified in classic KS, endemic African KS, and transplantation-related KS.[5] By PCR in-situ hybridisation and in-situ hybridisation, HHV8 DNA or RNA has been localised to spindle cells, endothelial cells, and monocytes in nodular KS.[6,7] Recent evidence suggests that surface heparan sulfate serves—in analogy to adeno-associated virus—as a cellular attachment receptor for HHV8 interacting with the K8.1 protein.[8] It has been suggested that HHV8-infected T cells and monocytes might be responsible for the circulation of HHV8, accounting for the multicentric nature of KS lesions.

Viral taxonomy, genome organisation, and molecular epidemiology

HHV8 is the first human γ-herpesvirus and has a tropism for lymphocytes, endothelial cells, keratinocytes, and possibly, marrow stromal cells. Its protein capsid structure is surrounded by an amorphous tegument and a lipid bilayer for a general size of 120–150nm. The HHV8 genome is ~165 kb long and contains a central region of low GC DNA (L DNA) flanked by multiple repetitive high GC DNA (H DNA).[9] Similar to other γ-herpesviruses, the L DNA segment of HHV8 has many open reading frames with significant homology to human genes. The genome is circular during latent infection and linear during the lytic phase. Like other herpesviruses, replication is by the rolling circle mechanism within the nucleus of the host cells.[9]

Molecular epidemiological studies suggest that HHV8 has existed in the human population at least for several centuries.[10] HHV8 has been phylogenetically classified into strains A, B, and C.[11,12] Strain A was more often seen in classic Mediterranean KS, and B and C were seen in African KS.[11]

Table 1. HHV8 lytic and latent genes and gene products

HHV8 genes	HHV8 protein	Possible function
Lytic		
ORF 74	vGPCR	Chemokine receptor homologue; constitutive signalling triggers other angiogenesis factors
ORF K6	vMIP-II	Chemokine homologue; triggers angiogenesis
ORF K1	vK1	Transmembrane signalling
ORF K9	vIRF-1	Blocks interferon-induced gene expression
ORF K8.1	gB	Binding to target cells
Latent		
ORF 73	LANA	Episome maintenance; plasmid stabilisation
ORF 72	vcyclin	Growth control; G1/S transition
ORF 71	vFLIP	Blocks cell-mediated apoptosis
ORF K12	Kaposin	May transform fibroblasts

FLIP=FLICE inhibitory protein (FLICE=Fas-associated death domain-like interleukin-1 beta converting enzyme); gB=surface glycoprotein B; GPCR=G protein-coupled receptor; IRF=interferon regulatory factor; LANA=latency-associated nuclear antigen; MIP=macrophage inflammatory protein; *ORF*=open reading frame.

On the aminoacid level, HHV8 has 30–50% homology to Epstein-Barr virus and herpesvirus saimiri sequences for 30–50%.[13] Like Epstein-Barr virus, herpesvirus saimiri, a virus of squirrel monkeys (*Saimiri sciureus*), can induce latent infection of peripheral blood lymphocytes, immortalise lymphocytes in vitro, and lead to the development of malignant lymphomas.

Viral genes

The list of HHV8 genes that affect binding, cell growth, proliferation, inflammation, and angiogenesis is long (table 1). They are expressed during latency or the lytic life cycle. These viral products induce cellular *bcl*-2, interleukin 6, cyclin D, an interleukin 8 receptor, interferon regulatory factor, and the complement-controlling protein CR2.[9,13–15] Thus, HHV8 seems to have developed alternative means of expressing proteins that overcome the usual responses to viral infection by helping to oppose cell-cycle arrest, apoptosis, and activation of cellular immunity. Some of these genes could be implicated in tumour induction because they are homologous to known cellular oncogenes, some of which have the ability to subvert normal cellular pathways involved in cell-cycle regulation, cell death, and/or signal transduction.[16]

The latency-associated nuclear antigen (LANA) of HHV8 (encoded by *ORF* 73) may act as a transcriptional regulator and modify gene expression of viral and cellular genes (table 1).[17] With an antibody (LNA-1) against LANA, latent HHV8-infection was detected in patch, plaque, and nodular KS.[18] Mature endothelial cells surrounding normal blood vessels were HHV8 negative. Although HHV8 is

present in less than 10% of the cells in early KS, it is seen in more than 90% of spindle cells in nodular KS. LANA is expressed in all latently infected cells and has been shown to transform primary rodent cells in conjunction with the oncogene H-*ras* by targeting both the p53 and retinoblastoma-E2F pathways.[19]

Latent HHV8 gene products induce tumour hyperplasia and spindle-cell proliferation, although the inciting triggers are still unknown. Several HHV8-expressed genes encode proteins that help HHV8-infected cells to escape immune surveillance—eg, by downregulation of the MHC. When latently HHV8-infected cells are exposed to inflammatory cytokines, especially interferon γ, the viral lytic cycle is induced; tumour necrosis factor (TNF) α, interleukin 1, interleukin 2, interleukin 6, granulocyte-macrophage-colony stimulating factor (GM-CSF), basic fibroblast growth factor (bFGF), and glucocorticoids do not induce lytic infection.[20–22] The lytic gene products seem important for the inflammatory and angiogenic component of KS lesions, although they do not immortalise cells or induce proliferation of spindle cells (table 1). Three genes—namely the viral interferon regulatory factor (K9, vIRF),[23] the viral G-protein-coupled receptor (*ORF* 74, vGPCR),[24,25] and *ORF* K1—transform rodent cells or cause tumours in animal models.[26] Of the homologues to cellular cytokines, the HHV8-encoded cytokine interleukin 6 (viral interleukin 6, encoded by *ORF* K2)[27,28] can, in many ways, simulate human interleukin 6. In animal models viral interleukin 6, like human interleukin 6, acts as a multifunctional cytokine and promotes haematopoiesis, plasmacytosis, and angiogenesis. The effects of viral interleukin 6 are triggered through the interleukin 6 receptor, via the human interleukin-6-induced signalling pathways, including JAK1, STAT1/3, STAT5, and the *Ras*-mitogen-activated protein kinase cascade. However, interleukin 6 and viral interleukin 6 display differences in receptor usage that may give rise to underlying qualitative and quantitative differences in the signalling pathways.[27]

Angiogenesis is important to any tumour growth and here again HHV8 seems to have pirated some relevant cellular genes. One of these is *ORF* 74, encoding a vGPCR that is homologous to the human interleukin 8 receptor types A and B.[24,25] This receptor can bind chemokines from the CXC and the CC family, but does not require ligand engagement for its activation.[29] Importantly, it was shown that signalling via vGPCR eventually leads to the upregulation of vascular endothelial growth factor (VEGF) expression, therefore inducing angiogenesis via paracrine mechanisms.[30] Indeed, a recent study in transgenic mice has shown that HHV8 vGPCR expression in haematopoietic cells leads to angioproliferative lesions exhibiting remarkable similarity to KS.[29]

Several other viral homologues of human genes are also important to understand the pathogenesis of KS. Apoptosis of infected host cells is an immunological strategy that viruses must elude if they are to establish persistent infection. HHV8 encodes a homologue of the cellular *bcl*-2.[31] The viral *bcl*-2 contains crucial conserved domains for interaction with other *bcl*-2 members and has been shown to inhibit apoptosis in vitro.[31,32] Viral IRFs of HHV8 have been seen to abolish IRF-1-mediated and strongly reduce TCR/CD3-mediated CD95L induction, thereby inhibiting activation-induced cell death.[33] The inhibition of CD95-dependent T-cell function could contribute to the immune escape of HHV8.[33] HHV8 also encodes the FLICE-inhibitory protein (vFLIP) that may serve to prevent TNF receptor-1 and Fas-mediated apoptosis.[34]

Like the cellular cyclin D, the viral cyclin can activate kinases that phosphorylate, and thereby inactivate pRB,[35] a checkpoint protein that inhibits entry into S-phase. Unlike its cellular counterpart, however, viral cyclin does not seem to be inhibited in the usual way, and can therefore sabotage a possible pRB-mediated defence against ongoing viral infection.[36]

In addition, HIV-transmission studies showed that the HHV8-encoded macrophage inhibitory protein (vMIP)-I is similar to human MIP chemokines in its ability to inhibit replication of HIV-1 strains that depend on the CCR5 coreceptor.[28,37] The vMIPs differ from human MIPs in that they are angiogenic in standard chick chorioallantoic assays.[37] These viral genes also participate in the response to host defences and could be relevant to HHV8 and HIV-1 interactions.[38]

Interferons mediate part of the innate immune response to viral infection. HHV8 encodes a homologue of the human interferon response factors (IRF), transcriptional factors that inhibit interferon-mediated effects.[23] Nevertheless, interferon α had a dose-dependent inhibitory effect on HHV8 induction.[20,22]

Cytokines

The exact role of cytokines in the pathogenesis of KS remains uncertain. The inflammatory cytokines that are upregulated in the serum of AIDS patients and are present in KS lesions seem to reactivate HHV8 infection.[20] This finding is important because it links two proposed factors for AIDS-KS: immune dysregulation leading to increased inflammatory cytokines, and infection with HHV8 leading to viral gene expression. Th1-type cytokines induce a generalised activation of endothelial cells leading to adhesion and tissue extravasation of lymphocytes/monocytes, spindle cell formation, and angiogenesis. These events are triggered or enhanced by infection with HHV8, which is, in turn, reactivated by the same cytokines.

Growth promotion of KS is stimulated by various cytokines and growth factors such as TNF α, interleukin 6, bFGF, platelet-derived growth factor, transforming growth factor-β, VEGF, and oncostatin M.[39] KS cells also express some of the corresponding receptors with high affinities for several of these cytokines.[25,28,40,41] These cytokines and their receptors further induce inflammation and angiogenesis in an autocrine way.[42] If KS is regarded as a reactive hyperplastic process, infection of monocytes with HIV and interaction of productively infected monocytes with endothelial cells could serve to disseminate the virus—along with the cytokines and growth factors—leading to the proliferation of KS cells and fibroblasts.[42,43] AIDS-KS cells have also been shown to grow in response to interleukin 6 and oncostatin M,[44] a cytokine derived from activated

lymphocytes. This growth response contrasts with normal mesenchymal cells such as endothelial or smooth muscle cells. Oncostatin M may be a transforming agent because it alters the histological appearance of KS cells in culture and makes endothelial cells more spindle-shaped.[44] Recently, Lagunoff et al[45] showed the infection of immortalised human dermal microvascular endothelial cells by KSHV virions. Latently infected endothelial cells did not display major morphological changes or growth transformation, and infection was lost from the culture on serial passage.[45] In this regard the culture model recapitulates the behaviour of spindle cells explanted from primary KS biopsies and should further enable comparative studies of the interrelation of HHV8 and spindle cells in vivo and in vitro.

AIDS-KS and classic KS lesions coexpress VEGF and bFGF.[40] The produced VEGF isoforms are mitogenic for endothelial cells but not for KS spindle cells, suggesting a prevailing paracrine effect. The different stimulation may be due to the level of VEGF-receptor expression that is downregulated in KS cells compared with endothelial cells. KS-derived bFGF and VEGF synergise to induce endothelial cell growth as shown in studies using both neutralising antibodies and antisense oligonucleotides against these cytokines.[40] It is likely that these molecules act to stimulate angiogenesis and excessive vascular permeability, resulting in the oedema commonly seen in KS.[46] The staining for VEGF receptor-3, a marker of lymphatic vessels, was similar to that for HHV8. The extensive expression of VEGF receptor-3 in early KS suggests that at this stage KS is either a proliferation of lymphatic endothelium or of immature endothelial cells (precursors).[47]

HIV

Although HIV infection is neither necessary nor sufficient for KS development, it is associated with an increased incidence and a more aggressive course of KS. HIV-infected cells produce several inflammatory and growth promoting cytokines (figure 1). Indeed, patients infected with HIV-1 were roughly 12-fold more likely to develop KS than patients infected with HIV-2.[48] The HIV-1 transactivating gene (*Tat*) produces Tat protein that stimulates proliferation of spindle cells and inhibits apoptosis, perhaps via interferon γ or transactivation of the interleukin 6 gene,[49–52] while spindle cells are not infected by HIV. The Tat protein was also shown to synergise with bFGF and to stimulate the expression of growth-promoting cytokines.[53] The role of HIV-1 Tat protein, as opposed to HIV-2 Tat protein, is rather complicated[49] because it also inhibits the activation of two interferon-induced enzymes (protein kinase and 2′-5′ oligoadenylate synthetase), showing evidence of a viral escape mechanism of HIV-1. In addition, *Tat* has been shown to directly act as an angiogenic factor that can interact with the VEGF-receptor, Flk-1/KDR.[54] The angiogenic effect of *Tat* from HIV-1 was shown to correlate with the expression of αvβ3-integrin on KS spindle cells that binds to the arginine-glycin-aspartic acid (RGD)-region of *Tat*, making αvβ3-competitors potential new targets for the treatment of AIDS-KS.[51,53] In a study examining the contribution of *Tat* to AIDS-KS, a human AIDS-KS cell line was injected into *Tat*-transgenic mice which subsequently manifested KS-like tumours that expressed the MMP gelatinase B and were associated with infiltrating leucocytes,[55] thus making MMP inhibitors attractive novel drugs for the treatment of KS.

There have been several reports of immortalised KS cell lines established from patient samples resembling the spindle-cell component of the tumour lesions. These KS cell lines have been reported to express markers of endothelial, lymphatic, macrophage, and smooth muscle lineages.[2,3] Implantation of these cell lines, or their conditioned media, into nude mice may not induce growth of a tumour but results in a strong angiogenic reaction.[54,56]

KS as a polyclonal inflammatory lesion

Another area of controversy is whether KS represents a clonal, neoplastic process or a polyclonal inflammatory lesion. In early KS the number of spindle cells is low compared with the surrounding inflammatory cells. Furthermore, KS cells in culture depend on exogenous growth factors and, when implanted into nude mice, can induce an inflammatory angiogenic reaction, but do not induce tumours as would fully transformed cells.[57] Regression of KS can happen spontaneously or when immunosuppression is relieved. These characteristics, along with the multifocality of KS lesions, suggest that the process is primarily one of dysregulated inflammation. However, X chromosome inactivation studies within a single lesion as well as comparison of multiple lesions from a single patient, support a clonal origin in a subset of advanced cases.[58] More recent studies have shown varying monoclonality, oligoclonality, and polyclonality from lesions of various patients.[59] One possibility is that KS starts as a hyperplastic polyclonal lesion that gives rise to a clonal cell population only under specific circumstances and selective pressures, with HHV8 infection preceding the clonal expansion and sarcoma development. This view is supported by Judde et al,[60] who measured the size heterogeneity of terminal repeats in HHV8 and showed that nodular KS displays all possible patterns of clonality. However, early KS and multiple lesions

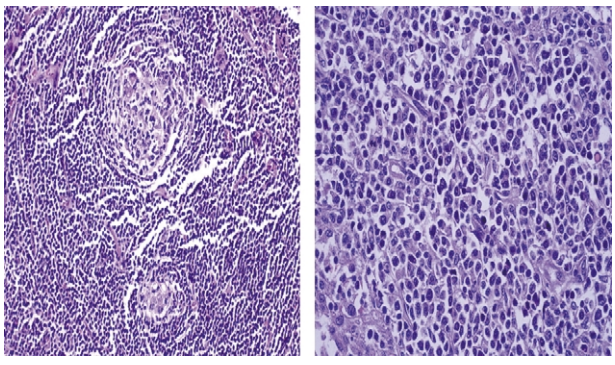

Figure 2. Hyaline vascular and plasmacytoid variant of Castleman's disease. Left: hyaline vascular type changes to lymphoid follicles in Castleman's disease (lymph node, hematoxylin and eosin, 200x). Right: plasmacytoid infiltrate in a lymph node of an HIV patient with (hematoxylin and eosin, 400x). Both photomicrografts were a kind gift of S David Hudnall, Department of Pathology, University of Texas Medical Branch, Galveston, Texas.

from the same patients were not analysed, nor was microdissection used to isolate spindle cells.

Immune responses to HHV8

Cellular immunity to HHV8 has been analysed in chronic and primary infection. Whereas none of 20 normal blood donors had T-cell proliferative responses to purified HHV8, eight of 19 (42%) HIV-negative, HHV8-seropositive homosexual men responded, as did four (16%) of 25 HHV8-seronegative homosexuals.[61] Among HIV-positive homosexual men, however, none of 21 HHV8-seropositives had T-cell responses to HHV8, even though most responded to common recall antigens and had more than 400 CD4 cells/μL.[61] In another study by Osman et al,[62] strong cellular HLA-class-I-restricted cytotoxic T-lymphocyte (CTL) responses against K1, K8.1, or K12 were detected in HHV8-seropositive men without KS, suggesting a protective effect. Another group identified an increasing frequency (fourfold) of HHV8-specific CTL following the institution of highly active antiretroviral therapy.[63] In addition, primary HHV8 infection in HIV-negative men was also found to elicit strong CTL responses to several lytic gene products.[64] The results suggested that HHV8 T-cell-proliferative responses were common in HIV-negative homosexual men and that HIV infection could be associated with diminished cellular immunity against HHV8, possibly before there is a substantial depletion of CD8 cells.

HHV8 was recently found to encode two novel proteins (modulators of immune response, MIR-1 and MIR-2) that block display of MHC-class-I chains on the cell surface by increasing their endocytosis, thus enabling immune evasion during viral infection.[65,66] HHV8-infected cells also needed higher concentrations of soluble peptides to induce sufficient CTL-mediated cell lysis than did control cell lines, and were unable to process and/or present intracellularly expressed antigen because of significantly reduced TAP-I expression.[67] It seems, therefore, that HHV8 can modulate HLA-class-I restricted antigen presentation to CTLs, which may allow latently infected cells to escape CTL recognition and persist in the infected host.

Multicentric Castleman's disease

Castleman's disease refers to a diverse group of lesions that are clinically classified as one of two forms: (i) localised Castleman's disease is a single, usually mediastinal, angiofollicular lymph-node hyperplasia that resolves after surgical resection; or (ii) multifocal or multicentric Castleman's disease with multisystem involvement accompanied by generalised lymphadenopathy. Histologically, Castleman's disease is classified as the more common hyaline vascular type or the plasma cell type (figure 2). Unlike KS and pleural effusion lymphoma, not all Castleman's disease has been found to contain HHV8 DNA,[68] and thus, there is likely to be more than one cause for this diverse group of lesions. Several case reports have described an association of the hyaline-vascular Castleman's disease (that is usually HHV8-negative) with paraneoplastic pemphigus.[69]

Multicentric Castleman's Disease is a lymphoproliferative disorder that has been reported in close association with KS in both HIV-seropositive and HIV-seronegative patients.[68,70] It has been shown frequently to contain HHV8 DNA.[68,70,71] Exacerbation of symptoms related to multicentric Castleman's disease correlated with an increased HHV8 viral load in peripheral blood mononuclear cells, supporting a role for HHV8 in multicentric Castleman's disease pathogenesis.[72] HHV8 may infect IgM-positive naive B cells and drive these cells to differentiate into plasmablasts without undergoing the germinal centre reaction, during which normal naive B cells mutate their rearranged immunoglobulin genes and differentiate into plasma or memory B cells.[73]

Multicentric Castleman's disease is clinically characterised by systemic manifestations that include fever, weakness, generalised lymphadenopathy, and hyper-gammaglobulinaemia due in part to raised serum

Table 2. Comparison of primary effusion lymphoma (PEL), Castleman's disease, and KS

	PEL	MCD	KS
Site	Body cavity, extranodal	Lymph nodes, spleen	Any site; mucocutaneous most likely
Morphology	Immunoblasts	Plasmablasts*	Spindle-shaped KS cells
HHV8	Positive; expressing mostly latency genes	Mostly negative; few cells strongly expressing lytic genes	Positive in all forms of KS; expressing mostly latency genes
Epstein-Barr virus	Positive in majority	Negative	Negative, except in African endemic KS
Cytoplasmic Ig expression	Absent	High level of IgM	NA
Ig light chain	Monotypic or mRNA	Monotypic Ig	NA
CD30	Positive	Weakly positive-negative	Negative
B-cell antigens	Absent	Weak or absent	Absent
Mutation in *Ig* genes	Hypermutated in majority	Absent	Absent
Cellular origin	Germinal centre or post-germinal centre B cells	Naive IgM-expressing B cells	Lymphatic endothelia
Clonality	Monoclonal	Oligo-polyclonal	Polyclonal; at nodular stage evidence for monoclonality

*Plasmablast is used to indicate a medium-sized cell with a moderate amount of amphophilic cytoplasm and a large vesicular nucleus containing up to three prominent nucleoli. By contrast with an immunoblast, the cytoplasm contains abundant immunoglobulin (modified after reference 73). MCD=multicentric Castleman's disease; NA=not applicable; Ig=immunoglobulin.

concentrations of interleukin 6.[70,72] Cytopenias, rashes, and intercurrent infections are not unusual and some patients with multicentric Castleman's disease have been reported to develop non-Hodgkin lymphoma.

Morphologically, the cells harbouring HHV8 in multicentric Castleman's disease resemble immunoblasts with prominent central or marginal nuclei.[60,74] These cells occur as isolated cells in the mantle zone of B-cell follicles, but may eventually form monoclonal microlymphomas.[73] They do not stain for T cell (ie, CD3, CD45R0) or dendritic cell markers (CD2, CD23), but stain with the B-lymphocyte marker CD20 and the memory B-cell marker CD27.[18,73] However, they lack expression of B-cell activation markers such as CD23, CD38, and CD30 (table 2).

Table 3. Expression of HHV8 proteins

HHV8 protein	PEL-derived cell lines*	KS	MCD	PEL
LANA	+	+	+	+
Viral IRF1	+	-	+	-
Viral interleukin 6	+	-	++	+

*BCBL-1, BCP-1, BC-1 (modified after reference 74). MCD=multicentric Castleman's disease.

Comparison of Epstein-Barr virus-induced with HHV8-induced lymphoproliferation

Multicentric Castleman's disease harbours HHV8 but it is negative for Epstein-Barr virus infection by immunohistochemistry or by in-situ hybridisation in any significant number of cells (table 2).[70,75] Growing evidence indicates that, as in Epstein-Barr virus-driven lymphoproliferative disorders after transplantation, the HHV8 DNA burden in peripheral blood mononuclear cells may represent the most accurate marker of disease activity.

It is well known that Epstein-Barr virus immortalises B cells in vitro and is associated with malignant lymphomas, including endemic Burkitt's lymphoma, AIDS-related non-Hodgkin lymphoma, lymphoproliferative disorders occurring after transplantation, and Hodgkin's disease.[76] These conditions are similar to those seen in lymphoproliferative disorders caused by Epstein-Barr virus in immunosuppressed patients, including polyclonal plasmacytic hyperplasia and monoclonal polymorphic and monomorphic B-cell lymphoma.[77] However, unlike HHV8-related lymphoproliferative disorders, the Epstein-Barr virus-triggered lymphomas are of germinal centre origin (table 2).

There is a marked correlation between the viral gene homologues in HHV8 and the genes induced by Epstein-Barr virus, in particular the Epstein-Barr virus nuclear antigens (EBNAs) and latent membrane proteins (LMPs).[10,76,78] B cells transformed by Epstein-Barr virus express the full set of latent proteins (EBNA 1-6, LMP 1-2) as do Epstein-Barr virus-associated B cell lymphomas.[10,77] EBNAs and LMPs are known to be crucial for the maintenance of viral latency and for growth transformation of cells infected by Epstein-Barr virus. HHV8 encodes an interleukin 6 homologue (v interleukin 6), while Epstein-Barr virus does not encode interleukin 6 or cyclin D homologues.[10,14,18,76] Cells surrounding the germinal centres of the follicles in Castleman's disease express v interleukin 6 in an onionskin-like distribution.[18] The expression rate of v interleukin 6 is much higher in multicentric Castleman's disease than in either pleural effusion lymphoma or KS (table 3),[71] suggesting that interleukin 6 overexpression is implicated in the pathogenesis of multicentric Castleman's disease.[71,73] In particular, HHV8 expresses predominantly lytic genes in multicentric Castleman's disease (table 3).[71,78]

Therapy of multicentric Castleman's disease

HHV8-related multicentric Castleman's disease is potentially lethal. It is often refractory to systemic therapy, including corticosteroids and chemotherapy. Dysregulated overproduction of interleukin 6 from affected lymph nodes is thought to be responsible for the systemic manifestations of this disease. Therefore, interference with interleukin 6 signal transduction may constitute a new therapeutic strategy for multicentric Castleman's disease. Recently, a humanised anti-interleukin 6 receptor antibody (rhPM-1) has been used to treat seven patients with multicentric Castleman's disease.[79] On treatment, fever, fatigue, anaemia, hypergammaglobulinaemia, and serum concentrations of C-reactive protein and lymphadenopathy were notably improved. Another report describes the administration of the anti-CD20 monoclonal antibody rituximab, allowing a 14-month remission of clinical symptoms and HHV8 viraemia.[80]

Interferon α has also been effectively used to treat HIV-associated Castleman's disease.[81] However, fatal courses of multicentric Castleman's disease have been recorded after the initiation of highly active antiretroviral therapy, potentially as immune-reconstitution disease.[82]

Primary effusion lymphoma

Primary effusion lymphoma or body cavity-based lymphoma belongs to the group of non-Hodgkin B-cell lymphomas and is characterised by pleural, pericardial, or peritoneal lymphomatous effusions in the absence of a solid tumour mass. HHV8 was exclusively identified in primary effusion lymphoma, but not in other non-Hodgkin lymphomas. Primary effusion lymphomas regularly contain both HHV8 and Epstein-Barr virus DNA (table 2). The HHV8 gene expression in primary effusion lymphoma parallels the one seen in KS with mostly latency-associated genes being expressed (table 2).[78]

Primary effusion lymphoma has an intermediate immunophenotype but B-cell genotyping showed clonal rearrangements of the immunoglobulin genes. However, primary effusion lymphoma lacks rearrangements of the c-*myc* gene.[83] It also lacks B or T lymphocyte antigens.[84] The primary effusion lymphoma tumour cells are usually positive for the epithelial membrane antigen CD30, resembling a plasmacytoid morphology (figure 3).[83]

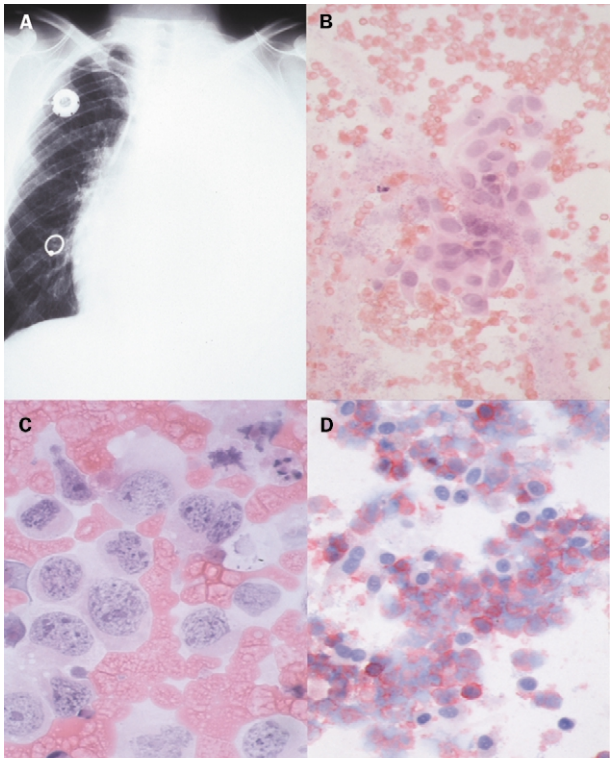

Figure 3. Primary effusion lymphoma of the pleural and pericardial space, in an HIV–infected patient (HHV8 positive). (A) Massive effusions in the pericardium (200 mL) and pleural space. The aspirate contained numerous clusters of tumour cells with large hyperchromatic nuclei (B, C), which stained positive for cutaneous lymphocyte–associated antigen (D) and lambda light chains (not shown), but negative for CD30, B–cell and T–cell markers. H+E stains of original magnification 400x (B and D) and 1000x (C) are shown.

In primary effusion lymphoma cells and in cell lines established from them, HHV8 episomes are present in every cell with a variable presence of viral particles.[45,85]

As in KS spindle tumour cells that are infected with HHV8, primary effusion lymphoma cells only express a minor population of lytic genes (tables 2 and 3). The difference in v interleukin 6 expression rates between the latently infected populations of KS and primary effusion lymphoma is notable (table 3).[71,78] This finding could signify the existence of at least two cell-type-specific programmes of latency for HHV8, for which a precedent exists in Epstein-Barr virus infection.[76] Variation in the rates of v interleukin 6 expression could be due to the tissue-specific and cell-specific environments of infection (transcription factors, cytokines, etc), as well as interactions with other infectious agents. The high concentrations of v interleukin 6 in few B cells could act by a paracrine mechanism to drive proliferation and differentiation of B cells in HHV8-associated multicentric Castleman's disease. It is noteworthy that 25% of patients with primary effusion lymphoma also have KS.[86] Although the coexistence of HHV8 and Epstein-Barr virus in KS lesions of primary effusion lymphoma patients raises the possibility that lesional B cells might be at risk of neoplastic transformation through the combined effect of these two viruses, no clonality of intralesional B cells has been shown.[85]

Search strategy and selection criteria

Searching for relevant articles was done in PubMed with "HHV8", "KSHV", and "Kaposi's sarcoma" as keywords. Publications in English were selected. Case reports were considered only if there was no larger study available. Selected previous reviews are included to give readers a more systematic overview.

More generally, differential expression of HHV8 genes in different cell types could be responsible for the heterogeneity of pathological states in these diseases. In that regard, the suspected association of HHV8 with multiple myeloma and monoclonal gammopathy of undetermined significance has not been confirmed.[87,88] However, some reports showed the presence of ORF 26 (KS330) expression from HHV8 subtype C3 and a single deletion in ORF 65 in some of these patients, suggesting the existence of another closely related virus.[89,90]

Comparison of primary effusion lymphoma with multicentric Castleman's disease

The plasmablastic lymphoma derived from HHV8-positive multicentric Castleman's disease is distinct from primary effusion lymphoma in many ways, although both are associated with HHV8 infection (table 2). HHV8-associated multicentric Castleman's disease and plasmablastic lymphoma are not associated with Epstein-Barr virus, whereas coinfection is common in primary effusion lymphoma (table 2). HHV8-positive plasmablasts express high concentrations of cytoplasmic IgM and are weakly positive or negative for CD30, whereas most primary effusion lymphomas lack cytoplasmic immunoglobulin but strongly express CD30. Furthermore, plasmablastic lymphomas harbour unmutated immunoglobulin genes and are derived from naive B cells; by contrast, primary effusion lymphoma bears mutated immunoglobulin genes and originates from germinal centre or postgerminal centre B cells. Although it remains to be tested whether the raised human interleukin 6 concentration in multicentric Castleman's disease is the result of HHV8 infection (table 3), and thus an important factor for HHV8-induced lymphoproliferation, it is notable that human interleukin 6 is produced by primary effusion lymphoma and promotes the growth of primary effusion lymphoma cells in vitro and in vivo.[73]

It is well known that Epstein-Barr virus can immortalise B cells in vitro, but Epstein-Barr virus alone may not be sufficient for tumour development as is exemplified by the complementation of the activated c-*myc* oncogene and Epstein-Barr virus in Burkitt's lymphoma.[91] Thus, it is possible that HHV8 acts in conjunction with Epstein-Barr virus to induce full transformation.[76,86]

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Conflicts of interest

The authors have no conflicts of interest, financial or otherwise, in relation to publication of this review.

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