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Human Herpesvirus 8 in Hematologic Diseases*

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Human herpesvirus type 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus (KSHV) is a new member of the γ-herpesvirus family. It is an unusual herpesvirus in that it carries a large number of genes that encode oncoproteins or cell signaling proteins. In addition to being the causative agent of both HIV-associated and non-HIV-associated Kaposi's sarcoma this DNA tumor virus has been implicated in the pathogenesis of several diseases. These include multiple myeloma (MM), Waldenström's macroglobulinemia (WM), multicentric Castleman's disease (MCD), body cavity-based lymphoma (BCBL), and various other conditions such as sarcoidosis and pemphigus. While the causative role of the viral infection is fairly certain in the development of BCBL and multicentric Castleman's disease, HHV-8 may act through a different mechanism to induce plasma cell malignancies. It has been suggested – though the finding is still controversial - that infection of bone marrow stromal dendritic cells by HHV-8 might be a key factor in the etiology and pathogenesis of monoclonal gammopathies. The aim of this review is to provide a short introduction into the tumorigenic potential of HHV-8 as well as to detail the available data and possible mechanisms on the involvement of this virus in different hematologic diseases. (Pathology Oncology Research Vol 5, No 1, 73–79, 1999)

Key words: HHV-8, tumorigenesis

Introduction

Genetic structure of HHV-8

Recently, molecular cloning of the BC-1 strain of HHV-8 was successfully completed. The structure of the BC-1 HHV-8 genome is similar to the model virus of the rhadinovirus family, *Herpesvirus saimiri* (HVS) in that it also has an approximately 140.5 kb long unique region (LUR) flanked by terminal repeats (TRs). The LUR shares the seven block organization of other herpesviruses with subfamily-specific or unique open reading frames (ORFs) present in the interblock regions. There are at least 81 identifiable ORFs on the LUR, an unusually high number in this virus family. They include conserved ORFs for presumed viral structural proteins (five conserved herpesvirus structural capsid and five glycoprotein genes) and enzymes involved in viral replication (e.g.: DNA polymerase, dihydrofolate reductase, thymidylate synthase), as well as two transactivator proteins (IA1A and B). Additionally, there are several genes of unknown function with existing homologs such as chicken nucleolin, a putative tegument protein, a secreted glycoprotein (gX), kaposin (a 60 aa peptide), and a U-RNA-like transcript called nut-1.[34]

A most interesting class of ORFs encode homologs to oncoproteins and cell signalling proteins. These include ORF-16 that encodes a functional Bcl-2-like protein, ORF-72 that encodes a functional cyclin D homolog (K-cyclin),

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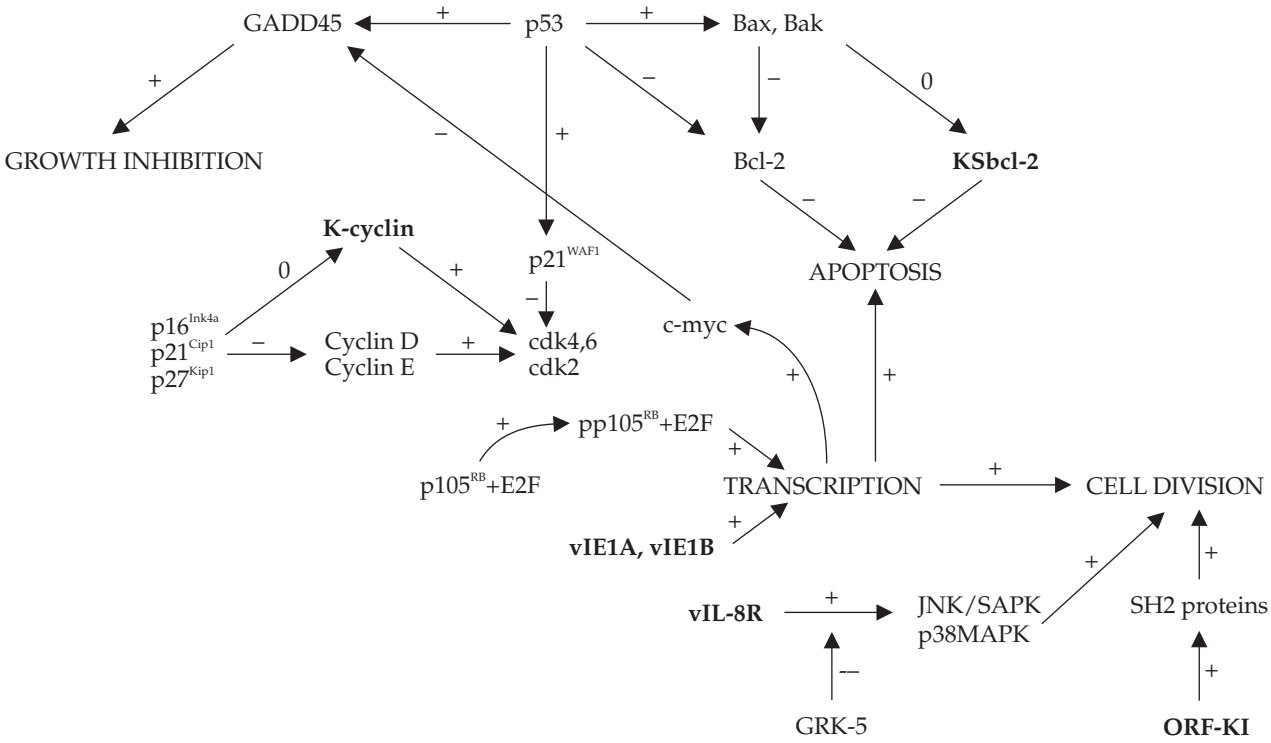

Figure 1. *Modulation of cell growth by HHV-8 proteins. A simplified network of cell division control is presented. HHV-8 encoded proteins are shown in boldface. Stimulatory actions are marked with "+" , inhibitory effects are denoted as "–" irrespective to the actual mode of action (transcription or protein level). Symbol "0" represents lack of expected interaction or modulatory activity.*

and ORF-74 encoding a functional G-protein-coupled receptor, a viral IL-8 receptor homolog (vIL-8R). HHV-8 also encodes a functionally active viral IL-6 (vIL-6, ORF-K2), two macrophage inflammatory proteins belonging to the β-chemokine family (MIPs, ORF K4 and K6). ORF-K9 (vIRF) encodes a protein with high homology to other interferon regulatory factors. Additional potential signal transduction homologs encoded by the virus are v-CBP (ORF-4) that represents a complement-binding protein and v-*adh* (ORF-K14), a neural cell adhesion molecule (NCAM) analogue.

Molecular basis of the oncogenic potential of HHV-8

Molecular mimicry by HHV-8 of cell cycle regulatory and signaling proteins is a prominent feature of this virus *(Figure 1)*. DNA tumor viruses have evolved mechanisms to coerce quiescent cells to enter the S phase in order to create a microenvironment containing the necessary proteins for viral replication. A similar deregulation of cell division control is seen in many tumors. All DNA tumor viruses possess some kind of mechanism to neutralize the potent antiproliferative actions of the retinoblastoma gene product ($p105^{RB}$). This includes production of the well-known SV40 T-antigen, adenovirus E1A, human papilloma virus E7, EBNA-3C from EBV, the *tax* gene of the human T-cell leukemia and lymphoma virus (HTLV-1 and 2), and the *x* gene of the hepatitis B virus. The common feature of these proteins are that they bind to and abrogate $p105^{RB}$ function. HHV-8 harbors a mechanism that acts upstream to this restriction point.[26] K-cyclin produced by HHV-8[6] efficiently forms a functionally active kinase complex with Cdk6 a protein that phosphorylates $p105^{RB}$ [6,38]. These complexes are especially highly active as they are resistant to inhibition by the cellular inhibitors of CDK, $p16^{Ink4a}$, $p21^{Cip1}$ or $p27^{Kip1}$ [6]. As a consequence, exogenous expression of K-cyclin by HHV-8 results in a highly effective bypass of a crucial checkpoint function that regulate G_1 arrest, therefore, it has a similar effect to the loss of $p105^{RB}$ on G_1 progression.

Bcl-2 is a potent cell-death suppressor and it represents a unique type of protooncogene in that it extends cell survival by inhibiting apoptosis rather than directly promoting cell proliferation. Both EBV and HHV-8 encode *bcl-2* homologs called *BHRF1* and *KSbcl-2*, respectively. The latter protein is especially important in maintaining viability of the infected cells, as cyclin D – in addition to its role in cell cycle progression and tumorigenesis – may also induce apoptosis. Therefore, an anti-apoptotic activity could potentially counteract the pro-death signal of excess cyclin D activity (induced by expression of K-cyclin). An especially interesting feature of of *KSbcl-2* is that it can

not heterodimerize with *Bax* or *Bak*, thus it could potentially escape negative regulation by these proteins. *KSbcl-2*, therefore, represents an even more powerful death-suppressor.[11]

To elaborate further on the comparison between HHV-8 and other DNA tumor viruses one may note that HHV-8 encodes many viral homologs of those cellular genes that are induced by EBV infection. This means that HHV-8 likely alters the similar signaling and regulation pathways to those induced by EBV after infection. Contrary to the intracellular action of EBV (i.e. induction of cellular genes for viral persistance and replication) HHV-8 introduces and utilizes exogenous genes from its own genome. The best example for this is ORF 74 protein (vIL-8R) that is analogous to EBI1 induced by EBV infection. vIL-8R is a constitutively active G-protein-coupled receptor that can activate two protein kinases, JNK/SAPK and p38MAPK and exploit cell signalling pathways to induce both transformation and angiogenesis (vascular endothelial growth factor, VEGF secretion) in the process of HHV-8 mediated tumor formation.[5]

Similar to other viruses, HHV-8 also employs molecular piracy of cellular regulatory genes as a mechanism to avoid antiviral responses within the cell. An example for this is the HHV-8 encoded viral interferon regulatory factor (vIRF) a gene product with homology to the IRF family of transcription factors. vIRF inhibits responses to type I and type II interferons and blocks IRF-1 mediated transcription. Interestingly, it does not compete with IRF-1 for binding to DNA or complex with IRF-1 directly, rather does it through a novel but still undetermined mechanism.[42]

Another aspect of tumorigenesis by HHV-8 is through the involvement of infected cells that may act in an autocrine/paracrine manner to aid the growth of malignant clones. This mechanism may take part in the clonal expansion of tumors where HHV-8 infected supporting cells contribute to malignant growth by secreting functionally active vIL-6 that promotes the proliferation of tumor cells that surround them.[7,18] This mechanism will be described in detail in a subsequent section dealing with the possible role of HHV-8 in the pathogenesis of multiple myeloma.

At a position equivalent to oncogenes in other transforming herpes viruses – i.e. the saimiri transforming protein (STP) of the Herpesvirus saimiri and the latent membrane protein-1 (LMP-1) of the Epstein-Barr virus (EBV) – HHV-8 contains a distinct open reading frame called K1. K1, STP, and LMP-1 exhibit similarities neither in their amino acid sequences nor in their structural organizations. Expression of the K1 gene in rodent fibroblasts induced transformation (morphologic changes and anchorage independent growth). Infection of T-lymphocytes with a recombinant herpesvirus – in which the STP gene was replaced by the K1 gene – immortalized the cells and induced lethal malignant lymphomas in marmoset monkeys. As a result, it was concluded that, similar to LMP-1 and STP, K1 is a major viral oncogene[21,22]. Protein products of these genes are transmembrane glycoproteins, which bind to as yet unknown ligands and participate in the signal transduction process. STP has been shown to activate the *ras* signal transduction pathway. By disrupting the connection between STP and *ras*, the transforming capacity of the Herpesvirus saimiri could be eliminated. The intracellular carboxyl end of LMP-1 has been shown to interact with tumor necrosis factor receptor (TNF-R) associated factors. In contrast to the extracellular (amino terminus) and the transmembrane domains, the intracellular carboxyl terminal region of the K1 genes from different HHV-8 isolates is highly conserved. It contains an immunoreceptor tyrosine-based activation motif that is able to interact with certain cytoplasmic second messengers and this interaction might be crucial for the transforming capacity of the K1 gene product.[22] Identifying the intracellular molecular collaborators of K1 might lead to a better understanding of the transforming mechanism of the K1 gene and it also raises the possibility of interfering with the molecular cascade that leads to cellular transformation. The variability of the extracellular domain, on the other hand, might make us consider that the several K1 alleles could bind alternative ligands on the cell surface.

Another important feature of HHV-8 in different malignancies is the expression of an abundant, latency-associated transcript referred to as T 0.7. T 0.7 encodes a unique ORF, K12, also known as kaposin. It has also been documented that transfection of kaposin into Rat-3 cells caused malignant transformation. Kaposin-transformed Rat-3 cells produced highly vascular undifferentiated sarcomas following subcutaneous injection into nu/nu mice.[27]

Most recently, infection and direct transformation of human microvascular endothelial cells by HHV-8 was demonstrated. Infection resulted in long-term proliferation and survival of cells and the acquisition of telomerase activity and anchorage independent growth. Interestingly, HHV-8 was present in only a subset of these cells and the rest of the cellular elements seemed to be stimulated by the infected cells in a paracrine fashion. It has been speculated that this stimulatory effect was carried out by the upregulation of the VEGF-receptor.[15]

HHV-8 and human tumors

It is now appreciated that development of a small but steadily increasing percentage of human malignancies involves the action of viruses. One of the newer additions to this growing population of tumorigenic viruses is HHV-8 originally called Kaposi's sarcoma herpesvirus (KSHV).[6] It is now well established that HHV-8 is responsible for both the endemic and HIV-related forms of Kaposi's sarcoma. There are some excellent recent reviews on this topic,[36,40]

therefore in this review we would like to concentrate on the other forms of human disease that have been shown to involve HHV-8 infection in their pathogenesis.

Body cavity based lymphoma

Lymphomatous effusions may occur with variable frequency as part of the presentation of non-Hodgkin's lymphoma (NHL) or any time along the course of the disease usually as contiguously spreading tumor masses. In the beginning of the 1990s, as a part of the growing AIDS epidemic, an unusual subset of AIDS-related NHL was noted in HIV-1 infected patients. In contrast to effusions complicating a tissue-based lymphoma, in these patients exclusive or predominant involvement of pleural, pericardial and/or peritoneal cavities was reported. As a result, this unusual form of disease was termed as body cavity based lymphoma (BCBL) or primary effusion lymphoma (PEL).[3,28] Further interesting features of BCBL are that their tumor cells usually exhibit indeterminate immunophenotypes, frequently associate with EBV, and consistently lack rearrangements of the c-*myc* gene. Characteristically, these cells are of B-cell lineage as confirmed by immunoglobulin light and heavy chain rearrangements and usually express CD45 and CD30 antigens.[4]

Following their description in HIV-positive patients, these unusual lymphomas were also described in non-HIV-positive elderly patients. At the same time these unusual tumors – in both subsets of patients – were shown to contain the herpesvirus that is involved in Kaposi's sarcoma denoted later as HHV-8. On the other hand, in the vast majority of cases coinfection of tumor cells with EBV was also detected and a dual herpesvirus infection and transformation model was proposed.[28] The close and specific association of HHV-8 and BCBL was further evidenced by epidemiological studies which showed that HHV-8 has an epidemiology similar to that of Kaposi's sarcoma, i.e. it primarily occurs in HIV-positive homosexual men. Rather unexpectedly, the presence of HHV-8 and rearrangement of the c-*myc* gene were found to be mutually exclusive molecular events when BCBL was compared to Burkitt-type effusions, suggesting that HHV-8 and c-*myc* activation may represent alternative molecular pathways in the development of malignant lymphomatous effusions.[28]

The involvement of cytokines in BCBL was studied in detail in order to understand the role of HHV-8 in tumorigenesis.[4] The main finding was that these cells express IL-6, IL-10 and IL-6 receptors, while do not express GM-CSF, IL-1β, IL-8, IL-12, bFGF, PDGF or c-*kit*. Interestingly, the high level of IL-6 produced was not the viral form of the cytokine (vIL-6). Moreover, IL-6 antisense oligonucleotides nearly 100% inhibited the clonal growth of these cells while IL-6 antibodies had no effect indicative of a possible intracellular shortcut pathway of IL-6 action. The growth control of BCBL cells is clearly different from that of other AIDS-related lymphomas; IL-10 and IL-12 that are autocrine growth factors in other AIDS-related lymphomas have no particular importance in BCBL. Taken together, it is tempting to speculate that the particular importance of IL-6 in BCBL is caused by the HHV-8 infection. In later stages of tumor development, however, the viral vIL-6 does not seem to be a direct autocrine stimulator. Alternatively, it might be possible that its presence in the early stage of tumor development may have primed the cells to utilize the IL-6 - IL-6 receptor based autostimulatory circuit[4].

In addition to primary effusion lymphomas a subset of secondary effusion lymphomas was also reported to harbor HHV-8. These are the secondary effusion lymphomas that follow lymphoma of the bowel[13]. Not much is currently appreciated about this rare form of HHV-8/EBV-positive lymphoma, nevertheless, it may provide a useful tool to determine the mechanism of lymphocyte homing to body cavities.

Castleman's disease

Multicentric Castleman's disease (MCD), also called multicentric angiofolliclar lymphoid hyperplasia, is a systemic lymphoproliferative disease characterized by fever, lymphadenopathy, and splenomegaly. The disease is associated with an increased risk for developing Kaposi's sarcoma and lymphoid malignancies. There are three histopathologic forms of this disease based on the degree of capillary proliferation, follicular involution, and plasmocytic infiltration of the affected lymph nodes: hyaline vascular form, plasma cell form, and mixed form. While the hyaline vascular form – also known as localized Castleman's disease (LCD) – is usually readily treatable by surgical resection the plasma cell variant frequently presents as multicentric lymphadenopathy accompanied by systemic symptoms.[20,29]

Castleman's disease may occur among HIV-positive persons and in this patient population it was shown that MCD is nearly always associated with the presence of HHV-8.[17] Moreover, HHV-8 is not only present in these patients but a high viral load is also detectable even if there are no microscopically detectable foci of Kaposi's sarcoma lesions in lymph nodes.[29] In HIV-negative patients, however, the association between the virus and the disease seems much murkier[17]. While some evidence supports that HHV-8 is associated with MCD also in the majority of HIV-negative patients it is probably not associated with LCD.[17,29]

IL-6 has a pivotal role in the pathogenesis of Castlemans disease.[29] Patients with MCD often have excess IL-6 production within lesions. Also, in situ IL-6 overexpression in a mouse model has led to polyclonal hypergamma-

Table 1. **HHV 8 positivity in plasma cell disorders**

Disease	*HHV-8 positive (# of patients studied)*	*Methodology*	*Reference*
Multiple myeloma	15 (15)	PCR on cultured stromal cells	31
	17 (20)	ISH and PCR on fresh core biopsies	35
	18 (20)	PCR on paraffin-embedded biopsies	7
	5 (10)	PCR on paraffin-embedded biopsies	1
	22 (27)	0RF65 immunoblot	2, 16
	1 (11)	PCR on apheresis dendritic cells	39
	1 (17)	PCR on fresh core biopsies	10
	0 (23)	Indirect immunofluorescence using patient sera	25
	2 (78)	ORF 65.2 ELISA	24
	0 (8)	PCR on apheresis dendritic cells	41
	4 (25)	Indirect immunofluorescence using patient sera	19
WM	6 (10)	PCR on fresh core biopsies	1
	4 (4)	PCR on CD68 and CD83 enriched cells	32
	1 (20)	PCR on fresh core biopsies	8
MGUS	2 (8)	ISH and PCR on fresh core biopsies	31
	1 (18)	ORF 65.2 ELISA	10
	0 (2)	PCR on cultured dendritic cells	41
	1 (3)	Indirect immunofluorescence using patient sera	25
AIDS-related plasmocytosis	2 (2)	PCR on fresh core biopsies	32
Primary amyloidosis	10 (11)	ISH on fresh core biopsies	32

globulinaemia with plasma cell hyperplasia mimicking human CD. Furthermore, treatment of MCD with an IL-6 monoclonal antibody has been shown to be of therapeutic benefit. HHV-8 has a potent viral IL-6 encoded in its genome and it was shown that HHV-8 infected and vIL-6 expressing lesions are more likely to be multicentric with a plasma cell morphology and often follow a rapidly fatal course[29]. It is common that these HHV-8 infected patients have autoimmune hemolytic anaemia or mono-/polyclonal gammopathy consistent with abnormal proliferation of plasma cells due to overstimulation by vIL-6. Interestingly, and furthering the importance of HHV-8 infection, in most of the patients with Castleman's disease without apparent vIL-6 production the disease remains localized and responds well to surgical excision therapy.

Multiple myeloma and Waldenström's macroglobulinemia

Multiple myeloma (MM) is a lymphoid malignancy characterized by the accumulation of transformed plasma cells in the bone marrow and the presence of a monoclonal immunoglobulin or immunoglobulin subunit produced by these cells in the serum or urine or both. Waldenström's macroglobulinaemia (WM) is a related but distinct clinical entity that involves proliferation of B-cells that synthesize and secrete IgM. A third related entity is monoclonal gammopathy of undetermined significance (MGUS), a disease characterized by the presence of monoclonal serum components, mild bone marrow plasmacytosis, and normal levels of serum immunoglobulins. Although it is impossible to predict the course of any individual patient with MGUS, and only approximately 25% of MGUS patients develop multiple myeloma, the condition is considered to be a precursor to myeloma.[18,31]

It has been known for some time that IL-6 is a growth factor for plasma cells including those in myeloma.[9] IL-6 is normally secreted by bone marrow stroma cells and act in a paracrine manner to stimulate growth of neoplastic plasma cells and also to prevent apoptosis of these cells. These stroma cells, therefore, play a major role in mediating the paracrine stimulation of myeloma cell growth. The discovery of HHV-8 and its ability to produce large amounts of potent vIL-6 has prompted several investigators to study its potential involvement in bone marrow plasma cell diseases. In tissue culture vIL-6 was shown to support the growth of MM cells that undergo apoptosis in the absence of IL-6.[9] To date, however, extensive studies addressing the potential role of HHV-8 in the etiology and pathogenesis of MM have yielded somewhat controversial results *(Table 1)*. First, there is no epidemiological link connecting Kaposi's sarcoma (or BCBL or MCD) to multiple myeloma. Second, prevalence of antibodies to HHV-8 latent nuclear antigen in MM patients was similar to that seen in the general population,[19] as opposed to Kaposi's

sarcoma, where all patients of classic and AIDS-related Kaposi's sarcoma had high titers of antibodies. Third, using polymerase chain reaction (PCR) HHV-8 was not detectable in bone marrow aspirates from MM patients. Four, in some studies functional dendritic cells obtained with apheresis from patients with MM did not harbor PCR-detectable HHV-8 DNA.[12,39,41]

On the other hand, in one study, bone marrow stroma cells obtained from MM patients and cultured *in vitro* harbored PCR-detectable HHV-8 in all of the investigated cases[28]. In MGUS the ratio of positivity was two out of eight patients but no virus was detected in bone marrow mononuclear cells.[28] These cultured bone marrow dendritic cells were also HHV-8 positive in *in situ* hybridization (ISH) assays.[28] In order to deny the possibility of a cell culturing artefact the study was later reproduced on fresh bone marrow core biopsies in which dendritic cells in 17 of 20 MM patients proved to be HHV-8 positive[32]. Similar confirmatory results were obtained from DNA extracted from paraffin-embedded bone marrow sections not only from MM[1,7] but also from WM patients.[1]

Recently, based on the available positive data, a model was created to explain the involvement of HHV-8 in the development of plasma cell malignancies. Since it has been known for a long time that IL-6 is one of the major growth stimulatory factors of myeloma cells it has been suggested that infection of bone marrow dendritic (stroma) cells by HHV-8 could propel the runaway growth of myeloma cells "by remote control".[18,31] In fact, the viral infection of these dendritic cells may eliminate the last existing physiological checkpoint that hinders the growth of the malignant cells.

In view of these conflicting results it might be difficult to interpret the existing positive and negative data and fit them into the proposed model. Apparently, both the currently available molecular and serological methods to detect HHV-8 infection are unreliable. For instance, in one study it was shown that approximately 25% of the general population carries antibodies to HHV-8; whereas, in another the ratio was 0/72.[23,30] Another source of conflicting data may stem from the variability of the virus itself: It can plague not only the PCR studies but also serology as some mutations cause frameshifts in the virally encoded antigen proteins. From previous studies it is also evident that the results of molecular detection are highly influenced by the probes used and the experimental method i.e. simple or nested PCR. Our own attempts to amplify HHV-8 sequences from bone marrow aspirates and peripheral mononuclear cells of 17 MM and 3 WM patients have also been unsuccessful (data not shown). Finally, in disease states with secondary deficiencies of antibodies, such as many plasma cell diseases, serological response to HHV-8 may be inadequate to reach detectability in the assay. Taken together, though the hypothesis of the master control herpesviral infection as a means to cause runaway malignant disease is intriguing, at this point the available data are insufficient to establish it as an unequivocally valid model of MM pathogenesis.

Possible involvement of HHV-8 in other diseases

The possible involvement of HHV-8 infection in the development of several diseases not yet mentioned has also been suggested. The most convincing, though still preliminary, data emerged in the case of sarcoidosis.[14] Sarcoidosis is characterized by a granulomatous immune response to as yet unknown sequestrate foreign antigens. In sarcoidosis patients HHV-8 DNA may be detected in sarcoid but not in non-sarcoid tissues. At this point it is not clear whether HHV-8 is specifically associated with the etiology of sarcoidosis or just with the granulomatous response (as non-sarcoid granulomas were not investigated) but the very high rate of HHV-8 positivity in sarcoid granulomas suggests a causative but yet unexplained role of the virus. Macrophage inflammatory cytokines encoded by HHV-8 might be implicated in the granulomatous response.

The other diseases that were thought to involve infection by HHV-8 have been studied in much less detail and lower patient numbers. Therefore, conclusions may be too preliminary. These diseases include primary amyloidosis[32] and pemphigus.[37] With the greater availability of HHV-8 detection techniques one may expect implication of viral infection in a lot more disease forms, nevertheless, proving direct causative relationships is expected to remain difficult for an extended period of time.

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