

HHV-8 infection and multiple myeloma

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Abstract: Human herpes virus 8 (HHV-8) also known as Kaposi's sarcoma-associated herpes virus has been strongly implicated in the pathogenesis of Kaposi's sarcoma (KS), primary effusion lymphoma, and multicentric Castleman disease. Recently, this gamma-herpes virus was also found in the nonmalignant bone marrow dendritic cells of the majority of myeloma patients. In addition, HHV-8 is also detectable in the peripheral blood of most myeloma patients. In contrast, this virus is rarely detected in close contacts of myeloma patients or healthy subjects. Furthermore, only about one-third of patients with monoclonal gammopathy of undetermined significance (MGUS) are infected with HHV-8. Sequencing of HHV-8 DNA isolated from myeloma patients shows both interpatient differences and conserved differences unique to myeloma compared to HHV-8 in other malignancies. Consistent expression of both the viral homologs of interferon regulatory factor and interleukin-8 receptor in myeloma suggests a possible role for these transforming viral genes in the pathogenesis of this disease. *J. Leukoc. Biol.* 66: 357-360; 1999.

Key words: Kaposi's sarcoma herpes virus · pathophysiology · monoclonal gammopathy of undetermined significance

INTRODUCTION

In myeloma, the role of the bone marrow microenvironment has been shown to be of increasing importance in supporting the malignant plasma cell [1, 2]. Specifically, nonmalignant stromal cells in the bone marrow from myeloma patients have been shown to promote the growth and prevent the apoptosis of malignant plasma cells largely by the production of interleukin-6 (IL-6) [1-3]. Our laboratory has recently demonstrated the presence of human herpes virus-8 (HHV-8), a new gamma herpes virus (Fig. 1), in the adherent nonmalignant stromal cells from long-term cultures of bone marrow of myeloma patients [4]. HHV-8 encodes a viral IL-6 (vIL-6) homolog capable of stimulating growth and preventing apoptosis of a murine myeloma cell line [5]. The vIL-6 homolog also stimulates growth of the INA-6 human myeloma cell line [6]. Moreover, approximately one-third of patients with MGUS also demonstrated virus in these bone marrow-derived stromal cells.

HHV-8 IN FRESH MYELOMA BONE MARROW AND PERIPHERAL BLOOD

HHV-8 was first found in long-term stromal cultures derived from fresh bone marrow aspirates from myeloma patients [4]. Characterization of the virally infected stromal cells suggests a dendritic cell phenotype. Additional studies performed on fresh bone marrow biopsies using both polymerase chain reaction and *in situ* hybridization techniques with viral specific primers and probes, respectively, demonstrated viral presence in most myeloma bone marrow biopsy samples. In contrast, biopsies from patients with lymphomas, other cancers infiltrating the bone marrow, or healthy subjects did not contain HHV-8 [7]. In patients with KS, the presence of circulating HHV-8 has been demonstrated [8]. In peripheral blood mononuclear cells (PBMCs) from myeloma patients, polymerase chain reaction (PCR) amplification detects HHV-8 in only a small minority of patients. However, enrichment of PBMCs for markers (CD68 or CD83) present on the virally infected bone marrow stromal cells allows detection of HHV-8 in most (65%) of the 115 cases analyzed (Table 1) [9]. Similarly, although HHV-8 can be detected in most patients' blood after enrichment for dendritic cells at diagnosis (76%), its absence appears to be associated with a lower tumor burden at diagnosis. Moreover, myeloma patients with active disease are more likely to exhibit HHV-8 infection than patients with inactive disease (75 vs. 51%, respectively). Recently, we developed a PCR-based assay to quantify viral load in both peripheral blood and bone marrow samples through the use of primers directed at the viral interferon regulatory factor (vIRF) open reading frame (ORF). This quantitative assay, which allows detection of a single viral copy, is based on our previous work using multiple replicates of serially diluted samples of blood or bone marrow from myeloma patients to quantify tumor load using patient specific immunoglobulin gene primers [10]. This technique will allow accurate measurement of viral load at diagnosis and monitor temporal changes in the HHV-8 burden during the course of the disease.

Abbreviations: HH8, human herpes virus 8; KS, Kaposi's sarcoma; MGUS, monoclonal gammopathy of undetermined significance; IL-6, interleukin-6; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; vIRF, viral interferon regulatory factor; ORF, open reading frame.

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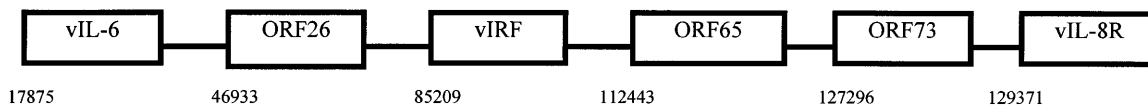


Fig. 1. Schematic of partial HHV-8 genome and locations of ORFK2 (vIL-6), ORF26, ORFK9 (vIRF), ORF65, ORF73, and ORF74 (vIL-8R). The positions of the ORFs of interest are indicated by open boxes and the nucleotide numbers marking their beginning are listed in the lower left corners.

TRANSMISSION OF HHV-8 AMONG FAMILY MEMBERS AND SEXUAL PARTNERS OF MYELOMA PATIENTS

HHV-8 is present in body secretions [11, 12], raising the issue of potential sexual transmission of HHV-8. Of note, in KS a close relationship between the presence of antibodies to HHV-8 and the number of sexual partners in homosexual men was demonstrated, and seroconversion was associated with the development of KS [13]. We sought to determine the transmissibility of HHV-8 among family members and sexual partners of myeloma patients. Although 66% of the 42 myeloma patients studied demonstrated HHV-8 in PBMCs enriched for dendritic cells, only 1 of the 54 close contacts showed viral presence in the enriched PBMCs [14]. The low incidence of HHV-8 in individuals at risk for viral exposure may reflect low viral infectivity, although it is also possible that the viral load is below the sensitivity of this PCR-based assay. It is highly unlikely that PCR artifact explains our results because studies of patients (mostly HHV-8 positive) and their family members (consistently HHV-8 negative) were done concurrently in a blinded fashion.

SEQUENCING OF HHV-8 ORFs IN MYELOMA PATIENTS

The association between HHV-8 and multiple myeloma remains controversial [15–17, 29, 30]. We explored the possibility that nucleotide variations unique to the HHV-8 strain found in myeloma could offer insights into this controversy, and possibly explain some of the difficulties in detecting serologic responses in these patients. All PCR assays were performed randomly and in a blinded fashion, using several virgin non-overlapping HHV-8 primers; at least two independently amplified PCR products were sequenced bidirectionally.

PCR-amplified product from ORF26 using the KS330₂₃₃ primers was sequenced from three different tissue sources (long-term cultures of adherent bone marrow stromal cells, PBMCs enriched for dendritic cells, and bone marrow core biopsies) from four patients. These three tissues showed identical sequences in the same patient except for a single base pair substitution in one sample. The ORF26 segments from 24 myeloma patients showed variable minor interpatient differ-

ences. Based on these data, 92% of myeloma patients are infected with the HHV-8 strain B' as described by Zong et al. [18], whereas the remaining patients are infected with the HHV-8 strain B.

Similar analysis of ORF65 also showed variable small interpatient differences. Of note, however, a unique consistent deletion was detected at position 112227 near the 3' end of this ORF. All 14 HHV-8-positive myeloma samples examined consistently demonstrated this deletion. In contrast, this deletion was not present in HHV-8 positive lymphomas or in KS tissue. This myeloma-specific deletion results in a reading frame shift that translates to the expression of a myeloma viral protein that is putatively 24 amino acids longer. Because this ORF is responsible for a major part of the serological response to HHV-8, deletion of a base pair, which likely results in changes in the expressed protein product may help explain the lack [19, 20] or low level [21] of serological response to this virus observed in myeloma patients. In further support of this hypothesis, despite using multiple primer pairs against ORF73, no amplified product could be demonstrated in myeloma samples otherwise readily positive using primers against ORF26. Furthermore, amplified product is easily detected in HHV-8-positive lymphomas using ORF73 primers. This ORF is largely responsible for the serological response to latency-associated nuclear antigen [22]. Primer pairs against other regions of the HHV-8 genome, including viral cyclin D, bcl-2, macrophage inflammatory proteins, IL-8R, and IRF successfully yield amplified product in the myeloma samples.

REPRESENTATIONAL DIFFERENTIAL ANALYSIS (RDA) USING BONE MARROW DENDRITIC CELLS FROM MYELOMA PATIENTS

To further explore the role of the myeloma bone marrow dendritic cell in the pathogenesis of myeloma, RDA was performed using bone marrow dendritic cells from myeloma patients and normal subjects [23]. It is interesting that the vIRF homolog from HHV-8 was identified in the myeloma sample along with a new member of the melanoma-associated antigen (MAGE) family [24]. MAGEs have been identified as antigenic targets on melanoma cells for cytolytic T lymphocytes [25]. The role of this new MAGE in myeloma pathogenesis is now the subject of intense investigation in our laboratory.

HHV-8 GENE EXPRESSION IN MYELOMA BONE MARROW

After RDA detection of vIRF, we performed reverse transcriptase (RT)-PCR on RNA derived from fresh bone marrow

TABLE 1. PCR Detection of HHV-8 Using the KS330₂₃₃ Primers in PBMCs of Patients with Myeloma or MGUS and Healthy Subjects

	Number PCR positive (%)
Myeloma patients (<i>n</i> = 115)	75 (65%)
MGUS patients (<i>n</i> = 28)	10 (36%)
Healthy subjects (<i>n</i> = 72)	2 (3%)

biopsies from myeloma patients and normal subjects to assess whether this viral gene is expressed. vIRF transcripts were, indeed, readily detected in fresh bone marrow from the myeloma patients but were absent in RNA from normal subjects. The expression of this gene in myeloma bone marrow dendritic cells may allow this virally infected population to “outgrow” uninfected stromal cells based on the observations that transfection of vIRF into murine fibroblasts leads to a transformed phenotype and induces formation of stromal tumors in nude mice [26]. Moreover, vIRF transfection of these cells inhibits the normal induction of the *p21* cyclin D kinase inhibitor, a growth inhibitor, by interferon- β , and results in a growth advantage for vIRF-transfected cells.

Additional studies to determine expression of HHV-8 specific genes have also shown the presence of the viral homolog of IL-8 receptor (vIL-8R) in fresh myeloma bone marrow samples. This viral gene also produces a transformed phenotype in transfected murine fibroblasts and leads to the formation of stromal cell tumors in nude mice [27, 28]. In addition, vIL-8R appears to stimulate angiogenesis by induction of vascular endothelial growth factor (VEGF). Studies of myeloma bone marrow show marked staining with CD31, a vascular endothelial cell marker, raising the possibility of VEGF induction in these HHV-8-infected samples, possibly mediated by vIL-8R.

In contrast, despite the expression of vIL-6 transcripts in the adherent stromal cells of long-term cultures from myeloma bone marrow, these transcripts are identified in only a minority of patients' fresh bone marrow biopsies. Recent studies show the relative lack of potency of this viral homolog compared to the human IL-6, down-playing the potential role of vIL-6 in the pathogenesis of myeloma [6]. Thus, other viral factors are likely involved in supporting the virally infected stromal cells as well as the malignant plasma cells.

Although these studies suggest a potential role for HHV-8 in the pathogenesis of multiple myeloma, further work is required to clearly establish this connection. The establishment of a direct or indirect causal effect of HHV-8 could lead to major changes in how clinicians approach this fatal malignancy.

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