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Herpesvirus and Multiple Myeloma  

HHV-8 Is Present in Multiple Myeloma Patients  

By James R. Berenson and Robert A. Vescio  

IN MYELOMA, THE ROLE of the bone marrow microenvironment has been shown to be of increasing importance in supporting the malignant plasma cell. 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). Recently, a new member of the human herpesvirus family, HHV-8, was discovered in a case of Kaposi’s sarcoma (KS) and was also found to be present in patients with a rare form of B-cell lymphoma, primary effusion lymphoma, and multicentric Castleman’s disease. We have recently found this virus associated with multiple myeloma. In the acquired immunodeficiency syndrome (AIDS) population in whom KS and HHV-8 infection are relatively common, a higher risk of multiple myeloma has been also observed in a recently published study. In further support of the connection between this new virus and multiple myeloma, HHV-8 was found to encode an IL-6 homologue that was capable of stimulating growth and preventing apoptosis of murine and human myeloma cell lines. Previously, our laboratory had demonstrated the presence of HHV-8 in the adherent nonmalignant cell population from long-term cultures of bone marrow from myeloma patients. Moreover, approximately one fourth of patients with monoclonal gammopathy of undetermined significance (MGUS) also demonstrated virus in these bone marrow-derived dendritic cells. Further characterization of the virally infected bone marrow stromal population showed dendritic cell features with a specific immunophenotype. Although some groups have been unable to detect virus in stromal cell populations derived from myeloma bone marrow, the phenotype of the cells generated in these cultures is unknown and could influence the results (see below).

In our initial study, we could not detect HHV-8 in the fresh aspirate from myeloma patients, although this virus was readily detected after long-term culture. Others have also recently reported the absence of HHV-8 using polymerase chain reaction (PCR)-based techniques on fresh marrow aspirates from these patients. Some of these studies also did not detect this virus in myeloma cell lines, consistent with our identification of the nonmalignant dendritic cell as the HHV-8-containing population in these patients. Because the infected cells were initially found only in long-term cultures and not in the fresh bone marrow aspirates, further studies were performed on fresh bone marrow biopsies using both PCR and in situ hybridization techniques with HHV-8–specific primers and probes, respectively. In studies of nearly 50 myeloma patients, viral presence could be demonstrated using these techniques in myeloma bone marrow biopsy samples from 80% of these cases, whereas biopsies from patients with lymphomas, other cancers infiltrating the bone marrow, and normal subjects did not contain HHV-8. A French group confirmed the presence of HHV-8 in the bone marrow biopsies derived from the majority of the myeloma patients evaluated using the KS330 primers, whereas it was not detected in any of the normal subjects’ bone marrow biopsies. Recent work has also shown the presence of HHV-8 in 80% of Turkish myeloma bone marrow specimens and none of the control samples. The presence of virus has now been confirmed using multiple primer pairs from different open reading frames (ORFs) of HHV-8 (ORF26, ORF65, ORF74, v-MIP-1, v-IRF, v-IL-6, T1.1, v-bcl-2, and v-cyclin D). In these studies, each of these HHV-8 primer pairs was optimized for temperature and buffer conditions. Importantly, without optimizing these conditions, the primers may be as much as three logs less sensitive to detect HHV-8. In addition, even after optimization of individual primer pairs, there are marked differences in sensitivity of primers from different HHV-8 ORFs.

To further confirm the presence of HHV-8 in myeloma bone marrow, reverse transcription-PCR (RT-PCR) using RNA from fresh marrow biopsies has shown consistent expression of two HHV-8 transforming genes, vIRF and ORF74, in myeloma but not normal subjects’ specimens, which was confirmed by sequencing the amplified product.

HHV-8 can be detected in the peripheral blood of most KS patients. The acquired immunodeficiency syndrome (AIDS) population in whom KS and HHV-8 infection are relatively common, a higher risk of multiple myeloma has been also observed in a recently published study. In further support of the connection between this new virus and multiple myeloma, HHV-8 was found to encode an IL-6 homologue that was capable of stimulating growth and preventing apoptosis of murine and human myeloma cell lines. Previously, our laboratory had demonstrated the presence of HHV-8 in the adherent nonmalignant cell population from long-term cultures of bone marrow from myeloma patients. Moreover, approximately one fourth of patients with monoclonal gammopathy of undetermined significance (MGUS) also demonstrated virus in these bone marrow-derived dendritic cells. Further characterization of the virally infected bone marrow stromal population showed dendritic cell features with a specific immunophenotype. Although some groups have been unable to detect virus in stromal cell populations derived from myeloma bone marrow, the phenotype of the cells generated in these cultures is unknown and could influence the results (see below).

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patients.\textsuperscript{18} Using PCR with multiple sets of HHV-8 primers, we and others have failed to find HHV-8 in whole peripheral blood mononuclear cells (PBMCs) from myeloma patients, except in a small minority of cases.\textsuperscript{1,12,19} However, after enrichment of PBMCs for cells bearing CD68 or CD83 surface markers that were present on the virally infected bone marrow stromal cells, we were able to detect virus in most of the 157 myeloma samples analyzed (70\%) and one fourth of the MGUS specimens, whereas only 1 of 63 normal subjects showed viral presence.\textsuperscript{19} Other studies have failed to detect HHV-8 in peripheral blood samples enriched for dendritic cells by tissue culture.\textsuperscript{20,21} However, these investigators have studied cells that lack the CD83 marker and bear other markers not present on the virally infected cell type identified in our studies.

HHV-8 has previously been demonstrated to be present in sexual and other body secretions,\textsuperscript{22} and antibodies to HHV-8 as well as KS have been found with a higher prevalence among homosexual males with a large number of sexual partners.\textsuperscript{23} Using the PCR assay with the ORF26 primers, a similar study among family members of myeloma patients was performed analyzing PBMCs enriched for CD68 or CD83. Although approximately three fourths of the 36 myeloma patients studied demonstrated HHV-8, only 2\% (1/46) of the family members showed viral presence in the enriched PBMCs.\textsuperscript{24} In these enriched PBMC samples, PCR amplification of the KS330,\textsuperscript{23} and vIRF primers gave identical results. These results suggest either that HHV-8 is absent in this group at risk for viral exposure or that the level is below the level of sensitivity of this PCR-based assay. Finally, these findings make PCR artifact highly unlikely as an explanation for our detection of HHV-8 in myeloma patients, because studies of patients (mostly HHV-8 positive) and their family members (consistently HHV-8 negative) were performed concurrently in a blinded fashion.

Some studies have shown interpatient differences in the sequence of HHV-8 from ORF26.\textsuperscript{25} Sequencing bidirectionally using multiple primer pairs specific for several HHV-8–specific ORFs from myeloma patients has recently been completed.\textsuperscript{26} First, PCR-amplified products from ORF26 using the KS330 primers were 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 4 patients. Although interpatient differences existed, these three tissues showed identical ORF26 sequences in the same patient except for a single basepair substitution in only 1 sample. Further evaluation of single tissue sources from an additional 12 patients’ ORF26 segment showed interpatient differences. In addition, compared with those sequences derived from both HHV-8–infected lymphomas and KS tissues, ORF26 derived from myeloma patients showed specific consistent changes. A similar analysis of ORF65 also showed interpatient differences as well as consistent changes compared with the sequence obtained from lymphoma and KS tissues. These findings make PCR artifact unlikely as an explanation for the amplified products produced using HHV-8 primers in myeloma samples. Moreover, because this latter ORF is responsible for a major part of the serological response to HHV-8, deletion of a basepair likely resulting in changes in the resulting protein product may help explain the lack\textsuperscript{27,28} or low level\textsuperscript{29} serological response to this virus observed in myeloma patients.

Thus, our studies confirm the presence of HHV-8 in the vast majority of myeloma patients and suggest that specific viral products may contribute to the pathogenesis of this disease. Attempts to detect this virus must rely on highly sensitive PCR techniques using samples that are known to contain higher levels of HHV-8 in these patients, including bone marrow biopsies and peripheral blood enriched for markers known to be present on the virally infected dendritic cells in these patients’ bone marrow. A major objective is to define the role of this virus in the pathogenesis of this B-cell malignancy.

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Kaposis’s Sarcoma-Associated Herpesvirus and Multiple Myeloma: Lack of Criteria for Causality

By Karin Tarte, Yuan Chang, and Bernard Klein

RETTIG ET AL.1 RECENTLY reported the detection of Kaposis’s sarcoma-associated herpesvirus (KSHV) DNA as well as KSHV viral interleukin-6 (vIL-6) transcripts in cultured bone marrow (BM) stromal dendritic cells (DC) of patients with multiple myeloma (MM). In a follow-up study, this same group used KSHV ORF72 (v-CYC) in situ hybridization and KSHV polymerase chain reaction (PCR) to detect the virus in fresh BM biopsies of MM patients.2 Given the general consensus that IL-6, mainly produced by BM stromal cells, is a major growth factor for malignant plasma cells in the active stage of the MM3 and because the viral homolog of IL-6 is able to sustain survival and proliferation of myeloma cells,4 these data provided a plausible and convenient model for MM pathogenesis. The putative role of KSHV in the emergence of MM was also emphasized by the detection of KSHV DNA in cultured stromal DC of 2 of 8 patients with monoclonal gammopathy of undetermined significance (MGUS),5 a finding that could be used to explain transformation to MM in 25% of MGUS.6 Berenson’s group also reported detection of KSHV DNA in peripheral blood DC6 and in apheresis cells from patients treated with cyclophosphamide and growth factors for hematopoietic precursor mobilization.6 Furthermore, Berenson’s group has claimed that KSHV not only is involved in the pathogenesis of MM, but also is involved in another late B-cell disorder, Waldenstrom macroglobulinemia.7

The determination of whether an infectious agent is causally related to a disease was first formally codified by Robert Koch in the famed Koch’s postulates. These criteria served early microbiologists well. However, in the present era, when newly discovered pathogens are not easily cultivated and the detection of agents may depend on exquisitely sensitive molecular techniques, scientists have looked to other guidelines. A.B. Hill’s epidemiologic criteria for causation, now widely used to distinguish a causal from a noncausal association, is one such set of guidelines.8 According to Hill’s criteria, evidence for causality depends on (1) strength of association, (2) specificity, (3) temporality, (4) consistency/reproducibility, (5) biologic...
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