

Hypothesis

HHV-8 in multiple myeloma: is this the first paracrine model of human tumorigenesis and do Koch's postulates apply?

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Human herpesvirus 8 (HHV-8) is now clearly implicated in the pathogenesis of Kaposi's sarcoma.¹⁻⁶ HHV-8 is also implicated in the pathobiology of effusion lymphoma⁷ and, recently, we and others have demonstrated this virus in effusion and non-effusion multiple myeloma,⁸⁻¹⁰ in pyothorax associated lymphoma, and in Castleman's disease.¹¹ HHV-8, a γ -II herpes virus, is unique in that it carries 16 different genes important in inflammation, the cell cycle, and apoptosis, including orf 16 (a bcl-2 homologue), viral interleukin 6 (v-IL-6), v-cyclin, vMIP I, and vMIP II.¹² vMIP I and vMIP II are inflammatory macrophage proteins, with sequence similarity to the human CC chemokines.

IL-6 is a growth factor for multiple myeloma.¹³ Indeed, it has been shown that transgenic C57BL/6 mice, which express an immunoglobulin enhancer activated IL-6 gene, develop polyclonal benign plasmacytosis.¹⁴ Interestingly, this strain is resistant to experimental plasmacytoma induction. In the context of the susceptible BALB/c strain, IL-6 transgenic mice develop malignant, monoclonal plasmacytomas that carry a rearranged

c-myc gene, consistent with the immunoglobulin/myc translocations found in BALB/c plasmacytomas induced by other means.

There is increasing evidence that IL-6 is not simply an autocrine growth factor in multiple myeloma, but is the product of other cells in the microenvironment of the bone marrow.⁸ IL-6 is also a growth factor for Kaposi's sarcoma, pleural effusion lymphoma, and multicentric Castleman's disease.¹⁵

Recently, Rettig and colleagues identified HHV-8 in bone marrow dendritic cells in a quarter of their patients with monoclonal gammopathy of undetermined significance, a condition that might progress to multiple myeloma, and in all their patients with multiple myeloma.⁸

Expansion of a tumour precursor population under the influence of a viral infection, followed by the successive emergence of genetically changed clones and subclones, is consistent with current concepts of virally assisted carcinogenesis. The novelty of the paracrine model for HHV-8 associated multiple myeloma (via the production of v-IL-6 and other HHV-8 encoded products; fig 1) lies in the fact that a non-malignant accessory cell would be responsible for the virally encoded production of a specific cytokine of cellular origin that expands the tumour precursor population at risk.

The concept of a particular disease being caused by a particular organism is embodied in Koch's postulates, which state that:

- (1) The organism should be found in all cases of the disease in question and its distribution in the body should be in accordance with the lesions observed.
- (2) The organism should be cultivated outside the body of the host, in pure culture, for several generations.
- (3) The organism so isolated should reproduce the disease in other susceptible animals.

Evidence for HHV-8 in the pathobiology of multiple myeloma

The model of HHV-8 carcinogenesis in multiple myeloma is based on the pre-existence of a monoclonal population of plasma cells upon which HHV-8 viral infection of bone marrow dendritic cells intersects itself. The expression

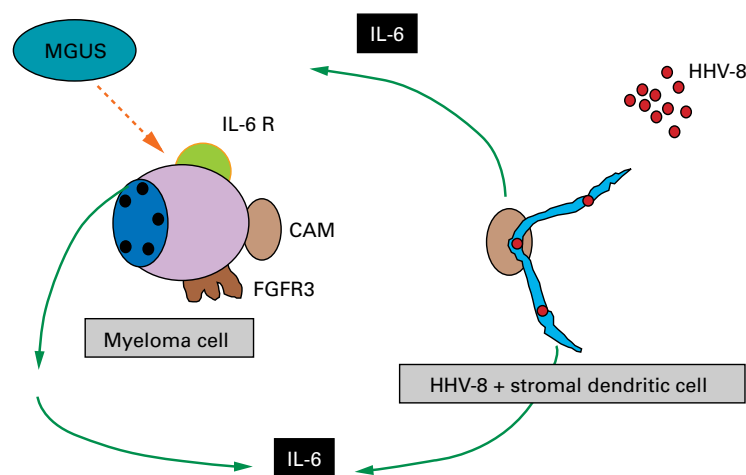


Figure 1 Schematic diagram of the possible role of HHV-8 in the pathogenesis of intramedullary multiple myeloma. The model assumes the existence of a premalignant population of cells (MGUS; monoclonal gammopathy of uncertain significance). HHV-8 colonises dendritic reticular cells in the bone marrow, with subsequent upregulation of HHV-8 v-IL-6 production. This interacts with the IL-6 receptor (IL-6 R) on the surface of a plasma cell. The plasma cell is also equipped with the fibroblast growth factor 3 receptor (FGFR3). The result is a paracrine loop of v-IL-6 production, which promotes plasma cell tumour growth.

and secretion of viral and human proteins (HHV-8 v-IL-6 and its human homologue, HHV-8 vMIP I, and HHV-8 vMIP II) might stimulate myeloma growth and predispose cells to secondary genetic alterations that ultimately result in the development of a clone capable of rapid expansion and accumulation. In essence, in intramedullary sites, HHV-8 might act as the viral "paracrine drive" for malignant plasma cells. Recently, we have shown that in effusion myeloma, malignant plasma cells are infected with HHV-8,⁹ and HHV-8 encoded products (v-IL-6, vMIP I, and vMIP II) might act as an "autocrine drive".

While this is an attractive hypothesis, it is currently the subject of much speculation and investigation. Rettig and colleagues⁹ identified HHV-8 in all cultured bone marrow stromal/dendritic cells from patients with multiple myeloma but not in the malignant plasma cell population. Ten out of 15 of these patients were untreated. Importantly, HHV-8 was not identified in bone marrow stromal cell DNA from 16 patients with other malignancies, including two cases of Hodgkin's disease and 12 cases of non-Hodgkin's lymphoma, or in 10 normal controls. In situ hybridisation localised HHV-8 in bone marrow stromal cells. Using a reverse transcription polymerase chain reaction (RT-PCR) assay, the authors identified HHV-8 v-IL-6 transcripts in three of three bone marrow stromal cell samples from patients with myeloma.

Recently, the same group has identified HHV-8 orf 72 DNA sequences in bone marrow biopsies by in situ hybridisation. Eighteen of 21 patients with multiple myeloma harboured HHV-8 sequences, as did two of two patients with AIDS associated plasmacytosis, and one of three patients with monoclonal gammopathy of undetermined significance. Six of seven fresh multiple myeloma bone marrow core biopsies contained HHV-8 DNA sequences. Again, the normal control and haematological malignancy cohort (n = 25) did not contain HHV-8. These results exclude an "artefact" result in cultured dendritic cells.

Recently, we have reported localisation of HHV-8 in seven of 12 fresh multiple myeloma biopsies, using real time Taq Man quantitative PCR and PCR in situ hybridisation (PCR-ISH) to orf 26 (major capsid protein), v-cyclin, and v-IL-6 encoding regions.⁹ We have confirmed the observation by Rettig *et al* of HHV-8 localisation in a minority cell population (dendritic cells) in bone marrow isolates. The copy number of HHV-8 in these lesions is low and we have convincingly demonstrated HHV-8 v-IL-6 production as well as vMIP I and vMIP II. In contrast, effusion myelomas contain higher quantities of HHV-8 as assessed by Taq Man PCR and quantitative competitive PCR.

A larger study of patients with multiple myeloma has recently reported finding HHV-8 in 32 of 74 patients.¹⁶ Similar results have also been reported by others.¹⁰

Evidence against HHV-8 in the pathobiology of multiple myeloma

Several groups have raised questions regarding the infection of bone marrow dendritic cells in

multiple myeloma. Much of the controversy has emanated from serological studies. Parravicini and colleagues have recently examined a series of 40 Italian patients with multiple myeloma, using a sensitive nested PCR assay and a serological assay to HHV-8 latency associated nuclear antigen (LANA) and orf 65 lytic genes.¹⁷ They identified antibodies against HHV-8 in only one of 20 patients. Concomitantly, they examined the antibody status to cytomegalovirus and hepatitis B, which showed positive serology in 18 of 20 and eight of 20 patients, respectively. Whitby and colleagues¹⁸ used immunofluorescence antibody screening against HHV-8 LANA-1 to test blood donors and patients with multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphoma. Their results show 11% positivity in multiple myeloma patients, 5% positivity in patients with monoclonal gammopathy of undetermined significance, 17% positivity in patients with Hodgkin's disease, 13% positivity in patients with non-Hodgkin's lymphoma, and 13% positivity in blood donors. Other researchers have also questioned the association between HHV-8 and multiple myeloma.^{19 20}

Unifying hypotheses

Clearly, a discrepancy exists between the serological and PCR assay results. The lack of serological evidence for HHV-8 in multiple myeloma might relate to a change in B cell ontogenicity. Patients with multiple myeloma have panhypogammaglobulinaemia, which could potentially reduce the titre of HHV-8 specific antibodies. However, the same should apply for all other viruses, which has not been shown to be the case.¹⁷ More importantly, however, might be the copy number of HHV-8 infection in patients with multiple myeloma. We have shown that the copy number in these patients is low (1–10 copies/1000 bone marrow cells).²¹ DNA loading experiments have shown a predicted increase in HHV-8 viral DNA copy number, with greater numbers of bone marrow cells examined. In multiple myeloma, neither the precise number of dendritic cells remaining in the neoplastic marrow or what proportion of these cells are infected with HHV-8 are known. We speculate that the numbers of individual dendritic cells infected with HHV-8 are low, and that the copy number of virus in any individual cell is between 1–3 copies, as assessed by PCR-ISH.²¹ Under such circumstances, it is not entirely surprising to find that HHV-8 serology may be negative. In contrast, in effusion related myelomas, a high copy number of HHV-8 is found, akin to effusion lymphomas (Tsang *et al* 1998, unpublished data).

Negativity for HHV-8 with solution phase PCR poses a problem. Heparin used in bone marrow biopsy samples inhibits Taq polymerase, so that when heparin treated samples are examined, heparinase should also be used. Secondly, polymorphisms within the KS Bam 330 regions at priming sites might account for some negative PCR results. It also appears that one specific strain of HHV-8 might be selectively involved in the pathogenesis of multiple myeloma.

Clearly, HHV-8 in multiple myeloma does not satisfy Koch's postulates, as is found for most infectious organisms identified by molecular biological assays. There are now several excellent models available for the propagation of HHV-8 in culture and recently we have shown that HHV-8 can serially infect endothelial cells.²⁴

In relation to HHV-8, we need to clarify further the exact prevalence of HHV-8 in multiple myeloma series from disparate geographical areas and gain more detailed sequence data on possible polymorphisms. In addition, the effect of treatment needs to be investigated urgently, as this could potentially alter the HHV-8 copy number. More importantly, HHV-8 viral DNA and the absolute expression profile of HHV-8 v-IL-6, vMIP I, and vMIP II in dendritic cells isolated from multiple myeloma cells need to be measured, and the effects of multiple myeloma proteins on HHV-8 copy number and expression profiles also need to be investigated. Finally, if HHV-8 is involved in a proportion of cases of multiple myeloma, then the effect of antiviral treatments, such as Foscarnet, which have dramatic influences on the outcome of Kaposi's sarcoma need urgent evaluation.

In summary, the jury is still out on the role of HHV-8 in multiple myeloma. However, we might be looking at the first example of a paracrine/autocrine model of viral carcinogenesis in humans.

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