Kaposi's Sarcoma–Associated Herpesvirus Infection and Multiple Myeloma

M. B. Rettig *et al.* (*1*) describe evidence of Kaposi's sarcoma–associated herpesvirus (KSHV) infection in long-term cultured bone marrow dendritic stromal cells in 15 out of 15 samples obtained from patients with multiple myeloma (MM) and in two out of eight samples from patients with monoclonal gammopathy of undetermined significance (MGUS). Rettig *et al.* used polymerase chain reaction (PCR) and in situ hybridization techniques. In the same study, three out of three long-term cultures of bone marrow stromal cells obtained from patients with MM were also found to contain KSHV transcripts coding for vIL-6, a viral protein homologous to human interleukin-6. Because vIL-6 may stimulate in vitro the proliferation of plasmocytoid cell lines, Rettig *et al.* postulate that infected bone marrow dendritic stromal cells may sustain in vivo the growth of uninfected plasma cell clones, promoting transformation from MGUS to MM. The validity of this attractive hypothesis is challenged, however, by the fact that Rettig *et al.*, using PCR, did not find KSHV in bone marrow aspirates from any of the 15 patients with MM. Rettig *et al.* ascribed this discrepancy between in vivo and ex vivo results to four concomitant factors: low number of stromal cells, heavy contamination by peripheral blood, presence of up to 90% uninfected neoplastic plasma cells in bone marrow aspirates, and insufficient sensitivity of the PCR assay.

To evaluate the proposed link between KSHV (also known as human herpes virus–8, or HHV-8) and MM without the limitations of a purely PCR-based experimental design, we studied a series of 40 Italian patients with MM by combining a nested PCR method that achieves single copy sensitivity (*2*) with serologic assays for antibodies against KSHV. For each case, 1 μg of DNA extracted from freshly purified bone marrow mononuclear cells (10 times the amount used by Rettig *et al.*) was tested in triplicate by nested PCR. Serum samples obtained at diagnosis (that is before therapy began) were available for half (20) of the patients and were tested by protein immunoblot (*3*) and immunoperoxydase (*4*) assays for the presence of KSHV-specific antibodies against *orf65* lytic and latency-associated nuclear antigens, respectively. In addition, all sera were screened for antibodies against cytomegalovirus (HCMV) and hepatitis B (HBV) (*5*). We performed these assays in order to exclude false negative results since, as Rettig *et al.* correctly observe (*1*), patients with MM often exhibit a profound inhibition of the humoral immune response (Table 1).

Our results show that none of the 40 patients with MM had KSHV DNA sequences that were detectable in bone marrow aspirates and that only one out of 20 (5%) had antibodies against KSHV (Table 1). By contrast, antibodies to HCMV and HBV were detectable in 18 out of 20 (90%) and 6 out of 15 (38%) of the sera samples, and seroprevalence rates were similar to those found in earlier studies of the Italian general population (*6*). The latter findings make it unlikely that the low prevalence of KSHV antibodies in MM patients was a result of concomitant inhibition of the humoral response, unless one assumes that this inhibition selectively involved the production of antibodies against KSHV antigens. In fact, KSHV seroprevalence rates are similar in MM patients (5%) and in Italian blood donors of similar geographic origin (4%) (*7*). Therefore, our findings indicate that most MM patients are not infected by KSHV and argue against there being a link between KSHV infection and the development of MM.

Table 1. Demographic and serologic features of 20 Italian patients with MM. Ab, antibody; Neg., negative; Pos., positive; ND, no data.

Patient	Sex	Age	Monoclonal component	Decreased serum polyclonal Ig classes*	KSHV$^+$ LANA	KSHV orf65	HBV Ab	HCMV Ab
1012	F	67	G,k	2	Neg.	Neg.	Neg.	Pos.
1013	F	74	G,k	2	Neg.	Neg.	Pos.	Pos.
1014	M	57	A,k	2	Neg.	Neg.	Neg.	Pos.
1015	F	71	A,k	ND	Neg.	Neg.	ND	Pos.
1017	F	80	G,λ	ND	Neg.	Neg.	ND	Pos.
1019	F	57	G,λ	1	Neg.	Neg.	Pos.	Pos.
1020	M	58	G,k	0	Neg.	Neg.	Neg.	Neg.
1021	F	54	A,k	0	Neg.	Neg.	Pos.	Pos.
1101	M	64	G,k	1	Pos.	Pos.	Neg.	Pos.
167	F	67	k	2	Neg.	Neg.	ND	Pos.
1734	M	65	A,k	0	Neg.	Neg.	Neg.	Pos.
1735	F	70	G,λ	1	Neg.	Neg.	Neg.	Pos.
1739	F	63	A,k	0	Neg.	Neg.	Pos.	Pos.
1740	F	67	A,λ	0	Neg.	Neg.	Neg.	Pos.
1745	F	79	A,λ	2	Neg.	Neg.	ND	Pos.
1746	M	45	G,k	2	Neg.	Neg.	Neg.	Pos.
1748	M	68	A,k	2	Neg.	Neg.	Neg.	Pos.
1749	F	45	G,k	0	Neg.	Neg.	Pos.	Pos.
1750	M	67	G,k	ND	Neg.	Neg.	Pos.	Neg.
1761	F	78	A,k	ND	Neg.	Neg.	Neg.	Pos.
Positive/total sample (Percent positive)					1/20 (5)	1/20 (5)	6/16 (38)	19/20 (90)

*Serum polyclonal Ig classes were evaluated by nephelometry (BNA, Behring, Dusseldorf, Germany); 0, no Ig reduction; 1, reduction of one serum polyclonal Ig class; 2, reduction of two serum polyclonal Ig classes.

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2. Bone marrow mononuclear cells were obtained from bone marrow aspirates by Ficoll density centrifugation and their DNA extracted with the use of standard protocols [J. Sambrook *et al.*, in *Molecular Cloning: A Lab-*

oratory Manual (Cold Spring Harbor Laboratory Press, New York, ed. 2, 1989)]. Thirty-cycle PCR using human β-globin specific primers [M. Bauer *et al.*, *J. Am. Med. Assoc.* **256**, 472 (1991)] and 1 μg of DNA as template was performed in order to rule out major Taq polymerase inhibitors and to verify the integrity of the extracted DNA. Nested PCR (35+35 amplification cycles) was performed as described in detail elsewhere [M. Corbellino *et al.*, *AIDS Res. Hum. Retrovir.* **12**, 651 (1996)]. One microgram of DNA was used as template, and each sample was tested in triplicate individual PCR reactions. This assay achieves single-copy sensitivity, as assessed by titration experiments and signal distribution analysis [Z. Wang and J. Spadoro in *94th Gen. Meet. Am. Soc. Microbiol.*, abstr. D256 (1994), p. 141]. According to the Poisson distribution, when the sensitivity of an analytical system achieves a single copy and the solution is repeatedly tested in this system, the concentration of the PCR target [which is referred to as Signal Generating Unit (SGU), or the smallest unit that gives a PCR-positive signal] is given by the formula SGU/PCR = −ln (no. of negative results/no. of replicates). Thus, if the hypothesis of Rettig *et al.* is correct (that all MM bone marrow aspirates contain KSHV-infected bone marrow dendritic stromal cells), given that we tested 40 MM specimens in triplicate (that is, a total of 120 replicates) and they were all negative, then the concentration of KSHV DNA sequences in bone marrow aspirates should be very low (for example, with 1/120 positive replicates the concentration of KSHV sequences is 1 KSHV-SGU per 17,924,896 cell equivalents).

3. *orf*65 KSHV recombinant protein was provided by Y. Chang and P. Moore (Columbia University, New York). Protein immunoblot assay was performed according to the method published by G. R. Simpson *et al.* [*Lancet* **348**, 1133 (1996)].
4. Immunoperoxydase assay against KSHV latency associated nuclear antigens (LANA) was performed on acetone-fixed cytospins of KSHV-infected BCBL-1 cells with the use of sera diluted 1:100, 1:500, 1:2500, and 1:10,000. Reaction was revealed by a peroxydase labeled antiserum specific for human immuno-globulin (IgG) (Dako, Glostrupp, DK) diluted 1:100, followed by tyramide signal amplification (Du Pont/NEN, Boston, MA). KSHV-infected BCBL-1 cells were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, from M. McGrath and D. Ganem [R. Rennie *et al.*, *Nature Med.* **2**, 342 (1996)]. Cells were cultured with RPMI-1640 (Gibco-BRL, Grand Island, NY). Patient sera and peroxydase-labeled rabbit antibody to human IgG were diluted in 0.05M TBS (tris-buffered saline, pH 7.2) containing 1% BSA (bovine serum albumin, Fraction V, Sigma Chemical, St. Louis, MO) and 0.001% Nonidet P-40 (Sigma Chemical, St. Louis, MO). BCBL-1 immunoperoxidase (IPA) is identical to previously reported immunofluorescence (IFA) assays [S. J. Gao *et al.*, *Nature Med.* **2**, 925 (1996); D. H. Kedes *et al.*, *ibid.*, p. 918] and represents a technical modification of this test. For both protein immunoblot and IPA tests, sera from patients with Mediterranean Kaposi's sarcoma and blood donors previously demonstrated to be KSHV seropositive and seronegative, respectively, were used as controls. IPA assay was effective in detecting latent KSHV infection in patients who developed Kaposi's sarcoma after receiving solid organ allograft [C. Parravicini *et al.*, *Blood*, **90**, 2826 (1997)].
5. Serological tests were performed for detecting IgG antibodies against cytomegalovirus, IMX System (Abbott Diagnostics, Science Park, IL) and antibodies against hepatitus virus B surface antigen, MEIA, AXSYM system (Abbott Diagnostics).
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KSHV is associated with Kaposi's sarcoma (KS), Castleman's disease, and a rare form of body cavity lymphoma (*1*–*4*). Rettig *et al.* (*5*) found KSHV (also known as HHV-8) in bone marrow stromal cells, but not malignant plasma cells, in all 15 patients with MM that they examined. Their results suggest that KSHV may be required for the transformation of plasma cells. However, their study was limited to examination of bone marrow stromal and mononuclear cells by PCR for KSHV and showed viral DNA only in the bone marrow stromal cells. Bone marrow cells before stromal cell isolation were found not to contain KSHV. Serology for KSHV was not given for these patients with MM (*5*).

We blindly analyzed serum samples from patients with MM or with epidemic (classic) KS, and from healthy adult donors for antibodies against γ [KSHV-8 and Epstein-Barr virus (EBV)] and β (HHV-6 and HHV-7) herpesviruses. We used (i) an enzyme-linked immunosorbent assay (ELISA) to detect whole virus lysate (2×10^9 viral particles per liter) that contained the majority of the viral structural proteins (1.5 mg of viral protein per milliliter) and (ii) an indirect immunofluorescence assay (IFA) to detect KSVH lytic antigens with the KS-1 cell line [which comes from human immunodeficiency virus (HIV)–and EBV-negative body cavity lym-

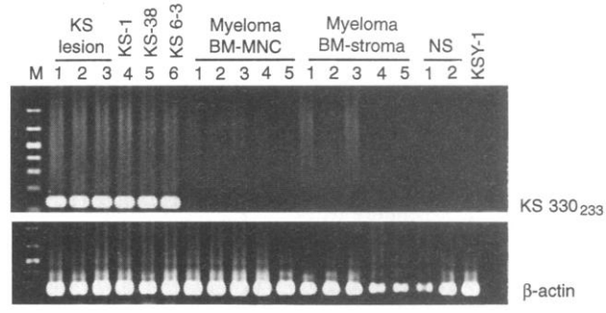

Fig. 1. KSHV DNA amplification of the $KS330_{233}$ sequence showing a PCR product of 233-bp in the upper panel and β-actin as control in the lower panel in KS primary tumor tissues (lanes 1 to 3), KS–1 (lane 4), early passage KS primary isolates (lanes 5 and 6), but not in myeloma bone marrow mononuclear cells (lanes 1 to 5, myeloma BM-MNC), myeloma bone marrow stromal cells (lanes 1 to 5, myeloma BM-stroma), normal bone marrow stromal cells (lanes 1 and 2, NS), or neoplastic KS cell line (KSY-1). PCR amplification of each test sample (250 ng of KSHV; 100 ng of β-actin) was performed for 35 cycles.

Table 1. Antibody assays of immune response.*

Virus	MM	KS	Blood donor
	Number positive/ number tested (percent positive)	Number positive/ number tested (percent positive)	Number positive/ number tested (percent positive)
KSHV	2/28 (7)	22/25 (88)	2/24 (8)
EBV	26/28 (93)	20/25 (80)	20/24 (83)
HHV-6	22/28 (79)	21/25 (84)	21/24 (88)
HHV-7	24/28 (86)	22/25 (88)	23/24 (96)

*More than 60% of the samples were evaluated by IFA as well as by ELISA. IFA samples were tested at a 1:20 dilution; ELISA samples were tested at a 1:100 dilution. Appropriate negative and positive control sera for each herpes virus were tested simultaneously.

Table 2. Detection of KSHV DNA by PCR or by IFA. PB, peripheral blood; MNC, mononuclear cell; BM, bone marrow; ND, not done.

Cell type	Method	MM	Hematologic malignancies
		Number positive/ number tested	Number positive/ number tested
PB-MNC	PCR	0/6	ND
BM-MNC	PCR	0/5	0/11
BM stroma*	PCR	0/5	0/11
Cell lines	PCR	0/2	0/5
BM stroma	IFA	0/5	ND

*Short-term cultures of bone marrow stromal cells were established and successive subpassages (P_1–P_3) were tested for presence of KSHV antigen by IFA with the use of polyclonal KSHV-positive and KSHV-negative human serum. Hematologic malignancy cell lines included HUT 78, Raji cells, U937, Daudi cells (all from the American Type Culture Collection), and 23-2 [a lymphoma cell line (*8*)]. Bone marrow mononuclear cells from hematologic malignancies consisted of chronic myelogenous leukemia and Hodgkin's disease.

phoma] as a substrate (6). The IFA to detect HHV-6, HHV-7, and EBV (also called HHV-4) was performed as described previously (7). We also examined peripheral blood mononuclear cells (six cases) and bone marrow mononuclear cells (five cases), bone marrow stromal cells (five cases) from patients with MM, and two myeloma cell lines for KSHV DNA with the use of PCR. Bone marrow stromal cells were isolated and studied as described previously (5) and were also analyzed for the presence of KSHV by IFA, with the use of the polyclonal antibody against KSHV (6). We did not observe any decline in KSHV antibody when we tested the polyclonal reference serum (which came from a patient with classic KS) for absorption of EBV antibodies with the EBV-producing cell line P3HR1 (Advanced Biotechnologies, Inc., Columbia, Maryland).

Antibodies against EBV, HHV-6, and HHV-7 IgG were detected in the majority of the sera from MM patients (Table 1). However, antibodies against KSHV were detected in only 7% of MM sera samples, as compared with 8% of samples from healthy adult donors, and over 85% of samples from patients with acquired immunodeficiency virus (AIDS) and KS. Thus, these patients with MM were able to mount an immune response to highly prevalent lymphotropic human herpesviruses (EBV, HHV-6, HHV-7). The low seropositivity to KSHV in MM is similar to that of the general population in North America and is not secondary to the lack of a humoral response. Furthermore, mononuclear cells (from peripheral blood and from bone marrow) from the 11 patients with MM that we tested were found not to contain KSHV DNA; all of these patients were also seronegative for KSHV serum antibodies (Tables 1 and 2). Last, the short term cultures of myeloma bone marrow stromal cells from five of these cases were found not to contain KSHV with the use of DNA PCR and IFA (Table 2, last row, and Fig. 1).

These data contradict the study by Rettig *et al.* in which KSHV DNA was detected with the use of PCR in all 15 samples of bone marrow stromal cells taken from MM patients (5).

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Rettig *et al.* propose (*1*) that KSHV (*2*) is involved in the development of MM from its more indolent precursor MGUS. They argue that KSHV may influence the switch from benign MGUS to malignant myeloma through the secretion and induction of autocrine growth factors, because the virus itself is not present in the clonal plasma cells. This is an attractive hypothesis because KSHV encodes a homolog of one of the cytokines, IL-6 (*3*), that is involved in MM pathogenesis. A further link might be that patients with multicentric Castleman's disease, another lymphoproliferation associated with KSHV (*4*), often have immunoglobulin dyscrasias, and occasionally develop MM.

The curious epidemiologic distribution of KS implies that an agent causing this disease would certainly not be the same agent inducing myeloma, which is a common hematological cancer with a much more even distribution throughout different populations. Furthermore, current molecular and serologic assays indicate that KSHV is, in northern Europe and the United States at least, predominantly sexually acquired and is not like most other herpesviruses, which are common pathogens (*5*) (*6*). The incidence rates of MM are homogenous in the various regions of northern and southern Italy (*7*), in contrast to the rate of classic KS (*8*), which correlates with the seroprevalence rates of KSHV in the different regions (*9*).

An immunofluorescence assay (IFA) that detects antibodies against the latent nuclear antigen (LNA-1) of KSHV is the most sensitive and specific serologic assay that we know of for revealing past or present infection with this virus (*10*). The decrease in polyclonal immunoglobulins associated with MM may be global or specific, and antibodies against KSHV may therefore not be detectable in infected patients, even if they have latent or lytic-specific antibodies against other herpesviruses. Antibodies against viruses in patients with MGUS are not suppressed, as a near normal immunoglobulin profile distinguishes MGUS from MM. If KSHV plays any major and common pathogenic role in MM and a subset of MGUS cases, we would expect to find increased serologic markers of infection, particularly in those with MGUS.

We therefore investigated the prevalence of antibodies against KSHV LNA-1 by IFA in patients with MM or MGUS from the Po valley of northern Italy, an area with one of the highest incidence rates of classic (HIV-negative) KS in the world. We compared the detection rate of antibodies against KSHV in these patients with that in patients with lymphoma (Hodgkin's disease and non-Hodgkin's lymphoma) and blood donors, all from the same Po valley area (Table 1). Sera were tested blindly at a dilution of 1:100 as previously described (*11*).

The prevalence of antibodies against KSHV in the sera of patients with MM, with MGUS, with lymphoma, and in sera from blood donors was not significantly different ($P > 0.01$; Table 1). The detection rate in Po valley blood donors is significantly higher (12.9% as opposed to <3.0%) than that found with the use of the same assay in blood donors in the United States or the United Kingdom (6) (*11*), which reflects the much higher incidence of classic KS there.

Four of the MGUS samples with no detectable antibodies were from patients who subsequently developed overt MM, whereas the only two MGUS patients with detectable KSHV antibodies had no evidence of MM after 36 and 48 months, respectively.

Thus, we did not find evidence for an increased detection rate of antibodies

Table 1. Results from sera of patients and blood donors living in the Po valley of Italy, tested with an IFA specific for antibodies against KSHV.

Sera type	Number tested	Number positive (percent positive)
Myeloma	37	4 (10.8)
MGUS	36	2 (5.5)
Hodgkin's disease	42	7 (16.7)
NonHodgkin's lymphoma	60	8 (13.3)
Blood donors	139	18 (12.9)

against KSHV in patients with MM, and even less so for those with MGUS. Our data do not support a role for KSHV in the evolution of MGUS to MM.

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12. Sera provided by G. Longo, Second Medical Clinic, Modena, and D. Vallisa and L. Cavanna, First Medical Division, Section of Hematology, Piacenza, Italy. Supported by the Medical Research Council and the Cancer Research Campaign.

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As a clinical dermatologist involved in the study of classic KS, I (F.C.) have followed about 200 cases of this disease over 20 years in the University Hospital of Sassari, which is the referral center for this disease in northern Sardinia. My epidemiological study has shown a standardized incidence of 1.58 per 100,000 inhabitants per year (2.43 for males and 0.77 for females) (*1*); this incidence is significantly higher than that observed in nine other Italian regions (1.05 for males and 0.27 for females) (*2*). I have found cases of KS associated with Hodgkin's disease, chronic lymphocytic leukemia, and Castleman's disease, but not with MM. Furthermore, the incidence of MM in the Sassari district is 4.51 per 100,000 inhabitants per year (4.57 for males and 4.45 for females), which is no different from that observed in seven other Italian regions where classic KS is not frequently detected (incidence range from 3.3 to 7.6 for males and from 3.7 to 7.3 for females) (*3*).

With regard to KSHV epidemiology in northern Sardinia, we found that 12 out of 13 KS patients had KSHV in their peripheral blood as detected by PCR (*4*). We have recently found that 95% of KS patients had sera with antibodies against KSHV and that the healthy northern Sardinian population has a higher prevalence of antibodies against KSHV in the serum (18%) than do people in the rest of Italy (4%) (*5*). If KSHV had a role in MM, as suggested by Rettig *et al.* (*6*), then we would expect to find an increased rate of MM in the Sassari district and, therefore, a more frequent association of classic KS with MM. Our observations (although made on a small number of cases because of the rarity of classic KS) do not seem to support such an association.

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7. I would like to thank an anonymous reviewer for helpful remarks.

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Contrary to the report by Rettig *et al.* (*1*), other groups have not found antibodies against KSHV in serum from patients with MM (*2*).

We took acetone-fixed and paraffin-embedded bone marrow biopsies from patients with MM and performed PCR analysis in an attempt to detect KSHV. We investigated 20 cases of MM (at different stages) with a protocol previously described (*3*) with the use of the primer set within open reading frame (ORF) 26 ($KS330_{233}$). We carried out two rounds of amplification (2 × 30 cycles). This protocol yielded a positive amplification of 233 base pairs (bp) in 18 out of 20 patients. We also analyzed 15 bone marrow biopsy samples from patients with follicular lymphomas and 5 samples from patients with reactive processes; these samples did not show the presence of KSHV. In all cases, the integrity of the DNA was confirmed after amplification of a 258-bp-long fragment corresponding to the *c-raf-1* gene as previously described (*4*). The specificity of the $KS330_{223}$ PCR was checked by sequencing the amplification products of five randomly selected cases. We consider our results to be specific because there were different point-mutations in each case as compared with the original sequence (*3*). These results are similar to those obtained by Rettig *et al.* (*1*). Our method would appear to eliminate technical problems such as contamination as a source of the results. Overall, we confirm that, at least in our French series of cases, KSHV is strongly associated with MM.

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Response: Several groups raise questions regarding our report that KSHV (also called HHV-8) infects the bone marrow dendritic cells of myeloma patients and may be involved in myeloma pathogenesis (*1*). Whitby *et al.* performed a serologic assay, and both Parravicini *et al.* and Massoud *et al.* used both serologic and PCR assays to conclude that a lack of evidence exists for the association of KSHV and myeloma. Cottoni *et al.* demonstrate a lack of clinical correlation between the incidence of classic KS and MM in a cohort of patients from Sardinia.

A possible mechanism for the lack of serological evidence for KSHV in MM relates to a change of environment for B cell development in these patients. Myeloma patients demonstrate panhypogammaglobulinemia (that is, decreased concentrations of antibodies other than the monoclonal antibody produced by the malignant plasma cells), which may reduce the titer of KSHV antibodies. In addition, the infection of dendritic cells within the bone marrow, the site of early B cell development, may lead to B cell tolerance to KSHV antigens. Clonal deletion, functional inactivation, and antigen receptor editing have been shown to be mechanisms for preventing the maturation of B cells that have been presented antigen in the bone marrow (*2*). Thus, the combination of global and viral antigen-specific immunological defects may contribute to the decreased seroprevalence to KSHV in myeloma patients.

With the use of PCR amplification, Parravicini *et al.* and Massoud *et al.* were unable to detect viral sequences in mononuclear cells obtained from aspirated bone marrow, peripheral blood mononuclear cells, or bone marrow stromal cells. The results from bone marrow mononuclear cells are consistent with our

original report. Similar to the work of Parravicini *et al.*, we have subsequently performed nested PCR on 1 μg of DNA from mononuclear cells from bone marrow aspirates and repeated the reaction up to 10 times for each patient; we have not detected the virus. Parravicini *et al.* alluded to some of the possible explanations for the inability to detect virus. They stated that the presence of *Taq* polymerase inhibitors were excluded because they amplified the β globin gene. However, the presence of heparin, which is the anticoagulant most often used for bone marrow aspirations, may inhibit the amplification of low copy number sequences by *Taq* polymerase (3). Moreover, heparin is not removed during standard DNA extraction techniques. We have used heparinase to remove contaminating heparin from our bone marrow mononuclear cell samples and have successfully amplified KSHV sequences in bone marrow mononuclear cell samples. Recently, the precise bone marrow culture conditions have been shown in an abstract to be essential for the detection of KSHV DNA (4). PCR signal for viral DNA may be lost with more prolonged culture, as has been seen in the case of KS.

To exclude the possibility that our observations of KSHV infection in dendritic cells were a result of an in vitro artifact of tissue culture, we have evaluated fresh uncultured bone marrow core biopsies for the presence of KSHV. Whereas none of the normal subjects ($n = 4$) or patients with other hematological malignancies ($n = 23$) demonstrated the presence of KSHV by in situ hybridization for ORF 72, 18 out of 21 myeloma patients showed viral staining (5). PCR for $KS330_{233}$ on these same tissue samples detected KSHV in 12 out of 16 myeloma patients (5). Our results have been replicated by Brousset *et al.*, as described in their comment. In our cohort of myeloma patients, immunohistochemical staining on serial sections showed that the distribution of the presence of KSHV corresponded to the distribution of cells containing long cytoplasmic processes. The latter stained for fascin, a marker for dendritic cells).

Similar to results seen by Parravicini *et al.*, we were unable to detect viral sequences in bone marrow mononuclear cells. Although we were also unable to detect KSHV in whole peripheral blood mononuclear cells, when we enriched these cells for macrophage and dendritic cell markers (CD68 and CD83, respectively) with the use of an immunomagnetic bead column, we identified viral sequences in 25 out of 32 myeloma patients, 0 out of 5 patients with other malignancies, and 1 out of 13 hematologically normal subjects (6).

Cottoni and Uccini discuss a cohort of classic KS patients in Sardinia in whom they did not detect an increased prevalence of myeloma. The extrapolation of these data to myeloma patients worldwide may not be valid. In addition to KSHV infection, other environmental and genetic factors may well contribute to the development of myeloma. By analogy, EBV is closely associated with nasopharyngeal myeloma in southern China and with Burkitt's lymphoma in Africa, yet the incidence of these malignancies among immigrants to the United States is equal to that of the general population. Three decades ago, Mazzaferri and Penn reviewed the literature and concluded that there was a correlation between KS and myeloma (7). Epidemiological studies in HIV-positive patients may provide important clinical information correlating KSHV infection, KS, and myeloma. As HIV patients live longer, they may manifest an increased incidence of myeloma.

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