Kaposi Sarcoma–Associated Herpes-Like Virus (Human Herpesvirus Type 8) DNA Sequences in Multicentric Castleman's Disease: Is There Any Relevant Association In Non-Human Immunodeficiency Virus–Infected Patients?

To the Editor:

Recently, Soulier et al[1] reported in *Blood* the detection of Kaposi sarcoma herpes virus like (KSHV) (also called human herpesvirus type 8)[2,3] DNA sequences in 14 of 14 cases of human immunodeficiency virus (HIV)-associated multicentric Castleman's disease (MCD), and in 7 of 17 MCD occurring in HIV$^-$ patients. They suggested that KSVH could play a role in the pathogenesis of MCD, an angio follicular lymphoid proliferation[4] often associated with KS (especially in HIV-infected patients).[4,5] We recently searched for such viral sequences in lymphoproliferative disorders including multicentric and localized Castleman's diseases occurring in non–HIV-infected patients, and MCD (also called follicular hyperplasia of type IB[6]) in HIV$^+$ patients. In addition, cases of the persistant adenopathy syndrome occurring in HIV-infected patients with follicular hyperplasia and variable degree of folliculolysis (Follicular Hyperplasia IA type[6]) and cases of non-Hodgkin's lymphoma (NHL) were studied including follicular in HIV$^-$ patients or Burkitt's type in HIV-infected patients (Table 1).

DNA was extracted from 43 cryopreserved tumor samples, mostly lymph nodes from 40 patients (Table 1). To detect KSHV sequences, we used two different sets of primers.[2] The first (labeled 81 to 82) amplified 233 bp whereas the second (84 to 85) corresponded to a fragment of 671 bp. The polymerase chain reaction (PCR) was performed as described[2] on 1 to 2 μg of DNA with an initial denaturation step at 94°C and then 35 cycles for 1 minute at 94°C, 1 minute at 55°C, and 2 minutes at 72°C. After electrophoresis, the PCR products were transferred to a nylon membrane and hybridized with two different P32 radiolabeled oligonucleotides (83 and 86) specific for the two amplified KSHV PCR products. DNA extracted from 2 KS cutaneous tumors or from peripheral blood mononuclear cells (PBMC) of HIV seronegative blood donors were used for PCR as positive and negative controls respectively. All DNA samples were also amplified by PCR for human β-globin sequences to show the integrity of the DNA specimen.

KSHV DNA sequences were detected in only one of the six Castleman's disease lymph node biopsies studied and occurring in HIV seronegative persons. This KSHV$^+$ case was a typical plasma cell type MCD occurring in a 57-year-old woman from Mauritania who was also diagnosed with a renal amyloidosis. None of these 6 cases had KS. In contrast, 3 cases out of 4 MCD cases occurring in HIV-infected patients contained KSHV sequences in the lymph nodes. Furthermore, such sequences were also detected in the PBMC DNA of one of these patients. It is worth noting that these 3 KSHV$^+$ patients (all homosexual) had developed KS. However, in these 3

Table 1. Epidemiological, Pathological, and Virological Features of the 40 Patients Studied

Histology	HIV Serology	Case	No.	Age/Sex Risk Factors	Tumor Localization	KSVH Sequence 81-82	KSVH Sequence 84-85*	Associated Diseases
Castleman's disease								
Multicentric Castleman PL	–	Tet	134	38/M	LN 1992	–	–	
Multicentric Castleman PL	–	Tet	119	38/M	LN 1995	–	–	
Multicentric Castleman PL	–	Duv	181	75/M	LN	–	–	
Multicentric Castleman PL	–	*Min*	185	59/F	LN	++	++	Renal Amyloidosis
Localized Castleman H/V	–	Yas	150	66/M	LN Mediast	–	–	
Localized Castleman PL	–	Iro	131	37/F	LN Mesenter	–	–	HTLV-1 seropositive
Localized Castleman PL	–	Mus	130	30/F	LN Mesenter	–	–	
MCD Hyp fol IB, Hyper fol IA	+	Del	127	39/M homosexual	LN 1988	++	++	Cutaneous Kaposi 1987
	+	Lan	179	45/M homosexual	PBMC	++	++	Kaposi 1991
MCD-Hyp fol IB, Hyper fol IA	+	Lan	133	45/M homosexual	LN 1991	++	++	Kaposi 1991
MCD-Hyp fol IB	+	Fan	135	34/M homosexual	LN 1994	++	++	Cutaneous Kaposi 1995
MCD-Hyp fol IB	+	Fan	146	34/M homosexual	LN 4/1995	++	++	Cutaneous Kaposi 6/1995
MCD-Hyp fol IB, Hyper fol IA	+	Yal	126	31/M IVDU	LN	–	–	
Persistent lymphadenopathy								
Hyp fol IA	+	Urr	161	36/M homosexual	LN	++	++	
Hyp fol IA	+	Oli	158	30/M not known	Tonsil	+	+	
Hyp fol IA	+	Pom	156	64/M homosexual	LN	+	+	
Hyp fol IA	+	Amr	116	23/M IVDU-homosexual	LN	+	+	Hodgkin disease, 1986
Hyp fol IA	+	Alv	132	32/M IVDU	LN	–	–	Hodgkin disease, 1992
Hyp fol IA	+	Tra	114	55/F heterosexual	LN	–	–	HTLV-I seropositive
Hyp fol IA	+	Leg	129	30/M IVDU	LN	–	–	
Hyp fol IA	+	Mav	122	32/M heterosexual	LN	–	–	
Hyp fol IA	+	Rou	117	23/M heterosexual	LN	–	–	
Hyp fol IA	+	Rob	159	33/M IVDU	LN	–	–	
Hyp fol IA	+	Bel	162	49/M not known	Tonsil	–	–	
Hyp fol IA	+	Heb	147	33/M IVDU	LN	–	–	
Non-Hodgkin's lymphoma								
NHL Burkitt	+	Hai	128	31/M homosexual	LN	–	–	
NHL Burkitt	+	Gaa	120	30/F IVDU-prostitution	LN	–	–	
NHL Burkitt	+	Bar	145	42/M homosexual	LN	–	–	
NHL Burkitt	+	Tck	115	40/M IVDU	LN	–	–	
NHL Burkitt	+	Bel	144	56/M IVDU?-homosexual?	LN	–	–	
NHL B CB-CC follicular	–	Lev	136	53/F	LN	–	–	
NHL B CB-CC follicular	–	Dec	137	76/F	LN	–	–	
NHL B CB-CC follicular	–	Pue	138	51/M	LN	–	–	
NHL B CB-CC follicular	–	For	139	75/F	LN	–	–	
NHL B CB-CC follicular	–	Der	140	61/M	LN	–	–	
NHL B CB-CC follicular	–	Kra	141	47/M	LN	–	–	
NHL B CB-CC follicular	–	Lav	142	50/F	LN	–	–	
NHL B CB-CC follicular	–	Hiv	143	22/F	LN	–	–	
NHL B CB-CC follicular	–	Bro	154	57/F	LN	–	–	
NHL B CB-CC follicular	–	Lai	149	51/F	LN	–	–	
NHL B CB-CC follicular	–	Ler	148	46/M	LN	–	–	
NHL B CB-CC follicular	–	Cla	153	63/F	LN	–	–	
NHL B CB-CC follicular	–	Mor	152	47/M	LN	–	–	

Abbreviations: IVDU, intravenous drug user; LN, lymphnode; PL, plasma cell type; H/V, hyalino vascular type; NHL, non Hodgkin's lymphoma; MCD, multicentric Castleman's disease; CB-CC, centroblastic-centrocytic.

* Sequences kindly provided by Dr J.T. Aubin, Pitié Salpétrière Hospital, Paris, France.

cases, KS was diagnosed in other organs and no detectable KS microscopic lesions could be observed in the lymph nodes. In one of these cases, the cutaneous KS lesions appeared 2 and 12 months, respectively, after the lymph node biopsies found strongly positive for KSHV sequences. It is of interest that no KSHV DNA sequences was detected in the only MCD case occurring in an HIV patient without any evidence of KS. This patient was a nonhomosexual intravenous drug user (IVDU). We also found KSVH sequences in 4 out of 12 cases of type IA follicular hyperplasia occurring in HIV-infected patients. Of possible relevance is that 3 of these 4 positive cases (2 lymph nodes and 1 tonsil biopsies) occurred in homosexual men, (the risk factor for HIV infection was unknown for the fourth case) whereas 7 out the 9 KSHV$^-$ cases of type IA follicular hyperplasia occurred in IVDU (4 cases) or in patients who acquired their HIV infection possibly heterosexually (3 cases). The 5 HIV positive Burkitt's NHL and the 13 HIV$^-$ follicular lymphomas were KSHV$^-$.

A semiquantitative PCR evaluation for KSVH sequences performed as previously described[7] showed a high viral load in the MCD lesions occurring in patients with or without HIV infection and in one patient with a type IA follicular hyperplasia, being equivalent or slightly lower (around 1 copy per 10 cells) to that found in KS lesions (around 1 viral copy per cell). Furthermore, the viral burden was lower in the PBMCs of patient Lan 133 and in the LN of one case of a type IA follicular hyperplasia.

These data confirm that KSHV DNA sequences are present, at a high viral load in most of the MCD occurring in HIV-infected patients even if there is no microscopically detectable foci of KS lesions in the lymph node. Furthermore, our findings also showed that KSVH can be found (possibly at a lower viral load) in lesions of the type IA follicular hyperplasia occurring in homosexual HIV-infected patients without any clinical evidence of KS. In our small series, this virus seems nearly exclusively found in homosexual patients. It would have been interesting to know the risk group of the HIV-infected patients reported by Soulier et al[1] especially for those who did not develop KS. Furthermore, our findings combined with those of Soulier et al[1] suggest that KSHV sequences are detectable in only a minority of the Castleman's disease lesions occurring in HIV-uninfected patients. Nevertheless, we believe that this virus may be associated, (presence of a high viral load), with such rare cases of multicentric but not localized Castelman's disease. However, the possible etiological role of KSVH in the pathogenesis of Castelman's disease remains to be determined by (1) molecular studies on KSHV and other human herpes viruses[8] on larger series of MCD cases comparing multicentric to localized diseases and plasma cell, hyalino-vascular, or mixed cell types[4] and (2) in situ-PCR with the aim of localizing the infected cells (B or T lymphocytes, macrophages, endothelial cells). Finally, the strength of the association of KSHV and Castleman's disease has to be compared in different populations (from several geographical locales) having various prevalence rates of KSHV infection, but this has to wait until specific serologic tests are available.

ACKNOWLEDGMENT

We thank Drs E. Dulmet, F. Galateau, and I. Bouchard for providing some lymph node biopsy samples and clinical information concerning some of the studied patients. Also, technical help by Serge Bain was greatly appreciated.

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