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Human Herpesvirus 8 Serological Markers and Viral Load in Patients with AIDS-Associated Kaposi's Sarcoma in Central African Republic

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Epidemic Kaposi's sarcoma (KS) is one of the most frequent types of cancer in several African countries; however, very few data are available on human herpesvirus 8 (HHV-8) markers in KS patients from Central Africa. In a series of 36 AIDS-KS cases from Central African Republic, we showed, using a real-time PCR quantitative assay, the high frequency (82%) of detectable HHV-8 DNA in peripheral blood mononuclear cells (PBMCs). We also found that the level of antibodies directed against lytic or latent HHV-8 antigens is not correlated to the amount of HHV-8 viral load in the PBMCs, and finally, we demonstrated a much higher viral load in tumoral skin lesions (6.07 log copies/μg DNA) than in unaffected skin (2.93 log copies/μg DNA) or in PBMCs (2.55 log copies/μg DNA).

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), is a γ-2 herpesvirus considered to be the etiological agent of all forms of Kaposi's sarcoma, a tumor occurring frequently during human immunodeficiency virus type 1 (HIV-1) infection (epidemic KS [AIDS-KS]) and in transplant recipients (iatrogenic KS) (4, 14). Such tumors also occur among non-HIV-infected individuals, predominantly either in aged men of Mediterranean and Middle East origin (classic KS) or in inhabitants from East and Central Africa (endemic KS) (4, 14). Epidemiological surveys indicate that HHV-8 is not a widespread ubiquitous virus but that its presence is mainly restricted to areas in which classic or endemic KS is highly endemic. HHV-8 infection is thus highly endemic in Central, East, and South Africa, areas in which the viral seroprevalence can reach up to 50% in the general population (11). In these areas, endemic KS cases have been reported for nearly a century, and such a tumor can account for 1 to 10% of all the diagnosed cancers. Since the dramatic expansion of the HIV-1 epidemic in Africa, KS is today one of the most frequent types of cancer in several African countries, indeed, representing in some regions, nearly 20 to 40% of all the reported cancers (9, 19).

Concerning the African continent, most of the studies on HHV-8 viral markers in KS patients have been performed in East and South African areas, especially in Zimbabwe (6–8), Uganda (21), and South African Republic (25, 26). Very few data on such viral markers in KS patients from West (1) and Central African countries exist.

Thus, the goals of our study were (i) to evaluate different HHV-8 viral markers in AIDS-KS patients from Central African Republic, a country in which both HHV-8 (16) and HIV-1 infections are highly endemic (18) (we focused especially on titers of HHV-8 antibody directed against lytic and latent antigens and on HHV-8 viral load in three different localizations [PBMCs, unaffected skin, and tumoral KS lesions]) and (ii) to search for any correlation between these viral markers themselves but also with demographic data, such as age and gender.

Thirty-six AIDS patients with histologically proven cutaneous KS were studied (Table 1). All patients were seen in the departments of dermatology and internal medicine of hospitals in Bangui, the capital city of Central African Republic, in 1998. Any inpatients or outpatients presenting with AIDS-KS were asked to participate in this virological study. After giving informed consent, those who agreed were enrolled. None of the 36 patients had received highly active antiretroviral therapy (HAART). For each patient, biological material for the KS diagnosis included a plasma sample and PBMCs obtained after Ficoll separation. Furthermore, in most cases, a biopsy of clinically unaffected skin, as well as a biopsy of a tumoral skin lesion, was performed after informed consent. All samples were kept frozen at −80°C. All the patients were African, of Central African Republic origin, particularly from Bangui or its surrounding neighborhood. There were 15 males and 21 females, with a mean age of 31.5 years (range, 8 to 50 years) (Table 1).

Concerning HHV-8 serology, two different immunofluorescence assays were used, as previously described (15, 24): (i) a commercially available test (HHV-8 immunofluorescence an-

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TABLE 1. Demographic data, HHV-8 serological markers, and viral loads for 36 AIDS-associated Kaposi's sarcoma patients from Central African Republic[a]

Patient (code no.)	Age (yrs)	Sex	Titer of antibody against indicated type of antigen in blood (by immunofluorescence antibody assay)		HHV-8 viral load (log copies/μg DNA) in indicated specimen by TaqMan PCR		
			Lytic	Latent	Blood	Unaffected skin	Tumoral skin
RCA 01	48	F	80	80	1.87	<SS	NA
RCA 02	32	F	320	<20	<SS	4.53	6.19
RCA 03	32	F	320	<20	2.71	3.32	NA
RCA 04	8	F	<20	<20	3.53	3.09	NA
RCA 05	30	F	320	<20	NA	<SS	NA
RCA 06	24	M	10,240	<20	4.36	<SS	NA
RCA 07	8	M	80	160	<SS	1.42	NA
RCA 08	27	F	640	<20	2.11	4.26	NA
RCA 09	34	M	160	40	4.77	3.92	6.27
RCA 10	46	M	1,280	160	3.58	4.04	NA
RCA 11	38	F	640	320	4.21	NA	6.35
RCA 12	28	M	160	20	2.39	NA	6.46
RCA 13	22	F	10,240	2,560	3.88	NA	5.29
RCA 14	28	F	1,280	160	<SS	2.69	6.07
RCA 15	46	M	5,120	1,280	1.48	3.04	NA
RCA 16	30	F	160	20	4.47	3.55	NA
RCA 17	30	F	2,560	<20	4.36	3.54	6.27
RCA 18	30	F	640	320	4.66	3.29	6.17
RCA 19	35	M	640	160	<SS	2.04	NA
RCA 20	50	M	320	40	3.29	4.38	NA
RCA 21	28	F	640	1,280	3.54	2.79	6.14
RCA 22	49	M	320	40	3.81	NA	6.04
RCA 23	35	M	320	<20	1.63	5.01	6.53
RCA 24	32	F	80	40	2.53	NA	NA
RCA 25	40	M	160	80	<SS	4.93	6.65
RCA 26	33	M	640	20	1.98	3.8	NA
RCA 27	21	F	640	1,280	3.35	2.44	5.88
RCA 28	28	F	320	20	3.85	3.8	6.56
RCA 29	32	F	640	320	2.19	2.17	NA
RCA 30	25	M	10,240	40	3.14	<SS	6.38
RCA 31	26	M	NA	NA	NA	2.72	5.67
RCA 32	30	F	<20	20	3.04	2.68	NA
RCA 33	37	F	NA	NA	NA	2.85	5.18
RCA 34	33	F	40	<20	1.05	3.87	5.59
RCA 35	13	M	1,280	640	<SS	3.98	5.93
RCA 36	45	F	320	<20	2.31	3.55	5.83

[a] F, female; M, male; NA, not available; <20, not detectable at 1/20 dilution; <SS, inferior to the detection threshold that is close to 1 copy in 50,000 cells for this TaqMan assay.

tibody assay; ABI, Columbia, Maryland), using the KS-1 cell line as a HHV-8 source of antigens and detecting antibodies directed mainly against lytic HHV-8 antigens and (ii) a test using BC-3 cells which express only HHV-8 latent antigens (LANA) (15). For both tests, anti-HHV-8 antibody titers were determined through successive twofold dilutions, beginning at 1/20.

Specific antibodies directed against HHV-8 lytic antigens were detected in 32 out of 34 (94%) plasma samples (Table 1). The detection of anti-LANA antibodies was less frequent, being present only among 24 out of the 34 (71%) plasma samples tested (Table 1). The geometric mean of the antibody titers directed against HHV-8 lytic antigens was of 1/346 (95% confidence interval [CI], 1/184 to 1/720). It was of 1/30 (95% CI, 1/13 to 1/71) for the anti-LANA titers. There was a positive significant correlation between the levels of antibody titers directed against lytic antigens and those directed against LANA ($P = 0.03$; coefficient correlation, 0.37). For both lytic and latent titers, there was no difference either according to gender (P values were 0.34 and 0.46) or according to age (P values were 0.28 and 0.54).

High-molecular-weight DNA was obtained, after classical extraction methods, from the 33 PBMCs and the 51 frozen cutaneous biopsy specimens (31 unaffected skin specimens and 20 tumor lesions) (Table 1) as previously described (16). The 84 DNA samples were first amplified by the human β-globin gene-specific primer pairs, demonstrating in all cases their integrity. A real-time (TaqMan) quantitative PCR on a conserved region of ORF26 was then performed, as previously described (22). The geometric mean of the HHV-8 viral load was 2.55 log copies/μg of DNA (95% CI, 2.02 to 3.08 log copies/μg of DNA) in the PBMCs, 2.93 log copies/μg of DNA (95% CI, 2.41 to 3.44 log copies/μg of DNA) in the unaffected skin, and 6.07 log copies/μg of DNA (95% CI, 5.18 to 6.65 log copies/μg of DNA) in the tumoral KS lesions (Fig. 1). Whereas there was no significant difference between the level of the

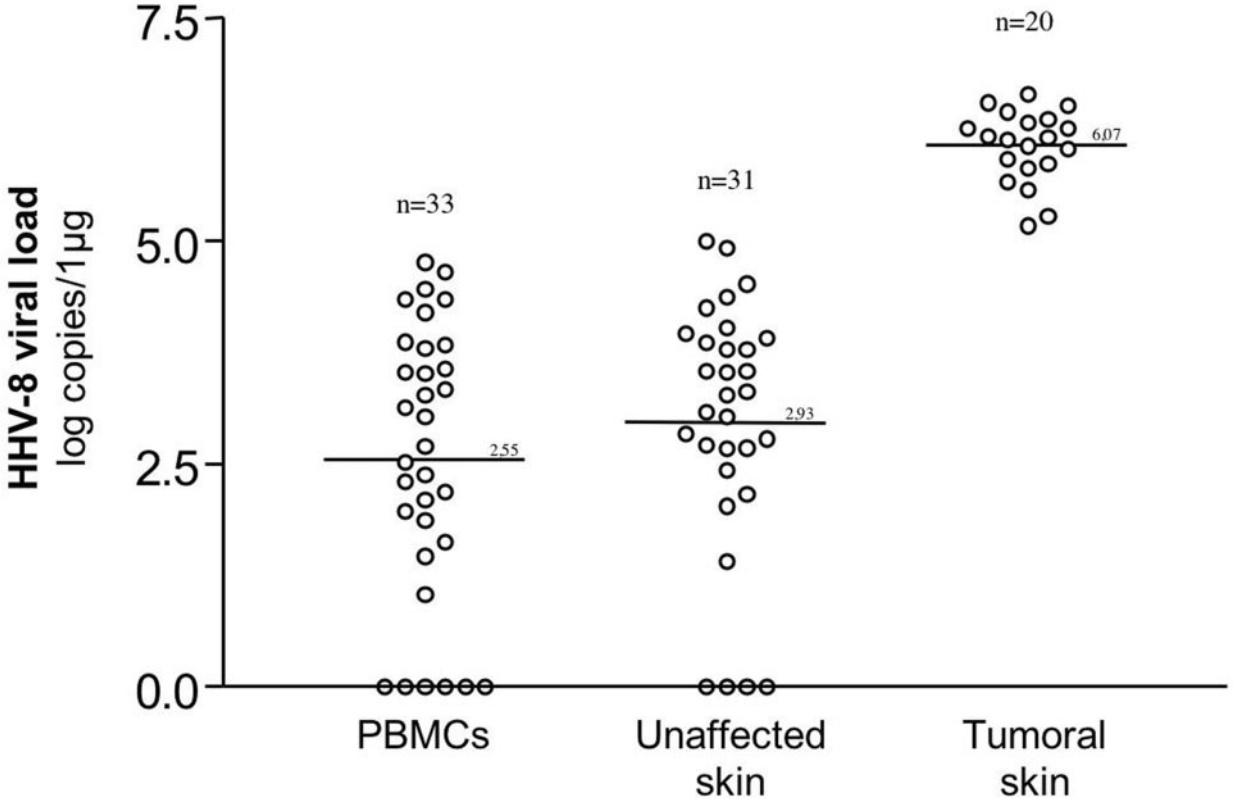

FIG. 1. Comparison of the HHV-8 viral loads in PBMCs ($n = 33$) and unaffected skin ($n = 31$) and tumoral skin ($n = 20$) samples from patients with AIDS-associated Kaposi's sarcoma from Central African Republic. The viral load quantification was performed using a real-time (TaqMan) quantitative PCR on a conserved region of ORF26 (22).

viral load in the PBMCs and that in the unaffected skin ($P = 0.32$), the viral load present in the tumoral lesions was much higher ($P = 10^{-4}$). Furthermore, there was no significant difference according to gender or age for the viral loads in the PBMCs, unaffected skin, and tumoral skin. Lastly, there was no significant correlation between any of the three viral loads (PBMCs, unaffected skin, and tumoral skin) or between the antibody titers (either latent or lytic) and any of the viral loads.

This study presents the first evaluation of HHV-8 serological and molecular markers in AIDS-KS patients from Central African Republic, a country in which both HHV-8 (16) and HIV-1 (18) infections are highly endemic. Our data extend thus to patients from Central African areas some features which have been reported recently in East and South African AIDS-KS patients. Indeed, we confirmed the high frequency of detectable virus in PBMCs of African patients with AIDS-KS with a quantitative PCR (7). Furthermore, the range of the number of copies of HHV-8 DNA in the PBMCs among our patients is quite similar to those reported previously for persons with AIDS-KS in Zimbabwe (7) and seems higher than in few reported series from European and United States patients (2, 5, 12). We also confirmed that in AIDS-KS patients, the levels of antibodies directed either to latent antigens or to lytic antigens were not correlated to the amount of cell-associated HHV-8 present in the PBMCs (6). Interestingly, other studies have reported, in contrast, the existence of a correlation between lytic and/or latent antibody titers and HHV-8 viral load; however, these results are difficult to compare, as some of these studies were performed either with healthy individuals or with HIV-infected individuals without KS (10). Moreover, some studies on viral transmission were focused on the HHV-8 load in saliva (10) and not in the PBMCs. We have, however, recently reported an absence of correlation of antibody level and buffy coat HHV-8 load in healthy HHV-8-infected individuals from Cameroon (13, 23).

In this study, we also provide new original data on the quantification of HHV-8 load in the tumoral skin lesions of KS patients. Indeed, very few papers have been published on this topic. A first study used a quantitative competitive PCR on a small series of biopsy specimens from seven AIDS-KS patients (3). However, these patients were treated by HAART, which is known to modify greatly such viral parameters (5). A second study was performed on 12 paraffin-embedded biopsy specimens from a group of United States patients, including both epidemic and classic KS patients (20). Their results indicated a high viral copy number that had, however, a very large range in relation to tumor burden, dissemination of the lesions, and HAART efficacy. Our study, which is the first one using quantification by real-time PCR indicated, as expected, a much higher viral load in tumoral skin lesions compared to unaffected normal skin and PBMCs. The quite similar levels of HHV-8 load measured in the PBMCs and in the unaffected skin biopsy DNA reflect very probably the presence of infected PBMCs in vessels of the skin. In contrast, the very high level of

HHV-8 load found in the lesions is the result of an active, specific recruitment and proliferation of spindle cells latently infected with HHV-8 in this viral driven tumor. Furthermore, the geometric mean of the HHV-8 viral load in the tumor lesion was of 6.07 log copies/μg (range, 5.18 to 6.65 log copies/μg), corresponding to around 7 copies by cell (range, 0.9 to 27 log copies). This is much less than the number obtained with PEL cells (10 to 50 copies by cell) by using comparable (17) or identical techniques (Duprez et al., unpublished data). Besides possible specific features of such tumors, such a difference in viral load may be due in part to the fact that while KS lesions are generally constituted by several cell types (including inflammatory mononuclear cells and endothelial cells, etc.), some of them not being HHV-8 infected, especially during the early steps of the lesion, PEL cells constitute a clonal homogenous, unique, population of pure HHV-8-infected cells.

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