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Detection of herpesvirus-like DNA by nested PCR on archival skin biopsy specimens of various forms of Kaposi sarcoma

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Abstract

***Aims*—To detect herpesvirus-like DNA sequences, defining a new herpesvirus, human herpesvirus 8 (HHV8), in paraffin wax embedded skin biopsy specimens of the various forms of Kaposi sarcoma.**

***Methods*—DNA was extracted from archival skin biopsy specimens of Kaposi sarcoma, other mesenchymal skin tumours and various inflammatory skin lesions of HIV seropositive and negative patients. HHV8 DNA was detected by using a nested PCR assay. Human β-globin DNA served as an internal control.**

***Results*—Twenty two samples of Kaposi sarcoma were analysed, comprising 12 of the endemic type, nine HIV associated and one transplantation related. HHV8 DNA was detected by nested PCR in all forms of Kaposi sarcoma. By contrast, no HHV8 DNA was detected in five mesenchymal skin tumours or nine biopsy specimens of unspecific inflammatory skin lesions of HIV seropositive and negative patients.**

***Conclusions*—Detection of HHV8 DNA in paraffin wax embedded tissue can be used to confirm a diagnosis of Kaposi sarcoma.**

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Keywords: Kaposi sarcoma, herpesvirus-like DNA, PCR, archival tissue.

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Since the first description of Kaposi sarcoma in 1872, several different clinical forms of this tumour have been described. The classic or endemic form of Kaposi sarcoma was originally found in older men of Eastern European or Mediterranean origin, usually showing an indolent clinical course. The recently described forms, however, have a more aggressive clinical course and are strongly associated with prolonged immunosuppression, as seen in solid organ tranplant recipients[1] and especially in patients infected with HIV.[2] The histological features of the these various forms are essentially the same.[3] In the early (or patchy) stage, the Kaposi sarcoma consists of jagged thin walled and dilated vascular spaces with interstitial inflammatory cells and extravasated red blood cells. In the more characteristic plaque or nodular stage, the tumour is composed of plump spindle cells with irregular slit-like vascular spaces lined by recognisable endothelium and filled with erythrocytes.

Among patients with AIDS, homosexual men are significantly more likely to develop Kaposi sarcoma: in the study by DesJarlais *et al*[4] 47% of newly diagnosed cases of Kaposi sarcoma were homosexual men compared with 3.9% of intravenous drug users. For unknown reasons, the incidence of this tumour has been reported to be declining in all patient groups.[5] Epidemiological data suggest that Kaposi sarcoma is an infectious, probably sexually transmitted viral disease[6 7] and a variety of viruses have been implicated, including cytomegalovirus and HIV.[8 9]

Recently, herpesvirus-like DNA sequences, defining a new herpes virus, Kaposi sarcoma associated herpes virus (KSHV) or human herpes virus 8 (HHV8), have been detected and characterised in patients with Kaposi sarcoma with and without AIDS.[10-14] Here, we describe the successful detection of HHV8 DNA in formalin fixed, paraffin wax embedded skin biopsy specimens of Kaposi sarcoma but not in other mesenchymal tumours or inflammatory lesions of the skin.

Methods

Skin biopsy specimens were retrieved from the files of the Departments of Pathology and Dermatology of the Cantonal Hospital, Basel, Switzerland, and the Department of Dermatopathology of the University "Federico II" of Naples, Italy. All biopsy specimens had originally been taken for diagnostic purposes. The tissue samples were fixed in 4% buffered formalin and paraffin wax sections were prepared and stained with haematoxylin and eosin. All patients with Kaposi sarcoma except one were tested for the presence of anti-HIV antibodies and if a negative result was obtained, the testing was repeated. One male patient with Kaposi sarcoma refused to be tested for HIV. However, this patient belonged to a typical risk group and showed clinical evidence of HIV infection and was therefore regarded as HIV positive. One tumour sample was from a male patient who developed Kaposi sarcoma two years after undergoing a renal transplant and was repeatedly negative for HIV antibodies.

Four skin biopsy specimens of HIV positive patients without clinical evidence of Kaposi sarcoma and 10 biopsy specimens of mesenchymal skin tumours and non-specific inflammatory lesions of the skin of patients without evidence of HIV infection served as controls

Table 1 Clinical and histological data of patients without Kaposi sarcoma

Histology	*n*	*Sex*	*Age (years)*	*HIV status*
Hemangiosarcoma	1	F	80	Negative
Angiokeratoma	1	F	71	Negative
Solitary glomus tumour	1	M	37	Negative
Capillary hemangioma	1	F	50	Negative
Histiocytoma	1	F	35	Negative
Granuloma pyogenicum	2	F, F	34, 37	Negative
Non-specific dermatitits	3	F, F, M	46, 47, 81	Negative
Eosinophilic folliculitis	1	M	46	Positive
Leucocytoclastic vasculitis	1	M	40	Positive
Non-specific dermatitits	2	M, M	47, 67	Positive

Table 2 Clinical and histological data of patients with Kaposi sarcoma (KS)

Disease	*n*	*Age (mean)*	*Sex (M:F)*	*HIV status*	*Histology (patch:plaque)*	*Detection of HHV8 DNA (%)*
HIV associated KS	9	40.2	9:0	Positive	6:3	100
Classic KS	12	60.3	12:0	Negative	0:12	100
Transplant associated KS	1	43	1:0	Negative	1:0	100
Other skin lesions	4	50.0	4:0	Positive	–	0
	10	53.2	3:7	Negative	–	0

(table 1). These patients were not tested for the presence of HIV antibodies but none had epidemiological or clinical evidence of HIV infection.

DNA EXTRACTION AND PCR

Two to four 5 μm sections were cut from the paraffin wax blocks under stringent conditions to avoid cross-contamination. Sections were deparaffinised by xylene, the xylene removed by ethanol, dried in a lyophiliser and the pellet digested over night at 37°C in a buffer containing proteinase K (200 μg/ml proteinase K, 50 mmol Tris-HCl (pH 8.0), 0.5% Tween 20, 1 mmol EDTA) followed by extraction in phenol/chloroform and ethanol precipitation. The pellet was finally dissolved in water.

Five microliters of DNA were used in each PCR reaction. The integrity of the extracted DNA was confirmed by amplifiying the human β-globin gene. A single step PCR assay was used to detect HHV8 DNA, as described previously.[10] To improve sensitivity, a nested PCR was performed using two outer primers (KS3: 3'-ACAGCAACACCCAGCTAGCA and KS4: 5'-AGATCGTCAAGCACTCGCAG) for the first round and the previously published primers KS330/233 for the second round.[10] PCR conditions were as follows: denaturation at 94°C for one minute, reannealing at 55°C for 45 seconds, extension at 72°C for 45 seconds, for 30 cycles. All PCR products were electrophoresed on a 2% agarose gel and visualised by staining with ethidium bromide. Stringent laboratory conditions and appropriate negative controls were used to avoid cross-contamination and false positve results.

Results

A total of 22 skin biopsy specimens of Kaposi sarcoma was analysed. Nine had been taken from patients with HIV infection, 12 from patients repeatedly negative for anti-HIV antibodies and one from a kidney transplant recipient (table 2). The plaque form of Kaposi sarcoma was found in all HIV negative patients on histological examination. In the HIV infected patients, six of nine biopsy specimens showed the patchy form of the tumour. This form was also seen in the patient with transplantation associated Kaposi sarcoma. Early lesions, characterised histologically by proliferation of minute vessels surrounding larger ectatic vessels, were found in three of 10 biopsy specimens with patchy Kaposi sarcoma.[3] The mean age of patients infected with HIV was significatly lower than that of those with classic Kaposi sarcoma (40.2 *v* 60.3 years; Student's t-test, $p < 0.001$). All patients with Kaposi sarcoma were male.

The one step PCR, performed as described previously,[10] produced a weak and inconsistent PCR product, and we therefore introduced a two step amplification procedure (fig 1). Using this nested PCR assay, HHV8 DNA was detected in all skin biopsy specimens with Kaposi sarcoma. No HHV8 DNA was found in the four biopsy specimens of HIV positive patients without clinical evidence of Kaposi sarcoma. HHV8 DNA was not detected in the 10 biopsy specimens of HIV negative patients with various mesenchymal skin tumours or unspecific inflammatory skin lesions (table 2). These biopsy specimens all gave a positive result for human β-globin, confirming adequate preservation and extraction of the DNA tested. Furthermore, amplification did not occur when cytomegalovirus infected fibroblasts or Epstein-Barr virus transformed lymphoid cell lines were used as a DNA template (data not shown).

Discussion

HHV8 DNA sequences, related to the γ-herpesviruses, Epstein-Barr virus and herpesvirus saimiri,[10] have been dedected in 90.9–

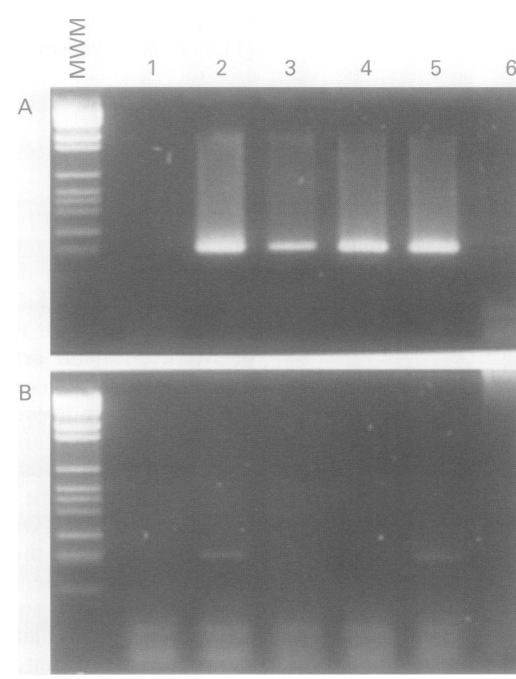

Figure 1 Detection of HHV8 DNA by PCR. Ethidium bromide stained agarose gels showing products of (A) nested and (B) single step PCR. Lane 1, molecular weight markers; lanes 2 and 3, classic Kaposi sarcoma; lanes 4 and 5, HIV associated Kaposi sarcoma; lane 6, angiosarcoma of the skin.

100% of HIV associated and in 87–100% of classic Kaposi sarcomas.[10 13 15] These values were obtained mainly from unfixed, native tissue. We were able to improve the sensitivity and to extend the applicability to formalin fixed material by introducing a nested PCR assay. Using adequate controls, the presence of HHV8 DNA was restricted to all cases of Kaposi sarcoma, irrespective of aetiology, including the classic, the HIV associated and the transplant related variants. HHV8 DNA was also detected in early lesions of Kaposi sarcoma. HHV8 DNA was not detected in other mesenchymal skin tumours nor in non-specific inflammatory skin lesions, even in the presence of active HIV infection.

Our findings supply further evidence of a possible aetiological role for HHV8 in the development of Kaposi sarcoma in conjunction with immunosuppression. Previous studies found HHV8 DNA in 36.3–89% of skin biopsy specimens adjacent to primary Kaposi sarcoma lesions.[12 14 15] Whether this is indicative of diffuse spread of the virus or whether this is a presarcomatous lesion has yet to be determined. At least for patients with AIDS, the presence of HHV8 DNA without clinical evidence of overt Kaposis's sarcoma may identify those at risk of developing Kaposi sarcoma at a later stage of their disease.[16]

In conclusion, our data show that HHV8 DNA can be detected by nested PCR in all biopsy specimens of Kaposi sarcoma but not in other mesenchymal tumours or non-specific inflammatory skin lesions. Given that the early vascular lesions of Kaposi sarcoma may be subtle and difficult to diagnose, the detection of HHV8 DNA by PCR will become a important tool to confirm the diagnosis of Kaposi sarcoma.

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