

Herpes Virus-like DNA (HHV-8) in Immunosuppressive Therapy-related, HIV-related and Classical Kaposi's Sarcoma in Norwegian Patients

PETTER JENSEN¹, YAO QI HUANG², JIAN JUN LI², OLE PETTER F. CLAUSEN³ and ALVIN E. FRIEDMAN-KIEN²

¹Department of Dermatology, Rikshospitalet, The National Hospital, University of Oslo, Oslo, Norway, ²Department of Microbiology, New York University Medical Center, New York, U.S.A. and ³Department of Pathology, Rikshospitalet, The National Hospital, University of Oslo, Oslo, Norway

The recently discovered human herpes virus 8 (Kaposi's sarcoma-associated herpes virus) has been implicated in the pathogenesis of Kaposi's sarcoma. Using polymerase chain reaction we detected DNA sequences of this herpes virus in 11 of 14 biopsy specimens from Kaposi's sarcoma in Norwegian patients, including the immunosuppressive therapy-related type (3 of 3), the HIV-related type (4 of 5), and the classical type (4 of 6). The results support the hypothesis of a role for human herpes virus 8 in all types of Kaposi's sarcoma independent of geographical area. Key words: Kaposi's sarcoma-associated herpes virus (KSHV); immunosuppression; organ transplantation; rheumatoid arthritis.

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Petter Jensen, Department of Dermatology, Rikshospitalet, University of Oslo, N-0027 Oslo, Norway.

Kaposi's sarcoma (KS) is a relatively rare form of neoplasm, mostly found in the skin (1). The classical form of KS is seen most often in older men, usually having an indolent course, and is more common in certain ethnic groups (i.e. Mediterranean, Arabic, Jewish). An endemic form of KS is seen in subtropical Africa. The more recently described forms of KS have a more aggressive course and are strongly associated with long-term immunosuppression, as seen in patients infected with HIV (2) and in organ transplant recipients (3, 4).

Epidemiological data, such as the clustering of KS in well-defined populations (5) and the occasional occurrence of KS in HIV-negative homosexual men (6), suggest an infectious agent other than HIV as the cause of KS in patients with AIDS (2).

Recently, herpes virus-like DNA sequences have been detected and characterized in KS lesions from patients with and without AIDS, defining a new herpes virus called human herpes virus 8 (HHV-8) or Kaposi's sarcoma-associated herpes virus (KSHV) (7–10). The investigations have mainly been done on fresh tissue material, but detection of HHV-8 DNA has also been done in formalin-fixed and paraffin-embedded skin biopsy specimens (11).

We wanted to determine whether HHV-8 could be detected in KS lesions found in organ transplant recipients and other patients on immunosuppressive therapy, as well as in classical KS, in Norway, where the prevalence of KS is very low.

MATERIAL AND METHODS

Skin biopsy specimens were retrieved from the archives of the Departments of Pathology at Rikshospitalet and Ullevål Hospital, Oslo,

Norway. All biopsies had originally been taken for diagnostic purposes between 1988 and 1994, and had been fixed in 4% buffered formalin, embedded in paraffin and stained with haematoxylin and eosin.

Tumour specimens were obtained from patients with various forms of KS. The diagnosis of KS were confirmed by an experienced pathologist. All patients with KS had been tested for the presence of anti-HIV antibodies. Five biopsy specimens from haemangiomas of the skin were used as controls. The serological status of these patients was unknown.

Between 10 and 20 sections (5 µm) were cut from each paraffin block under stringent conditions to prevent cross-contamination. The sections were treated with xylene and DNA extracted as previously described by our group (8). Polymerase chain reaction (PCR) was performed in a volume of 50 µl containing 10 pmol of each primer specific for HHV-8 (8), 2.5 units of Taq DNA polymerase, 50 µl of each dNTP, 25 mM KCl, 1.5 mM MgCl₂ and 20 mM Tris-HCl (pH 8.3). After 35 cycles of denaturation (94°C, 1 min), annealing (55°C, 1 min) and extension (72°C, 1 min), 20 µl of each PCR product containing 233 base pairs (KS330₂₃₃) was electrophoresed on a 1.5% agarose gel and stained with ethidium bromide. The gel was blotted on a membrane, followed by hybridization with a ³²P labelled probe specific for HHV-8 (8). Positive signals were observed after membrane exposure to Kodak film (Fig. 1).

DNA was amplified from a total of 14 skin biopsy specimens of KS (Table I). Three skin biopsy specimens were from patients on long-term immunosuppressive therapy, including one 68-year-old male patient with a renal transplant and two patients with severe rheumatoid arthritis, one 65-year-old female and one 75-year-old male. Five specimens were from patients with AIDS (all male between the ages of 29 and 54 years), and six from patients with classical KS (three female, three male, between 79 and 96 years). The duration of immunosuppressive therapy or of AIDS was unknown.

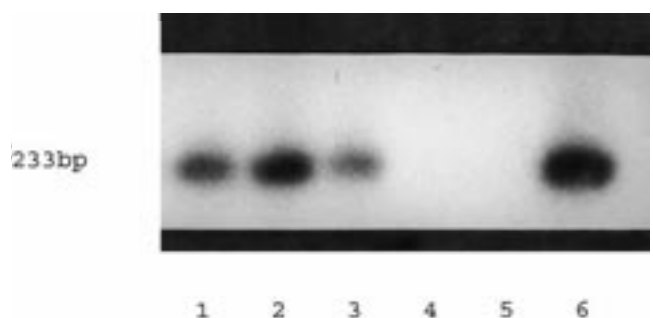


Fig. 1. Detection of human herpes virus type 8 (HHV-8) DNA sequences (KS330₂₃₃) by polymerase chain reaction, showing representative results of hybridization analyses in Kaposi's sarcoma and control samples. Lanes 1–3: Positive bands from three different KS samples. Lane 4: Negative results from a sample. Lane 5: Negative control. Lane 6: Positive control.

Table I. Detection of human herpes virus type 8 DNA sequences (HHV-8) in specimens of various types of Kaposi's sarcoma.

Type of lesion	No.	HIV status	HHV-8
Kaposi's sarcoma	14		11/14
Immunosuppressive therapy-related	3	Negative	3/3
AIDS-related	5	Positive	4/5
Classical	6	Negative	4/6
Haemangiomas	5	Unknown	1/5

RESULTS

HHV-8 DNA was detected in 11 of the 14 skin biopsy specimens with KS, including immunosuppressive therapy-related type (3 of 3), HIV-related type (4 of 5), and classical type (4 of 6) (Table I). Of five skin biopsy specimens from haemangiomas, used as controls, HHV-8 was detected in one.

DISCUSSION

This study shows that HHV-8 is present not only in HIV-related KS and in classical KS, but also in KS tissue from iatrogenically immunocompromised patients, such as renal transplant recipients and patients with rheumatoid arthritis. HHV-8 being previously detected in KS of transplant recipients in the United Kingdom (10) and in South Africa (12), this is to our knowledge the first report on HHV-8 in KS of immunosuppressed rheumatoid arthritis patients.

In transplant recipients, KS lesions have been reported to regress when the immunosuppressive therapy is discontinued or lowered (3). Specimens taken from normal skin lack HHV-8, except for some of those taken from HIV-positive patients (7, 8). HHV-8 is generally not found in skin tumours other than KS (13).

We have no certain explanation for the presence of HHV-8 in one haemangioma. The serological status of this patient is unknown and unavailable. It is possible that the lesion was excised to rule out the diagnosis of KS in a HIV-positive patient, as normal skin may contain HHV-8 in HIV-infected patients (7, 8), although contamination cannot be ruled out.

This study of KS in patients from a geographical area with low prevalence of KS lends support to the hypothesis that HHV-8 play a role in the etiology and/or pathogenesis of the different subtypes of KS, including those in patients on systemic immunosuppressive therapy.

Although KS is rarely seen even in solid organ transplant recipients (4), epidermal skin cancers are not. Transplant recipients are at a highly increased risk of developing cutaneous squamous cell carcinomas and basal cell carcinomas (14). Except for long-term immunosuppression and sun exposure,

no other cause for these skin cancers has been established. Efforts to implicate a role for virus (i.e. human papilloma virus) have so far been unsuccessful. Research on HHV-8 and KS, including the present study, indicates that certain viruses under immunosuppressive conditions can be activated and play a causative role in the development of cancer, such as KS.

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