

Non-AIDS-related Kaposi Sarcoma Tissues do not Contain DNA Sequences of HHV-6, HHV-7, Epstein-Barr Virus, Cytomegalovirus and HSV

Sir,

The possible role of human herpesvirus (HHV)-8 has monopolized the scientific debate on the etiopathogenesis of Kaposi sarcoma (KS) in recent months (1). Studies on other HHVs, are very few and inconclusive. We analysed DNA in tissue samples from patients with histologically proven KS for the presence of HHV-6, HHV-7, Epstein-Barr virus (EBV), cytomegalovirus (CMV) and HSV DNA sequences.

The study involved 23 patients (49 to 84 years of age, 17 men and 6 women) with Mediterranean KS and 3 renal transplant male patients (56 to 64 years of age) who had developed KS after being treated with prednisone and cyclosporine. All were negative for HIV antibodies by ELISA and Western blot.

The DNA was extracted from fresh tissues or from sections of formalin-fixed paraffin-embedded skin specimens collected between 1983 and 1997. The samples were tested for the presence of HHV-6, HHV-7, EBV, CMV and HSV sequences by polymerase chain reaction (PCR) with specific primers (2–4).

DNA was obtained from skin by proteinase K sodium-dodecyl-sulphate digestion followed by phenol-chloroform extraction and ethanol precipitation of nucleic acids. The HHV-6 sequences of the outer primer were 5'-CCGCAATCGAATC-CACCTAGCGG-3' and 5'-GTGAGAACGGATTCGAA-CAGTGCTG-3'. Inner primers were 5'-CCCATTTACGATT-TCTGCACCACCTCTCTGC-3' and 5'-TTCAGGGAC-CGTTATGTCATTGAGCATGTGC-3'. The thermocycling procedure (Thermal Cycler 9600 Perkin-Elmer Cetus, Norwalk, CT) consisted of initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 2 min. The same cycles were used for the bath inner primer sets. HHV-6 positive control DNA was obtained by standard phenol extraction of HHV-6 strain GS-infected HSB-2 cells. Negative control DNA was extracted from uninfected HSB-2 cells.

The HHV-7 sequences of the outer primer were 5'-AGTTC-CAGCACTGCAATCG-3' and 5'-CACAAAAGCTCGC-TATCAA-3'. The inner primers were 5'-CGCATAC-ACCAACCCTACTG-3' and 5'-GACTCATTATGGGGATC-GAC-3'. The HHV-7 DNA thermal condition included initial denaturation at 94°C for 2 min, denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec for 30 cycles, and final extension at 72°C for 2 min. The same cycles were used for the bath inner primer sets. HHV-7 positive control DNA was obtained by extraction of HHV-7 strain-infected Sup-T1 cells. Negative control DNA was extracted from non-infected Sup-T1 cells.

Nested PCR products of HHV-6 and HHV-7 were visualized by electrophoresis on 2% agarose gels (in TBE buffer and containing 1 µg of ethidium bromide). From sequence analyses, the sizes of the products after nested PCR were expected to be 186 pb for HHV-6 and 264 pb for HHV-7. PCR conditions for the detection of HSV, CMV and EBV were as previously described (2). Sequences of HHV-7, HHV-6, HSV, EBV and CMV DNA were not detected in patients' lesional skin.

In KS, the ultimate evidence of a causal role for HHV-8 is still lacking. Among many aspects of HHV-8 biological properties still to be clarified are its serological prevalence in the general population, in which of the host cells the virus may remain latent and those in which it may multiply, and the factors disturbing the virus–host balance.

Other HHVs, therefore, may be important in KS. For example, CMV, EBV, HHV-6 and HHV-7 alone or in association may act as co-factors and transactivators. Only a handful of studies have verified such a possibility. CMV, HHV-6 and HHV-7 have been detected in skin specimens from AIDS-associated KS (5), though their prevalence was similar or lower than in normal skin of AIDS patients without KS. In 7 of 22 classic KS, EBV has been found in the lesional skin and in 4 of them it was associated with HHV-8 (6). Lastly, such an association has also been found in a single patient with iatrogenic KS (7).

In our study, which is probably the largest one on non-AIDS-associated KS for HHVs other than HHV-8, we were unable to confirm that HSV, CMV, EBV, HHV-6 and HHV-7 play an aetiological role.

REFERENCES

1. Cohen J. Controversy: Is KS really caused by new herpesvirus? *Science* 1995; 268: 1847–1848.
2. Mitchell SM, Fox JD, Tedder RS, Gazzard BG, Lightman S. Vitreous fluid sampling and viral genome detection for the diagnosis of viral retinitis in patients with AIDS. *J Med Virol* 1994; 43: 336–340.
3. Klotman ME, Lusso P, Bacchus D, Corbellino M, Jarrett RF, Berneman ZN. Detection of human herpesvirus 6 and human herpesvirus 7 by PCR amplification. In: Persing DH, Smith TF, Tenover FC, White T, eds. *Diagnostic molecular microbiology. Principles and applications*. Washington, Am Soc Microbiol 1995: 501–510.
4. Okuno T, Oishi H, Hayashi K, Nonogaki M, Tanaka K, Yamanishi K. Human herpesviruses 6 and 7 in cervixes of pregnant women. *J Clin Microbiol* 1995; 33: 1968–1970.
5. Kempf W, Adams V, Hassam S, Schmid M, Moos R, Briner J, et al. Detection of human herpesvirus type 6, human herpesvirus type 7, cytomegalovirus and human papillomavirus in cutaneous AIDS-associated Kaposi's sarcoma. *Verh Dtsch Ges Pathol* 1994; 78: 260–264.
6. Luppi M, Barozzi P, Maiorana A, Collina G, Ferrari MG, Marasca R, et al. Frequency and distribution of herpesvirus-like DNA sequences (KSHV) in different stages of classic Kaposi's sarcoma and in normal tissues from an Italian population. *Int J Cancer* 1996; 66: 427–431.
7. Henghold II WB, Purvis SF, Schaffer J, Leonard DGB, Giam CZ, Wood GS. Kaposi sarcoma-associated herpesvirus/human herpesvirus type 8 and Epstein-Barr virus in iatrogenic Kaposi sarcoma. *Arch* 1997; 133: 109–110.

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