

CASE REPORT

HHV8 associated Kaposi's sarcoma during triple immunosuppressive treatment with cyclosporin A, azathioprine, and prednisolone for ocular Behçet's disease and complete remission of both disorders with interferon α

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Behçet's disease is a multisystem vasculitis with oral and genital aphthous ulcers, cutaneous vasculitis, uveitis, and arthritis as its main features.¹ Ocular manifestations, especially panuveitis, have a poor prognosis, resulting in blindness in most patients after five years, irrespective of treatment. Recent reports have shown significant improvement of visual prognosis by early and aggressive immunosuppressive treatment.² However, immunosuppressive treatment bears a risk of opportunistic infections, especially herpesvirus reactivation and increased incidence of malignancies.³

Kaposi's sarcoma is a rare malignant tumour (incidence 0.01%) of endothelia and vascular smooth muscle cells which has a markedly increased incidence in patients with AIDS (0.9%)⁴ and iatrogenic immunosuppression (0.52%).⁵ Recently, human herpesvirus 8 (HHV8) was identified as the causative agent.

We describe a patient with severe ocular Behçet's disease who developed disseminated, HHV8 positive Kaposi's sarcoma during triple immunosuppressive treatment with prednisolone, cyclosporin A, and azathioprine.

Case report

In 1990 HLA-B51 positive Behçet's disease was diagnosed in a 29 year old man of Turkish origin. He had a history of severe painful oral aphthosis. Panuveitis of both eyes with hypopyonitis was first diagnosed in June 1989. In July 1990 he was doing well while receiving 50 mg prednisolone daily. Clinical examination showed no signs of Behçet's disease, except ocular inflammation. No serological evidence of infection with HIV, Epstein-Barr virus (EBV), herpes simplex virus (HSV), and human cytomegalovirus (HCMV) was found. Cyclosporin A (5 mg/kg body weight orally daily) was added to prednisolone owing to occlusive retinal vasculitis. Because of relapses of bilateral uveitis with further loss of vision azathioprine (150 mg orally daily) was added in November 1992. This resulted in complete remission of ocular inflammation. In December 1993 the patient complained of epigastric

pain, had lost 10 kg in weight, and was anaemic. Gastroscopy disclosed a patchy reddish induration of the gastric corpus and duodenal mucosa. The histological picture of angiomatosis, present in biopsy specimens from both locations, suggested Kaposi's sarcoma. In February 1994 multiple tumours emerged on the patient's skin and hard palate (fig 1A). Histologically, Kaposi's sarcoma was diagnosed. A chest x ray showed multiple bilateral nodular infiltrates and lymphangiomatosis (fig 1B). The CD4 cell count was 400/ μ l (CD4/CD8 ratio 1.0). Virological tests were still negative for HIV 1/2, EBV, and HSV, but IgM specific for CMV was positive, and CMV was cultured from urine and saliva. Cyclosporin A and azathioprine were discontinued. Two days later, a relapse of bilateral panuveitis occurred. Treatment with interferon α 2a (IFN α 2a; Roferon) was started with a maximum dose of 18 million units daily. After two weeks this had to be reduced to 12 million units daily because of leucopenia. Ocular inflammation improved after four weeks. Five months later, skin tumours and ocular inflammation had completely resolved. Oral mucosa appeared normal. Gastrointestinal and pulmonary lesions (fig 1C) had improved. IFN α was reduced to 10 million units every other day. In February 1995, gastrointestinal and pulmonary lesions had cleared. Since then the doses of IFN α and prednisolone have been further reduced and in June 1999, the patient was still in complete remission for both diseases without IFN α , which was discontinued in January 1998.

Histological, virological, and molecular biological examinations of Kaposi's sarcoma tissue

Histology of the reddish lesion of the hard palate showed typical Kaposi's sarcoma (fig 2A). A biopsy from caecum, disclosed an unspecific colitis (fig 2B). HHV8-specific nested polymerase chain reaction (PCR) assays with the DNA extracted from paraffin embedded, formalin fixed tissues were performed according to a previously described protocol.^{6–8} This

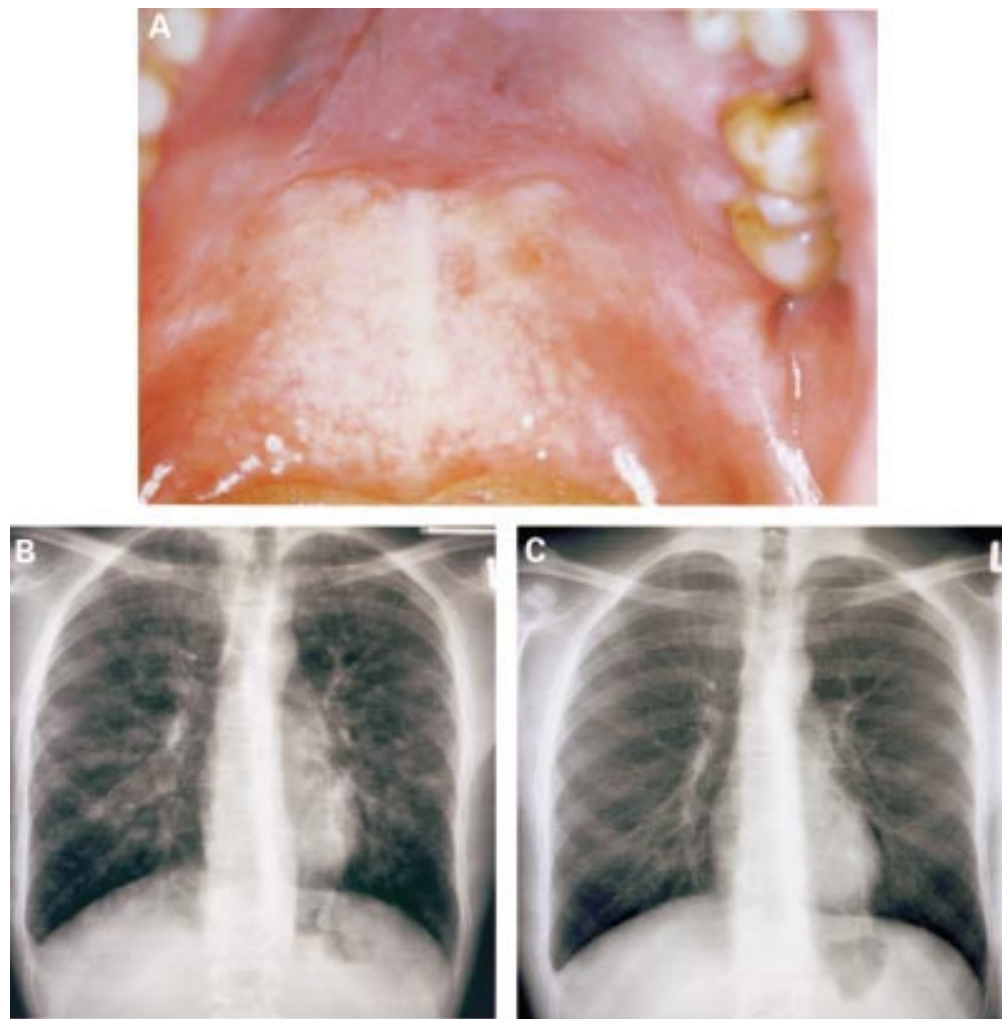


Figure 1 (A) Tumour at the hard palate of the patient (reddish lesion). (B) Chest x ray in February 1994, showing multiple nodular infiltrates and lymphangiosis. (C) Chest x ray in May 1994, showing resolution of the nodular infiltrates and lymphangiosis.

PCR results in a 160 bp nested PCR amplifycate. A second, seminested PCR was established for the variable domain of the open reading frame K1. This gene has an unknown function in HHV8 and there are no known cellular or viral homologues. The primers used were K1P5A (5'-ATGTTTCCTGTATGTTG TCTGCAGTC-3') and K1P3A (5'-CAAAGT AACATGCTGACCACAAGTG'), those for the nested PCR assay were K1P5N (5'-GTT TGCTTTTCGAGGACTATTAAGCC-3') and again K1P3A. The material from the hard palate showed a positive result in both HHV8-specific PCRs (fig 3, lanes 6 and 10), but the material from the caecum was negative for HHV8. A nested PCR assay for detection of the immediate early gene region of human cytomegalovirus (HCMV) (primer sequence for the first PCR: RZ-IE3: 5'-CGA CAT CTT TCT CGG GGT TCT CG-3', 23mer: 172408-172430, RZ-IR4: 5'-GAC ACG ATG GAG TCC TCT GCC-3', 24mer: 172771-172751. Nested PCR: RZ-IE1: 5'-CGG CCA ACT CTG GAA ACA GCG GG-3', 23mer: 172474-172496, RZ-IE2: 5'-CCC TGA TAA TCC TGA CGA, GGG CCC-3', 24mer: 172736-172713; Kandolf *et al*, unpublished data) showed HCMV infection of the caecum

(fig 3, lane 1), whereas no HCMV-specific sequences were detected in the lesion of the palate (fig 3, lane 2).

Discussion

The prognosis of ocular Behçet's disease has significantly improved since the institution of early and aggressive immunosuppression, often consisting of a combination of several immunosuppressants—for example, steroids, azathioprine, and cyclosporin A.² In the patient described here this combination was the only immunosuppressive treatment which suppressed his severe, sight-threatening ocular vasculitis.

In iatrogenic immunosuppression an increased risk, especially for Kaposi's sarcoma, has been shown for cyclosporin A.⁵ and there is evidence that high doses of immunosuppressants or a combination of several agents with different modes of action (for example, cyclosporin A, azathioprine, and prednisolone) has a cumulative risk of neoplasia.⁵⁻⁹ In our patient the latency period from institution of azathioprine as third immunosuppressant until the occurrence of Kaposi's sarcoma was 14 months, which is in agreement with data from a previous study.¹⁰

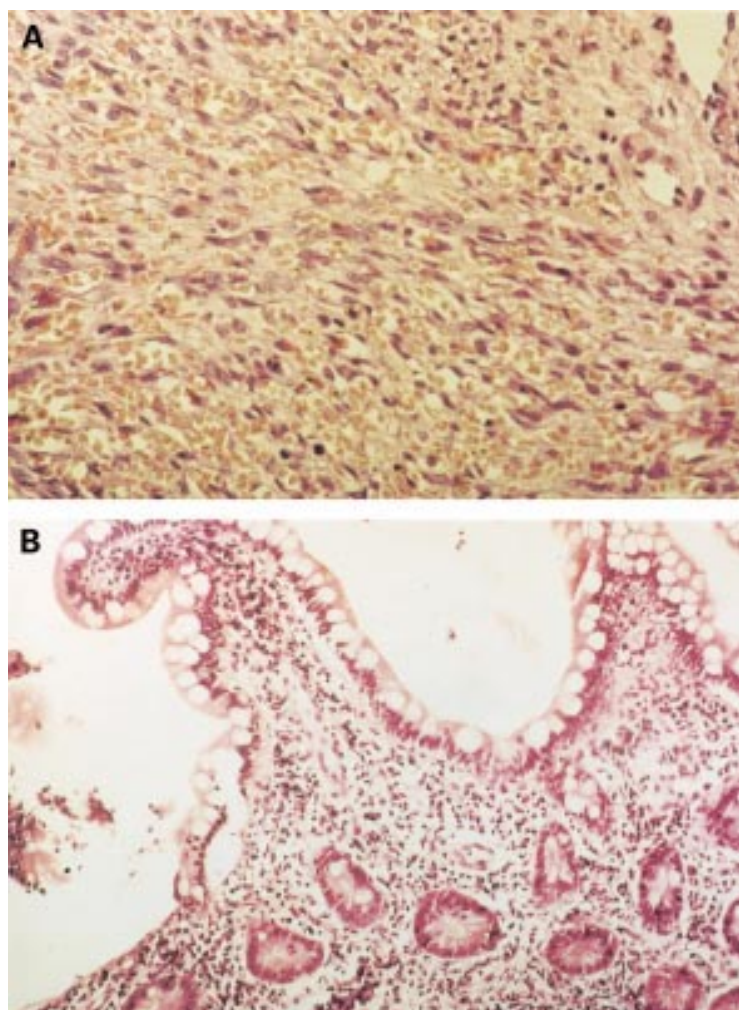


Figure 2 (A) Histology of the lesion from the hard palate, showing features of Kaposi's sarcoma with spindle shaped cells. (B) Histology of the caecum showing unspecific colitis.

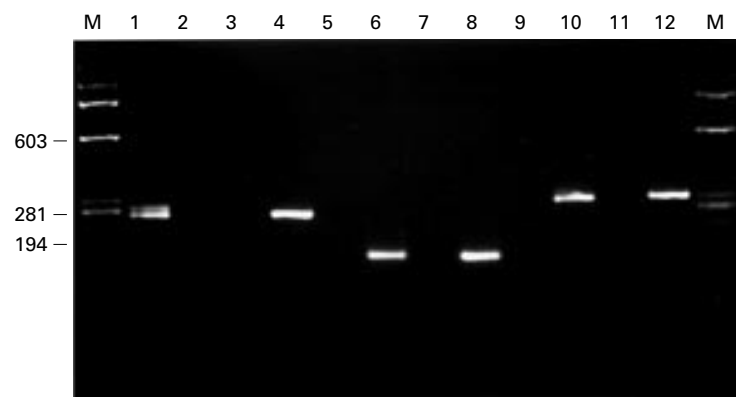


Figure 3 Polymerase chain reaction (PCR) amplicates are shown by ultraviolet illumination of an ethidium bromide stained 2% agarose gel. Lanes 1-4 show the HCMV-IE gene-specific PCR amplicons obtained with 200 ng DNA extracted from the caecum (lane 1), hard palate (lane 2), negative and positive controls (lanes 3 and 4). Lanes 5-8 represent the HHV8-specific amplicons according to Lin *et al.* The sequence of materials is the same as in lanes 1-4. Lanes 9-12 represent the respective K1 (HHV8-K1)-specific amplicons. M is a PhiX Hae III digested DNA marker. The numbers at the left indicate the respective length of the restriction fragments in base pairs.

Recently, HHV8 was shown to be present in almost all cases of Kaposi's sarcoma,⁶ underlining its role as putative causative agent. In our patient, HHV8 was present in lesions of the hard palate, but not in the colon. This result

correlates with the respective histology in the biopsy material (figs 2A and B). Interestingly, our patient had a systemic, though subclinical, HCMV infection. This may reflect a more general reactivation of, or alternatively, a new infection with, herpesviruses during triple immunosuppressive treatment.

Possibly, discontinuation of immunosuppression alone would have led to regression of Kaposi's sarcoma¹¹ by normalisation of the immune status. Because of relapse of uveitis, some kind of treatment without immunosuppressive effects was urgently needed in order to treat Behçet's disease but not to promote progression of Kaposi's sarcoma. IFN α was chosen because it is effective in 30-50% cases of Kaposi's sarcoma, though durable responses are rare,^{12,13} and preliminary data showed efficacy in non-ocular Behçet's disease. Recently, IFN α has been described as an effective agent in ocular manifestations.¹⁴⁻¹⁷ In these studies the most severe side effects of IFN α consisted of depression (4%), induction of autoimmune phenomena (occurrence of anti-nuclear and antithyroid antibodies with overt thyroiditis or even systemic lupus erythematosus in some patients (10%)), and worsening or induction of psoriasis (4%). It remains to be shown if IFN α is as effective and has less severe side effects than the conventional immunosuppressive treatment for Behçet's disease.

Its efficacy in this case may be regarded as consistent with a possible viral cause of Behçet's disease. Herpes viruses have been implicated as causative agents in Behçet's disease since its first description.¹⁸ Nevertheless, HHV8 is unlikely to be this cofactor, because to our knowledge, no other coincidences of Kaposi's sarcoma and Behçet's disease have been reported. This should be the case if patients with Behçet's disease, often immunosuppressed by treatment, were infected with HHV8. The mode of action of IFN α in Behçet's disease is still unclear. It enhances HLA class I antigen expression, T and NK cell cytotoxicity, and diverts the T cell response in the direction of T helper 1 (Th1), which seems to be paradoxical, because Behçet's disease itself is Th1 mediated.¹⁹ All these effects may be helpful in improving the elimination of foreign antigens. Thus the surprisingly positive results of IFN α treatment in Behçet's disease may have implications for further clinical trials and should stimulate research to determine the cause of Behçet's disease.

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