Progressive HHV-8-positive classic Kaposi’s sarcoma: rapid response to interferon α-2a but persistence of HHV-8 DNA sequences in lesional skin

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Summary
The pathogenesis of Kaposi’s sarcoma (KS) is often attributed to an infectious agent. In particular, the human herpesvirus 8 (HHV-8) was currently shown to be closely related to all known KS types, including HIV-associated KS, European classic KS, African endemic KS and iatrogenic KS. We report here on an HIV-negative, German patient of neither Jewish nor Mediterranean descent with disseminated classic KS showing unusual rapid progression into the tumour stage. After systemic administration of interferon α-2a over 4 weeks all tumour lesions cleared completely. Interestingly, HHV-8 DNA sequences detected by nested polymerase chain reaction in KS lesions before the onset of treatment were still present in lesional skin after complete remission of the tumour. No recurrence was seen after a follow-up period of 6 months.

Classic Kaposi’s sarcoma (KS) is a multilocular neoplasm of vascular origin. The disease primarily affects males of mainly Mediterranean or eastern European descent over the age of 60 years. Cutaneous involvement shows macular, papular and nodular lesions, with typically acral and symmetrical localizations, spreading centripetally and usually with slow evolution. Common sites of visceral involvement, which occurs in only about 10% of all cases, include the gastrointestinal tract, liver, lungs and the lymph nodes. Very rarely, classic KS becomes manifested first in visceral organs without cutaneous involvement. Classic KS patients survive an average of 10–15 years before dying of usually unrelated causes. Secondary malignancies, especially of the lymphoreticular system, may be found in more than 35% of the cases.

Various studies have suggested that the pathogenesis of KS is possibly related to a sexually transmitted viral agent inducing increased cell proliferation by expression of transforming genes with oncogenic potential. A number of DNA viruses including certain herpes viruses might carry apoptosis-inhibiting gene sequences allowing them to escape an apoptotic host response. Sero- logical evidence of cytomegalovirus (CMV) infection in patients with KS as well as the presence of CMV-DNA in KS tissue have been investigated, but no consistent association between CMV infection and classic KS could be established. Testing for relations between KS and other viral agents (Epstein–Barr virus, hepatitis B virus) led to similar conclusions. Human papillomavirus (HPV) was also suggested to be a sexually transmitted cofactor for KS. In 1994, short DNA sequences associated with KS were identified by representational difference analysis. These DNA sequences were significantly homologous to capsid and tegument protein genes of herpes virus saimiri and Epstein–Barr virus, which belong to a group of herpes viruses with oncogenic potential (sub-family gammaherpesvirinae). It has been suggested that this newly identified human herpes virus (HHV-8) may play a causative role in KS development and pathology. HHV-8 DNA sequences were detected in lesional skin, blood mononuclear cells, and sensory ganglia of patients suffering from various KS types including HIV-associated KS, classic KS, African endemic KS and iatrogenic KS, whereas the evaluation of possible HHV-8 sequences in semen of immunocompetent men or HIV-infected men showed controversial results. HHV-8 shows slight sequence variations for different KS populations, but the various clinical KS types are probably due to differences in host immune response rather than nucleic acid alterations.
In this paper we report on an unusual case of a rapidly developing, HHV-8-positive, disseminated KS in an elderly German patient who was HIV-negative and not otherwise immunodeficient. Interestingly, the tumour cleared completely after systemic treatment with interferon α-2a over a period of 4 weeks, whereas, the HHV-8 DNA sequences were still detectable in lesional skin 1 day after completion of treatment.

Case report

An 89-year-old, otherwise healthy male of German parentage who was born in Berlin and had never travelled abroad, presented himself with a 6-week history of rapidly erupting purplish-brown nodules and plaques in acral localization. The lesions first developed on the inner surface of the right, then the left foot, followed by the right knee and both hands (Fig. 1). There was no clinical evidence of immunosuppression.

In addition, our patient suffered from recurring eczema on the lower limbs and he had been repeatedly treated with topical and short intervals of low-dose systemic corticosteroids. The last dose of systemic treatment, however, had been given 9 months before the development of the tumour. He also reported having had a total excision of the rectum for rectal cancer in 1992, without subsequent chemotherapy or radiation.

Skin lesions were histologically identified as classic KS with capillary angioproliferation and bundles of partly atypical spindle cells lining vascular slits; at places, extravasated cells and degenerating erythrocytes were seen. Electron microscopy showed phagocytosis of extravasated erythrocytes by atypical endothelial cells.

All routine laboratory investigations showed normal blood parameters. Serological testing with enzyme immunoassays revealed the presence of IgG antibodies directed against Epstein–Barr virus (anti-EBNA) and cytomegalovirus (anti-CMV-IgG), while anti-VCA IgG, anti-VCA-IgM and anti-CMV-IgM were absent. HLA-DR 5, usually associated with classic KS, could not be detected. HIV-associated KS was excluded by constantly negative HIV 1/2 serology and absence of any related risk factors in the patient’s history. Gastrointestinal tract, liver and lung examinations revealed no evidence for systemic involvement.

Biopsies of normal and lesional skin were assayed for HHV-8 DNA sequences by nested polymerase chain reaction (PCR). Two nested sets of oligonucleotide primers were designed in order to detect a 233 bp region within the minor capsid protein gene (ORF 26) of HHV-8 (Table 1). It was found that a specific amplificate could be obtained from biopsies of KS lesions while unaffected skin was negative (Fig. 2). The nucleotide sequences of the obtained PCR products were determined by automated cycle sequencing and were found to be identical with the published DNA sequence of HHV-8 in that particular genomic region (GenBank, accession no. U75698, nucleotide position 47287–47519).

Table 1. Nested polymerase chain reaction (PCR) oligonucleotide primers for the detection of the central portion of the minor capsid protein gene (ORF 26) of human herpesvirus 8. The expected size of the PCR amplification products is 293 bp (external primer set) and 233 bp (internal primer set), respectively.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence</th>
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<tbody>
<tr>
<td>HHV8293 external forward</td>
<td>5'-GGACAGCAAACACCAGCTAGC-3'</td>
</tr>
<tr>
<td>HHV8293 external reverse</td>
<td>5' - CACGCAGGCCAGCTAGCTAGG-3'</td>
</tr>
<tr>
<td>HHV8233 internal forward</td>
<td>5' - AGCCGAAAGGATTCACCATT-3'</td>
</tr>
<tr>
<td>HHV8233 internal reverse</td>
<td>5' - TCGTGGTGTCTACGTCCAGA-3'</td>
</tr>
</tbody>
</table>
During his stay in the hospital the patient was treated with interferon α-2a, $6 \times 10^6$ IU three times a week over a total period of 4 weeks. On this treatment complete remission of all KS lesions was seen and no relapse was found over a 6-month follow-up period. Nevertheless, HHV-8 DNA sequences were again detected by nested PCR in the area of lesional skin after completion of the treatment (Fig. 2).

**Discussion**

There have only been a few clinical reports on HHV-8 positive, classic KS. The possible relations between the intrallesional evidence of HHV-8, the clinical involvement and the course of the disease have not yet been established. Our case seems to be of interest because HHV-8 was detected here in an HIV-negative patient of non-Jewish, non-Mediterranean descent with disseminated classic KS and unusually rapid tumour progression.

The aetiological significance of HHV-8 for the development of KS has to be further evaluated as HHV-8 DNA sequences were not only found in various forms of KS lesions but also in HIV-associated lymphoma. AIDS-related multicentric Castleman’s disease, body cavity-based lymphoma in both HIV-positive and HIV-negative patients, and even in a case of pemphigus vulgaris in an HIV-negative patient. Benign vascular lesions were shown to be negative for HHV-8. Concerning angiosarcoma contradictory results could be found, with occurrence or absence of HHV-8 DNA sequences. While viral DNA isolates from KS lesions appear to be in a latent circular form, viral replication seems to occur in peripheral blood cells, where linear replicative forms of the viral DNA can be detected. The same DNA sequences were found in the blood of allograft patients and healthy donors. These findings indicate that HHV-8 could have a wider distribution than predicted for the putative KS agent. Overall, whether HHV-8 is a passenger or acts as a driver in KS is still unknown: recently, however, the virus could be cultured from KS lesions. HHV-8 might also be present at the site of inflammation as an epiphenomenon. The fact that in our case HHV-8 was still detectable in the former location of the tumour after its full remission is in agreement with the biological properties of other gamma-herpes viruses with virus latency in the infected cells. Upon reactivation of the latent virus the infected cells proliferate and become prone to immortalization by viral gene products and/or virus-induced DNA recombination events.

There are various therapeutic options for KS including radiotherapy, surgical intervention, cryotherapy or laser evaporation and intrallesional application of cytostatic substances such as vinblastine or vincristine, as well as intrallesional or systemic application of recombinant interferon α. In cases of classic KS, systemic treatment with interferon α-2a is poorly described as the drug is often applied as an intralesional injection. In our patient systemic administration was chosen because of our good experiences with systemic treatment of HIV-associated KS with interferon α-2a. The response was dramatic and all lesions cleared completely in a period of 4 weeks.

There is evidence that interferon α increases the expression of major histocompatibility complex class I antigens on KS tumour cells making them sensitive to interferon-α-primed natural killer cells. Therefore, the anti-KS potential of interferon α may result from immunomodulatory rather than from antiproliferative effects. Recently, it was shown that HHV-8 encodes a functional homologue of Bcl-2, which is a cellular gene product involved in the regulation of apoptosis. The dysregulation of the cellular Bcl-2 gene is believed to contribute to neoplastic cell expansion via an anti-apoptotic effect. Our findings suggest that there is no direct link between the presence of HHV-8 DNA sequences encoding the minor capsid protein and the development of KS lesions. But it seems likely that HHV-8 can either persist in a
silent or KS-promoting stage, which cannot be discriminated on the molecular level at this time.

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References