Kaposi’s Sarcoma–Associated Herpesvirus/Human Herpesvirus 8 Is Not Detectable in Peripheral Blood Mononuclear Cells of the Relatives of Sporadic KS Patients

To the Editor:

Chang et al (1994) have identified in 90% of Kaposi’s sarcoma (KS) lesions of human immunodeficiency virus-1 (HIV-1)-infected individuals a new human herpesvirus, provisionally defined with the descriptive name of Kaposi’s sarcoma herpesvirus (KSHV) (Chang et al, 1994), and now in the course of designation as human herpesvirus 8 (HHV8) (Moore et al, 1996). KSHV/HHV8 has been subsequently demonstrated in the skin lesions of Mediterranean KS (Dupin et al, 1995), in African KS (Huang et al, 1995), and in a patient who developed KS after immunosuppressive therapy for allogeneic bone-marrow transplantation (Gluckman et al, 1995). KSHV/HHV8 DNA sequences have been also demonstrated in peripheral blood mononuclear cells (PBMC) of more than 50% of KS patients (Whitby et al, 1995).

Little is known about the routes of transmission of KSHV/HHV8. Viral DNA sequences have been found by nested polymerase chain reaction (PCR) in the semen of more than 90% of HIV-1-infected homosexual men and in 23% of the healthy donors (Lin et al, 1995). Moreover, KSHV/HHV8 DNA sequences have been infrequently detected in other biologic fluids of HIV-1-positive KS patients. KSHV/HHV8 was found in the saliva of one of 27 KS patients, in the throat swabs of one of 21, and in none of 18 stool samples of KS patients, suggesting that cell-free KSHV/HHV8 is unlikely to be shed. In contrast, KSHV/HHV8 DNA sequences were detected in plasma and serum of 16% and 46%, respectively, of KS patients (Whitby et al, 1995).

The aim of our study was to investigate the presence of KSHV/HHV8 in the peripheral blood mononuclear cells of the relatives of classic KS patients. As reported in Table I, PBMC were isolated on a Ficoll–Hypaque gradient from 13 KS patients and 18 relatives. The relatives were 11 wives and seven children (three males and four females) showing no evidence of KS. Control PBMC were obtained from 12 patients affected by non-KS cutaneous diseases (Table I). Total DNA was extracted from PBMC through the use of Microturbogen DNA extraction kit (Invitrogen, San Diego, CA). Samples were assayed for KSHV/HHV8 sequences by nested PCR using primers KS4 and KS5 for the first round of amplification, followed by a second round of KS1 and KS2 (Boshoff et al, 1995). PCR products were analyzed by Southern blot hybridization using the KS330-233 fragment 5'-end-labeled as a probe (Chang et al, 1994). DNA extracted from a KS skin biopsy was used as positive control for PCR. As negative control for PCR, we used a body-cavity-based lymphoma in an HIV-negative patient. N Engl J Med 333:943, 1995


Table I. Detection of KSHV/HHV8 DNA Sequences in PBMC from KS Patients, from Their Relatives and from Patients Affected by Non-KS Cutaneous Diseases a

<table>
<thead>
<tr>
<th>PBMC DNAs</th>
<th>No. of Cases</th>
<th>Sex</th>
<th>Age (range)</th>
<th>KSHV/HHV8</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS patients</td>
<td>13</td>
<td>13 M/0 F</td>
<td>61–86</td>
<td>12/13 (92%)</td>
</tr>
<tr>
<td>Wives</td>
<td>11</td>
<td>59–84</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>7</td>
<td>35–50</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Non-KS patients</td>
<td>12</td>
<td>9 M/3 F</td>
<td>32–81</td>
<td>1/12 (8%)</td>
</tr>
</tbody>
</table>

a DNA extracted from PBMC was assayed for KSHV/HHV8 DNA sequences by nested PCR, and the products were analyzed by Southern blot hybridization.

DNA extracted from PBMC previously shown to be negative for KSHV DNA sequences.

KSHV/HHV8 DNA sequences were detected in PBMC of 12 of 13 KS patients, in none of the 18 relatives, and in one of 12 patients with non-KS cutaneous diseases (Fig 1). The positive patient was a 64-y-old female with a previous diagnosis of mycosis fungoides (MF). KSHV/HHV8 does not seem to be related to MF, however, because PBMC from an additional MF patient and four MF cutaneous lesions were negative for KSHV/HHV8 DNA sequences (data not shown). Serial dilutions of a plasmid bearing the KS330-233 bp fragment were used as a template for PCR amplification (Chang et al, 1994). Upon hybridization, up to 60 molecules could be detected (data not shown).

It has been shown that KSHV/HHV8 infects circulating B lymphocytes (Ambrozak et al, 1995; Mesri et al, 1996). Therefore, we have investigated the possibility that a profound alteration in the number of circulating B lymphocytes in KS patients could be responsible for our findings. Apparently this was not the case, because cytofluorimetric analysis of PBMC revealed that the number of CD19+ B lymphocytes of six KS-patients (12.7 ± 7, range

Figure 1. KS1/KS2 nested PCR Southern blot of DNA from PBMC of classic KS patients and their relatives. LANES: 1-2:5,8,11,14; KS patients; lanes 3-6:9,12,15,16,17: relatives; lanes 4,7,10,13,19: no samples; lane 1: MW marker. 18 negative control; 20 positive control.

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Abbreviations: HIV-1, human immunodeficiency virus-1; KSHV, Kaposi’s sarcoma herpesvirus; HHV8, human herpesvirus 8; MF, mycosis fungoides; PBMC, peripheral blood mononuclear cells.
6–24) was in the same range as that of seven relatives (10 ± 6, range 4–18).

KS patients had 5–10 y of disease history and only cutaneous localization. Epidemiologic analysis of the 13 families revealed that 11 of 13 were living in the rural areas around the city of Sassari in the North-West of Sardinia. Seven of 13 KS patients were farmers and shepherds. The couples lived together for an average period of 30 y, and the children shared family life for approximately 25 y. Our results confirm the close association between KS and KSHV/HHV8 and are in keeping with the results reported in a recent study in which antibodies to KSHV/HHV8 were found only in sera obtained from AIDS-KS patients and not from HIV-1-infected patients without KS (Moore et al., 1996). Our observation may indicate that KSHV/HHV8 behaves as a poorly transmissible virus. Alternatively, the possibility exists that the virus is transmitted from KS patients to their relatives and is eliminated thereafter by the host immune defense. Serologic studies aimed to demonstrate the presence of antibodies directed against KSHV/HHV8 antigens will help to clarify this issue.

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REFERENCES


Diacylglycerol-Induced Tanning

To the Editor:

We read with interest the recent publication of Allan et al (1995) demonstrating that topically applied diacylglycerols (DAG) increase pigmentation in guinea pig skin. The work of Allan et al (1995) reconfirms and extends our earlier study (Agin et al, 1991) showing that DAG induces pigmentation in hairless pigmented mice. Because Allan et al (1995) omitted referencing our earlier findings (Agin et al, 1991), it is appropriate that the experiments, results, and conclusions from both studies be compared and discussed.

First, the maximum effective DAG dose to induce pigmentation determined by Allan et al (1995) in guinea pig skin was about 100 times the maximum effective dose to induce pigmentation in hairless mouse skin (Agin et al, 1991). One explanation for the apparent 100-fold difference in sensitivity is a difference in skin penetration due to the vehicles used in the two studies, i.e., acetone for mice (Agin et al, 1991) and propylene glycol for guinea pigs (Allan et al, 1995). Alternatively, guinea pig skin may be less sensitive to DAG-induced stimulation of melanogenesis compared to mouse skin.

Second, DAG caused skin irritation in guinea pigs whereas it did not in mice. Higher DAG doses might have caused irritation in mice, but the lower doses used in our study (Agin et al, 1991) produced a dose-dependent increase in pigmentation and then a saturation of the response. Allan et al (1995) propose that DAG-induced irritation plays a role in stimulating melanogenesis. Our results (Agin et al, 1991), however, clearly demonstrate that DAG-induced irritation is not necessary to induce pigmentation.

Third, when we applied DAG for 1 wk and then waited for 2 more weeks, the initial DAG-induced increase in dihydroxyphenylalanine (DOPA) oxidase activity translated to a later increase in melanization, implying that the DAG effect was mainly on stimulating DOPA oxidase activity (Agin et al, 1991). In the guinea pig (Allan et al, 1995), DOPA oxidase activity was not measured. Because peak pigmentation in guinea pigs was observed about 50–55 d after the 5- or 10-d DAG application had ceased, it is possible that DAG only stimulated DOPA oxidase, as is the case in mice (Agin et al, 1991).

Finally, we found that ultraviolet light synergizes with DAG to induce pigmentation in mice (Agin et al, 1991) as it does in cultured melanocytes (Friedman et al, 1990). It would be interesting to determine whether this synergism also applies to guinea pig skin.

In conclusion, we were very pleased to see studies in another animal model confirm our earlier work suggesting a role for protein kinase C in regulating melanogenesis. These in vitro studies support the more direct in vivo experiments by Friedman et al (1990), and Gordon and Gilchrest (1989), which demonstrate this mechanism of action in cultured melanocytes.