Serological and virological evidence of non-sexual transmission of human herpesvirus type 8 (HHV8)

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SUMMARY

To evaluate whether or not human herpesvirus 8 (HHV8) can be transmitted through a non-sexual route a serological survey was carried out in a group of 51 catholic nuns. The seroprevalence rate and the geometrical mean antibody titre to anti-latent HHV8 antigen were similar in nuns and in a group of 60 women, matched by age, in the general population (27 ± 24% vs. 1028 ± 1575, respectively). Moreover, by using nested polymerase chain reaction (PCR), HHV8 DNA sequences were detected in 7 of 16 (43.8%) saliva and peripheral blood mononuclear cells (PBMC) from patients with classical Kaposi’s sarcoma (KS) and in 3 out of 7 (42%) AIDS-KS patients. None of 5 HIV positive persons who did not have KS tested positive for HHV8 DNA. HHV8 DNA sequences were also detected in 2 of 12 (17%) saliva and 1 PBMC sample out of 12 healthy HHV8 positive individuals (age range: 30–80 years old). This paper suggests that non-sexual transmission of HHV8 is operating in our geographical setting and saliva may be a potential source of HHV8 spreading in the general population.

INTRODUCTION

Kaposi’s sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8), was initially discovered in AIDS-related Kaposi’s sarcoma (KS) by representational difference analysis [1]. Subsequently, by polymerase chain reaction (PCR) and serology, it was shown that HHV8 is strongly associated with all epidemiological forms of KS (human immunodeficiency virus (HIV) associated, classic, endemic and post-transplantation) [2].

A serological survey in Sicily, where the incidence of classical KS was high before the AIDS epidemic, showed that HHV8 infection is widespread in the general population [3]. The definitive route(s) of HHV8 transmission is still not well established, but epidemiological and serological data suggest that multiple modes may play a role.

Although HHV8 seropositivity has been shown to be more closely linked to groups postulated a priori to have a high risk of acquiring sexually transmitted diseases (STD) [3, 4], antibodies to HHV8 were also detected in prepuberal children in Sicily and in Africa, where KS is endemic [3, 5].

To provide further information about a non-sexual transmission of HHV8 infection, we carried out a serological survey in a group of catholic nuns, living in a religious rest house, in order to compare their relative HHV8 antibodies prevalence with that detected in a group of women, matched by age, in the general population. We also conducted a molecular study to detect HHV8 DNA in saliva and peripheral
blood mononuclear cells (PBMC) of HHV8 antibodies positive individuals with and without KS and HIV infection.

**METHODS**

**Patients and specimens**

Between March and May 1999, 52 nuns who were living in a religious retirement home in Palermo were invited to participate in the study. All were elderly (age range 70–90 years, median 78 years) and had taken the veil as young adults (ages 18–22 years). Fifty-one of the 52 gave their consent to participate in the study; one declined because of ill health. Of the 51, 24 had been elementary school teachers, 12 had been local nurses, 4 had worked in foreign missions, and 11 had provided general services to their convents (2 tailors, 5 gardeners, 4 cooks). None had received an organ transplant, and only three had been transfused in the previous 20 years. HHV8 seroprevalence in the 51 nuns was compared to that in 60 age-matched women in the general population, whose residual sera were obtained following the completion of routine chemistry analyses. All gave their consent to be included into the study.

Saliva and PBMC were collected from 16 classical KS (mean CD4+ cell count: 590/mm³), 7 HIV-positive with KS (mean CD4+ cell count: 261/mm³), 5 HIV-infected subjects without KS (mean CD4+ cell count: 288/mm³) and 12 adult blood donors (mean CD4+ cell count: 823/mm³). All subjects were HHV8 antibody positive as determined by an indirect immunofluorescence assay using the BCBL1 cell line as described [3]. None of the KS patients had oral lesions.

**IFA**

Antibodies to latent and lytic antigens of HHV8, were detected as previously described [3] by using BCBL-1 cell line. To induce HHV8 activation, the BCBL-1 were treated with 20 ng/ml tetradecanoyl phorbol ester acetate (TPA, Sigma Aldrich, Italy) and 200 U/ml human recombinant interleukin 6 (Boehringer–Mannheim, Germany) for 5 days.

Uninduced and TPA-induced BCBL-1 cells were collected, washed in PBS pH 7-4, spotted on slides, air dried and fixed in cold acetone for 10 min. Fixed slides were incubated with human sera diluted 1 in 120 and then stained with rabbit anti human IgG-fluorescein isothiocyanate conjugate (Sigma Aldrich, Italy).

**PCR analysis**

DNA from 100 µl of unstimulated whole saliva samples was extracted by boiling with 25% w/v Biorad Chelex 100 [6]. PBMCs, isolated by ficoll-hypaque centrifugation of heparinized blood, were processed for DNA extraction by phenol-chloroform as described previously [7]. The presence of HHV8-DNA was determined by PCR analyses: 1 µg and 15 µl of the DNA, respectively from PBMC and saliva, were used for each PCR reaction. Samples were screened for HHV8 open reading frame (ORF) 26 by nested-PCR amplification [7]. All positive PCR samples were also confirmed by second nested-PCR that amplified a 197 bp fragment of ORF 72 (viral cyclin D gene) [8].

To ensure that negative results were not due to non-specific inhibition of PCR, an internal positive control for human β-globin gene was included in each saliva specimen and tested by PCR, using β-globin primers.

**Statistical analysis**

Comparison of geometrical mean titre (GMT) of HHV8 antibodies was made with Student t test.

**RESULTS**

Table 1 shows the results of anti HHV8 antibodies detected in sera of nuns and age-matched healthy women. Fourteen of the 51 (27%) nuns had antibodies to HHV8 antigens.

This result is quite similar to that detected in the age-matched women in the general population (15 of 60, 25%). Moreover, sera of nuns showed a GMT (1029) to latent HHV8 antigen that was similar to the general population (1575) (P > 0.5).

In the molecular study, we found HHV8 DNA sequences in saliva from 7 (43.8%) of 16 patients with classical KS, and in 3 (42%) of 7 patients with AIDS-KS, but not in the saliva of 5 HIV-positive individuals without KS (Table 2).

We also analysed the saliva of 12 healthy HHV8 infected donors, divided in 2 age groups: 6 donors in the age range (30–49 years) of the HIV positive individuals with and without KS, and 6 in the age range (50–80) of the classical KS patients. The same
frequency of HHV8 DNA detection (16.6%) was observed in the saliva of these healthy HHV8-infected donors regardless of age (Table 2). HHV8 DNA sequences also were detected in PBMC of the HHV8 infected individuals. Although nearly half of the subjects with classical KS or AIDS-KS had HHV8 sequences in their PBMC, compared to only 1 of the 17 subjects without KS lesions.

The presence of virus in saliva did not always correlate with its detection in PBMC. In fact only three subjects with classical KS and one with AIDS-KS had HHV8 DNA detected in both saliva and PBMC. There was no association between the CD4+ cell count and detection of HHV8-DNA either in saliva or PBMC in HIV infected individuals.

**DISCUSSION**

Serological assays have revealed great geographic variability in the prevalence of HHV8 infection. Although to date there is no serological ‘gold standard,’ an international pattern of HHV8 seroprevalence is apparent. There are substantial studies showing a very low viral circulation in the general populations of the United States and northern Europe [9, 10] whereas in the countries of the Mediterranean, HHV8 infection begins in childhood and increases steadily with age, reaching approximately one-quarter of the elderly populations [3]. In Africa, HHV8 infection also begins in childhood, increasing steeply with age such that the majority of young adults are antibody positive [5].

What it is not yet well established are the modes of transmission of virus throughout the general population. In a previous paper [3], we found that high seroprevalence rates in Sicily were observed in the older individuals, reaching 22.3% of those who were 51–70 years old. Among younger subjects, seroprevalence also was significantly increased among homosexual men and female prostitutes, suggesting that sexual contact may be a major mechanism for HHV8 transmission. However, HHV8 antibodies also were present in prepuberal children.

In this study we have sought HHV8 antibodies in a group of Catholic nuns as an indirect effort to assess whether or not HHV8 can be transmitted through a non-sexual route. These elderly women are assumed to have had little or no sexual activity. They had lived their entire adult lives in convents and had worked with children and in hospitals. Our data demonstrate that HHV8 seroprevalence was as high in the nuns as it was among presumably sexual active women in the

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**Table 1. Prevalence of HHV8 antibodies in a community of nuns matched by age (70–90 years old) to healthy women of general population**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. tested</th>
<th>No. (%) with HHV8 antibodies</th>
<th>GMT* latent HHV8 antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuns</td>
<td>51</td>
<td>14 (27.4%)</td>
<td>1028†</td>
</tr>
<tr>
<td>Healthy women</td>
<td>60</td>
<td>15 (25.0%)</td>
<td>1575†</td>
</tr>
</tbody>
</table>

* Geometric mean titre.  
† Student t test.  
t fl 0.46 \( P < 0.5 \).

**Table 2. HHV8-DNA detection by PCR in saliva and peripheral blood mononuclear cells from HHV8 antibodies positive subjects with and without Kaposi’s sarcoma and HIV infection**

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>HHV8-DNA in saliva</th>
<th>HHV8-DNA in PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number positive/tested</td>
<td>%</td>
</tr>
<tr>
<td>HIV-negative with KS</td>
<td>7/16</td>
<td>43.8</td>
</tr>
<tr>
<td>HIV-positive with KS</td>
<td>3/7</td>
<td>42</td>
</tr>
<tr>
<td>HIV-positive without KS</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>Age range: 30–49</td>
<td>1/6</td>
</tr>
<tr>
<td>Age range: 50–80</td>
<td>1/6</td>
<td>16.6</td>
</tr>
</tbody>
</table>
general population. This suggests that non-sexual transmission is operating in Sicily.

Several reports have cited saliva as the main vehicle of transmission [11–14]. In our study, HHV8 DNA was detected in saliva from nearly half (43.8%) of patients with classical and AIDS-associated KS. The source of viral production is unknown. No patient had an oral lesion, and therefore it is unlikely that the viral shedding was due to the local viral production by the spindle cells.

The detection of the virus in saliva might be due to infected PBMC in the mouth. We found, however, that the virus was not always present in an individual’s circulating PBMC and saliva. Therefore, HHV8 detection in saliva does not merely reflect its presence in PBMCs. Perhaps, like Epstein–Barr virus, HHV8 replicates in oropharynx epithelial cells.

The presence of the virus in the saliva may be due to its primary replication or its reactivation as occurs in other herpesviruses infections. As we noted direct correlations of age with both antibody seroprevalence and antibody titres to HHV8 latent antigen [3, 15] we suggested that HHV8 reactivation may be common in elderly healthy HHV8 infected individuals.

This hypothesis was not supported by our current results, detection of the virus in saliva was unrelated to age. However, we cannot exclude the possibility that the virus may undergo reactivation and replication in other sites. The virus, indeed, was present in PBMC. HHV8 has been previously identified in PBMC of AIDS-KS and classical KS patients, and its detection in HIV patients was suggested to be predictive for their eventual development of KS [16]. Nevertheless, the main reservoir of the virus has yet to be determined.

Although this and other studies demonstrated the presence of virus in saliva [11–14] the epidemiological and medical implications of salivary shedding are at present difficult to evaluate. One recent study, however, detected a higher prevalence and copy number of HHV8 in saliva compared with semen [14], suggesting that oral contact might carry more of a risk for transmission than intercourse [17]. Larger and well designed longitudinal studies are need to assess the role of HHV8 at different sites for the transmission of the virus in the general population.

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