Respective Roles of Serological Status and Blood Specific Antihuman Herpesvirus 8 Antibody Levels in Human Herpesvirus 8 Intrafamilial Transmission in a Highly Endemic Area

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ABSTRACT

Transmission of human herpesvirus 8 (HHV-8), the etiological agent of Kaposi’s sarcoma, occurs mainly during childhood in endemic countries and, to a large extent, through intrafamilial contacts. To additionally investigate this familial transmission, and especially the role of plasma anti-HHV–8 antibody titers, we conducted a large survey in a village from Cameroon, Central Africa, including 92 families (608 individuals). Plasma samples were tested for specific IgG directed against HHV-8 lytic antigens by immunofluorescence assay, and titers were determined by 2-fold dilutions. Global HHV-8 seroprevalence was 60%, raising from 32% under 9 years up to a plateau of around 62% between 15 and 40 years. The familial correlation patterns in HHV-8 seropositive/seronegative status showed strong dependence from mother to child and between siblings. In contrast, no familial correlation in anti-HHV–8 antibody levels was observed among infected subjects. In particular, no relationship was observed between the anti-HHV–8 antibody titer of HHV-8 seropositive mothers and the proportion of their HHV-8 seropositive children. Furthermore, a random permutation study of the anti-HHV–8 antibody titers among HHV-8 infected subjects showed that the main risk factor for infection was the HHV-8 serologic status and not the antibody level. In addition, no correlation was found between anti-HHV–8 antibody levels and bloody coat HHV-8 viral loads in a subsample of 95 infected subjects. Overall, these results strongly suggest that, in this highly endemic population from Central Africa, HHV-8 transmission mainly occurs from mother to child and between siblings, and it is independent of plasma antibody levels of HHV-8 infected relatives.

INTRODUCTION

Human herpesvirus 8 (HHV-8) is the etiological agent of Kaposi’s sarcoma (1). In nonendemic countries (United States, Northern Europe, Asia...), HHV-8 infection essentially occurs in homosexual men. In this specific population, the main identified risk factors are HIV seropositivity, increased number of sexual partners, and history of sexually transmitted diseases (2–5) suggesting HHV-8 contamination during sex. In such countries, heterosexual HHV-8 contamination is relatively infrequent, but it is reported in women with the same risk factors (6–8). In contrast, in HHV-8 endemic countries (Meditteranean basin, Africa, South America), most individuals get infected during childhood, because HHV-8 seroprevalence increases markedly from 1 to 15 years old to reach rates close to those observed in adults [e.g., 15% in French Guiana (9), 48% in Cameroon (10), 50% in Uganda (11), 58% in Egypt (12), and 70% in Tanzania (13)].

Our previous work done in a population from African origin from French Guiana, an intermediate HHV-8 endemic area with 13% of infected individuals, showed strong correlations in the HHV-8 seropositive/seronegative (HHV-8 +/−) status between mother and child and between siblings (9). The sib-sib correlation remained significant after adjustment for mother-child dependence, and it was stronger in pairs in which the two siblings had close ages. Several authors reported similar results regarding mother-child and sib-sib correlations (12–15), strongly supporting the hypothesis of HHV-8 transmission during childhood through close contacts (probably via saliva) in endemic countries. Less consistent results were observed with respect to the relative magnitude of HHV-8 heterosexual transmission in these endemic areas. Epidemiologic studies done in peculiar populations (prostitutes or patients attending a sexually transmitted disease clinic) found either a weak association between HHV-8 infection and sexually transmitted disease history and/or high numbers of sexual partners (16–18) or no association (19, 20). Familial studies in general populations also provided divergent results by finding either some evidence for a correlation in HHV-8 +/− status among spouses (13, 15) or no correlation (9).

On the other hand, few studies have been done to investigate the role of antibody levels against lytic or latent HHV-8 antigens in HHV-8 transmission. Blood specific antibody levels against a given pathogen is a classical marker of the infectious burden (21–25), frequently correlated with individual infection transmission, as shown for example in human T-cell virus type-1 infection for mother-child transmission (26, 27). With respect to HHV-8, most studies investigating the correlation between antibody titers and the viral load were done in individuals coinfected with HIV often suffering from Kaposi’s sarcoma (KS). They provided controversial results, although generally in favor of the absence of correlation (28–30). Another study done in Cameroon also showed no difference in specific antibody titers of HHV-8 infected children according to their negative/positive status for detection of HHV-8 DNA by PCR in the blood (10). However, two studies in South Africa (31, 32) concluded that the proportion of HHV-8 infected children increased with increasing maternal antibody titers, suggesting a possible correlation between anti-HHV8 antibody levels and the risk of mother-child transmission.

To additionally investigate familial transmission of HHV-8, we conducted a large survey in a highly endemic general population from Cameroon, Central Africa (10), an area where endemic Kaposi’s sarcoma is frequent. The first goal of the present study was to estimate familial correlations regarding the HHV-8 +/− status in this peculiar population and to confirm results previously obtained in French Guiana, a less endemic population for HHV-8. The second goal was to explore the role of antibody titers in familial transmission of the virus with two strategies: (1) we investigated familial correlations of...
anti-HHV–8 antibody titers and tested whether or not peripheral blood anti-HHV–8 antibody levels could influence the familial transmission of the virus, especially between infected mothers and their children; and (2) we studied the relationship between anti-HHV–8 antibody titers and PCR viral detection in buffy coat of HHV-8 healthy carriers from the same population.

MATERIALS AND METHODS

Studied Population

The survey was carried out in an isolated village located in the rain forest of the Ntem region in Southern Cameroon. This work, initiated since 1998, is a part of a multidisciplinary project (anthropological, medical, and economical). All individuals living in this village were included. Information on familial relationships was obtained on the basis of several interviews. Full pedigrees, constructed according to this information and validated with concerned individuals, comprised a total of 887 subjects from the Bantou ethnic group, mainly from the Fang tribe. To link the families and build the pedigrees, we included a substantial proportion of ancestors or relatives who were dead or had left the village. Thus, complete biological and epidemiologic data were finally available for 608 subjects, 287 males and 321 females, aged from 1 to 88 years (median 18 years), and clustered in 92 families ranging from 2 to 24 members. This survey was done after authorization of the national (Ministry of Health and Ethic committee of Cameroon) and local authorities (village chief) with information to each participant. Informed consent was obtained from adults or parents for minors. Furthermore, all of the participants underwent a medical examination and were treated, if necessary, according to the local medical facilities.

Biological Methods

A 10 mL blood sample was taken on EDTA from the 608 included subjects for HHV-8 determination. Plasma samples were tested at a 1:20 dilution for HHV-8–specific IgG by immunofluorescence assay (HHV-8 IFA, ABI, Columbia, MD; ref. 33). This assay, which uses KS-1 cell line as source of HHV-8, detects antibodies directed mainly against lytic HHV-8 antigens (33). It is well adapted to epidemiologic studies and does not react with any other known human herpesviruses, including Epstein-Barr virus. Anti-HHV–8 antibody titers were determined through successive 2-fold dilutions. DNA of high molecular weight was extracted from the buffy coat of HHV-8 seropositive individuals with the QIAamp DNA Blood Mini Kit (Qiagen, Courtaboeuf, France). Quantitative PCR detection of a fragment of HHV-8 open reading frame 26 gene was done by using a Taqman technique as described previously (34).

Statistical Methods

Phenotypes of Interest. We first studied a binary phenotype defined as HHV-8 seropositive or seronegative (HHV-8+/−) status. A sample was considered positive when showing a cytoplasmic reactivity on immunofluorescence at 1:20 dilution. The antibody titer directed against HHV-8 lytic antigens was then studied as a quantitative variable. Two quantitative analyses were successively done. The first one included only HHV-8 seropositive subjects, and the second one included also seronegative ones. In this latter case, we assigned a “negative” antibody titer of either 1:10 (half of the first positive dilution) or 1:1 (extreme negative value) to HHV-8 seronegative subjects, and both coding schemes were used in the study. All of the analyses with the quantitative variable were done on log-transformed antibody titer values.

Risk Factors

The first step was to determine the main risk factors influencing the phenotype under study in this highly endemic population. The risk factors tested in this analysis were sex and age considered both as a continuous (age in years) and a categorical variable. Different age coding schemes were tested, and the best fitting model was obtained with three age classes (<10 years old, 10 to 39 years old, and 40 and over). The effect of these risk factors on HHV-8+/− status and anti-HHV–8 antibody titers was assessed by logistic regression and linear regression, respectively. Because individuals within familial clusters are not independent, conventional regression could not be used, and regression analyses were carried out with first-order estimating equation (EE1) technique for both binary and quantitative variables (35). The estimating equation method is an alternative to the classical maximum likelihood approach, which provides asymptotic unbiased estimates of the usual regression parameters and their standard errors in the analysis of correlated data. All analyses were done with the Proc Genmod procedure of the SAS software, version 6.12 (SAS institute, Cary, NC).

Familial Dependencies

The second step was the estimation of four kinds of familial dependencies for the HHV-8 phenotype under study: spouse-spouse (father–mother), father-child, mother-child, and sib-sib. For the HHV-8+/− binary status, these familial dependencies were estimated in terms of odds ratio (OR). We first computed naïve ORs by the use of 2 × 2 tables with the distribution of all of the possible pairs of a given familial dependence (e.g., mother–child) according to the HHV-8 status of each member of the pair. Although this approach is a useful tool for identifying familial aggregation, it does not provide valid estimates for ORs because of the nonindependence of the observations (e.g., a mother with more than one child could be counted several times), and it does not allow to adjust OR values for other relevant risk factors. Therefore, we estimate valid familial ORs by use of the second-order estimating equation technique (denoted as EE2; refs. 36, 37), as already described elsewhere (9). We also used the same method as proposed in ref. 9 to adjust the sib-sib OR on mother-child dependence. For the anti-HHV–8 antibody titers, familial dependences were expressed in terms of standard Pearson correlation coefficients, denoted as ρ. Correlation coefficients between antibody titers within family clusters were estimated without assuming any distribution for the data by the EE2 technique developed for quantitative variables (38, 39). This technique also allows to simultaneously estimate the usual regression parameters to adjust for the relevant covariate effects (age was the only one in the present analysis). To estimate a sib-sib ρ independent of any mother-child correlation, the mother anti-HHV–8 antibody titer was simply included as an explanatory variable in the regression model. All EE2 analyses were carried out with the EE2 program described in Tréguet et al. (39).

Additional analyses were done to study the relationship between the anti-HHV–8 antibody titers of the mothers and the proportion of their HHV-8 seropositive children. To compare proportions, we used classical (Pearson) χ2 test and χ2 test for trend (Proc Freq procedures of SAS software, version 6.2, SAS Institute, Cary, NC). To compare antibody titers, we used distribution-free ANOVA based on ranks (Proc Rank and Proc Anova procedures of SAS software). This latter method was also used to analyze anti-HHV–8 antibody titers in function of viral loads.

RESULTS

Descriptive Analysis of HHV-8 Infection in a Highly Endemic Area, Central Africa. Fig. 1 presents the influence of age and gender on HHV-8 seroprevalence. The overall seroprevalence was 59.9% (364 HHV-8+ subjects) with no significant difference between males (59.9%) and females (59.8%). HHV-8 seroprevalence was at 32% under 9 years old, then rose up to a plateau of ~62% between 10 and 39 years, with a final increase (~8%) in older age groups (~40 years). As mentioned in the Materials and Methods, this coding scheme in three classes (<10, 10 to 39, and ≥40 years old) was the best fitting model to account for the age effect (P < 0.0001) and was used for additional analyses. In the 364 HHV-8+ subjects, there was no overall significant effect of age and gender on anti-HHV–8 antibody titers (Fig. 2). However, we noted that the oldest male group (~40 years) had significantly higher antibody levels (1:236 [95% confidence interval (CI) 1:166 to 1:337]) than males <40 years (1:154 [95% CI 1:125 to 1:216], P = 0.037), as already observed in French Guiana (40).

Familial Correlations According to the HHV-8 Serologic Status. Familial dependences between spouses, father-child, mother-child, and sib-sib were first investigated for the HHV-8+/− serologic
status. ORs obtained by the naïve approach indicated the presence of highly significant mother-child and sib-sib correlations (Table 1). To account for the age effect and dependence among pairs, valid familial ORs were then estimated with the EE2 approach. The same pattern of dependences was observed with strong correlations between mother and child \[ OR = 4.68 \ (95\% CI, 1.86–11.74) \], \[ P = 0.001 \] and between sibs \[ OR = 8.44, \ (95\% CI, 2.83–25.16) \], \[ P = 0.0001 \]. In contrast, no evidence of dependence between spouses \[ OR = 1.22 \ (95\% CI, 0.39–3.87) \] nor between father and child \[ OR = 1.08, \ (95\% CI, 0.51–2.21) \] was detected. To additionally investigate the sib-sib correlation, additional analyses were conducted. First, after adjustment for mother-child dependence, the HHV-8 sib-sib correlation remained significant \[ OR = 7.93 \ (95\% CI, 2.46–25.55) \], \[ P = 0.0005 \], suggesting that the sib-sib dependence in HHV-8 serologic status was independent of the mother to child spread. Then, we found that the sib-sib OR (adjusted for mother-child dependence) was higher for sib-pairs in which the two sibs had an age difference \[ \geq 5 \text{ years} \] \[ OR = 13.94 \ (95\% CI, 3.59–54.0) \], \[ P = 0.0001 \] than for sib-pairs including sibs with an age difference \[ < 5 \text{ years} \] \[ OR = 4.09 \ (95\% CI, 1.22–3.74) \], \[ P = 0.026 \]. These results, quite similar to our previous findings, reported in a much lower HHV-8 endemic area (9) strongly support the view that close interpersonal (especially mother to child and sib to sib) contacts are a major factor of HHV-8 transmission during childhood.

Familial Correlations According to Anti-HHV–8 Antibody Titers. Taking advantage of the high proportion of HHV-8 seropositive subjects in this population, we also studied familial correlations for anti-HHV–8 antibody titers directed against lytic antigens. In a first step, only the 364 seropositive subjects were considered in the analysis. Using the EE2 approach, no evidence was found for any familial correlation (Table 2), indicating that in familial pairs, including two infected relatives, anti-HHV–8 antibody titers were independent of each other. In a second step, we did an EE2 analysis including HHV-8 seronegative individuals with an anti-HHV–8 antibody titer assigned as mentioned in Materials and Methods (1:1 or 1:10). Because both coding schemes led to the same results (data not shown), only those obtained with an antibody titer at 1:1 are presented in Table 2. Results showed significant positive correlations between mother and child \[ p = 0.22 \ (95\% CI, 0.07–0.36) \], \[ P = 0.003 \] and between sibs \[ p = 0.36 \ (95\% CI, 0.15–0.54) \], \[ P = 0.0008 \]. In addition, neither father-mother correlation \[ p = -0.08 \ (95\% CI, -0.32 to 0.16), \ P = 0.51 \] nor father-child correlation \[ p = 0.21 \ (95\% CI, -0.06 to 0.46), \ P = 0.37 \] were found. Sib-sib correlation remained significant after adjustment for titer of the mother \[ p = 0.29 \ (95\% CI, 0.10–0.47), \ P = 0.003 \]. This pattern of correlation is very similar to that observed previously for the serologic status, suggesting that the most important risk factor in HHV-8 familial correlations among the two analyzed is infection per se (HHV-8+/−).

To additionally explore this hypothesis, we did a permutation study of the antibody titers within infected subjects. The 364 observed antibody titers were classified into three groups according to the age of the infected subject (<10, 10 to 39, and ≥40 years) and then randomly assigned to an infected subject belonging to the corresponding age class. HHV-8 seronegative subjects kept their negative anti-HHV–8 antibody titer (1:1 in the presented results). Twenty replicates were generated and analyzed leading to a mean mother-child corre-
analysis and confirmed that the main risk factor for infection is the titer of seropositive subjects, which have no influence in the observed distribution. The CI obtained with the real data (0.07 to 0.36 and 0.15 to 0.54, for both mother-child and sib-sib correlations were included within the 95% CI of the means. 

<table>
<thead>
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<th>Type of pair</th>
<th>(+, +)</th>
<th>(+, -)</th>
<th>(-, +)</th>
<th>(-, -)</th>
<th>Total</th>
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<td>10</td>
<td>3</td>
<td>2</td>
<td>45</td>
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<td>2. Father-child</td>
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<td>72</td>
<td>10</td>
<td>4</td>
<td>160</td>
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<td>63</td>
<td>21</td>
<td>54</td>
<td>243</td>
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<td>96</td>
<td>34</td>
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<td>392</td>
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<table>
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<tr>
<th>Type of pair</th>
<th>$\phi$ (95% CI)</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td>1. Father-mother</td>
<td>-0.08 (-0.47 to -0.32)</td>
<td>0.69</td>
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<tr>
<td>2. Father-child</td>
<td>0.12 (0.12 to 0.35)</td>
<td>0.35</td>
</tr>
<tr>
<td>3. Mother-child</td>
<td>0.05 (-0.14 to 0.24)</td>
<td>0.60</td>
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<tr>
<td>4. Sib-sib</td>
<td>0.04 (-0.26 to 0.18)</td>
<td>0.23</td>
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</tbody>
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Table 2 Distribution of pairs according to the familial relationship and the HHV-8 serological status (+/-) in Ntem Valley, Cameroon

Table 2 Familial correlations in anti-HHV-8 antibody titers ($\rho$), 95% CI and $P$ values, estimated by EE2 in Ntem Valley, Cameroon.

Table 2. Familial correlations in anti-HHV-8 antibody titers ($\rho$), 95% CI and $P$ values, estimated by EE2 in Ntem Valley, Cameroon.

**Fig. 3.** Geometric means of anti-HHV-8 antibody titers (determined by 2-fold dilution) among seropositive mothers according to the number of their children <10 and the HHV-8 serologic status of these children. Bars, 95% CI of the means.
peripheral blood anti-HHV-8 antibody titers could influence transmission within family. Together with the former serologic results, the analyses done on this quantitative measurement, and especially the permutation study among seropositive subjects, clearly showed that the main risk factor for infection was the HHV-8 status, and not the peripheral blood antibody levels. This finding is well illustrated by the specific study on mother-child transmission. Although no correlation was observed between maternal anti-HHV-8 antibody titers and the HHV-8 status of their children when only seropositive mothers were considered, this correlation became significant when seronegative mothers were included in the analysis. This absence of relationship between maternal antibody levels and HHV-8 status of children seems to be different from the results of two previous studies (31, 32). However, it can be easily seen from Table 1 and Table 2 of these respective papers (31, 32) that the reported increasing probability of mother-to-child HHV-8 transmission with increasing maternal antibody titers is entirely because of the presence of the HHV-8 seronegative mothers, and that the data of Sitas et al. (31) and Dedicat et al. (32) are therefore consistent with our present findings. However, it should be stressed that these studies made the implicit assumption that the considered anti-HHV-8 antibody levels reflect the maternal antibody levels during the viral exposure of the children. This latter hypothesis remains to be validated by large longitudinal studies.

We did not find any evidence for HHV-8 peripheral blood antibody titers being correlated with HHV-8 buffy coat viral load detection or quantification, which is consistent with their absence of role in HHV-8 transmission. Similar results were reported in a previous study from Cameroon, focusing on a few HHV-8 infected children, who displayed no difference in specific antibody titers according to their negative/positive PCR status (10). We did not have enough data to test whether or not buffy coat HHV-8 viral load could directly influence mother-child HHV-8 transmission, and this question remains to be addressed. Search for better markers of HHV-8 infectious burden, which can be an important risk factor for intrafamilial transmission, is currently done by several laboratories. In a very recent study, Dedicat et al. (32) have investigated the level of viral DNA in the saliva of the mother as a potential risk factor for mother to child HHV-8 transmission. The prevalence of lytic HHV-8 antibody titers only increased in the small sample of children born to mothers with the highest HHV-8 DNA viral load in saliva as compared with children born to mothers with no detectable saliva HHV-8. Further studies are needed to appreciate the importance of this risk factor in HHV-8 intrafamilial transmission and to explore the variability overtime of this marker.

Finally, we did not find in this hyperendemic population any evidence for a father-mother correlation in the HHV-8 status, confirming our previous results in the population from French Guiana (9), but done in a lower HHV-8 endemic area. This suggests that heterosexual transmission (through saliva and/or sperm) does not seem to be a predominant mode of HHV-8 infection in endemic populations. However, two other studies reported some evidence for a correlation in HHV-8 status between spouses, one done in Jewish families from Israel recruited through one hepatitis B virus infected individual and the second in highly endemic families from rural Tanzania. These discrepancies may be explained in part by differences in analysis methods (the EE2 technique with age adjustment seems to be, to our knowledge, the most appropriate method to analyze this kind of data). In any case, the simple observation that, in endemic countries, HHV-8 seroprevalence remains roughly steady or increases very moderately between 15 and 40 years strongly argues against a major role of heterosexual transmission in HHV-8 infection in these areas. This plateau of seroprevalence does not exclude the existence of heterosexual transmission but supports the hypothesis that a significant proportion of the population may be resistant, or at least less susceptible, to HHV-8 infection, whereas more susceptible subjects have already been infected during childhood. In the population from French Guiana, a lower HHV-8 endemic population than Central Africa, we already showed evidence for the presence of a recessive gene predisposing to HHV-8 infection and explaining the observed variation of HHV-8 seroprevalence with age (46). Genetic studies are ongoing to investigate whether or not the same pattern of genetic susceptibility exists in the present HHV-8 hyperendemic population from Cameroon and to identify the responsible genes.

ACKNOWLEDGMENTS

The authors wish to express thanks to the interviewers of the village of Ntem Valley. We would also like to thank Jocelyn Thonnon and Eric Nerienott of the Centre Pasteur du Cameroun for their support.

REFERENCES


Table 3 Relation between the anti-HHV–8 antibody titer of the mothers and the proportion of their HHV-8-seropositive (HHV-8 +) children aged <10 years old in Ntem Valley, Cameroon

<table>
<thead>
<tr>
<th>Maternal antibody titer</th>
<th>Number of mothers</th>
<th>HHV-8 +/total number of children (%)</th>
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<tr>
<td>&lt;1/20 (negative)</td>
<td>21</td>
<td>4/39 (10%)</td>
</tr>
<tr>
<td>1/20 to 1/80</td>
<td>14</td>
<td>10/28 (36%)</td>
</tr>
<tr>
<td>1/160</td>
<td>10</td>
<td>13/23 (57%)</td>
</tr>
<tr>
<td>≥1/320</td>
<td>15</td>
<td>9/27 (33%)</td>
</tr>
</tbody>
</table>

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Cancer Res 2004;64:8782-8787.

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