HHV-8/KSHV: Clinical and molecular Epidemiology and clonality of Kaposi’s sarcoma.

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Kaposi’s Sarcoma-Associated Herpesvirus/Human Herpesvirus 8 KSHV/HHV-8

- First identified in 1994, by the Representational Difference Analysis technique, (Chang et al. Science 1994) in a biopsy tumor from an AIDS related KS.

- Herpesviridae family, Gammaherpesvirinae subfamily, Rhadinovirus (or γ2-herpesvirus) genus.

- Complete sequence obtained in 1996 from a PEL strain (BC1 cell line).

- Long unique central region (LUR) with more than 90 ORFs and flanked by terminal repeats (TR) in variable number.

- Human tumor virus associated with KS, PEL and a subset of MCD and few other rare lymphoproliferative disorders.
Kaposi’s Sarcoma

Pathological Features of KS with 3 diagnostic criteria:
- vascular proliferation
- extravasated erythrocytes
- presence of spindle cells (“tumor cells”)

Expression of Latency-Associated Nuclear Antigen in Spindle Cells

Anti-LANA (ORF73)

Early stage in the fusiform-shaped cells forming the walls of the neo-formed, slit-like vessels,

Late stage (in spindle cells)

Idiopathic multiple pigmented sarcoma (KS), published in 1872

Moritz Kohn was born in Kaposvar, Hungary on 23 October, 1837. He matriculated at the University of Vienna in 1856 and graduated with the degrees of Doctor of Medicine in 1861 and Doctor of Surgery in 1862. His interest in dermatology began while working in the Department of Syphilology at the Allgemeines Krankenhaus and he transferred to Ferdinand von Hebra’s Department of Dermatology in 1866. Kohn changed from the Jewish to the Roman Catholic faith in 1869, changed his surname to Kaposi in 1871 and married von Hebra’s daughter. Among his many distinguished contributions to dermatology, Kaposi was the first to describe xeroderma pigmentosum and idiopathic multiple pigmented sarcoma. Moritz Kaposi died in 1902. (Ober W. Kaposi: The Man and the Sarcoma. In: Kaposi’s sarcoma: A Text and Atlas. 1988. Edited by GJ Gottlieb, AB Ackerman. Lea and Febiger Publishers, Philadelphia, USA.)
## Clinical and Epidemiological Forms of Kaposi’s Sarcoma

<table>
<thead>
<tr>
<th>Form</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classic (1872…)</strong></td>
<td>Elderly men of Eastern European or Mediterranean origin</td>
</tr>
<tr>
<td></td>
<td>- Slow chronic evolution</td>
</tr>
<tr>
<td></td>
<td>- Localized lesions</td>
</tr>
<tr>
<td></td>
<td>- Lower extremities</td>
</tr>
<tr>
<td><strong>Endemic (1914-1950..)</strong></td>
<td>Adults but also some children in Central, East and South Africa.</td>
</tr>
<tr>
<td></td>
<td>1) Slow</td>
</tr>
<tr>
<td></td>
<td>2) Aggressive disseminated lesions</td>
</tr>
<tr>
<td></td>
<td>3) Children form, lymph-node</td>
</tr>
<tr>
<td>**Immunosuppression-</td>
<td>Organ-transplant recipients</td>
</tr>
<tr>
<td>associated/iatrogenic (1970…)</td>
<td>1) Chronic</td>
</tr>
<tr>
<td></td>
<td>2) Rapide aggressive evolution</td>
</tr>
<tr>
<td></td>
<td>3) Regressive in some cases after removal of immunosuppression</td>
</tr>
<tr>
<td>**AIDS associated</td>
<td>Persons infected with HIV</td>
</tr>
<tr>
<td>Epidemic (1981….)</td>
<td>- Homosexual (Occident)</td>
</tr>
<tr>
<td></td>
<td>- Disseminated lesions</td>
</tr>
<tr>
<td></td>
<td>- Visceral mucosal involvement</td>
</tr>
<tr>
<td></td>
<td>- lymphnode</td>
</tr>
</tbody>
</table>
Clinical Aspects of KS

Endemic

Slow chronic evolution
- Localized lesions
- Lower extremities

HIV-Infected Patients: Epidemic Form.

Most frequent neoplasms in AIDS patients

Disseminated lesions

Classic

Mucosal involvement
Comparison of the HHV-8 viral loads in PBMCs, unaffected skin and tumor skin from patients with AIDS-associated KS from Central African Republic.

Quantification was performed using a real time PCR (Taqman) on a conserved region of ORF 26.
Geographical variations in relative frequency of Kaposi’s sarcoma among cancers in Africa before and after the AIDS epidemic

Kaposi Sarcoma represents one of the most frequent tumors in Africa, and 1% of all cancer world-wide.
Analysis of KSHV/HHV-8 Terminal Repeats as a Marker of Clonality in Kaposi’s Sarcoma

Duprez et al., JNCI, 2007
1) Clonality is a central issue in understanding cancers pathogenesis.

2) Regarding KS, the assessment of clonality has been difficult and studies based on the method of the pattern of X chromosome inactivation, specifically looking at the methylation status of the androgen receptor gene, have yielded conflicting results.

3) Regarding herpesviruses, the size of the EBV fused TR region has been used as a molecular marker for clonality (NPC, Burkitt’s lymphoma and some NHLs are EBV induced monoclonal tumors).

4) Our goals was to use a similar approach, studying the size heterogeneity of HHV-8/KSHV fused TR region as a clonality marker in a panel of KS.
KSHV/HHV-8 Terminal Repeats Southern Blot Analysis
(Pulse-Field Gel Electrophoresis used due to large number and large size of TR)

A) **Productive infection (lytic)**

- Linear genome
- TaqI
- TR
- K1
- K15
- 800 bp probe

\[ \Rightarrow \text{ladder of heterogeneous termini} \]

B) **Latent Infection**

- Episomal genome
- TaqI
- TR
- Left end
- Right end
- 800 bp

\[ \Rightarrow \text{homogeneous fused termini « monoclonal »} \]

\[ \Rightarrow \text{heterogeneous fused termini « Oligoclonal »} \]
Models for Herpesvirus Associated Tumor Pathogenesis and Clonality

1- **CLONAL EXPANSION MODEL**

- Infection of target cells
- Transformation
- Expansion of a single infected cell: clonal
- Tumour

Homogenous TR pattern

2- **« PASSENGER » MODEL**

- Infection of tumor cells
- Different viruses
- Different cells

Heterogenous TR pattern

Epidémiologie et Physiopathologie des Virus Oncogènes
Main features of the 139 samples from the four clinico-epidemiological forms of Kaposi Sarcoma studied

<table>
<thead>
<tr>
<th>Form of KS</th>
<th>Nb of patients</th>
<th>sex/ratio M/F</th>
<th>mean age (year)</th>
<th>age range</th>
<th>Nb of biopsies Skin</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS - KS</td>
<td>60</td>
<td>31/29</td>
<td>men : 37</td>
<td>9 - 54</td>
<td>56</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>women : 34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E - KS</td>
<td>10</td>
<td>10/0</td>
<td>69</td>
<td>42 - 86</td>
<td>28</td>
<td>1</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C - KS</td>
<td>21</td>
<td>16/5</td>
<td>men : 65</td>
<td>23 - 93</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>women : 69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I - KS</td>
<td>7</td>
<td>7/0</td>
<td>56</td>
<td>42 - 86</td>
<td>17</td>
<td>1</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>98</td>
<td>63/35</td>
<td>men : 50</td>
<td>19 - 93</td>
<td>127</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>women : 39</td>
<td></td>
<td></td>
<td>139</td>
</tr>
</tbody>
</table>
Quantification of HHV-8 load by Taqman assay according to the level of spindle cell infiltration in the studied biopsies

Anatomo-pathology classification
Level of spindle cell infiltration
HE coloration

LANA staining

HHV-8 viral load (copies/cell) Taqman for ORF26

60 informative samples with high level of infiltration by HHV-8 infected SC were studied for clonality.
Most of the highly infiltrated tumor KS lesions, whatever the epidemiological form, are either mono- or mostly oligo-clonal expansions of spindle cells latently infected by HHV-8.

A clonal HHV-8 pattern was obtained for 59/62 samples tested.

Eleven samples were found monoclonal and 48 oligoclonal.
We wanted to determine if multicentric (disseminated) KS lesions correspond to metastatic lesions or to expansions of independent clones.
### Multicentric Advanced Lesions of Kaposi Sarcoma

<table>
<thead>
<tr>
<th>ID11</th>
<th>ID73</th>
<th>ID78</th>
<th>ID85</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS12</td>
<td>KS13</td>
<td>KS98</td>
<td>KS90</td>
</tr>
<tr>
<td>KS96</td>
<td>KS97</td>
<td>KS14</td>
<td>KS12</td>
</tr>
<tr>
<td>KS98</td>
<td>KS99</td>
<td>KS15</td>
<td>KS13</td>
</tr>
<tr>
<td>KS108</td>
<td>KS109</td>
<td>KS105</td>
<td>KS14</td>
</tr>
<tr>
<td>KS107</td>
<td>KS122</td>
<td>KS123</td>
<td>KS124</td>
</tr>
<tr>
<td>KS126</td>
<td>KS125</td>
<td>KS126</td>
<td>KS126</td>
</tr>
</tbody>
</table>

26 lesions of disseminated KS from 6 patients were studied. Lesions were either monoclonal or oligoclonal. They harbor HHV-8 episomes of different sizes between samples.
Kaposi’s Sarcoma Clonality Studies Conclusion

1) True monoclonal expansions of HHV-8 infected SC are present in some KS lesions. HHV-8 was present prior to the expansion of a tumor clone of SC, sustaining thus strongly the etiological role of HHV-8 infection in SC proliferation \textit{in vivo}.

2) However, most of the advanced KS lesions appear to be rather oligoclinal expansions of HHV-8 infected cells.

3) Such herpesviral induced multigenotypic proliferation recalls EBV post-transplant lymphoproliferative diseases (PTLD) which can be polyclonal, oligoclonal or monoclonal in origin.

4) KS lesions, occurring at different anatomic sites in a given patient, generally harbor HHV-8 episomes of different sizes.

5) This suggests that \textbf{disseminated lesions represent multiple distinct primary expansions of HHV-8 infected SC (originating from different infectious events) rather than metastatic proliferations}.
KSHV/HHV-8 epidemiology

(Determined by presence of specific antibodies directed against latent and/or lytic antigens)

• KSHV/HHV-8 is **not a widespread ubiquitous virus.**

• It is mainly **restricted to areas of high endemcity for classic or endemic forms of KS.**

• KSHV/HHV-8 infection **is rare (<5%)** in the adult populations of **North America** and of the Northern and Western part of Europe.

• It is **more prevalent (5-20%)** in comparable populations of some **Mediterranean countries** such as Italy, Greece, Egypt, and Israel.

• KSHV/HHV-8 **is very frequent in Africa** especially in East / Central areas, where its seroprevalence reaches 70% among adults.
Known Geographical Repartition of KSHV/HHV-8 Seroprevalence

HHV-8 / KSHV seroprevalence in general population
- High 40-80 %
- Medium 10-30 %
- Low < 5 %

High KSHV/HHV8 seroprevalence foci (10-60 %) in the male homosexuel population

At least >> 300 millions of infected persons.
Transmission modes of KSHV/HHV8

• In non-endemic areas, among homosexual men, the HHV-8 transmission during sex has been established (penile oral intercourse with a man -> role of saliva?).

• Heterosexual transmission seems low.

• However, in endemic areas, the occurrence of KS in children as well as the high seroprevalence of HHV-8 infection in children in Central and East Africa indicate the existence of non sexual routes of HHV-8 transmission (role of saliva?).

• It can occur during organ transplantation.

• No clear evidence for important transmission through blood transfusion.
Seroprevalence of antibodies anti-HHV-8 according to age in 258 children and 189 pregnant women in Yaoundé (Cameroun).

Infection occurs during mainly in childhood in high endemic areas

From Gessain et al., Int. J. Cancer. 1999
Natural History of HHV-8 Infection in an Endemic Population: Evidence for Mother-Child and Sib-Sib Transmission: Study in French Guiana

A population based sero-epidemiological survey was conducted in a village of French Guiana including 1337 subjects from African origin aged from 2 to 91 years with reliable genealogical data.

All plasma were tested for HHV-8 specific IgG, using an IF assay (lytic antigens).

Risk factors and familial correlations for HHV-8 seropositivity were studied.
Age dependant KSHV/HHV-8 seroprevalence rate according to age in the Noir-Marrons population of MPA/PPI in French Guyana.

The overall HHV-8 seroprevalence was 13.2% with no difference according to sex. HHV-8 seropositivity was strongly age dependent: at 1.2% under 5 years, the HHV-8 seroprevalence rose up to a plateau around 15 % between 15 and 40 years and displayed an increase above 27% over 40 years.
### KSHV/HHV-8 Transmission Modes Studies

**Strong familial aggregation of KSHV/HHV8 seroprevalence**

<table>
<thead>
<tr>
<th>Type of pairs</th>
<th>Number of pairs</th>
<th>Total</th>
<th>Odds ratio† (95 % CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+,+</td>
<td>+,-</td>
<td>-,+</td>
<td>-,-</td>
</tr>
<tr>
<td>1 Father-mother</td>
<td>4</td>
<td>24</td>
<td>21</td>
<td>77</td>
</tr>
<tr>
<td>2 Father-child</td>
<td>12</td>
<td>92</td>
<td>39</td>
<td>286</td>
</tr>
<tr>
<td>3 Mother-child</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child &lt;10 ans</td>
<td>6</td>
<td>48</td>
<td>5</td>
<td>246</td>
</tr>
<tr>
<td>Child ≥10 ans</td>
<td>20</td>
<td>54</td>
<td>35</td>
<td>303</td>
</tr>
<tr>
<td>All children</td>
<td>26</td>
<td>102</td>
<td>40</td>
<td>549</td>
</tr>
<tr>
<td>4 Sib-Sib</td>
<td>38</td>
<td>123</td>
<td>74</td>
<td>905</td>
</tr>
</tbody>
</table>

-This pattern of familial aggregation, together with the variation of HHV8 seroprevalence with age indicate that in such an endemic populations, **HHV8 transmission mainly occurs from mother to child and between siblings during childhood and adolescence.**

-There is no evidence of heterosexual transmission in such a population (different to HTLV-1)
To investigate whether host genetic factors could explain in part these findings, a genetic epidemiological study with a segregation analysis was performed in 81 families (1623 subjects) living in this village.

- Evidence for a recessive major gene predisposing to HHV-8 infection in children (Inserm U550 L. Abel)

- Linkage studies with genetic markers are ongoing to locate this major gene (CNG)

- Another study is currently ongoing in Cameroon to replicate such findings
Genetic variability and molecular epidemiology of KSHV/HHV-8 (1)

• Initial studies, focused on 3 small gene fragments of the ORF 26 (minor capsid gene) and ORF 75 (tegument region) mainly from KS specimens were not very informative.

• Studies exploiting the higher level of genetic variability of the K1 gene were then developed.

• The ORF K1, located at the left-hand end of the genome, encodes for a transmembrane, highly glycosylated protein, that exhibits some similarities with the Ig receptor gene family.

870 bp with 2 highly variable regions VR1 and VR2
Genetic variability and molecular epidemiology of KSHV/HHV-8 (2)

Five major K1 molecular subtypes (called A, B, C, D and E) which appear linked to the geographical origin of the samples have been found.

• A and C are related to each other and are mostly found in Europe and emigrants.
• B strains are found in sub-saherian Africa.
• D strains are restricted to Pacific island heritage.
• E strains are present in Amerindians.

We performed studies on HHV-8 genetic variability to gain new insights into
1) The biodiversity of HHV-8 in different populations.
2) The search for specific KS risk for a given molecular subtype (E?).
3) Use it as a molecular mean to better understand population migrations.

Genetic variability of K1 gene
Genetic variability (subtypes) of K1 gene of KSHV/HHV-8 in Africa
(Net work of the Institut Pasteur)

Data based on complete or partial K1 genes

Number of studied samples
- 1 sample
- 2-4 samples
- 5-10 samples
- >10 samples

Black patients of African origin (Creole, Afro-american, Haitian) living in the America

10 B 3 A5 1 C 1 A

11 P 3 M

from:
Cook et al. AIDS 1999, 13 : 1165-1176
Lacoste et al. Virology 2000, 278, 60-74
Duprez et al. Virology 2006, 353,121-132

Epidémiologie et Physiopathologie des Virus Oncogènes
1) A first study of 38 KSHV/HHV-8 strains from Patients from Moscow (25 Classic, 6 Post-transplant, 7 AIDS KS). All were A/C with most of them being closely related in a specific A subgroup.

2) A second «Russian study» was performed in South Siberia in the Buryats Population

745 persons were studied (1995) IF latent using BC3 cells

High prevalence increasing with age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>[25-43]</td>
<td>5/60 (10.0)</td>
<td>23/167 (13.8)</td>
<td>28/217 (12.9)</td>
</tr>
<tr>
<td>[44-50]</td>
<td>9/60 (15.0)</td>
<td>26/112 (23.2)</td>
<td>35/172 (20.3)</td>
</tr>
<tr>
<td>[51-60]</td>
<td>17/69 (24.6)</td>
<td>22/104 (21.2)</td>
<td>39/173 (22.5)</td>
</tr>
<tr>
<td>[61-98]</td>
<td>35/77 (45.5)</td>
<td>50/106 (47.2)</td>
<td>85/183 (46.4)</td>
</tr>
<tr>
<td>Total</td>
<td>66/256 (25.8)</td>
<td>121/489 (24.7)</td>
<td>187/745 (25.1)</td>
</tr>
</tbody>
</table>

Search for the geographic source of HHV-8 D and E genotype

Epidémiologie et Physiopathologie des Virus Oncogènes

Cassar O. et al., Emerg.Inf. Dis., 2010 in press
18 HHV-8 strains - K1 gene - (red labeling) from Siberian individuals (Buryats) were analyzed together with 66 sequences from GenBank database (586 bp fragment)

No E or D subtype strain was found.

However most of the Siberian strain form a specific clade, suggesting a common origin.

We are planning to go more east, north and south to search the origin of E and D subtype.
Studies in Amerindians: 1) In French Guyana. (Kazanji M. et al., 2005)

Studies in Amerindians : 2) Kaposi Sarcoma in Peru :

(Collaborative studies between Eduardo Gottuzo team, Pathologists and our Unit in Paris).

Cassar et al., in preparation

Series of 45 parafin blocks of KS
with 25 HHV-8 strains detected
and partially studied.
Including A, B, C, and E subtypes
reflecting the great diversity of
the peopling of this country from
the Conquistadors (A/C) to the Indiens
(E) and the Black Slaves (B).
First E subtype in a KS.
Ongoing perspectives

**Oceania** with Melanesian vs Polynesian and populations of South East Asia.

**Central Africa** with Pygmées vs Bantous

**Futures studies will be multidisiplinary combining HHV-8 and HTLV-1 genetic variability with data based on human genetics as mitochondrial DNA and Y chromosome on the same specimens.**

**Ongoing collaboration with the team of Lluis Quintana-Murci at the Institut Pasteur**
E.P.V.O
Institut Pasteur
Calatitni S
Chevalier S A
Afonso P V
Tortevoye P
Duprez R
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Ozden S
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