KSHV and EBV Associated Germinotropic Lymphoproliferative Disorder

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Short title: KSHV associated germinotropic lymphoproliferative disorder

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Abstract

Kaposi’s sarcoma-associated herpesvirus (KSHV) is known to be associated with three distinct lymphoproliferative disorders: primary effusion lymphoma (PEL), multicentric Castleman’s disease (MCD) and MCD-associated plasmablastic lymphoma. We report three cases of a previously undescribed KSHV associated lymphoproliferative disorder. The disease presented as localized lymphadenopathy and showed a favorable response to chemotherapy or radiotherapy. Histologically, the lymphoproliferation is characterized by plasmablasts that preferentially involved germinal centers of the lymphoid follicles, forming confluent aggregates. They were negative for CD20, CD27, CD79a, CD138, BCL6 and CD10, but showed monotypic κ or λ light chain. Clusters of CD10+ CD20+ residual follicle center cells were identified in some of the follicles. The plasmablasts were positive for both KSHV and EBV, and most of them also expressed viral IL6. Unexpectedly, molecular analysis of whole tissue sections or microdissected KSHV positive aggregates demonstrated a poly- or oligoclonal pattern of Ig gene rearrangement. The plasmablasts showed somatic mutation and intraclonal variation in the rearranged Ig genes, and one case expressed switched Ig heavy chain (IgA), suggesting that they originated from germinal center B-cells. We propose calling this distinctive entity “KSHV associated germinotropic lymphoproliferative disorder”.
Introduction

Kaposi’s sarcoma-associated herpesvirus (KSHV) was initially identified from Kaposi’s sarcoma and plays a causative role in the genesis of this disease. KSHV is distantly related to another human gamma herpesvirus, EBV, and is also known as human herpesvirus 8 (HHV8). Like EBV, KSHV is lymphotropic and has been shown to be associated with three distinct lymphoproliferative disorders: primary effusion lymphoma (PEL), multicentric Castleman’s disease (MCD) and MCD-associated plasmablastic lymphoma.

PEL occurs in patients with human immunodeficiency virus (HIV) infection and involves primarily body cavities and occasionally extranodal sites. The lymphoma is composed of KSHV positive immunoblasts with plasmacytoid cytoplasm, which are also commonly co-infected by EBV. The tumor cells usually do not express immunoglobulin and B-cell markers, but express syndecan-1 (CD138) and harbor hypermutated rearranged immunoglobulin (Ig) genes, suggesting that they originate from germinal center or postgerminal center B-cells. In contrast, KSHV associated MCD occurs in patients with and without HIV infection and mainly involves lymph nodes and spleen. In patients with MCD, KSHV induces a range of lymphoproliferative lesions from polyclonal isolated plasmablasts in the mantle zone of B-cell follicles and microlymphoma to monoclonal microlymphoma and frank plasmablastic lymphoma. Irrespective of these different lesions, KSHV positive plasmablasts are monotypic, expressing exclusively IgM. They express high levels of cytoplasmic Ig and surface CD27, therefore resembling mature B-cells. However, they originate from naive B-cells as their rearranged Ig genes lack somatic mutation. Both KSHV associated PEL and MCD usually pursue an aggressive clinical course.
This report describes three cases of a previously undescribed KSHV associated lymphoproliferative disorder characterized by plasmablasts that are co-infected by KSHV and EBV, and preferentially involving the germinal centers of B-cell follicles.

**Materials and Methods**

**Case history:** The clinical features are summarized in Table 1. The patients presented with localized lymphadenopathy, and were otherwise healthy. Two patients tested negative for HIV, and they showed a favorable response to chemo- or radiotherapy.

**Histology and immunohistochemistry:** Histologic sections of the lymph node biopsies were reviewed. Immunostaining for KSHV LNA-1 (LANA) encoded by viral open reading frame (ORF) 73 and viral IL6 (vIL6) was carried out with rat monoclonal antibody LN53 and a rabbit polyclonal antibody respectively (Advanced Biotechnologies Incorporated, Columbia, U.S.A.) as described previously. Consecutive sections were stained with antibodies to Ig heavy chains α, δ, γ, and μ, and light chains κ and λ, CD10, CD20, CD21, CD27, CD30, CD38, CD79a, CD138 (Dako, High Wycombe, U.K.), BCL2, BCL6 (PharMingen) and Ki67 (Dako, High Wycombe, U.K.).

**DNA preparation and microdissection:** DNA samples were prepared from whole sections of formalin-fixed and paraffin-embedded tissues. To study KSHV positive cells, sections of lymph nodes were first stained for LNA-1. Confluent KSHV positive cells (1000-4000) from the same focus of consecutive sections were microdissected and pooled together. DNA was extracted as described previously.
PCR and sequence analysis of the rearranged Ig genes: To assess clonality, the rearranged Ig heavy chain and light chain (both κ and λ) genes were amplified from the framework 3 (FR3) to the joining (J) regions by PCR as described previously. For analysis of somatic hypermutation in the rearranged Ig genes, the region from FR2 to the J segment was amplified. FR2-JH PCR products were analyzed on 6% polyacrylamide gels, while FR3-JH products were examined on 10% polyacrylamide gels.

To study somatic mutation of the rearranged Ig genes, FR2-JH PCR products were cloned and sequenced. The variable (V), diversity (D) and joining (J) segments were identified by sequence comparison to the V Base using online DNAPLOT (MRC Center for Protein Engineering, http://www.mcr-cpe.cam.ac.uk/imt-doc/vbase-home-page.html).

EBER in situ hybridization: In situ hybridization was carried out with a PCR generated DNA probe labeled with digoxigenin, followed by incubation with anti-digoxigenin conjugated with alkaline phosphatase (Boehringer Mannheim, Germany) and visualization with BCIP and NBT.

Results and Discussion

All three cases presented as localized lymphadenopathy and none had a history of Kaposi’s sarcoma, immunodeficiency or immunosuppression. Two cases were treated by chemo- or radiotherapy and responded favorably, one in complete remission for 7 years and the other for 15 years.

Lymph node biopsies from all three cases showed similar histologic features. Overall architecture of the lymph node was preserved. In some of the follicles, the germinal centers were replaced partially or completely by large cells with a moderate amount of amphophilic
cytoplasm and large eccentric vesicular nuclei containing 1 or 2 prominent nucleoli. Most of
them were similar to the plasmablasts described in KSHV associated MCD, others showed
more bizarre anaplastic features (Figure 1A). These plasmablasts occurred as clusters and
often coalesced to form confluent aggregates, comparable to the KSHV positive
microlymphomas in MCD. Clusters of CD10+ CD20+ residual follicle center cells were
identified in some of the involved follicles. Plasmablasts were found neither in the mantle
zones, which appeared to be normal, nor in the interfollicular zone. However, mantle zone B-
cells were seen encroaching into the follicle centers in the involved follicles, reminiscent of
progressive transformation of germinal center. Uninvolved lymphoid follicles had reactive
follicle centers and did not exhibit features of Castleman’s disease. The interfollicular zone
showed prominent plasmacytosis.

All plasmablasts showed stippled nuclear staining for KSHV LNA-1 and were co-infected by
EBV as revealed by EBER in situ hybridization (Figure 1A). The vast majority of KSHV
positive plasmabalsts expressed vIL6. They were CD20+, CD27+, CD30++, CD38++, CD79a−,
CD138−, BCL6−, CD10−, BCL2−, and expressed monotypic Ig light chain, λ in two cases and κ
in one (Figure 1A). Plasmablasts in cases 1 and 2 expressed IgM/D and IgA respectively
(Figure 1A), while case 3 failed to show immunostaining for heavy chains. The majority of
KSHV positive plasmablasts were Ki67+, indicating that they were in cell cycle. CD21
staining showed that KSHV positive cells occurred within meshworks of follicular dendritic
cells (Figure 1A).

Despite the expression of monotypic Ig light chain by KSHV positive plasmablasts, FR3-JH,
FR3-Jκ and FR3-Jλ. PCR of DNA samples from whole tissue sections showed a weak
dominant band in a polyclonal background in case 1 but a polyclonal pattern in case 3 (Figure
1B). On both Ig heavy and light chain gene PCR analysis of microdissected KSHV positive
cell aggregates, an oligoclonal pattern was observed in case 1, and a polyclonal pattern was observed in case 3 (Figure 1A). Thus, despite being monotypic in terms of Ig light chain expression, these KSHV positive plasmablasts are genotypically polyclonal. PCR analysis of case 2 failed because of poor DNA quality.

To understand the cell of origin of KSHV positive plasmablasts, the microdissected KSHV positive cells were subjected to FR2-JH PCR to analyze somatic mutation of the rearranged VH gene. Successful PCR was achieved only in case 1 and which showed 2-3 dominant bands. Cloning and sequencing of the PCR products confirmed the presence of oligoclonal B-cells in each of the two foci examined and showed that different foci contained distinct clones despite being from the same tissue section and showing identical light chain restriction (Table 2). Furthermore, the rearranged VH region in all clones harbored somatic hypermutation and clone DP88 from focus 1 showed intraclonal variation. Thus, although the KSHV positive plasmablasts in this case express both IgM and IgD, they most likely originated from germinal center B-cells. This may represent a general feature of this lymphoma entity since case 2 expressed IgA, indicating the plasmablasts had undergone switch recombination, which occurs after somatic mutation during the germinal center reaction.

Based on the above findings, we propose calling this lymphoproliferative disorder “KSHV associated germinotropic lymphoproliferative disorder (GLD).” We selected this term because the lymphoproliferation is polyclonal and individual KSHV positive foci have potential to develop into monoclonal microlymphoma or even frank lymphoma, reminiscent of EBV associated post-transplant lymphoproliferative disorder and KSHV related MCD. Lymphoma with marked tropism for germinal centers has been previously described in three cases of large B-cell lymphoma of the mediastinum. These lymphomas show similar
clinical presentation and histological features to those of KSHV associated GLD, but express CD20 and are therefore unlikely to be KSHV associated GLD although KSHV and EBV infection were not investigated.13

There are similarities as well as important differences between KSHV associated GLD, PEL and MCD-associated plasmablastic lymphoma (Table 3). KSHV associated GLD occurs in immune competent individuals, presents as localized lymphadenopathy and responds favorably to therapy. In contrast, PEL and MCD predominantly occur in immunodeficient patients, commonly pursue an aggressive clinical course and respond poorly to current treatments. KSHV associated GLD differs from MCD in that the plasmablasts are co-infected by EBV, may express any heavy or light chain, preferentially invade germinal centers, and harbor mutated Ig gene.

Although the exact role of viral infection in the pathogenesis of KSHV associated GLD is unclear, it is noteworthy that vIL6 is expressed by the vast majority of the KSHV+ cells, which is much higher than that in PEL and KSHV associated MCD (10-20%).7,14 Activation of IL6 receptor signaling through vIL6 and human IL6 may play an important role in this disease as in PEL and KSHV associated MCD.7,15,16 Nonetheless, the pathogenic potential seems to be constrained by a competent immune system. Despite the co-infection by EBV and KSHV, the GLD presents as a localized disease and responds favorably to conventional therapy.
Figure legends:

Figure 1A: Histological and immunophenotypic features of KSHV associated GLD in case 1: The germinal centers are replaced by large cells with a moderate amount of amphophilic cytoplasm and a large vesicular nucleus containing 1 or 2 prominent nucleoli. These large cells coalesce and form confluent aggregates. They express high levels of cytoplasmic Ig (monotypic Ig\(^\lambda\), IgM/D) and are therefore termed as plasmablasts. Plasmablasts are positive for both KSHV and EBV and most of them express vIL6. CD21 staining shows that KSHV positive cells occur within meshworks of follicular dendritic cells.

1B: Clonality analysis of KSHV associated GLD in case 1: FR3 JH PCR analysis of DNA samples from whole tissue sections shows a weak dominant band in a polyclonal background. FR2-JH PCR analysis of microdissected KSHV positive foci (A1 and A2) shows an oligoclonal pattern.
Table 1: Clinical, histological, immunophenotypic and genotypic features of KSHV associated GLD.

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>61</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>Axillary (6 x 6 x 6 cm) and cervical lymph node enlargement for 6 years; Staging revealed right perirenal lymphadenopathy; Bone marrow trephine and aspirate were normal.</td>
<td>Submandibular and inguinal lymph node enlargement for 4 years; Staging showed slightly enlarged spleen and right “paratracheal” lymphadenopathy;</td>
<td>Presented with left leg swelling and paraesthesia. Enlarged para-aortic lymph node (5 x 4 x 3.5 cm).</td>
</tr>
<tr>
<td>Viral serology</td>
<td>KSHV+, EBV+, HCV+, HIV–.</td>
<td>KSHV+, EBV+ HIV–.</td>
<td>Not available</td>
</tr>
<tr>
<td>Treatment and outcome</td>
<td>6 cycles of CHOP, complete remission for 7 years</td>
<td>Surgical excision and radiotherapy, complete remission for 15 years.</td>
<td>New case</td>
</tr>
<tr>
<td>Ig light chain</td>
<td>λ</td>
<td>λ</td>
<td>κ</td>
</tr>
<tr>
<td>Ig heavy chain</td>
<td>IgM* D⁺</td>
<td>IgA⁺</td>
<td>IgM⁺, IgD⁺, IgG⁺, IgA⁻</td>
</tr>
<tr>
<td>Clonality</td>
<td>Whole tissue section</td>
<td>Weak dominant band in a polyclonal background</td>
<td>PCR failed</td>
</tr>
<tr>
<td>KSHV+ aggregate</td>
<td>Oligoclonal</td>
<td>PCR failed</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>VH gene mutation</td>
<td>Yes</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>
Table 2: PCR and sequence analysis of the rearranged IgH gene of KSHV positive foci in case 1

<table>
<thead>
<tr>
<th>Lesion</th>
<th>No. of clones</th>
<th>Clones with identical VH and CDR3</th>
<th>Mutation in VH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focus 1</td>
<td>12</td>
<td>4 (DP77); 7 (DP88); 1 (Yac7)</td>
<td>5R, 3s; 2R, 2s; 4R</td>
</tr>
<tr>
<td>Focus 2</td>
<td>4</td>
<td>3 (DP58); 1 (DP63)</td>
<td>2R, 4R, 2s</td>
</tr>
</tbody>
</table>

*R: replacement mutation; s: silent mutation.
Table 3: Comparison of KSHV associated lymphoproliferative disorders.

<table>
<thead>
<tr>
<th></th>
<th>PEL</th>
<th>MCD and associated plasmablastic lymphoma</th>
<th>GLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical presentation</td>
<td>In immunodeficient patients, with systemic symptoms, poor prognosis</td>
<td>Predominantly in immunodeficient patients, with systemic symptoms, poor prognosis</td>
<td>In immune competent patients with localized lymphadenopathy, favorable response to therapy</td>
</tr>
<tr>
<td>Sites</td>
<td>Body cavities, extranodal sites</td>
<td>Lymph nodes, spleen</td>
<td>Lymph nodes</td>
</tr>
<tr>
<td>Morphology</td>
<td>Immunoblasts with pleomorphic nuclei and abundant plasmacytoid cytoplasm</td>
<td>Plasmablasts, preferentially residing in the mantle zone</td>
<td>Plasmablasts, preferentially invading germinal centers</td>
</tr>
<tr>
<td>EBV</td>
<td>Present in HIV associated patients; absent in HIV negative patients</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Cytoplasmic Ig expression</td>
<td>Absent</td>
<td>High, always IgM</td>
<td>High, any heavy chain</td>
</tr>
<tr>
<td>Ig light chain mRNA</td>
<td>Monotypic κ or λ.</td>
<td>Monotypic λ.</td>
<td>Monotypic κ or λ.</td>
</tr>
<tr>
<td>CD30</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Variable</td>
</tr>
<tr>
<td>B-cell antigens</td>
<td>Absent</td>
<td>Weak or absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Mutation in Ig genes</td>
<td>Mutated in majority</td>
<td>Absent</td>
<td>Mutated</td>
</tr>
<tr>
<td>Cellular origin</td>
<td>Germinal centre or post germinal center B-cells</td>
<td>Naive IgMλ expressing B-cells</td>
<td>Germinal center B-cells</td>
</tr>
</tbody>
</table>
References


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