Kaposi sarcoma–associated herpesvirus infects monotypic (IgMλ) but polyclonal naive B cells in Castleman disease and associated lymphoproliferative disorders

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In a previous study, it was shown that the Kaposi sarcoma–associated herpesvirus (KSHV) was specifically associated with monotypic (IgMλ) plasmablasts in multicentric Castleman disease (MCD). The plasmablasts occur as isolated cells in the mantle zone of B-cell follicles but may form microlymphoma or frank plasmablastic lymphoma. To determine the clonality and cellular origin of the monotypic plasmablasts, the rearranged *Ig* genes in 13 patients with KSHV-related MCD, including 8 cases with microlymphomas and 2 with frank lymphomas, were studied. To investigate the role of the interleukin 6 (IL-6) receptor signaling in the pathogenesis of MCD and associated lymphoproliferative disorders, viral IL-6 and human IL-6 receptor expression was examined. KSHV-positive plasmablasts were polyclonal in MCD-involved lymphoid tissues in all cases and microlymphomas in 6 of 8 cases. Monoclonal KSHV-positive plasmablasts were seen in microlymphomas of 2 cases and in both frank lymphomas. Despite their mature phenotype, KSHV-positive plasmablasts did not harbor somatic mutations in the rearranged *Ig* genes, indicating origination from naive B cells. Viral IL-6 was expressed in 10% to 15% of KSHV-positive plasmablasts, whereas the human IL-6 receptor was expressed in most KSHV-positive cells. Thus, KSHV infects monotypic but polyclonal naive B cells and is associated with a range of lymphoproliferative disorders from polyclonal isolated plasmablasts and microlymphomas to monoclonal microlymphoma and frank plasmablastic lymphomas in MCD patients. Activation of the IL-6 receptor signaling pathway may play a role in differentiation of KSHV-infected naive B cells into plasmablasts and development of lymphoproliferative lesions. (Blood. 2001;97:2130-2136)

Introduction

Multicentric plasma cell variant Castleman disease (MCD) is a lymphoproliferative disorder of unknown etiology and is associated with development of secondary B-cell lymphoma.[1-4] Most cases of MCD are associated with infection by Kaposi sarcoma–associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8), that occurs in nearly 100% of human immunodeficiency virus (HIV)-associated cases and 40% to 50% of HIV-negative cases.[5-8] Using a monoclonal antibody against a KSHV latent nuclear antigen (LNA), we recently showed that KSHV is specifically associated with plasmablasts that localize mainly in the mantle zone of B-cell follicles.[9] These KSHV-positive plasmablasts express high levels of cytoplasmic IgM and show exclusively λ light-chain restriction. In contrast, KSHV-negative mature plasma cells in the interfollicular region, a prominent feature of MCD, are IgM negative and usually polytypic. The KSHV-positive plasmablasts in MCD typically occur as isolated cells but may coalesce to form microscopic aggregates that show λ light-chain restriction and therefore have been referred to as microlymphomas.[9] In some cases, KSHV-positive plasmablasts may form frank plasmablastic lymphoma.[9] In all lesions in 35 patients studied so far, KSHV-positive plasmablasts uniformly express IgMλ.

The finding that KSHV-positive plasmablasts in MCD are monotypic is intriguing and raises the question whether this is indicative of monoclonality or biased targeting of λ-expressing B cells by the virus. We previously performed polymerase chain reaction (PCR) analysis of the rearranged *Ig* heavy-chain genes and found monoclonal patterns in 2 of 3 KSHV-associated plasmablastic lymphomas, but polyclonal patterns in microdissected microlymphomas and unfractionated lymphocytes from MCD-involved lymphoid tissue.[9] It is unclear whether the failure to demonstrate monoclonality in these monotypic lesions was due to somatic mutations in the primer target region of the rearranged *Ig* genes, which may prevent the primer annealing and therefore PCR amplification. The clonal relationships among frank plasmablastic lymphoma, microlymphomas, and scattered individual plasmablasts as well as their cellular origins remain unknown. To address these questions, we studied both the rearranged *Ig* heavy- and light-chain genes of KSHV-positive plasmablasts by PCR, cloning, and sequencing in 13 patients with KSHV-associated MCD. To understand the role of KSHV in the pathogenesis of MCD and associated lymphoproliferative lesions, we also studied viral interleukin 6 (vIL-6) and human IL-6 receptor (hIL-6R) expression

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because the IL-6R signaling appears to be important in the pathogenesis of MCD.[10-13]

Patients, materials, and methods

Patients and tissue samples

Tissue from 13 patients with KSHV-positive MCD was retrieved from the surgical files of the Department of Histopathology, Royal Free and University College Medical School, London; the Department of Pathology, Höpital Cochin, Paris; and the Department of Pathology, Hopital Saint-Louis, Paris. Eleven patients were HIV positive and 2 were negative (Table 1). Two patients had developed plasmablastic lymphoma. Formalin-fixed and paraffin-embedded tissue was available in all cases (lymph nodes, n = 13; spleen, n = 6). In one case complicated by plasmablastic lymphoma, frozen nodal lymphoma and splenic tissues were available. Standard hematoxylin/eosin-stained sections were prepared from all samples and the histology was reviewed.

Immunohistochemistry

Immunostaining for KSHV LNA-1 (LANA) encoded by viral open reading frame (ORF) 73 was carried out with mouse monoclonal antibody LN53 (Advanced Biotechnologies, Columbia, MD) using the streptavidin-biotin method preceded by heat retrieval of antigen as described previously.[9] LN53 can detect 100% KSHV-positive cells under these conditions. The vIL-6 was stained with a rabbit polyclonal antibody (Advanced Biotechnologies) using similar methods. The hIL-6R was immunostained on frozen sections with a rabbit polyclonal antibody (R & D Systems Europe, Abingdon, UK). Consecutive sections were stained with antibodies to Ig heavy chains μ, α, and γ, and light chains κ and λ (Dako, High Wycombe, UK) and with antibody to CD27 (Pharmingen, San Diego, CA). In some cases where indicated, double staining for both KSHV LNA and CD27, vIL-6, or hIL-6R was carried out.

DNA preparation and microdissection

In each case, DNA samples were prepared from whole sections of formalin-fixed and paraffin-embedded tissues. To study KSHV-positive cells, sections of lymph nodes and spleens were first stained for LNA-1. Confluent KSHV-positive cells were microdissected and DNA was extracted as described previously.[14]

PCR of the rearranged *Ig* genes

To assess clonality, both the rearranged *Ig* heavy-chain and λ light-chain genes were amplified from the framework 3 (Fr3) to the joining (J) regions. For analysis of somatic hypermutation in the rearranged *Ig* genes, the regions from Fr1 or Fr2 to the J segment of the heavy-chain and λ light-chain genes were amplified.

For *Ig* heavy-chain gene PCR, the Fr1-JH region was amplified using individual Fr1 family-specific (VH1-VH6) and JH consensus primers; the Fr2-JH and Fr3-JH regions were amplified with consensus primers using seminested protocols as described previously.[15,16] The rearranged *Ig* λ light-chain gene was amplified with novel primers designed according to the consensus sequences of individual light-chain gene families (Table 2). The Fr1-Jλ region was amplified using mixed VL family-specific (VL1, VL2, VL3, VL4ab, VL5-9-10, VL6, and VL7-8) and Jλ primers (JL1 or JL2-3-7) in 2 separate reactions; the Fr3-Jλ region was amplified using consensus Fr3 (VLFR3) and Jλ primers (JL1 or JL2-3-7) in 2 separate reactions (Table 2). For Fr1-Jλ PCR, a hot-start touchdown protocol was used as follows: 95°C for 2 minutes, hold and add Taq polymerase at 65°C, 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 45 seconds for one cycle, then similar cycles with annealing temperature at 64°C for 2, 62°C for 3, 60°C for 4, 58°C for 5, and 55°C for 40 cycles with a final extension at 72°C for 5 minutes. For Fr3-Jλ PCR a hot-start protocol was used as follows: 95°C for 5 minutes, hold and add Taq polymerase at 58°C, 93°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute for 40 cycles, final extension at 72°C for 5 minutes. In each PCR, standard reaction conditions were applied with 1.5 mM $MgCl_2$. Fr3-Jλ PCR for clonality analysis was shown to be reliable on a range of neoplastic and reactive lymphoid samples in a separate study.[17]

All PCRs were performed with appropriate positive and negative (without template DNA) controls in each experiment and all samples were analyzed in duplicate. Fr1-JH, Fr2-JH, and Fr1-Jλ PCR products were analyzed on 6% polyacrylamide gels; Fr3-JH and Fr3-Jλ were examined on 10% polyacrylamide gels. Fr3-Jλ PCR products were first denatured at

Table 1. Histologic, immunophenotypic, and PCR findings in KSHV MCD

Case no.	HIV	EBV	Diagnosis and KSHV-associated lesions	Plasmablasts	KSHV LNA	KSHV* vIL6	Plasmablasts Ig	Fr3-JH	Fr3-Jλ
			MCD					P	P
1	−	−	Microlymphoma (Sp)	+	+	+	IgMλ	M	M
			Plasmablastic lymphoma (LN)					M	M
			MCD					P	P
2	+	−	Microlymphoma (Sp)	+	+	+	IgMλ	P	P
			MCD					P	P
3	−	−	Microlymphoma (LN)	+	+	+	IgMλ	P	P
			MCD					P	P
4	+	−	Microlymphoma (Sp)	+	+	+	IgMλ	M	M
			MCD					P	P
5	+	−	Microlymphoma (Sp)	+	+	+	IgMλ	P	P
			Plasmblastic lymphoma (LN)					M	P
			MCD					P	P
6	+	−	Microlymphoma (Sp)	+	+	ND	IgMλ	P	P
			MCD					P	P
7	+	−	Microlymphoma (Sp)	+	+	ND	IgMλ	P	ND
			MCD					ND	ND
8	+	−	Microlymphoma (Sp)	+	+	ND	IgMλ	ND	ND
			MCD					P	ND
9	+	−	Microlymphoma (LN)	+	+	+	IgMλ	P	ND
10	+	−	MCD	+	+	+	IgMλ	P	ND
11	+	−	MCD	+	+	ND	IgMλ	P	P
12	+	−	MCD	+	+	+	IgMλ	P	P
13	+	−	MCD	+	+	ND	IgMλ	P	ND

MCD indicates multicentric Castleman disease; LN, lymph node; Sp, spleen; P, polyclonal; M, monoclonal; ND, not done.
*KSHV vIL-6 was expressed in 10% to 15% of KSHV-positive plasmablasts.

Table 2. Primers used for Ig λ light-chain PCR

Target region	Primer*	
	Name	Sequence
	VL1	5′ CAGTCTGTGCTGAC(T/G)CAGCC 3′
	VL2	5′ CAGTCTGCCCTGACTCAGCC 3′
	VL3	5′ TCCTATGAGCTGAC(A/T)CAG 3′
Fr1	VL4ab	5′ CAGCCTGTGCTGACTCAATC 3′
	VL5-9-10	5′ CAG(C/G)CTGTGCTGACTCAGCC 3′
	VL6	5′ AATTTTATGCTGACTCAGCC 3′
	VL7-8	5′ CAG(A/G)CTGTGGTGACTCAGGA 3′
Fr3	FR3	5′ GA(T/C)GAGGCTGA(T/C)TATTACTG 3′
Jλ	JL1	5′ AGGACGGTGACCTTGGTCCC 3′
	JL2-3-7	5′ AGGACGGTCAGCT(G/T)GGT(C/G)CC 3′

*Primers were designed according to the consensus sequences of family members of individual light-chain genes.

95°C for 5 minutes then cooled on ice for at least 45 minutes (heteroduplex analysis) prior to electrophoresis.

Cloning and sequencing of PCR products

To study somatic mutation of the rearranged *Ig* genes, the Fr1-JH or λ and Fr2-JH PCR products were cloned and sequenced. PCR products were purified from 1.5% agarose gels using QIA Quick Gel Extraction Kit (QIAGEN, West Sussex, UK), then ligated into the pGEM-T vector and transformed into JM109 competent cells (Promega, Southampton, UK). The transformed cells were selected on LB-ampicillin agar plates containing X-gal and IPTG. White colonies were screened using PCR with vector primers (Sp6 and T7). The PCR products showing the expected insert size were sequenced in both directions using an ABI sequencer with dRhodamine terminators (Perkin-Elmer, Foster City, CA). At least 7 PCR clones from each sample were 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).

Epstein Barr virus in situ hybridization

In situ hybridization for Epstein Barr Encoded small RNA (EBER) was carried out with a PCR-generated DNA probe labeled with digoxigenin, followed by incubation with Anti-Digoxingenin-AP (Boehringer Mannheim, Lewes, UK) and visualization with 5-Bromo-4-chloro-3-indolylphosphate and 4-Nitro blue tetrazolium chloride, as described previously.[18]

Results

KSHV is specifically associated with monotypic (IgMλ) plasmablasts

All 13 cases of KSHV-positive MCD showed classic histologic features of MCD including variably hyalinized follicle centers, expanded mantle zones, and prominent infiltration of IgM-negative polytypic plasma cells in the interfollicular region.[9] In addition to these classic features, the mantle zone of all cases contained plasmablasts that were positive for KSHV, expressed high levels of cytoplasmic IgM, and showed exclusively λ light-chain restriction.[9] The number of plasmablasts (all KSHV positive) ranged from 1% to 15% of the total cell population in lymph nodes and spleens affected. In 8 cases, KSHV-positive plasmablasts coalesced to form microscopic lymphomas either adjacent to or partially replacing lymphoid follicles. In common with the isolated plasmablasts in the mantle zone, these microlymphomas exclusively expressed high levels of cytoplasmic IgMλ as shown previously.[9] The 2 plasmablastic lymphomas were composed of confluent sheets of plasmablasts with identical immunophenotypic features to those of KSHV-positive mantle zone plasmablasts and microlymphomas (Figure

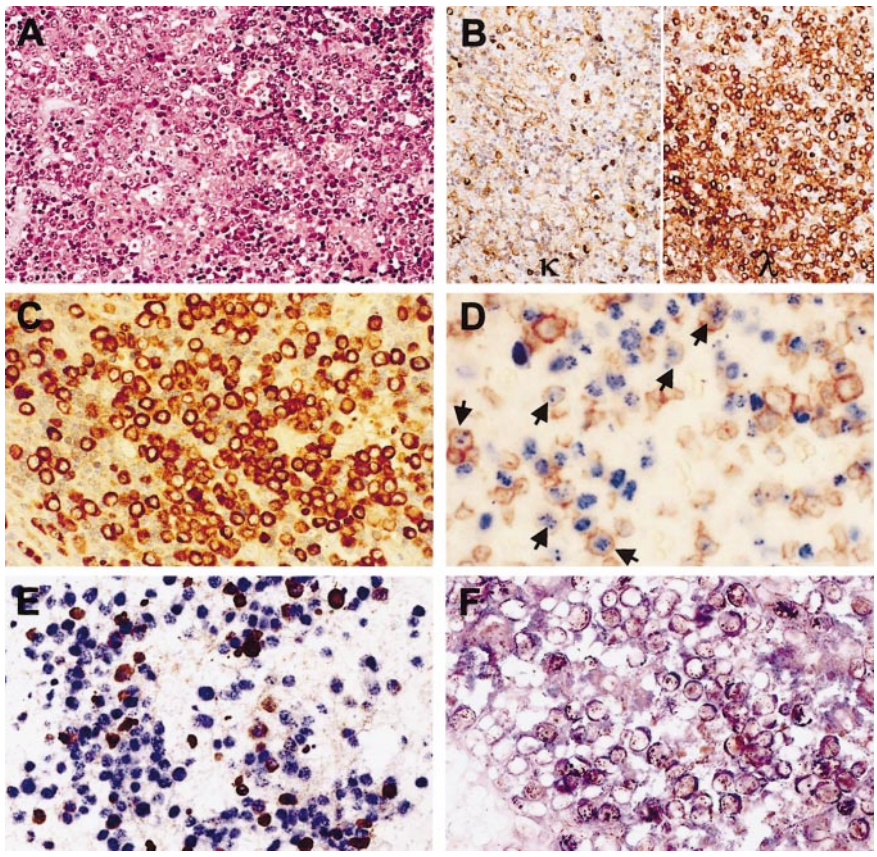

Figure 1. Histologic and immunohistochemical study of KSHV-positive plasmablasts in lymphoproliferative lesions in case 1. Plasmablastic lymphomas show confluent sheets of plasmablasts (A, hematoxylin-eosin) that express exclusively λ light chain (B) and high levels of cytoplasmic IgM (C). CD27 (brown), a marker for mature B cells, is expressed in 20% to 30% of KSHV-positive (blue) plasmablasts (D). Double staining shows that vIL-6 (brown) is strongly expressed in 10% to 15% KSHV-positive (blue) plasmablasts (E); hIL6-R (blue) is expressed in most KSHV-positive (brown) cells (F).

1A-C). Thus, KSHV-positive plasmablasts were consistently monotypic (IgMλ) in all lesions in all patients (Table 1).

Double immunohistochemical staining was carried out in cases 1, 6, and 9 for KSHV LNA-1 and CD27, a cell surface marker for memory B cells.[19,20] CD27 was expressed in 20% to 30% of the KSHV-positive plasmablasts (Figure 1D). The level of CD27 expression in these plasmablasts was similar to that in the KSHV-negative plasma cells in the interfollicular zones (Figure 1D).

Monotypic KSHV-positive plasmablasts in MCD and associated lymphomas are polyclonal or monoclonal and derive from naive B cells

Analysis by PCR of unfractionated lymphocytes from whole sections of MCD-involved lymphoid tissues consistently showed polyclonal patterns despite the presence of prominent monotypic KSHV-positive plasmablasts, which were well above the minimal requirement (1%) for demonstrating monoclonality by these PCR-based methods (Table 1, Figure 2).[21] Microdissected microlymphomas in 6 of 8 cases also displayed polyclonal patterns, but the remaining 2 cases (1 and 4) exhibited a weak dominant band (Table 1, Figure 2). Both plasmablastic lymphomas showed monoclonal patterns with Fr3-JH PCR (Table 1, Figure 2), but only 1 of the 2 plasmablastic lymphomas displayed a monoclonal pattern with Fr3-Jλ PCR (Table 1).

Cloning and sequencing of Fr1-JH (case 1) and Fr2-JH (cases 2-4) PCR products confirmed the presence of a dominant clonal cell population in the plasmablastic lymphoma and microlymphomas in cases that were monoclonal by Fr3-JH and Fr3-Jλ PCR analysis (Table 3). For example, in case 1, 5 of the 8 IgH clones sequenced from the frank plasmablastic lymphoma of lymph node were identical, and 4 of 8 and 2 of 9 IgH clones from 2 microlymphomas of the spleen, respectively, were identical (Table 3). However, the dominant clones of the 2 microlymphomas were different from each other and were unrelated to the clones, including the dominant clone, detected in the plasmablastic lymphoma of the same case. Interestingly, the plasmablastic lymphoma clone was present at a low frequency in the microlymphomas, suggesting that KSHV-positive plasmablasts had circulated. Similar results were seen from cloning and sequencing analysis of Fr1-Jλ PCR products of the plasmablastic lymphoma and microlymphoma in case 1 (Table 4).

In microlymphomas in which a polyclonal pattern was seen by Fr3-JH and Fr3-Jλ PCR analysis, sequencing of Fr2-JH PCR products revealed either multiple unrelated clones or multiple clones with one weakly dominant (Table 3). For instance, in case 2, all 8 IgH clones from microlymphoma 3 were different, whereas 2 of 10 clones from microlymphoma 2 were identical and the remaining 8 clones were unrelated. More importantly, different microlymphomas of the same case always showed different clones.

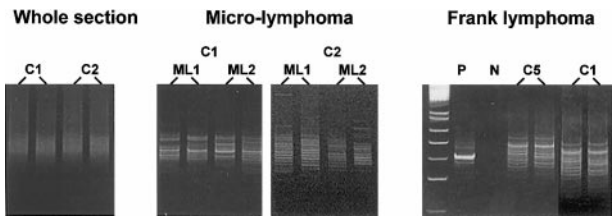

Figure 2. Fr3-JH PCR analysis of KSHV-positive plasmablasts in MCD, microlymphoma, and frank plasmablastic lymphoma. Whole sections of MCD-involved spleen show polyclonal patterns; microdissected microlymphomas display polyclonal (C2) or monoclonal (C1) patterns; frank plasmablastic lymphomas show monoclonal patterns. C1, C2, and C5 indicate different cases; P, positive control; N, negative control; ML, microlymphoma.

Thus, monotypic KSHV-positive plasmablasts in MCD were polyclonal although KSHV-positive microlymphomas sometimes contained a weak dominant clone.

To determine the cellular origin of KSHV-positive plasmablasts, their rearranged *Ig* V_H genes were compared with V_H germlines of the V base. Fewer than 2 mutations were consistently found in KSHV-positive plasmablasts irrespective of whether they were from microlymphoma or frank lymphoma (Table 3). Most mutations were localized outside the CDR regions and were silent changes. The frequency of these mutations (0.1%-0.4%) was indistinguishable from the estimated replication error rate (0.2%) during PCR (with *Taq* polymerase) and cloning.[22] Moreover, analysis of the rearranged λ light-chain genes from the frank plasmablastic lymphoma and one microlymphoma in case 1 showed similar results to those seen from V_H (Table 4). The data indicate that KSHV-positive plasmablasts originated from naive B cells despite their mature phenotype.

There was no apparently biased usage of a particular V_H gene family member by KSHV-positive plasmablasts. However, some V_H germlines were recurrently used, at a low frequency, by multiple clones. In case 1, the V_H germline DP75 used by the dominant clone of plasmablastic lymphoma was also found in 4 of 16 unrelated clones (Table 3). Similarly, DP47 was found in 4 of 26 and 4 of 17 clones in cases 2 and 3, respectively (Table 3). There was also no biased usage of D segments by KSHV-positive plasmablasts. J_H4, J_H5, and J_H6 were more commonly used than J_H1-3.

Analysis of Vλ germline usage in the plasmablastic and one microlymphoma in case 1 showed similar results (Table 4).

Expression of vIL-6 and hIL-6R by KSHV-positive plasmablasts

The KSHV vIL-6 was immunostained on paraffin-embedded sections in 8 cases (Table 1) and was expressed in both plasmablasts and cells undergoing apoptosis, which account for approximately 5% to 10% of the cells positive for vIL-6. Double immunostaining showed that vIL-6 was highly expressed in only 10% to 15% of the KSHV-positive cells (Figure 1E).

The hIL-6R was immunostained on frozen tissue sections in case 1. In contrast to vIL-6 expression, hIL-6R was moderately or strongly expressed by the vast majority of KSHV-positive plasmablasts (Figure 1F). In addition, hIL-6R was also expressed by KSHV-negative plasma cells.

EBV in situ hybridization

EBER was positive in occasional scattered lymphocytes, but the KSHV-positive plasmablasts in all cases were EBER negative.

Discussion

Our initial observation of monotypic KSHV-positive plasmablasts in MCD and associated microlymphomas and frank plasmablastic lymphomas was interpreted as evidence for monoclonality.[9] The present findings, however, indicate that KSHV infection invokes a monotypic but polyclonal B-cell proliferation in MCD patients and that in some cases KSHV-positive plasmablasts may form polyclonal or monoclonal microlymphomas or develop into frank monoclonal lymphomas. These events are similar to those in lymphoproliferative disorders caused by EBV in immunosuppressed patients.[23,24] EBV is another lymphotropic human γ-herpesvirus that induces a morphologically heterogeneous group of lymphoproliferative lesions including polyclonal plasmacytic hyperplasia and monoclonal polymorphic and monomorphic B-cell

Table 3. Sequence analysis of the rearranged *IgH* gene in KSHV-positive cells

Case no.	Lesion	Total no. of clones	Clones with identical V_H	Clones with identical CDR3	Mutation in V_H
1	Lymphoma (LN)	8	8 (DP75)	5	1s
	Microlymphoma 1 (Sp)	8	5 (DP14)	4	1s
			1 (DP75)	Identical to the dominant lymphoma clone	1s
			4 (DP10)	2	1R
	Microlymphoma 2 (Sp)	9	3 (DP14)		1s
			1 (DP75)	Identical to the dominant lymphoma clone	1s
			2 (V3-64)	2	—
			2 (DP77)	2	1R
			1 (DP49)		—
	Microlymphoma 1 (Sp)	8	1 (DP47)		2R
			1 (V3-53)		1R
			1 (Cos-8)		—
			3 (DP47)	2	—
			2 (DP77)		—
2	Microlymphoma 2 (Sp)	10	2 (DP38)		—
			1 (DP54)		—
			1 (DP53)		2s
			3 (DP42)		—
			2 (8−1B+)		1s
	Microlymphoma 3 (Sp)	8	1 (DP58)		—
			1 (DP63)		—
			1 (Cos-8)		1R, 1s
			2 (DP71)	2	3s
			3 (DP77)		2R
			1 (DP49)		1s
3	Microlymphoma 1 (LN)	10	2 (DP47)		—
			1 (V3-64)		1R
			1 (DP54)		1R
			3 (DP49)		—
			2 (DP47)		—
	Microlymphoma 2 (LN)	7	1 (DP63)		1R
			1 (4.30)		1R
			4 (DP78)	4	—
4	Micro-lymphoma 1 (Sp)	10	3 (DP46)	3	1R
			1 (DP47)		—

Clones with frequent mutations (> 10) were occasionally seen. They are most likely from reactive B cells rather than KSHV-positive plasmablasts and are not included.
S indicates silent mutation; R, replacement change; —, no mutation; LN, lymph node; Sp, spleen.

lymphoma.[23,24] However, these lymphomas, unlike KSHV-related lymphoproliferative disorders in MCD patients, are of germinal center or postgerminal center origin.[23-25]

Our observation that KSHV-positive plasmablasts in MCD are polyclonal based on PCR analysis of the rearranged *Ig* genes is further supported by clonal analysis of KSHV episomes. Judde and coworkers[26] studied the clonality of KSHV in 5 MCD patients by Southern blotting analysis of the viral terminal repeat and showed polyclonal KSHV episomes in each case. Although KSHV clonality analysis has not been done in KSHV-associated plasmablastic lymphomas, clonality studies of primary effusion lymphoma (PEL) by PCR-based *Ig* gene analysis and Southern blotting of the viral terminal repeat showed a good agreement between the 2 methodologies.[26]

Because both the rearranged IgH and λ light-chain genes in KSHV-positive plasmablasts are functional rearrangements, it is

Table 4. Sequence analysis of the rearranged *Igλ* gene in KSHV-positive cells

Case no.	Lesion	Total no. of clones	Clones with identical Vλ	Clones with identical CDR3	Mutation in Vλ
			6 (DPL11)	6	1s
			1 (DPL15)		1R
	Lymphoma (LN)	10	1 (DPL8)		1R
			1 (DPL18)		—
1			5 (DPL3)		1s
				3	1s
			3 (DPL19)		
	Microlymphoma 1 (Sp)	17	3 (DPL11)	3 identical to the dominant lymphoma clone	2R
			1 (DPL3)		—
			1 (G7)		—
			1 (DPL5)		2R, 1s

Clones with frequent mutations (> 5) were occasionally seen. They are most likely from reactive B cells rather than KSHV-positive plasmablasts and are not included.
S indicates silent mutation; R, replacement change; —, no mutation; LN, lymph node; Sp, spleen.

Table 5. Comparison of PEL- and MCD-associated plasmablastic lymphoma

	PEL	Plasmablastic* lymphoma
Site	Body cavity, extranodal	Lymph nodes, spleen
Morphology	Immunoblasts	Plasmablasts
KSHV	Positive	Positive
EBV	Positive in majority	Negative
Cytoplasmic Ig expression	Absent	High level of IgM
Ig light chain	Monotypic κ or λ mRNA	Monotypic λ Ig
CD30	Positive	Weakly positive
B-cell antigens	Absent	Weak or absent
Mutation in *Ig* genes	Hypermutated in majority	Absent
Cellular origin	Germinal center or post-germinal center B cells	Naïve IgMλ-expressing B cells

*The term plasmablast is used to connote a medium-sized cell with a moderate amount of amphophilic cytoplasm and a large vesicular nucleus containing up to 3 prominent nucleoli. In contrast to an immunoblast, the cytoplasm contains abundant Ig.

most likely that λ light chain is associated with IgM. It has been shown that κ light chain is more often associated with IgM than λ light chain in human splenic B cells.[27] The reasons why KSHV is overwhelmingly associated with polyclonal λ light-chain–expressing B cells in MCD are not clear. In PEL, a KSHV-associated lymphoma, the tumor cells may express functionally rearranged κ or λ light-chain transcripts.[28,29] In MCD, occasional KSHV-positive κ-expressing B cells were also observed (data not shown). Two hypotheses may explain the preferential association of the virus with λ light-chain–expressing B cells in MCD. First, KSHV may preferentially target λ light-chain–expressing B cells. However, the frequent finding of functionally rearranged κ light-chain transcripts in PEL argues against this hypothesis.[29,30] The present study also failed to show any apparently biased usage of Ig heavy- or light-chain family members in KSHV-positive plasmablasts, suggesting that the virus does not selectively target a subset of λ light-chain–expressing B cells. Second, KSHV may naturally target both κ and λ light-chain–expressing B cells without bias but only λ cells expand preferentially due to their intrinsic proliferative response to the viral infection. The finding of κ light chain-positive PEL does not argue against this hypothesis because the majority of PEL are also coinfected with EBV, which may override the differential response of κ and λ light-chain–expressing B cells to KSHV infection. It is noteworthy that the 3 EBV-negative PEL with light chain data from the literature could be λ light-chain–expressing cells.[28,29]

The other unexpected finding in the present study was that KSHV-positive plasmablasts did not harbor somatic mutation in the rearranged *Ig* gene. This indicates that they originate from naive B cells despite their mature phenotype as shown by expression of CD27, a cell surface marker for memory B cells,[19,20] and high levels of cytoplasmic Ig. These findings are in line with their preferential localization in the mantle zones of B-cell follicles in MCD and lack of detectable FDC meshwork within the microlymphoma sites even though they are often adjacent to or partially replace B-cell follicles (data not shown). Thus, KSHV may infect IgM-positive naive B cells and drive these cells to differentiate into plasmablasts without undergoing the germinal center reaction, during which normal naive B cells mutate their rearranged *Ig* genes and differentiate into plasma or memory B cells.[31]

The plasmablastic lymphoma derived from KSHV-positive MCD is distinctive from PEL in many ways although both are associated with KSHV infection (Table 5). KSHV-associated MCD and plasmablastic lymphoma are not associated with EBV,[9] whereas PEL is commonly coinfected with the virus.[28,30] KSHV-positive plasmablasts express high levels of cytoplasmic IgMλ and are CD30 weakly positive or negative,[9] whereas the majority of PEL lack cytoplasmic Ig but strongly express CD30. Our current study reveals further differences in the genotype and cellular origin between the 2 KSHV-associated lymphomas: plasmablastic lymphomas harbor unmutated *Ig* genes and are derived from naive B cells; in contrast PEL bears mutated *Ig* genes and originates from germinal center or postgerminal center B cells.[28,30]

How can KSHV drive IgM-positive naive B cells to differentiate into plasmablasts and form lymphoproliferative lesions? KSHV has a number of genes that encode homologues to cellular proteins involved in signal transduction, cell cycle regulation, and apoptosis inhibition as well as homologues to cellular cytokines and cytokine response genes, and could play a role in cellular proliferation and transformation.[32,33] Three genes, namely the viral interferon regulatory factor (*K9*, *vIRF*),[34] the viral G-protein–coupled receptor (*ORF 74*, KSHV *GPCR*),[35] and *ORF K1*,[36] transform rodent cells or cause tumors in animal models. However, their role in KSHV-induced oncogenesis remains unclear because they are not expressed in latent viral infection.[33,37] LNA-1 (encoded by *ORF73*) is expressed in all latently infected cells and has been shown to transform primary rodent cells with the oncogene H-*ras*, and target both the p53 and retinoblastoma-E2F pathways.[38,39] Of the homologues to cellular cytokines, the KSHV-encoded cytokine IL-6 (vIL-6, encoded by *ORF k2*) can, in many ways, simulate hIL-6. In in vitro experiments, vIL-6 supports the growth of both mouse and human IL-6–dependent cell lines.[40-42] In animal models, vIL-6, like hIL-6, acts as a multifunctional cytokine and promotes hematopoiesis, plasmacytosis, and angiogenesis.[43] The effects of vIL-6 were triggered through the IL-6R, but unlike hIL-6, vIL-6 preferentially binds to the gp130 subunits of the IL-6R complex.[44] Furthermore, vIL-6 activates all of the known hIL-6–induced signaling pathways including JAK1, STAT1/3, STAT5, and the Ras–mitogen-activated protein (MAP) kinase cascade.[41]

We have shown that vIL-6 is highly expressed in 10% to 15% of the KSHV-positive plasmablasts, which is consistent with that of a previous report,[6] whereas hIL-6R is strongly expressed in the vast majority of KSHV-positive cells. We believe that activation of the IL-6 signaling pathway may play an important role in driving KSHV-infected naive B cells to differentiate into plasmablasts and develop various lymphoproliferative lesions. VIL-6 may directly stimulate both vIL-6–positive and –negative cells infected with KSHV by autocrine and paracrine mechanisms. Alternatively, vIL-6 may exert its effect on KSHV-positive cells through indirect pathways. It has been shown that vIL-6 can induce high levels of production of vascular endothelial growth factor (VEGF) in mice.[43] VEGF induces microvascular endothelial cells to secrete hIL-6.[35] Thus, KSHV could induce host cells (not necessarily KSHV-positive plasmablasts) to produce hIL-6 through expression of vIL-6. In fact, elevated serum hIL-6 has been demonstrated in patients with MCD and the level of hIL-6 correlated with the clinical presentation of the disease and high KSHV viral load in peripheral blood mononuclear cells.[10,11,45] Overproduction of hIL-6 is thought to be responsible for the systemic manifestation of MCD because blockage of IL-6 signaling by antibody can dramatically alleviate both clinical and histologic presentations of the disease.[12,13] Although it remains to be tested whether the elevated hIL-6 in MCD is the result of KSHV infection and is an important factor for KSHV-induced lymphoproliferation, it is noteworthy that hIL-6 is produced by PEL and promotes the growth of PEL cells in vitro and in vivo.[46,47]

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