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Distinct cellular origins of primary effusion lymphoma with and without EBV infection

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Abstract

Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with three distinct lymphoproliferative disorders: primary effusion lymphoma (PEL), multicentric Castleman's disease (MCD) and germinotropic lymphoproliferative disorder (GLD). KSHV positive lymphocytes in GLD and in most cases of PEL are co-infected by Epstein-Barr virus (EBV) and these viral double positive cells harbour mutated rearranged immunoglobulin (Ig) genes, suggesting that they originate from germinal centre or post-germinal centre B-cells. In contrast, KSHV positive cells in MCD are invariably negative for EBV, do not carry Ig gene mutation and are believed to originate from naïve IgMλ expressing B-cells. Interestingly, one EBV negative PEL (BC3) also lacks Ig gene mutation, raising the question whether KSHV preferentially targets naïve B-cells in the absence of EBV. We compared the cellular origin of PEL with and without EBV infection by analysis of Ig gene mutation. High molecular weight DNA from 17 PELs was subjected to PCR of the rearranged Ig heavy and light chain genes. Successful amplification was achieved from eight cases (four EBV positive and four EBV negative) and the PCR products were sequenced. All four EBV positive PEL showed variable levels of mutation in their rearranged V_H or V_L genes, ranging from 4 to 7%. In contrast, two of the four EBV negative PELs including BC3 displayed absence of mutation in their rearranged Ig genes. Our results indicate that EBV positive PELs are derived from germinal centre or post-germinal centre B-cells, whereas EBV negative PELs may originate from either germinal/post-germinal centre or naïve B-cells.

Keywords: Primary effusion lymphoma; EBV; Ig gene; Mutation

1. Introduction

Epstein-Barr virus (EBV), a human γ-herpesvirus, is causally associated with several lymphoproliferative disorders including Burkitt's lymphoma, Hodgkin's disease, human immunodeficiency virus (HIV) associated central nervous system lymphoma and post-transplant lymphoproliferative disorders [1]. Interestingly, all of these EBV associated lymphomas harbour mutated rearranged Ig genes and are thus believed to originate from germinal centre or post-germinal centre B-cells [2]. Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus-8 (HHV-8), is distantly related to EBV. Like EBV, KSHV is lymphotropic and has been shown to be associated with three distinct lymphoproliferative disorders: primary effusion lymphoma (PEL) [3], multicentric Castleman's disease (MCD) [4] and germinotropic lymphoproliferative disorder (GLD) [5].

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 and microlymphoma to monoclonal microlymphoma and frank plamablastic lymphoma [6]. In all of these different lesions, KSHV positive plasmablasts are monotypic, expressing exclusively IgMλ [6,7]. Phenotypically,

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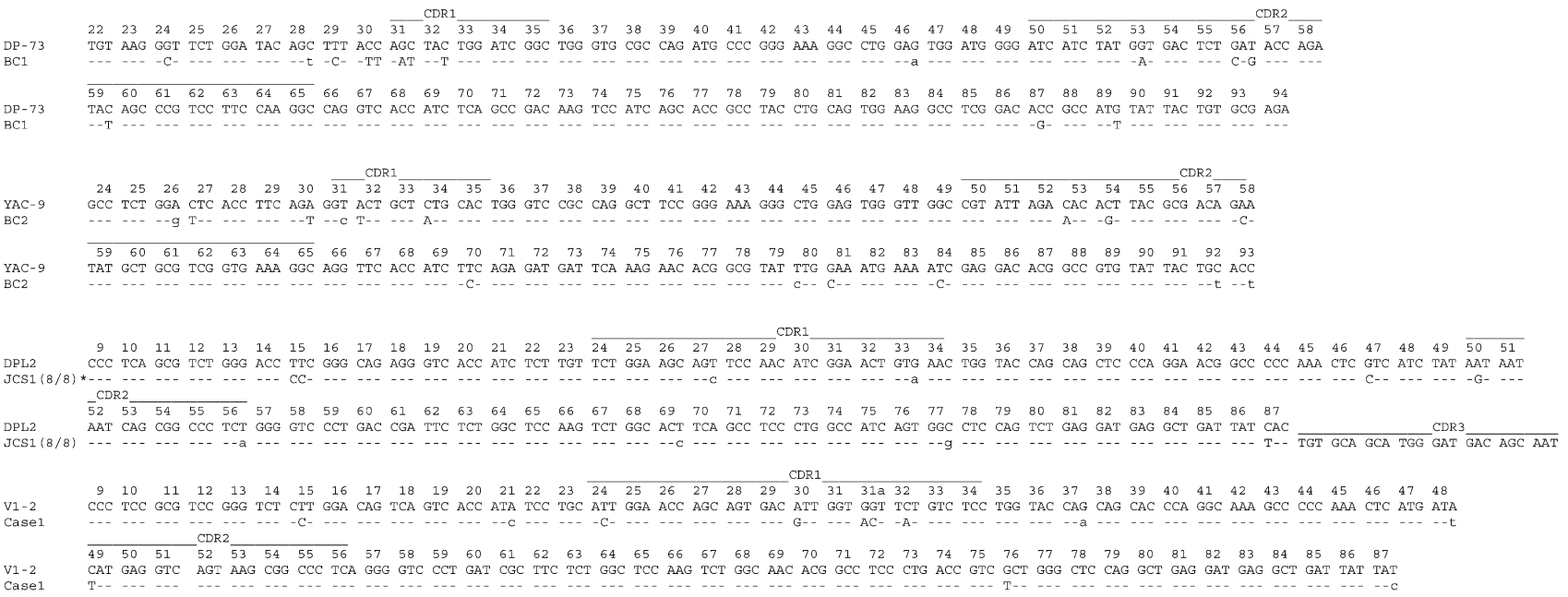
EBV positive PEL



(a)

Fig. 1. Alignment of the rearranged V_H or V_L of PEL to the closest germline segments. (∗) Number in bracket indicates identical clones of the total sequenced. Uppercase: replacement mutation; Lowercase: silent mutation.

EBV negative PEL

```
                                      CDR1                                                                                CDR2
           23  24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45  46  47  48  49  50  51  52  53  54  55  56  57  58
DP-79      ACT GTC TCT GGT GGC TCC ATC AGC AGT TAC TAC TGG GGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG TGG ATT GGG AGT ATC TAT TAT AGT GGG AGC ACC TAC
BC3(IgH)   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

           59  60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80  81  82  83  84  85  86  87  88  89  90  91  92  93  94  95
DO-79      TAC AAC CCG TCC CTC AAG AGT CGA GTC ACC ATA TCC GTA GAC ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG ACC GCC GCA GAC ACG GCT GTG TAT TAC TGT GCG AGA CAT
BC3(IgH)   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                 CDR1
           17  18  19  20  21  22  23  24  25  26  27  28  29  30  31  31a 31b 31c 32  33  34  35  36  37  38  39  40  41  42  43  44  45  46  47  48  49  50  51
DPL8       CAG AGG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC ATC GGG GCA GGT TAT GAT GTA CAC TGG TAC CAG CAG CTT CCA GGA ACA GCC CCC AAA CTC CTC ATC TAT GGT AAC
BC3(Igλ)   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
               CDR2
           52  53  54  55  56  57  58  59  60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80  81  82  83  84  85  86  87  88
DPL8       AGC AAT CGG CCC TCA GGG GTC CCT GAC CGA TTC TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC ACT GGG CTC CAG GCT GAG GAT GAG GCT GAT TAT TAC TGC
BC3(Igλ)   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                          CDR1                                                                     CDR2
           24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45  46  47  48  49  50  51  52  53  54  55  56  57  58  59
DP-8       GCT TCT GGA TAC ACC TTC ACC GGC TAC TAT ATG CAC TGG GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA TGG ATC AAC AAC AGT GGT GGC ACA AAC TAT
Case2(8/8) --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

           60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80  81  82  83  84  85  86  87  88  89  90  91  92  93  94  95
DP-8+      GCA CAG AAG TTT CAG GGC TGG GTC ACC ATG ACC AGG GAC ACG TCC ATC AGC ACA GCC TAC ATG GAG CTG AGA TCT GAC GAC ACG GCC GTG TAT TAC TGT GCG AGA GAA
Case2(8/8) --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                                       CDR2
           43  44  45  46  47  48  49  50  51  52  52a 53  54  55  56  57  58  59  60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80  81
DP-49      AAG GGG CTG GAG TGG GTG GCA GTT ATA TCA TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA
Case3(4/7) --- --- --- --- --- --- --- -G- --C --- AG- --- --- GTC --- --- C-G --- C-T --- --- --- -G- --- --- -A- --- --- --- -AG --- -C- --- -GC --- --c --c -T- --- --C
Case3(3/7) --- --- --- --- --- --- --- -G- --C --- AG- --- --- GTA --- --- C-G --- C-T --- --- --- -G- --- --- -A- --- --- --- -AG --- -C- --- -GC --- --c --c -T- --- --C

           82  82a 82b 82c 83  84  85  86  87  88  89  90  91  92  93
DP-49      ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT GCG                                CDR3
Case3(4/7) --T --t GA- --- --- C-- -g- --- --- --c AGA --- --t --- A-- ATG GCT TCC GCA AAA CAG AGG TCT TTT TTG GGG GGA TTG GGG AGA TGG CTG CAA TCT TGG GGC CCC TAC TAT TTT GAC
Case3(3/7) --T --t GA- --- --- C-- -g- --- --- --c AGA --- --t --- A-- --- --- --- --- --- --- --- --- --- --- --- ------ --- --- ------ --- --- ------ --- --- ------ --- ---

                                      CDR1                                                                     CDR2
           24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45  46  47  48  49  50  51  52  52a 53  54  55  56  57  58  59
DP-47      GCC TCT GGA TTC ACC TTT ACC GAC TTT GCC ATG GGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTA GAG TGG GTC TCC ACC ATT AGT GGT AGT GGT GAT TTC ACA TAC TAC
BCP1       --- --- --- --- --- --- --- -G- AG- -A- --- --- A-- --- --- --- --- --- --- --- --- --g --- --- --- --A G-T --- --- -C- --- --- -G- AG- --- --- ---

           60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80  81  82  83  84  85  86  87  88  89  90  91  92  93  94  95
DP-47      GCA GAC TCC GTG AAG GGC CGC TTC ACC GTC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTA CAA ATG AGA GCC GAG GAC ACG GCC ATC TAT TAC TGT GCG AAA GAC
BCP1       --- --- --- --- --- --- --g --- --- A-- --- --- --- --- --- --- --- --- --- --- --g --- --- --- --- --- --- --- --- G-A --- --- --- --- --- ---
```

(b)

Fig. 1. (*Continued*).

KSHV positive cells resemble mature B-cells, but genetic evidence suggests that they originate from naïve B-cells as their rearranged Ig genes lack somatic mutation [6,7]. The KSHV positive cells in MCD are always negative for EBV [6,7].

KSHV associated GLD is a newly identified entity [5]. The disease occurs in immunocompetent patients and presents as localized lymphadenopathy. The KSHV positive cells preferentially invade germinal centres of B-cell follicles and are monotypic, but polyclonal in terms of Ig gene rearrangement [5]. They most likely originate from germinal centre B-cells since they harbour mutated Ig genes and show Ig class switching [5]. The KSHV positive cells in GLD are co-infected by EBV in all cases reported so far [5].

PEL usually occurs in patients with HIV infection and is commonly associated with EBV, while in a small proportion, PEL occurs in patients without HIV infection and may not be associated with EBV [8,9]. PEL primarily involves body cavities and occasionally extranodal sites [10]. The lymphoma cells are characterized by immunoblasts with plasmacytoid cytoplasm, which do not express immunoglobulin and B-cell markers, but are syndecan-1 (CD138) positive. All EBV positive PELs examined so far harbour hypermutated Ig genes, suggesting that they originate from germinal centre or post-germinal centre B-cells [10–12]. Notably, one EBV negative PEL (BC3) lacks Ig gene mutation [12], raising the question of whether KSHV preferentially targets naïve B-cells in the absence of EBV. To examine this, we compared the cellular origin of a series of PELs with and without EBV infection by PCR and sequencing analysis of the rearranged Ig genes.

2. Material and methods

2.1. Patient and clinical data

Fresh frozen tumour cells or high molecular weight DNA samples were obtained from seven PEL patients and 10 PEL cell lines (BC1, BC2, BC3, BCP1, BCBL1, JCS1, CRO-AP/2, CRO-AP/3, CRO-AP/5 and CRO-AP/6). The clinical and histological details have been described in previous studies [13,14]. Nine PELs including four primary cases and five cell lines were negative for EBV, the remaining eight were EBV positive.

2.2. DNA preparation

High molecular weight DNA was prepared from fresh frozen cells using standard methodologies.

2.3. PCR and sequence analysis of the rearranged antigen receptor genes

High molecular weight DNA was subjected to PCR for both rearranged Ig heavy and light chain genes. For the rearranged IgH gene, different regions were amplified separately. Leader-J_H and Fr1-J_H were amplified using leader or family specific V_H and consensus J_H primers, respectively [15], while Fr2-J_H and Fr3-J_H regions were amplified with consensus primers as described previously [16].

Both Ig light chains were amplified. For κ light chain, Fr3-J_κ was amplified using two V_κ and two J_κ consensus primers in a single tube as previously described [17]. For λ light chain, Fr1-J_λ was amplified using mixed V_λ family specific (V_λ1, V_λ2, V_λ3, V_λ4ab, V_λ5–9–10, V_λ6 and V_λ7–8) and J_λ primers (J_λ1 or J_λ2–3–7) in two separate reactions [6]. Fr3-J_λ was amplified using one consensus Fr3 and two J_λ primers (J_λ1 or J_λ2–3–7) in two separate reactions [17].

In addition to the rearranged Ig genes, the rearranged TCRγ chain gene was also amplified using two sets of primers as previously described [18].

All PCR reactions were performed with appropriate positive and negative (without template DNA) controls in each experiment. All samples were analysed in duplicate. Leader-J_H, Fr1-J_H, Fr2-J_H and Fr1-J_λ PCR products were analysed on 6% polyacrylamide gels, while Fr3-J_H, Fr3-J_κ, Fr3-J_λ and TCRγ products were examined on 10% polyacrylamide gels. For Fr3-J_κ and Fr3-J_λ PCR products, the samples were first denatured at 95 °C for 5 min then cooled on ice for at least 45 min (heteroduplex analysis) prior to electrophoresis [17].

Cloning and sequencing of PCR products. Where indicated, PCR products were cloned and sequenced. In 5 cases, the PCR product was directly sequenced from both directions using dRhodamine dye terminators on an ABI Prism 377 sequencer (PE Applied Biosystems, Foster City, CA).

The variable (V), diversity (D) and joining (J) segments were identified by sequence comparison to the V Base using online DNAPLOT (MRC Centre for Protein Engineering, http://www.mrc-cpe.cam.ac.uk/DNAPLOT.php).

3. Results and discussion

Of the 17 cases of PEL examined, the rearranged IgV_H or IgV_L gene from Fr2 or upstream to J_H could be amplified in eight, including four EBV negative and four EBV positive cases (Table 1). These PCR products were sequenced directly or after cloning. All four EBV positive PELs showed somatic mutation in their rearranged Ig genes, ranging from 4 to 7% (Table 2). In contrast, two of the four EBV negative PELs including BC3 displayed no somatic mutation in their rearranged Ig genes (Table 2) (Fig. 1). In BC3, our present study further demonstrated that the rearranged Ig light chain gene did not harbour somatic mutations, which is in line with the finding of a lack of somatic mutation in the rearranged IgH [12]. Our data indicate that EBV positive PELs may be derived from germinal centre or post-germinal centre B-cells, while EBV negative PEL may originate from either naïve or germinal/post-germinal centre B-cells.

Table 1
PCR analysis of the rearranged antigen receptor genes

No. of cases	Sample	HIV	EBV	IgH				Igκ (Fr3-Jκ)	Igλ		TCRγ
				Leader-J_H	Fr1-J_H	Fr2-J_H	Fr3-J_H		Fr1-Jλ	Fr3-Jλ	
1	BC1	+	+	+	(+)	−	+	−	−	−	−
2	BC2	+	+	+	(+)	+	+	−	(+)	+	−
3	JCS1	+	+	−	−	−	−	−	(+)	+	−
4	Case 1	+	+	−		+	−	+	(+)	+	−
5	BC3	−	−	−	(+)	+	+	+	(+)	+	−
6	Case 2	+	−	−	(+)	+	+	−	+	+	−
7	Case 3	+	−	+	+	(+)	−	−	−	−	−
8	BCP1	−	−	−	(+)	+	+	+	−	+	−
9	Case 4	+	+	−	−	−	−	−	−	−	Weak band
10	CRO-AP/2	+	+	−	−	−	−	−	−	−	−
11	CRO-AP/5	+	+	−	−	−	−	−	−	−	−
12	Case 5	+	+	−	−	−	−	−	−	−	−
13	CRO-AP/3	+	−	−	−	−	−	−	−	−	−
14	CRO-AP/6	+	−	−	−	−	−	−	−	+	−
15	BCBL1	+	−	−	−	−	−	−	−	+	−
16	Case 6	−	−	−	−	−	−	−	−	−	−
17	Case 7	+	−	ND	−	−	−	−	−	−	ND

Positive and those in brackets are sequenced (+); negative (−); not done (ND).

Taken together, it appears that KSHV may preferentially target naïve B-cells in the absence of EBV infection, but commonly infects germinal centre or post-germinal centre B-cells in the presence of EBV. KSHV is less virulent than EBV in its ability to infect and immortalise B-cells and it is only weakly infectious to normal peripheral blood B-cells but more capable of infecting those positive for EBV [19]. In the presence of EBV, KSHV can infect peripheral blood B-cells and the viral infected cells are always positive for both viruses [19]. These data indicate that EBV positive B-cells are more susceptible to KSHV infection. It is possible that in KSHV associated lymphoproliferative disorders that are EBV positive, EBV infection precedes KSHV and thus dictates the cell population targeted by KSHV, whereas in those that are EBV negative, the cell population targeted should reflect the intrinsic nature of KSHV infection. It has been shown that EBV preferentially infects germinal centre or post-germinal B-cells that bear mutated Ig genes than naïve B-cells in infectious mononucleosis [20,21].

Of the 17 cases examined, 7 cases failed to show amplification of the rearranged Ig gene by all primer sets targeting both rearranged heavy and light chain genes and a further 2 cases only showed amplification with Fr3-J_λ primer set. For these failed cases, the BIOMED PCR primers were employed and this additional attempt also failed to amplify the rearranged Ig genes. Failure of PCR amplification of the rearranged Ig gene in PEL has been reported previously [22]. The failure rate of Ig PCR in PEL is much higher than that in other lymphomas, and is unlikely to be due solely to inappropriate primer annealing as a result of poor primer design or somatic mutation of the rearranged Ig gene. Since PEL does not commonly express B cell markers, this raises the question of whether the cases in which Ig PCR failed might have not yet undergone Ig gene rearrangement. Southern blot analysis should decipher whether these cases harbour rearranged Ig genes. Unfortunately, lack of sufficient DNA samples hindered such analysis.

In summary, our results indicate that EBV positive PELs are derived from germinal centre or post-germinal centre B-cells, whereas EBV negative PELs may originate from either germinal/post-germinal centre or naïve B-cells.

Table 2
Summary of Ig somatic mutation in PEL

No. of cases	Sample	EBV	Sequence methods	Identical clone of total sequenced	Germline V	Homologue to germline (%)	Mutation in CDR		Mutation in FR	
							R	S	R	S
1	BC1	+	Directly sequenced	–	DP73	93.1	7	0	6	2
2	BC2	+	Directly sequenced	–	YAC-9	92.8	5	1	5	4
3	JCS1	+	Cloned and sequenced	8/12	DPL2	95.7	1	3	4	2
4	Case 1	+	Directly sequenced	–	V1–2	94.9	6	0	2	4
5	BC3	−	Directly sequenced	–	DP79, V1–2	100, 100	–	–	–	–
6	Case 2	−	Cloned and sequenced	8/12	DP8	100	–	–	–	–
7	Case 3	−	Cloned and sequenced	7/12	DP46	78.0	12	0	16	6
8	BCP1	−	Directly sequenced	–	DP47	91.7	10	0	5	3

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