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Molecular histogenesis of plasmablastic lymphoma of the oral cavity

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Summary. Plasmablastic lymphoma (PBL) of the oral cavity is an aggressive B-cell lymphoma associated with human immunodeficiency virus infection. Although the lymphoma phenotype is consistent with late B-cell maturation, the molecular histogenesis of PBL is unknown. We investigated PBL of the oral cavity ($n = 12$) for mutations of immunoglobulin variable heavy chain (IgV_H) and *BCL*-6 genes, which are acquired by B cells at the time of germinal centre (GC) transit, and for expression of BCL-6, MUM-1 and CD138, which distinguish GC B cells from post-GC B cells. Somatic IgV_H hypermutation occurred in 4/10 PBL whereas 6/10 PBL displayed germline IgV_H genes. Among PBL carrying hypermutated IgV_H genes, the pattern of IgV_H mutations was consistent with antigen stimulation in two cases. Mutations of the *BCL*-6 gene were restricted to 1/12 patients with PBL of the oral cavity. All cases of PBL of the oral cavity displayed the BCL-6^-/MUM-1^+/$CD138^+$ phenotype that is consistent with late stage of B-cell differentiation. Overall, these data indicate that, despite a common phenotype and an apparently similar degree of differentiation, PBL of the oral cavity are characterized by histogenetic heterogeneity. A subset of PBL of the oral cavity carried the molecular clues of GC transit and conceivably originated from a B-cell subset corresponding to post-GC B cells. Conversely, another fraction of these lymphomas were devoid of somatic IgV_H mutations and appeared to originate from naive B cells that have undergone preterminal differentiation independent of GC transit.

Keywords: plasmablastic lymphoma of the oral cavity, histogenesis, immunoglobulin, BCL-6, AIDS.

Plasmablastic lymphoma (PBL) of the oral cavity is a peculiar type of B-cell lymphoma that has been recognized in association with acquired immunodeficiency syndrome (AIDS) and is classified as an individual nosological entity by the World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues (Delecluse *et al*, 1997; Carbone *et al*, 1999; Jaffe *et al*, 2001; Flaitz *et al*, 2002). PBL originate in the mucosa of the oral cavity, often involve the gingiva and may infiltrate the adjacent bone. In most patients, the tumour is confined to the oral cavity at the time of diagnosis, but extends to distant sites shortly afterwards, during the clinical course of the disease. All PBL display an aggressive clinical course and the prognosis is poor despite therapy (Delecluse *et al*, 1997; Carbone *et al*, 1999; Tirelli *et al*, 2000; Jaffe *et al*, 2001; Flaitz *et al*, 2002). Histologically, PBL are composed of rapidly growing, large neoplastic cells displaying a marked degree of plasma cell differentiation (Delecluse *et al*, 1997; Carbone *et al*, 1999; Jaffe *et al*, 2001; Flaitz *et al*, 2002). Phenotypically, PBL display an unusual profile characterized by weak or absent expression of conventional B-cell markers coupled to strong immunostaining with the plasma cell markers CD138/syndecan-1 and VS38c (Carbone *et al*, 1997, 1999; Delecluse *et al*, 1997; Jaffe *et al*, 2001; Flaitz *et al*, 2002).

Whereas the molecular histogenesis of several AIDS-related lymphomas has been elucidated in detail (Larocca *et al*, 1998; Gaidano *et al*, 2000; Carbone *et al*, 2001a,b), the histogenetic derivation of PBL of the oral cavity is currently unclear. Recently, the field of AIDS-related lymphoma histogenesis has received impetus following the availability of histogenetic markers, allowing the distinction

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Table I. Clinical characteristics of patients of PBL of the oral cavity.

Patient number	Age	Sex	Stage	Therapy*	HAART†	Response	Relapse‡	Vital status	Cause of death§
2438	33	M	IVA	ACVBP	No	Not evaluable	n.a.	Dead at 1 month	HCV
2439	25	M	IIA	ACVBP	No	Complete response	No	Dead at 16 months	OI
2440	29	M	IB	CHOP-like	No	Partial response	n.a.	Dead at 6 months	OI
2441	34	M	IVA	CHOP	No	Partial response	n.a.	Dead at 18 months	Lymphoma
2442	32	F	IVB	CHOP-like	No	Progression	n.a.	Dead at 4 months	Lymphoma
2443	33	M	IVB	ACVBP	Yes	Complete response	No	Alive at 51 months	n.a.
2444	37	M	IVA	CHOP	No	Progression	n.a.	Dead at 6 months	Lymphoma
2445	37	M	IA	CHOP	Yes	Complete response	No	Alive at 54 months	n.a.
2862	31	F	IA	CHOP	Yes	Complete response	No	Alive at 26 months	n.a.
2926	57	M	IA	CHOP	Yes	Complete response	Yes	Dead at 28 months	Lymphoma

*CHOP: cyclophosphamide, adriamycin, vincristine, prednisone; ACVBP: adriamycin, cyclophosphamide, vindesine, bleomycin, prednisone.
†HAART, highly active antiretroviral therapy.
‡n.a., not applicable.
§OI, opportunistic infection; n.a., not applicable.

of mature B cells into different compartments, namely virgin B cells, germinal centre (GC) B cells and post-GC B cells. Genotypic markers of B-cell histogenesis are represented by mutations of immunoglobulin variable (IgV) genes and of *BCL*-6, which are somatically acquired by B cells at the time of transit through the GC (Pasqualucci *et al*, 1998; Shen *et al*, 1998; Stevenson *et al*, 1998, 2001; Küppers *et al*, 1999; Peng *et al*, 1999). Positivity for IgV and/or *BCL*-6 mutations indicates that a given lymphoma derives from GC or post-GC B cells. Phenotypic markers are represented by the BCL-6, MUM-1 and CD138/syndecan-1 proteins, and enable the distinction between GC and post-GC B cells (Cattoretti *et al*, 1995; Carbone *et al*, 2000, 2001b; Falini *et al*, 2000). In fact, expression of BCL-6 clusters with the GC stage of differentiation, MUM-1 positivity clusters with B cells exiting the GC and with post-GC B cells, and CD138/syndecan-1 is a marker of preterminal B-cell differentiation. On this basis, AIDS-related lymphomas may be schematically distinguished into: lymphomas devoid of somatic IgV and *BCL*-6 hypermutation, which derive from pre-GC B cells; lymphomas associated with somatic IgV and/or *BCL*-6 hypermutation and BCL-6 expression, which closely reflect GC B cells; and lymphomas associated with somatic IgV and/or *BCL*-6 hypermutation and MUM-1 and CD138/syndecan-1 positivity, representing lymphomas of post-GC B cells (Carbone *et al*, 2001a).

The aim of this study was to investigate the molecular histogenesis of PBL of the oral cavity. This lymphoma was found to be histogenetically heterogeneous, as a subset of cases derived from post-GC-like B cells, whereas other cases originated from naive B cells that have undergone preterminal differentiation independent of GC transit.

MATERIALS AND METHODS

Patients. Twelve cases of PBL of the oral cavity formed the basis of this study. Eight cases had been referred to the INT-Centro di Riferimento Oncologico, Aviano, Italy, two cases had been referred to the Division of Pathology, Hospital Clinic, University of Barcelona, Spain, and two cases had been referred to the Institute of Pathology, Catholic University of the Sacred Heart, Rome, Italy. All cases of PBL of the oral cavity included in this study had developed in HIV-positive individuals. Ten patients were men and two were women. Age at diagnosis ranged between 25 and 68 years. Additional clinical information was available in detail for 10 patients and is summarized in Table I. Diagnosis of PBL of the oral cavity was based on the criteria originally reported by Delecluse *et al* (1997) and reflected the criteria adopted by the World Health Organization Classification of Tumours of the Haematopoietic and Lymphoid Tissues (Jaffe *et al*, 2001). A representative histology of PBL of the oral cavity is shown in Fig 1. All cases were negative for CD20, CD10, CD43. All but two cases were negative for CD79a.

DNA extraction. Genomic DNA was isolated by cell lysis followed by digestion with proteinase K and purification

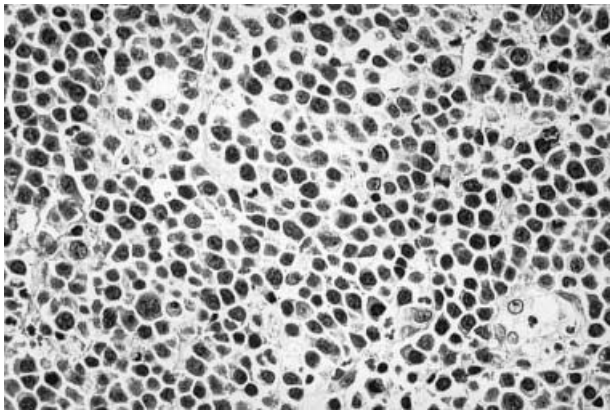

Fig 1. AIDS-related plasmablastic lymphoma of the oral cavity (patient 2442). Morphology (haematoxylin & eosin, original magnification ×250).

using a commercial kit (QIAamp DNA mini Kit; Qiagen, CA, USA), according to the manufacturer's instructions.

Analysis of IgVH genes. IgV_H gene rearrangements were amplified with a set of six V_H gene family-specific primers that hybridize to sequences in the framework region (FR) 1 and a J_H-degenerated primer, in separate reactions for each V_H primer. The sequences of the V_H FR1 primers utilized have been reported previously (Fais *et al*, 1998, 1999; Capello *et al*, 2000a,b). The sequence of the degenerated J_H primer was: 5′-CTY ACC TGA RGA GAC RGT GAC C-3′. Polymerase chain reaction (PCR) was performed for 45 cycles with an annealing temperature of 60°C. Samples for which no clonal IgV_H gene rearrangement was obtained with FR1 primers were further amplified, with a set of V_H primers that hybridize to sequences in FR2 and a J_H-degenerated primer, in three separate reactions. The V_H FR2 primers used in the first reaction were: 5′-GGA CAA RGG CTT GAG TGG AT-3′ (V_H1·1); 5′-GGA MAA SGS CTT GAG TGG AT-3′ (V_H1·2); 5′-GGG AAR GGV CTG GAG TGG AT-3′ (V_H4-5). V_H FR2 primers used in the second reaction were: 5′-GDT CCG CCA GGC TCC AG-3′ (V_H3·11); 5′-GGT CCG SCA AGC TCC AG-3′ (V_H3·12); 5′-GAT CCG TCA GCC CCC AG-3′ (V_H2). V_H FR2 primers used in the third reaction were: 5′-GGA AAA GGT CTG GAG TGG GT-3′ (V_H3·21); 5′-GGG AAG GGT CTG GAG TGG GT-3′ (V_H3·22); 5′-GGG AAA GGG CTG GAG TGG GT-3′ (V_H3·22a); 5′-TCG AGA GGC CTT GAG TGG-3′ (V_H6). PCR products were directly sequenced using a commercially available kit (ThermoSequenase; Amersham Life Sciences, Amersham, UK) as reported (Capello *et al*, 2000b). [α-^{33}P]-labelled terminator dideoxynucleotides (Amersham Life Sciences) were included in the sequencing mixtures. Sequences were compared with the V-BASE sequence directory [Medical Research Council (MRC) Centre for Protein Engineering, Cambridge, UK] using MACVECTOR 6.0.1 software (Oxford Molecular Group PLC, Oxford, UK) for comparison of the rearranged IgV_H genes with the most homologous germline sequences.

To define the occurrence of antigen stimulation and selection in IgV_H genes utilized by PBL of the oral cavity carrying IgV_H mutations, two statistical methods were used: the Chang–Casali binomial distribution model and the multinomial distribution model (Chang & Casali, 1994; Lossos *et al*, 2000).

Molecular analysis of BCL-6. Mutations of BCL-6 5′ non-coding regions were assessed by PCR DNA direct sequencing performed on three partially overlapping PCR fragments (E1·10, E1·11, E1·12), corresponding to a 739-bp region located downstream of the first BCL-6 non-coding exon (Capello *et al*, 2000b). This 739 bp region has been shown to harbour > 95% of BCL-6 5′ mutations detected in B-cell lymphoma. The sequences of the oligonucleotides used as primers, as well as PCR conditions, have been reported in detail previously (Capello *et al*, 2000b). DNA PCR products were purified using a commercially available kit (QIAquick gel extraction kit; Qiagen). Subsequently, DNA direct sequencing was performed with appropriate primers, using a commercially available kit (Thermosequenase). [α-^{33}P]-labelled terminator dideoxynucleotides were included in the sequencing mixture. For each DNA fragment analysed, sequencing of both strands was performed on independent PCR reactions.

Immunohistochemical studies. Deparaffinized tissue sections were used for immunophenotyping and lineage assignment of PBL of the oral cavity. The sources and specificities of the antibodies used in this study have been reported in detail previously (Carbone *et al*, 2001b). Immunohistochemistry was performed by the avidin–biotin peroxidase complex (ABC-px) or alkaline phosphatase anti-alkaline phosphatase (APAAP) methods as previously described (Hsu *et al*, 1981; Cordell *et al*, 1984).

CD138 expression was assessed using the B-B4 monoclonal antibody (mAb) (Wijdenes *et al*, 1996) (Serotec, Oxford, UK). The BCL-6 protein was detected by the PG-B6 mAb (Dakopatts, Glostrup, Denmark) (Flenghi *et al*, 1996). The expression of MUM1 was investigated with an affinity-purified polyclonal goat antibody (ICSAT/M-17) specific for the MUM1 protein (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The M-17 antibody reacts with MUM1 of mouse, rat and human origins, although it does not crossreact with other members of the interferon regulatory factor (IRF) protein family. All antigens were tested on paraffin-embedded tissue sections. For MUM1 and BCL-6 assessment, paraffin-embedded sections were treated in a microwave oven at 250 W for 30 min in EGTA solution (1 mmol/l, pH 8). Immunostaining for MUM1 and BCL-6 was performed on an automated immunostainer (Nexes; Ventana Medical Systems, Tucson, AZ, USA), according to a modified version of the company's protocols. Immunostaining for CD138 was performed using the APAAP method (Cordell et al, 1984). The percentage of antigen-positive neoplastic cells was assigned to one of the following categories: 0, less than 10%, 10–25%, 25–50%, 50–75% and > 75%.

Analysis of viral infection. Determination of tumour infection by Epstein–Barr virus (EBV) was performed by EBER (Epstein–Barr early RNA) *in situ* hybridization and/or by PCR DNA analysis of the EBNA-1 gene, as described (Carbone *et al*, 1996). In the case of EBV-positive samples, immunostaining for latent membrane protein-1 (LMP1) was performed with a LMP1-specific antibody (Dakopatts) on Bouin or formalin-fixed paraffin-embedded tissue sections, as described above.

RESULTS

Analysis of IgV_H genes

IgV_H amplimers obtained from 10 PBL of the oral cavity were found to represent functional Ig gene rearrangements upon DNA direct sequencing (Table II). In the remaining two patients, no IgV_H amplimer could be obtained. The IgV_H gene families utilized by PBL of the oral cavity displayed no bias for a specific V_H family and included VH1 (2/10 patients), VH3 (3/10 patients), VH4 (2/10 patients), VH5 (2/10 patients) and V_H6 (1/10 patients) (Table II). The D segment utilized could be confidently assigned in only five patients (patients 2439, 2440, 2441, 2443, 2929) (Table II). Analyses of J_H gene segments revealed under-representation of the most commonly used JH4 segment, which was identified in only one out of 10 patients (patient

Table II. Molecular analysis of IgV_H and *BCL-6* genes in AIDS-related plasmablastic lymphoma of the oral cavity.

	IgV_H								
Patient number	V_H family*	V_H gene*	D_H gene*	J_H gene*	Functional*	% somatic mutations*†	Antigen stimulation*‡	Antigen selection*‡	*BCL-6*§
2438	ne	ne	ne	ne	ne	ne	ne	ne	–
2439	VH3	b28e	D3-10	J_H6b	+	–	–	–	–
2440	V_H5	DP-73	D3-16	J_H6b	+	–	–	–	–
2441	V_H1	DP-7	D2-15	J_H6b	+	–	–	–	–
2442	V_H5	DP-73	D4-11	J_H6b	+	–	–	–	–
2443	V_H1	DP-88	D3-10	J_H6b	+	–	–	–	–
2444	V_H6	DP-74	ne	J_H6b	+	7·6	–	–	–
2445	V_H3	VH3-8	ne	J_H6b	+	–	–	–	–
2862	V_H3	b37	ne	J_H4b	+	10·8	–	–	+
2863	ne	ne	ne	ne	ne	ne	ne	ne	–
2926	V_H4	DP-63	ne	J_H5b	+	15·6	+¶	–	–
2929	VH4	DP-63	D3-3	J_H3a	+	5·9	+**	–	–

*ne, not evaluable.

†–, absence of IgV_H somatic mutations or frequency of IgV_H somatic mutations < 2%.

‡Antigen stimulation and selection were analysed by two independent statistical methods (binomial and multinomial distribution analysis). Antigen stimulation was considered positive if the number of R mutations within the FR was significantly lower than would be expected to arise by chance alone. Antigen selection was considered positive if the number of R mutations within the CDR was significantly higher than would be expected to arise by chance alone.

§–, germline *BCL-6* sequence; +, mutated *BCL-6* sequence.

¶*P*-values are $P = 0{\cdot}0208$ for the multinomial distribution model and $P = 0{\cdot}0328$ for the binomial distribution model.

***P*-values are $P = 0{\cdot}0002$ for the multinomial distribution model and $P = 0{\cdot}0002$ for the binomial distribution model.

2862). Seven out of 10 patients with PBL of the oral cavity utilized the J_H6b gene (Table II).

In 6 patients, the identified IgV_H gene was scored as germline, as it was either 100% homologous to germline IgV_H sequences or displayed a nucleotide divergence < 2%. Conversely, somatic hypermutation of IgV_H genes was positive in four patients with PBL of the oral cavity (Table II). In these patients, sequencing analysis demonstrated that the diversity between the IgV_H sequences utilized by PBL of the oral cavity and the closest germline genes ranged from 5·9% to 15·6% of the nucleotides examined (Table II). This diversity range corresponded to the rate of IgV_H mutations that had been somatically acquired by PBL of the oral cavity.

In the four PBL of the oral cavity harbouring somatically mutated IgV_H genes, statistical analysis of mutations was performed by the binomial (Chang–Casali) and the multinomial distribution methods (Table II). The results of the binomial and the multinomial statistical methods were superimposable (Table II). Evidence of antigen stimulation was observed in two patients, as defined by a lower number of replacing (R) mutations within the FR than would be expected to arise by chance alone. None of the PBL of the oral cavity displayed clues of antigen selection, as no patient displayed a significantly higher number of R mutations in complementarity determining regions (CDRs) than would be expected to arise solely by chance.

Based on the available data (Table I and Table II), the IgV_H mutation status did not apparently associate with specific clinical characteristics of PBL of the oral cavity.

Mutational analysis of BCL-6 gene

All 12 patients with PBL of the oral cavity were subjected to mutation analysis of the *BCL-6* gene. Mutations of *BCL-6* were restricted to one patient with PBL (case 2862), which harboured a single mutation (G756C) in the heterozygous state (Table II).

Expression of BCL-6, MUM-1 and CD138

The phenotypic profile could be assessed in 10 patients with PBL of the oral cavity for which sufficient material was available. Results are detailed in Table III and represented in Fig 2. All (10/10) patients were negative for BCL-6 but positive for MUM-1 and CD138, thus defining that all PBL of the oral cavity share the $BCL6^-/MUM\text{-}1^+/CD138^+$ phenotype.

Analysis of viral infection

Infection by EBV, as defined by EBER studies, occurred in 10/12 patients with PBL of the oral cavity (Table III). Expression of the EBV-encoded LMP-1 antigen was negative in all patients analysed (Table III).

DISCUSSION

This study aimed at refining the molecular histogenesis of PBL of the oral cavity, a lymphoma displaying features of preterminal B-cell differentiation, and primarily arising in the oral mucosa and gingiva (Delecluse *et al*, 1997; Carbone *et al*, 1999; Tirelli *et al*, 2000; Jaffe *et al*, 2001). At presentation, the tumour was limited to the oral cavity or

Table III. Expression of BCL-6, MUM-1, CD138 and LMP-1 in plasmablastic lymphoma of the oral cavity.

Patient number	BCL-6*	MUM-1*	CD138*	EBV-EBER*	EBV-LMP1*
2438	–	+ (> 75%)	+ (> 75%)	+	–
2439	–	+ (> 75%)	+ (> 75%)	–	–
2440	–	+ (> 75%)	+ (> 75%)	+	–
2441	–	+ (> 75%)	+ (50–75%)	+	–
2442	–	+ (> 75%)	+ (> 75%)	+	–
2443	–	n.e.	+ (> 75%)	–	–
2444	–	+ (> 75%)	+ (> 75%)	+	–
2445	–	+ (10–25%)	+ (25–50%)	+	–
2862	–	+ (> 75%)	+ (50–75%)	+	–
2863	–	+ (> 75%)	+ (> 75%)	+	–
2926	–	n.e.	+ (10–25%)	+	n.e.
2929	–	n.e.	+ (> 75%)	+	n.e.

*–, negative expression; +, positive expression (for MUM-1 and CD138, the percentage of positive cells is reported in brackets); n.e., not evaluable.

infiltrated the adjacent bone, although extension to distant sites and other organs could be identified shortly after diagnosis. In patients not receiving highly active antiretroviral therapy (HAART), the lymphoma displayed an aggressive clinical behaviour and prognosis was poor despite chemotherapy.

The molecular features of PBL of the oral cavity revealed a certain degree of histogenetic heterogeneity within this category of lymphoma. Although all PBL reflect a preterminally differentiated phenotype, defined by the BCL-6^-/MUM-1^+/$CD138^+$ profile characteristically associated with this tumour, our data suggest that a fraction of PBL derive from post-GC B cells, whereas other cases originate from apparently naive B cells that have undergone preterminal differentiation independent of the GC reaction.

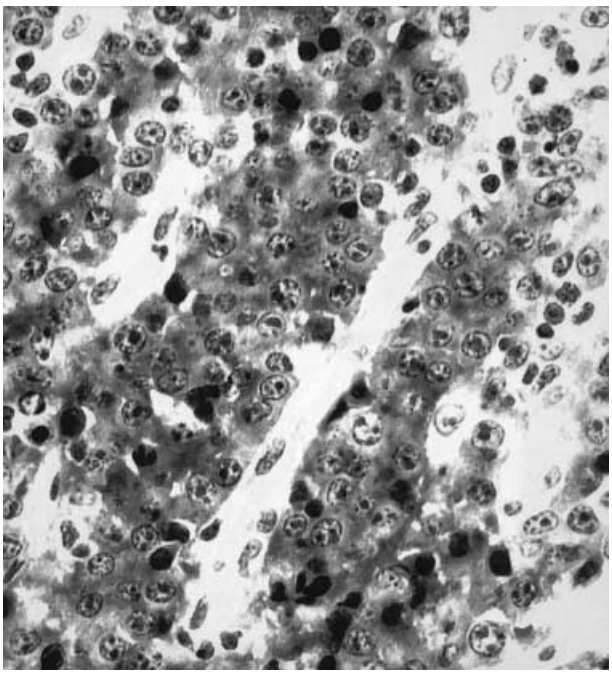

Fig 2. AIDS-related plasmablastic lymphoma of the oral cavity (patient 2443). All neoplastic cells are strongly labelled by the anti-CD138/B-B4 antibody (APAAP method, haematoxylin counterstain, original magnification ×250).

The histogenesis of a first subset of PBL of the oral cavity may be ascribed to post-GC B cells, as these lymphomas harbour somatic hypermutation of IgV_H genes that are acquired at the time of the GC reaction (Stevenson *et al*, 1998, 2001; Küppers *et al*, 1999). The precursor cells of this PBL subset may have migrated to the oral cavity after transiting through the GC or, alternatively, may have undergone the GC reaction and further differentiation directly in the microenvironment of the oral cavity. Overall, the histogenesis of this subset of PBL of the oral cavity appears to be similar to that of the majority of other AIDS-related lymphomas, including Burkitt's lymphoma, diffuse large cell lymphoma, primary central nervous system lymphoma and primary effusion lymphoma (Gaidano *et al,* 2000; Carbone *et al,* 2001a). Also, the derivation from GC-related B cells likens the histogenesis of this subset of PBL of the oral cavity to that of the vast majority of B-cell lymphomas in immunocompetent hosts (Stevenson *et al*, 1998, 2001; Küppers *et al*, 1999).

A second subset of PBL of the oral cavity appears to follow a different histogenetic pathway. Because PBL belonging to this group are devoid of both IgV_H mutations and of *BCL*-6 mutations, which denote GC transit, the origin of this subset of PBL of the oral cavity can be traced to naive B cells that have not experienced the GC reaction and microenvironment (Pasqualucci *et al*, 1998; Shen *et al*, 1998; Stevenson *et al*, 1998, 2001; Küppers *et al*, 1999; Peng *et al*, 1999). These cells, however, have been able to acquire phenotypic features of preterminally differentiated B cells independent of GC transit, as PBL of the oral cavity stained consistently positive for MUM-1 and CD138, two well-established markers of post-GC B cells advancing towards the plasma cell pathway of differentiation (Wijdenes *et al*, 1996; Falini *et al,* 2000; Carbone *et al,* 2001b).

The derivation of a significant fraction of PBL of the oral cavity from pre-GC B cells is unusual among B-cell disorders displaying the BCL-6$^-$/MUM-1$^+$/CD138$^+$ phenotype. In fact, somatic hypermutation of IgV_H genes is found in the overwhelming majority of immunoblastic lymphomas, primary effusion lymphomas and multiple myelomas, which are typically BCL-6$^-$/MUM-1$^+$/CD138$^+$ (Pasqualucci *et al*, 1998; Shen *et al*, 1998; Stevenson *et al*, 1998, 2001; Küppers *et al*, 1999; Peng *et al*, 1999; Jaffe *et al,* 2001). Conversely, absence of somatic IgV_H mutations is shared by few types of lymphomas, namely mantle cell lymphomas, a substantial fraction of B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma and a subset of splenic marginal zone lymphomas (Stevenson *et al*, 1998, 2001; Küppers *et al*, 1999; Algara *et al,* 2002). However, consistent with their pre-GC origin, and in contrast to PBL of the oral cavity, both mantle cell lymphoma and B-cell chronic lymphocytic leukaemia/small lymphocytic lymphoma stain negative for both MUM-1 and CD138 (Falini *et al,* 2000; Jaffe *et al,* 2001). Taken together, these findings indicate that development of PBL of the oral cavity may represent transformation of B cells at different stages of ontogeny and may provide a unique model of preterminal B-cell differentiation, occurring independently of the GC reaction.

In contrast to many types of lymphoma (Stevenson *et al*, 1998, 2001; Küppers *et al*, 1999), antigen stimulation and selection appears to play a minor role in the pathogenesis of PBL of the oral cavity. In fact, among the cases of post-GC origin, molecular evidence of antigen selection was negative in all patients and that of antigen stimulation was restricted to two patients. Also, it is notable that all but one PBL of the oral cavity with unmutated IgV_H genes utilized a JH6 segment. The excess of J_H6 genes utilized in productive but unmutated IgV_H rearrangements of PBL of the oral cavity reflects normal B-cell physiology, as, in normal physiology, the frequency of J_H6 is increased in B cells carrying unmutated productive rearrangements and significantly diminished in B cells carrying mutated V_HDJ_H rearrangements. (Brezinschek *et al*, 1997; Rosner *et al,* 2001). The analysis of CDR3 length of V_HDJ_H rearrangements of normal B cells suggests that longer CDRs, including JH6, may favour positive selection in the preimmune unmutated B-cell repertoire, whereas they may be disadvantageous for expansion by antigen selection (Brezinschek *et al*, 1997). On these bases, future studies are required to define whether the pathogenesis of PBL of the oral cavity requires oligoclonal expansions of pre-GC-unmutated B cells induced by HIV infection and clonally related to subsequent lymphoma development.

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