



New monoclonal antibodies against B-cell antigens: Possible new strategies for diagnosis of primary cutaneous B-cell lymphomas

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ABSTRACT

Reactivities of the monoclonal antibodies (mAbs) of the 9th Human Leukocyte Differentiation Antigen Workshop, in order to define specific antigenic expression of the primary cutaneous B-cell lymphomas (PC-BCL), were analyzed by immunohistology on human tonsil and on PC-BCL, such as follicular centre B-cell lymphomas (FCL), marginal zone lymphomas (MZL) and diffuse large B-cells lymphomas leg-type (DLBL-LT). We identified some subgroups of mAbs that were exclusively or preferentially positive in one lymphoma cell type: the PC-FCL subgroup of mAbs includes PD1/CD279, GCET-1, hFCRL1/CD307a, FCRL2/CD307b, CXCR5/CD185, B7-DC/CD273, MRC/CD200, CD130, CXCR4/CD184, Siglec-5/14, CD150, on the other hand subgroup of mAbs in PC-MZL includes BTLA/CD272, BLIMP-1, hCD38. No specific subgroup of mAbs was found to label PC-DLBCL. This study may be useful to better define specific antigen profile of different PC-BCL entities leading to a correct diagnosis.

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1. Introduction

Primary cutaneous B-cell lymphomas (PC-BCL) represent approximately 20–25% of all primary cutaneous lymphomas. In the last World Health Organization (WHO) classification for cutaneous lymphomas in 2008, uniform terminology and classification for this rare group of neoplasms was introduced. During the last two decades it has become clear that some subtypes of B-cell non-Hodgkin lymphoma can exclusively present in the skin. These PC-BCL are much less common, than PC-T-cell lymphomas (TCL). The WHO classification distinguishes three main types of PC-BCL: marginal zone B-cell lymphoma (MZL), follicle centre lymphoma (FCL) and diffuse large B-cell lymphoma, leg-type (DLBCL-LT) [1]. It is difficult to distinguish these three entities according only to their clinical and histopathologic characteristics, so it is fundamental the use of immunohistochemistry. As already known CD20, CD79a and PAX5 are useful for confirmation of B-cell lineage and CD3 for the amount of reactive T-cells. Cytoplasmic immunoglobulins (Igs) detection on frozen and paraffin sections confirm B-cell lineage and monotypic Ig expression. Polymerase chain reaction (PCR) is useful

to demonstrate clonal rearrangement of immunoglobulin genes. Moreover, every single type of PC-BCL presents different clinical and immunohistochemical features [2] (erythematous plaques, nodules or tumours); dissemination to extracutaneous sites is uncommon. The incidence of PC-FCL within PC-BCL is about 60%. Neoplastic cells express strongly BCL-6; while CD10 is positive only in cases of PC-FCL with a follicular growth pattern. Usually BCL-2 is not expressed on large cleaved cells or is weakly positive in a minority of neoplastic B-cells [3]. Recent studies report BCL-2 expression in a significant minority of PC-FCL with a follicular growth pattern. Staining for MUM-1/IRF4 and FOXP1 is negative in most but not all cases. PC-DLBCL-LT, instead, shows red tumours on the lower legs; frequently it disseminates to extracutaneous sites. The incidence within PC-BCL is about 10%. Nearly always the neoplastic B-cell of PC-DLBCL-LT express strongly BCL-2, MUM-1/IRF4, and FOXP1. BCL-6 is weakly expressed in some cases. Finally, PC-MZL shows erythematous plaques or nodules on legs and arms; rarely it disseminate to other organs. It represents about 30% of PC-BCL; neoplastic B-cells of PC-MZL express CD21 and CD35 (DRC) and plasmacytes are monotypic for kappa or lambda chains. In about 50% of cases these cells express also CD43 and weakly CD11c; rarely PC-MZL is CD5+ [1].

Although successful application of flow cytometry in the diagnosis of PC-BCL has been reported, it is not widely used and cannot be considered as a substitute for immunohistochemistry. Disadvantages of this approach are the difficulties to obtain sufficient viable neoplastic single cell suspensions, due to the vulnerability

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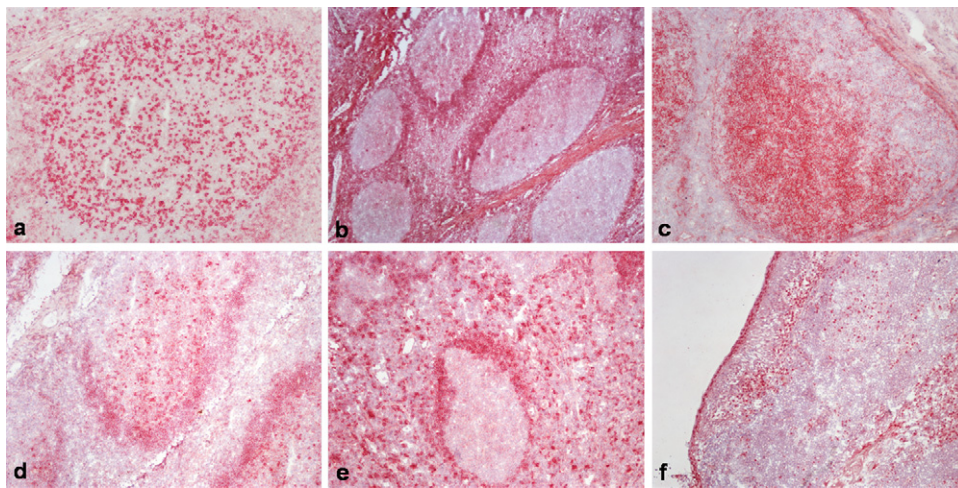


Fig. 1. Examples of reactivity on tonsil. (a) PD1(12): intrafollicular activated cells; (b) FCRL1(20): mantle zone cells, plasmacytes and stromal cells; (c) CD200 (40): germinal centre cells; (d) CXCR5 (50): intrafollicular activated cells and mantle zone of the follicles; (e) CD48 (88): mantle zone of the follicles, macrophages and dendritic cells; (f) BLIMP-1 (103): strong nuclear staining of plasmacytes and epithelial cells. The numbers in parentheses refer to the 9th HLDA antibody number.

of the cutaneous B-cells, the lack of architectural information and the need of additional fresh tissue material.

In our study we have tested, on the three PC-BCL cited above, monoclonal antibodies (mAbs) of 9th Human Leukocyte Differentiation Antigen (HLDA) Workshop in order to evaluate their immunoreactivity and to identify new markers useful for the differential diagnoses of PC-BCL.

2. Materials and methods

2.1. Patients

We evaluated 10 PC-FCL, 10 PC-MG and 5 DLBCL-LT by using mAbs of the 9th HLDA workshop. Skin specimens previously collected were sectioned for testing the new mAbs.

2.2. Immunohistochemistry on paraffin-embedded tissue and cryostat sections

Tissue samples were fixed in buffered formalin, dehydrated, embedded in paraffin wax and sectioned. After deparaffinizing and rehydrating, each tissue section was immersed in EDTA 0.05 M pH 8, boiled 3 times for 5 min in a pressure cooker and washed with TBS buffer according to Cattoretti et al. [4]. Each section was placed on the Dako cytometry automated immunostainer and incubated with the specific monoclonal antibody at room temperature for 45 min, washed with TBS pH 7.6 and incubated in a biotinylated goat anti-mouse anti-rabbit immunoglobulins (Dako REAL™, cod.K5005, Dako cytometry, Glostrup, Denmark) at room temperature for 30 min. After incubation with the secondary antibody and a new wash with TBS pH 7.6, sections were incubated with streptavidin conjugated to alkaline phosphatase (Dako REAL™,

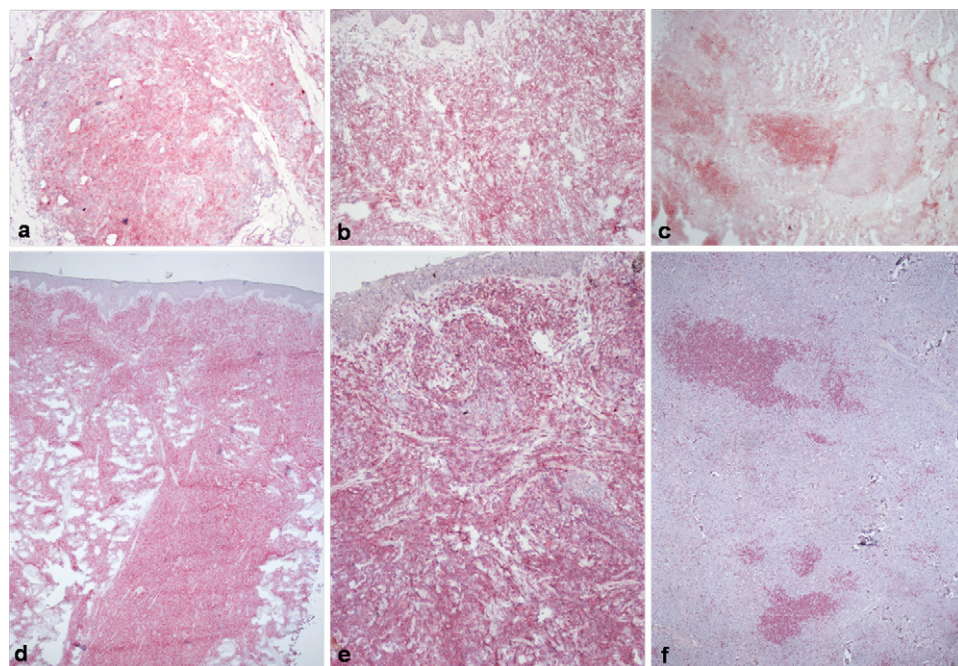


Fig. 2. Reactivity on frozen sections of PC-BL. (a) CD126(36): variable positivity on PC-FCL; (b) BTLA-CD272 (47): staining of PC-FCL cells; (c) CXCR5 (50): positive PC-FCL; (d) CD229 (86): staining of PC-DLBCL; (e) NTBA (93): positivity of PC-DLBCL; (f) TCL-1 (97): nuclear staining of mantle zone in PC-MZL. The numbers in parentheses refer to the 9th HLDA antibody number.

Table 1
mAbs reactivity on primary cutaneous b-cell lymphomas and tonsil.

Specificity	HLDA9 mAb number	Cluster Differentiation (CD)	PC - Follicular centre B-cell lymphomas	PC - Marginal zone B-cell lymphomas	PC - Diffuse large B-cell lymphomas leg-type	Reactivity on normal tonsil
hTRAIL-R2/DR5	11	CD262	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern
PD1	12–66	CD279	Positive	Negative	Negative	Intrafollicular activated cells
BTLA	13–107	CD272	Negative	Positive on reactive follicles and plasmacytes	Negative	Plasmacytes, Mantle zone of the follicles, macrophages and epithelial cells
GCET-1	47	CD272	Positive	Positive	Negative	Not tested
	14	None	Positive	Negative	Negative	Germinal centre cells and epithelial cells
KLHL6	18	None	Positive	Negative	Positive	Germinal centre cells
hFCRL1	20	CD307a	Negative	Negative	Negative	Mantle zone cells, plasmacytes and stromal cells
FCRL2	108	CD307a	Positive	Negative	Negative	Plasmacytes
	21–110	CD307b	Positive	Negative	Negative	Germinal centre cells, epithelium and diffuse stromal reactivity
IL-6R α	36	CD126	Variable positive	Variable positive	Variable positive	Activated T and B-cells
B7-DC	38	CD273	Positive	Negative	Negative	Germinal centre cells
MRC	40	CD200	Positive	Negative	Negative	Germinal centre cells
CD130	41	CD130	Positive	Negative	Negative	Germinal centre cells
CXCR4	46	CD184	Positive	Negative	Negative	Germinal centre cells
CCR6	48	CD196	Negative	Negative	Negative	Mantle zone cells and epithelial cells
CXCR5	50	CD185	Positive	Negative	Negative	Intrafollicular activated cells and marginal zone of the follicles
B7-H4	70	None	Positive	Negative	Positive	Germinal centre cells
Siglec 5/14	77	None	Positive	Negative	Negative	Mantle zone of the follicles and macrophages
Ly9	86	CD229	Positive	Positive	Positive	Positive pan leukocyte, non B-restricted pattern
BLAST-1	88	CD48	Negative	Positive	Variable positive	Mantle zone of the follicles, macrophages and dendritic cells
NTBA	93	None	Positive	Positive	Positive	Positive pan leukocyte, non B-restricted pattern
TCL1	97	Nuclear	Negative	Negative	Variable positive	Nuclear staining of the mantle zone of the follicles
BLIMP-1	103–104–105	Nuclear	Negative	Nuclear plasmacytes	Variable positive	Strong nuclear staining of plasmacytes and epithelial cells
hCD150	106	CD150	Positive	Negative	Negative	Activated T and B-cells
BLK154	114	None	Positive	Positive	Positive	Germinal centre, mantle zone of the follicles
hCD22	119	CD22	Positive	Positive	Positive	Germinal centre and mantle zone of the follicles
hCD38	128	CD38	Negative	Plasmacytes	Negative	Plasmacytes
AID	223	None	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern
BAFF-R	235	CD268	Positive	Positive	Negative	Mantle zone of the follicles
EVI2b	263	CD361	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern
LSP1	268	None	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern	Positive pan leukocyte, non B-restricted pattern

cod.K5005, Dako cytometry, Glostrup, Denmark) at room temperature for 30 min. A red chromogen solution was prepared as indicated by Dako REAL™ data sheet and used as enzyme substrate according to Cordell et al. [5]. Finally, each section was counterstained in Mayer's Hematoxylin solution and coverslipped using Vectamount (Vector Laboratories).

Cryostat sections of frozen tissues were dried for 12 h, fixed 10 s or 10 min in acetone and stained by an avidin-biotin immunoperoxidase method (DAKO) as described above.

3. Results and discussion

We used normal human tonsil as lymphatic positive tissue control (Fig. 1) and normal skin to look for cross-reactivities. In case of mantle zone reactivity, mAbs were also tested on spleen sec-

tions to distinguish marginal or mantle specificity. Different cells type and tissue structures involved other than neoplastic cells are summarized in Table 1.

Results were heterogeneous and some mAbs were not useful for the objective of our study, because they were negative in all the entities or were reactive with several cell types or tissue stroma. We tried therefore to identify some subgroups of mAbs that were exclusively or preferentially positive in one lymphoma cell type (Figs. 2 and 3). Thus we have not considered mAbs that showed broad positivities, but better defined mAbs identifying new clusters of differentiation or nuclear specific reactivities. From these data we have identified subgroups of mAbs positive, respectively on PC-FCL or PC-MZL, while we have not found any mAbs showing a selective staining for PC-DLBCL. The PC-FCL subgroup of mAbs includes: PD1/CD279, GCET-1, hFCRL1/CD307a,

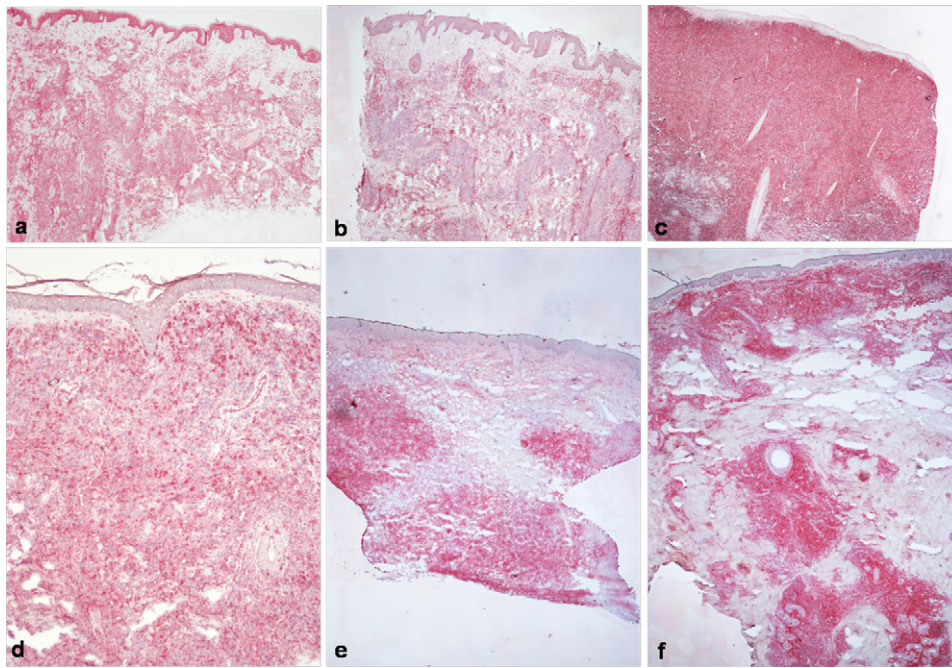


Fig. 3. Reactivity on formalin-fixed sections of PC-BL. (a) BTLA (13): positivity on PC-MZL; (b) BLIMP1 (103): strong staining of plasmacytes in PC-MZL; (c) LSP1 (268): pan-leukocyte reactivity on PC-DLBCL; (d) PD1 (12): positivity of PC-FCL; (e) BLK154 (114): positive PC-FCL cells; (f) EVI2b (263): diffuse staining of PC-MZL. The numbers in parentheses refer to the 9th HLDA antibody number.

FCRL2/CD307b, CXCR5/CD185, B7-DC/CD273, MRC/CD200, CD130, CXCR4/CD184, Siglec-5/14, CD150.

On the other hand subgroup of mAbs in PC-MZL includes: BTLA/CD272, BLIMP-1, hCD38.

Interestingly, in the group of mAbs showing nuclear reactivity, TCL1 and BLIMP-1 displayed a variable nuclear positivity on PC-DLBCL-LT. Our data demonstrate that also PC-DLBCL may express markers of immature/resting mantle B-cell (TCL-1) or of the marginal zone B-cell differentiation (BLIMP-1) as previously reported, respectively in some post-germinal-centre derived non-cutaneous B-cell lymphomas [6] and in aggressive large B-cell lymphomas with plasma cells differentiation [7], probably due to a disturbed terminal B-cell differentiation.

4. Conclusion

In the last WHO classification were defined new PC-BCL entities. Even if clinical presentation and histopathology are very helpful for a correct diagnosis, immunohistochemical analysis and molecular studies are increasingly necessary. This has important implications in the diagnosis, prognosis and treatment approach. We hope that our study, using the new markers of the 9th HLDA workshop, may

be useful to better define antigen profiles and biology of the different PC-BL entities.

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