Immunology Letters 134 (2011) 157–160

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/

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

D. Fanoni[a], S. Tavecchio[a], S. Recalcati[a], Y. Balice[a], L. Venegoni[a], R. Fiorani[a], C. Crosti[a], E. Berti[a,b,*]

[a] U.O. Dermatologia, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Dipartimento di Anestesiologia, Terapia Intensiva e Scienze Dermatologiche, Università degli Studi di Milano, via Pace 9, 20122 Milan, Italy
[b] Dipartimento di Medicina Clinica e Prevenzione, Università degli Studi di Milano-Bicocca, via Cadore 48, 20052, Monza, Italy

ARTICLE INFO

*Article history:*
Received 22 September 2010
Received in revised form
27 September 2010
Accepted 29 September 2010
Available online 15 October 2010

*Keywords:*
Cutaneous B-cell lymphoma
Monoclonal antibodies
Human Leukocyte Differentiation Antigen Workshop

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.



## 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 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 plasmocytes 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

* Corresponding author at: U.O. Dermatologia, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Dipartimento di Anestesiologia, Terapia Intensiva e Scienze Dermatologiche, Università degli Studi di Milano, via Pace 9, 20122 Milan, Italy.
*E-mail address:* immunopatologiacutanea@unimi.it (E. Berti).

0165-2478/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2010.09.022

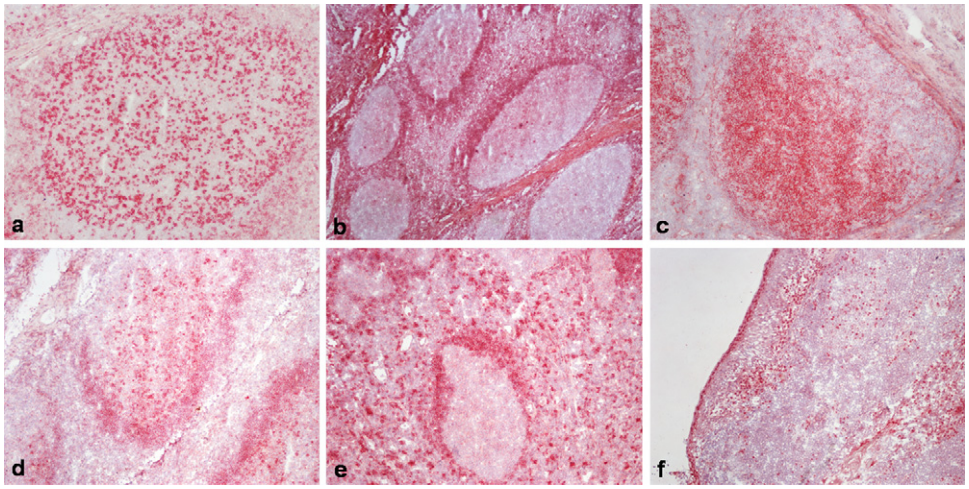


**Fig. 1.** Examples of reactivity on tonsil. (a) PD1(12): intrafollicular activated cells; (b) FCRL1(20): mantle zone cells, plasmocytes and stromal cells; (c) CD200 (40): germinal centre cells; (d) CXCR5 (50): intrafollicular activated cells and mantle zone of the follicles; (e) CD48 (88): mantle zone of the follicles, macrophages and dendritic cells; (f) BLIMP-1 (103): strong nuclear staining of plasmocytes 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 cytomation 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$^{TM}$, cod.K5005, Dako cytomation, 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$^{TM}$,

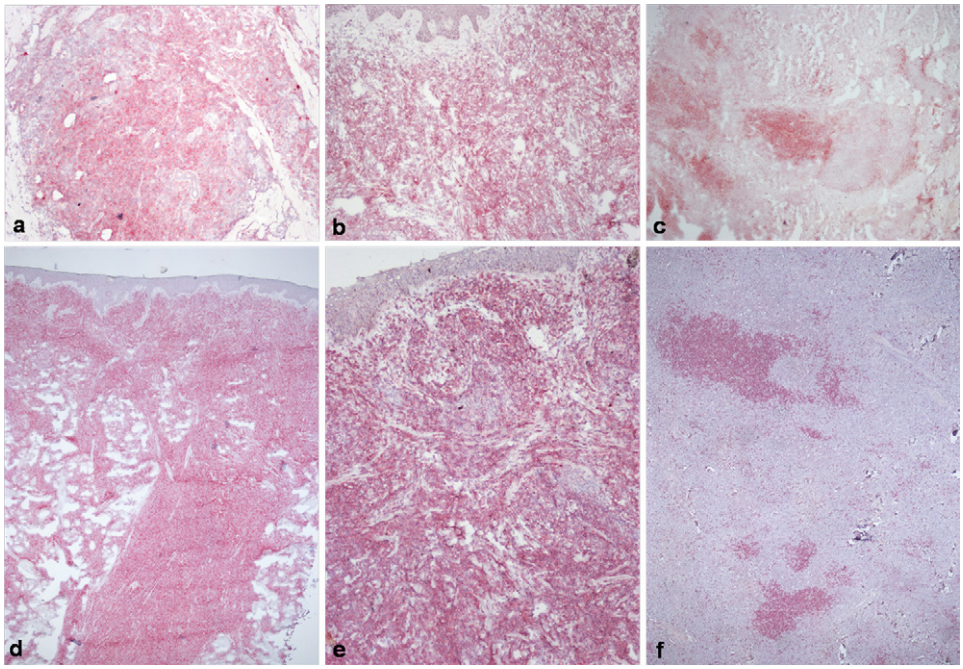


**Fig. 2.** Reactivity on frozen sections of PC-BL. (a) CD126(36): variable positivity on PC-FCL; (b) BTLA-CD272 (47): staining of PC-FCL cells; (c) CXCR5 (50): positive PC-FCL; (d) CD229 (86): staining of PC-DLBCL; (e) NTBA (93): positivity of PC-DLBCL; (f) TCL-1 (97): nuclear staining of mantle zone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