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CD200 expression may help in differential diagnosis between mantle cell lymphoma and B-cell chronic lymphocytic leukemia

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ABSTRACT

Chronic lymphocytic leukemia (B-CLL) and mantle cell lymphoma (MCL) share many features and their differential diagnosis may be challenging, especially when a leukemic picture alone is present. Monoclonal antibody panels are often useful, with CD23 being the most reliable. However, MCL diagnosis should be confirmed by immunohistochemical cyclin D1 detection, sometimes with equivocal or even negative results. Other cytofluorimetric, cytogenetics or molecular techniques are reliable but not widely available.

B-CLL leukemic cells express CD200, a membrane glycoprotein belonging to the immunoglobulin superfamily. We investigated its expression on fresh neoplastic cells of 93 patients with a CD5+ lymphoproliferative disease (79 selected B-CLL and 14 MCL in leukemic phase). Although these data cannot be generalized, all B-CLL samples we examined were positive, with CD200 present on the vast majority of the cells while, in MCL patients, CD200 was expressed by a small minority of CD5+ cells in three subjects and totally absent in the remaining 11.

We then examined CD200 expression on paraffin-embedded lymphoid tissues and bone marrow (BM) trephine biopsies from 23 B-CLL and 44 MCL patients. Again, all B-CLL cells were CD200+ both in lymph nodes and in BM while all MCL cells were negative. Adding CD200 in routine panels could be of diagnostic utility in excluding MCL diagnosis.

1. Introduction

The French-American-British (FAB) classification [1] in the past and, more recently the World Health Organization (WHO) [2] have set the criteria to distinguish between different lymphoproliferative disorders. However, there is a wide spectrum of B-cell tumours that present in leukemic phase and can be misdiagnosed as B-CLL because of overlapping disease features. In particular, in the presence of a CD5 positive B-cell lymphocytosis, it is important to differentiate B-CLL from mantle cell leukemia as the latter is a more aggressive disease and is generally treated differently than B-CLL. In the presence of a leukemic picture, differential diagnosis between B-CLL and MCL is assessed by immunophenotypic analysis on freshly isolated cells and in this respect CD23 is considered a reliable marker because of its positivity in CLL and negativity in MCL [3–7]. However, some cases where CD23 is not discriminant have been reported [5,6,8,9]. In any case the diagnosis of MCL should be confirmed by demonstration of cyclin D1 positivity or by the presence of the t(11;14) chromosomal translocation detected by cytogenetics, fluorescence in situ hybridization (FISH), Western blot or polymerase chain reaction (PCR) analysis. However, cyclin D1 detection can be easily accomplished on tissue biopsy only, while its determination in cell suspension by flow cytometry is cumbersome [10]. On the other hand, cyclin D1 negative MCL do actually exist [11,12] and, by working on fixed tissues (especially by B5 solution), CD5 and cyclin D1 analysis can give equivocal or even negative results [13] even though detection of cyclin D1 has become simpler since the availability of new antibodies [14]. In addition, the presence of the chromosomal translocation t(11;14)(q13;q32) is not pathognomonic for MCL. In fact, mantle cell lymphomas without t(11;14) have been reported [15] and the same translocation can be found in B-CLL and other lymphoproliferative disorders [16–18].

Therefore, there is a need for new markers that allow an easier differential diagnosis between CLL and MCL. It has been recently

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demonstrated that B-CLL are uniformly positive for CD200 [19]. CD200 (previously referred to as OX2) is a membrane glycoprotein, belonging to the immunoglobulin superfamily [20,21], expressed on a subset of T and all CD19+ B lymphocytes (but not on NK cells), dendritic cells [22] and highly on central and peripheral nerve tissue. Its expression has also been reported on human myeloma plasma cells [23], acute myeloid leukemias [24] and other non-hematological malignancies [25]. CD200 might play an immunosuppressive role [26] and regulate myeloid cell activity in a variety of tissues [22,27]. We investigated the expression of CD200 on neoplastic cells of patients affected by CLL and MCL and we found that there is a clearcut between B-CLL where CD200 is highly expressed versus MCL cells in which this antigen is negative.

2. Patients and methods

2.1. Patients

In this study 98 patients affected by B-cell chronic lymphocytic leukemia (B-CLL), and 52 CD5+ mantle cell lymphoma MCL, were enrolled. Other cases of CD5+ lymphoproliferative disorders were not included in the study. Diagnosis of B-CLL was made by flow cytometry according to the latest version of the Matutes score [28,29] and patients with the highest score (4 or 5) only were selected for the study (51 patients scored 5, the remaining 47 scored 4). Diagnosis of MCL was based on morphology and immunohistochemical detection of CD5 and cyclin D1 in tissue biopsies, except for one patient where diagnosis was made by FISH and PCR. In MCL patients, Matutes score was calculated only for patients in leukemic phase, corresponding to the 14 patients studied by immunophenotypic analysis: two patients scored 0, 8 had score 1 and for the remaining four the score was 2. Immunophenotypically atypical B-CLL cases with more than one deviation from the expected expression pattern of CD19, FMC-7, CD79b, CD5, CD23 expression of surface immunoglobulin were excluded from analysis (two and three patients with score 2 and 3, respectively).

2.2. Immunophenotypic analysis

We investigated the expression of CD200 on fresh neoplastic cells of 91 patients (79 B-CLL and 14 MCL in leukemic phase) by flow cytometry, using a mouse IgG1 anti-human moAb (MRC OX-104, BD Pharmingen, U.S.A.). All other monoclonal antibodies (CD5, CD19, CD23, CD79b, FMC7, surface Immunoglobulins, cyclin D1 were purchased from Immunotech, Marseille, France. Immunophenotyping was determined by flow cytometry on whole blood. In brief, 2 ml of peripheral blood were collected in EDTA and then washed twice in 20 ml of phosphate buffer saline (PBS) + 0.2% bovine serum albumin (BSA). White cell count concentration was adjusted to 5×10^6/ml, then 100 mcl aliquots of this cell suspension were stained with 10 mcl of the moAb for 15 min at room temperature. After lysing red cells with FACS Lyse (Becton Dickinson), according to manufacturer indications, cells were washed once more with 0.2% BSA PBS and flow cytometry analysis was performed, using a Cytomics FC500 (Beckman Coulter). An antigen was considered positive when at least 20% of the cells expressed that antigen [9,30,31]. The fluorescence intensity was measured according to Gong et al. [5], using a logarithmic scale with signal intensity ranging from 10^0 to 10^4. In brief, a cell population was considered negative when the mean fluorescence intensity (MFI) was not significantly different from that of cells labeled with a matched isotype moAb, assumed as negative control; dimly positive when the population was partially overlapping and with a MFI within 1 log of the control; moderately positive when the labeled cells were distinct from and with MFI about 1 log brighter than the control; brightly positive when the population was at least 1.5–2 logs brighter than the control.

2.3. Immunohistochemistry

Immunohistochemistry was performed retrospectively in archival material of cases with a well defined diagnosis of B-CLL or MCL. We collected material from 23 patients who had been diagnosed as B-CLL (Matutes score 4 or 5), four of whom were also included in the above described series studied by flow cytometry, and 45 patients with a diagnosis of MCL, seven of whom were also analyzed by flow cytometry.

All the formalin-fixed paraffin embedded material that was reviewed consisted of 41 bone marrow trephine biopsy (bilateral in three cases), 20 lymph nodes from various sites (axillary, cervical, supraclavicular, inguinal, mesenteric, intraparotid), two spleens, two tonsils, two mucosal biopsies (oral and conjuntival) and eight gastro-intestinal biopsies; 11 patients (four B-CLL and seven MCL) had both immunohistochemistry on fixed tissue and flow cytometry analysis on peripheral blood.

The histological specimens from all tissues, except bone marrow, were fixed in 10% neutral buffered formalin and paraffin wax embedded; the bone marrow biopsies were fixed and decalcified in Lowy's solution. Four micron sections were dewaxed and antigen retrieval was carried out by treating sections for 30 min in pH 6 citrate buffer at 98 °C.

Table 1
Immunophenotypic (percentage of positive cells in peripheral blood) and clinical characteristics of B-CLL and leukemic MCL patients.

	B-CLL (98 pts.)	Leukemic MCL (14 pts.)
CD200+ (mean, median, S.D.)	92.4, 98.6, 13.4	4.0, 0.2, 7.4
CD19+ (mean, median, S.D.)	77.7, 81, 13.8	60.7, 72.7, 29.7
CD5+/CD19+ (mean, median, S.D.)	73.3, 76.7, 15.8	54.1, 55.1, 31.9
CD23+ (mean, median, S.D.)	72.9, 74.1, 14.4	18.5, 8.9, 24.7
FMC7+ (mean, median, S.D.)	21, 12, 22.1	47.6, 48, 30.6
CD79b+ (mean, median, S.D.)	19.2, 3.4, 26.7	55.4, 73, 33.1
sIg (bright/dim)	3/95	9/5
WBC (mean, median, S.D.)	29 100, 18 600, 32 600	19 600, 12 400, 21 000
% Ly (mean, median, S.D.)	69.9, 70.5, 16.4	65.2, 66, 19.1
Age (median, range)	68, 38–87	69, 36–82
Sex (M/F)	52/46	11/3
Untreated/pretreated	74/24	10/4

Immunohistochemistry was performed using a goat anti-human CD200 IgG affinity purified polyclonal antibody (R&D Systems, U.S.A.) at 1:50 dilution for 30 min at room temperature; streptavidin–biotin peroxidase complex method was used for immunostaining with a DAKO Autostainer (LSAB+ system with DAB, DAKO, Denmark), according to standardized manufacturer procedures.

2.4. Statistics

A non-parametric two-way contingency table test (Fisher exact test) was employed, using StatView for Windows software, ver. 5.0.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Immunophenotypic analysis

In our series, CD200 was present on neoplastic cells of all 79 B-CLL (expression on 40–100% of CD5+ cells, mean 96%, standard deviation 7%). On the contrary, in MCL patients in leukemic phase CD200 showed a dim positivity in a small minority (less than 20%) of CD5+ cells in three subjects (4%, 7%, and 16%) and was totally absent in the remaining 11 (two-sided Fisher's exact test $p < 0.0001$). Typical cases of B-CLL and MCL are shown in Fig. 1.

CD23 is indicated as one of the master markers for the differential diagnosis between B-CLL (+) and MCL (−) and, as expected, it was positive in all B-CLL cases of our series since we have selected patients with a definite diagnosis of B-CLL (Matutes score 4 or 5). However, six out of 14 MCL samples showed a positivity for CD23 > 20% (range 23–48, median 29%), albeit at low intensity of expression. Immunophenotypic findings are reported in Table 1.

3.2. Immunohistochemistry

All B-CLL neoplastic cells showed diffuse intense membrane immunostaining with CD200 both in lymph nodes (Fig. 2B) and in BM (Fig. 2C) while in all MCL cases there was no immunostaining on atypical B-cells (two-sided Fisher's exact test $p < 0.0001$) both in lymphoid tissues (Fig. 2E) and in BM (Fig. 2F).

However, in all MCL CD200-negative lymphoid tissue biopsies, it was possible to observe CD200 + residual reticulum dendritic cells, as expected.

In addition, it has always been possible to obtain evaluable results with anti-CD200 in old archival samples and even in non-perfectly prepared tissues referred to us from other Centers. On the contrary, cyclin D1 immunostaining or CD5 detection did not give univocal results in these samples.

3.3. CD200+ MCL patients

As already mentioned, three MCL patients showed a dim positivity for CD200. The patient with the highest number of CD200