Differential Expression of CD200 in B-Cell Neoplasms by Flow Cytometry Can Assist in Diagnosis, Subclassification, and Bone Marrow Staging

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ABSTRACT

Objectives: To analyze CD200 expression by flow cytometry in a large series of B-cell neoplasms in a variety of tissue types in comparison with benign B-lineage cells.

Methods: We measured CD200 expression levels in 505 peripheral blood (PB), bone marrow (BM), and lymphoid tissue biopsy specimens, including 364 cases positive for B-cell leukemias and lymphomas.

Results: CD200 expression in chronic lymphocytic leukemia cases was as bright as or brighter than normal PB B cells in nearly all cases, while mantle cell lymphoma (MCL) cases were usually dim or negative. However, rare MCL cases (about 5%) were moderately bright for CD200. Marginal zone lymphomas varied by subtype, with nodal cases brighter, splenic cases dimmer, and extranodal cases heterogeneous for CD200 expression. Follicular lymphoma (FL) cells were brighter for CD200 in BM specimens than in lymph nodes. In some BM specimens, dim CD200 could distinguish FL cells from background hematogones. Large B-cell lymphomas of the non–germinai center type tended to be brighter for CD200 than those of the germinal center type, while Burkitt lymphomas were negative.

Conclusions: CD200 staining by flow cytometry can be useful in the differential diagnosis of B-cell neoplasms and in their detection in the BM.

Immunophenotypic characterization of lymphoid neoplasms is important for diagnosis, subclassification, and staging and can also play a role in monitoring minimal residual disease. However, the differential diagnosis may be difficult to resolve in some cases, for example, between B-cell neoplasms with full or partial CD5 expression. CD200 has recently been identified as a potentially useful antigen for flow cytometric immunophenotyping of lymphoid neoplasms, particularly those of the B lineage.

CD200 is a transmembrane type Ia glycoprotein originally identified by the antibody MRC OX-2, encoded by the CD200 gene mapped to chromosome 3q13.2. CD200 belongs to the immunoglobulin superfamily and is composed of a light chain–like structure with two extracellular variable- and constant-like domains followed by a transmembrane segment and a cytoplasmic tail. CD200 is expressed by various cell types, including B cells, a subset of T cells (including activated T cells), thymocytes, endothelial cells, and neurons. CD200 generates an immunosuppressive signal by binding to its cognate receptor, CD200 receptor 1 (CD200R1), which is expressed specifically in granulocytes and monocytes and in a subset of T cells. CD200 appears to play a role in the regulation of antitumor activity, and these findings are the basis for ongoing clinical trials using anti-CD200 therapy for chronic lymphocytic leukemia (CLL).

Several previous studies have examined differential CD200 expression in B-lineage neoplasms, initially focusing on the distinction between CLL, usually brightly positive for CD200, and mantle cell lymphoma (MCL), usually CD200 negative. Brunetti et al showed increased CD200 expression in hairy cell leukemia (HCL). Subsequently, others using flow cytometry and immunohistochemical (IHC)
analysis have shown that CD200 is expressed at different levels in a range of B-cell neoplasms. In some studies of other hematolymphoid malignancies, CD200 gene expression has been correlated with a poor prognosis.

In this study, we investigated CD200 expression by using flow cytometry immunophenotyping in a large cohort of B-cell lymphomas and normal hematolymphoid tissue samples. Our goals were to assess (1) a large number of cases with a broad range of low- and high-grade B-cell neoplasms and determine whether various types of B-cell lymphoma show consistent differential expression of CD200, (2) CD200 expression levels in B-cell neoplasms at different anatomic sites, and (3) CD200 in a variety of normal B-cell subsets. In addition, in a large group of CLL cases, we correlated CD200 expression levels with other known prognostic markers, including cytogenetic data with results of fluorescence in situ hybridization (FISH) studies, and somatic hypermutation status of the immunoglobulin heavy chain gene.

Materials and Methods

Patients

Patient samples for initial diagnosis, staging, or restaging submitted to the Clinical Flow Cytometry Laboratory in the Department of Hematopathology at the University of Texas MD Anderson Cancer Center (Houston, TX) were included in this study. The study group included 364 peripheral blood (PB), bone marrow (BM) aspirate, and fine-needle aspirate or lymph node excisional biopsy samples from patients with various B-cell neoplasms. The B-cell neoplasms included CLL (n = 119), follicular lymphoma (FL; n = 90), MCL (n = 61), large B-cell lymphoma (LBCL; n = 52), marginal zone lymphoma (MZL; n = 26), HCL (n = 7), Burkitt lymphoma (BL; n = 6), and lymphoplasmacytic lymphoma (LPL; n = 3). All cases were diagnosed using the 2008 World Health Organization classification criteria.

We also analyzed normal B-cell subsets, including CD10– extra–follicle center B cells (n = 49) or CD10+ follicle center B cells (n = 32) from lymph nodes, many with reactive follicular hyperplasia, benign bone marrow B-cell precursors (“hematogones,” n = 50), and normal peripheral blood B cells (n = 42). This study was approved by the Institutional Review Board at the University of Texas MD Anderson Cancer Center.

Immunophenotypic Analysis

Samples were assessed with a large panel of antibodies designed for characterizing B-cell lymphomas as part of the usual workup. In addition, we included a seven-color tube containing CD43 FITC, CD200 PE, CD38 PerCP-Cy5.5, CD19 PE-Cy7, CD10 APC, CD20 V450, and CD45 V500. All antibodies were purchased from BD Biosciences (San Jose, CA), and the anti-CD200 clone used was MRC OX-104. One million cells were processed using a standard stain-lyse-wash protocol, incubated with antibodies for 10 minutes at 4°C, lysed with 2 mL BD Pharmingen Lyse buffer (BD Biosciences), and washed once with 1× phosphate-buffered saline (PBS)/0.1% sodium azide using a Sorvall Cell Washer 2 (Thermo Fisher Scientific, Waltham, MA). The pellet was suspended in 1× PBS. Three-laser, eight-color FACSCanto II cytometers (BD Biosciences) were used to acquire the data for 30,000 events.

Data were analyzed using FlowJo software (TreeStar, Ashland, OR). Doublets were excluded by gating with forward and side light scatter parameters. In all cases, B cells were gated on the basis of CD19 and CD20 expression. Neoplastic B cells were separated from normal background B cells based on differential expression of CD10 and/or CD43. In reactive lymph node specimens, follicle center cells were identified based on expression of CD10 and bright CD38. In BM specimens, hematogones were identified based on coexpression of CD10 and CD43. CD200 expression was quantitated by calculating the mean fluorescence intensity (MFI) of CD200 for neoplastic B-cell populations or for normal reactive or immature B-cell subsets. In qualitative terms, CD200 expression levels were assessed as bright, dim, or moderate (similar) in comparison with normal mature B cells in PB and BM, in line with recommended reporting guidelines. Cases were considered negative when the MFI was similar to the level of autofluorescence in a control tube with no phycoerythrin-conjugated antibody (MFI, 20).

Conventional Cytogenetic Studies

Cytogenetic analysis was performed on conventional G-banded metaphase cells prepared from PB or BM aspirate specimens by using standard procedures described previously. Twenty metaphases were assessed and the results described by using the current International System for Human Cytogenetic Nomenclature. FISH was also performed on interphase cells of CLL cases using a five-probe panel (Vysis/Abbott, Des Plaines, IL). The panel of probes is designed to detect deletion 13q14.3, deletion 13q34, trisomy 12, deletion of TP53 at 17p13, and deletion of ATM at 11q22.3.

Statistical Analysis

Prism 6 software (GraphPad Software, La Jolla, CA) was used for statistical analysis. The paired two-tailed t test was used for comparisons of CD200 MFI values between different specimen sites from the same patients, and the unpaired two-tailed t test was used for comparisons between all other groups. A P value of less than .05 was considered statistically significant.
CD200 Expression in Benign B-Cell Subsets

Circulating B cells in the PB and CD10+ hematogones in BM showed relatively bright and homogeneous expression of CD200, with an MFI from 1,000 to 4,500 in nearly all samples. Hematogones at different stages of maturation showed similarly bright CD200 positivity (data not shown). A minority of mature B cells were dim for CD200, but the CD10+ subset included virtually no CD200 dim cells in normal BM specimens. In lymph node biopsy specimens with reactive follicular hyperplasia, CD10– extra–follicle center B cells showed slightly dimmer CD200 expression (median MFI, 1,089) than did B cells in the PB or BM. CD10+ follicle center B cells were consistently dim (median MFI, 211) (Figure 1).

CD200 Expression in CLL

All 119 cases of CLL were positive for CD200 (Figure 1). In nearly all cases, CD200 expression was moderate to bright (i.e., at least as bright as benign peripheral blood B cells), and only two CLLs had a CD200 MFI of less than 1,000. There was a significant difference in median CD200 expression between CLL specimens involving BM and PB (median MFI, 9,334 vs 5,975; \( P = .002 \)) but the ranges of expression were largely overlapping. There was no significant difference in CD200 expression levels between CLL cases with mutated or unmutated immunoglobulin heavy chain variable region genes (\( P = .41 \)). CLL cases with trisomy 12 showed significantly dimmer CD200 expression than did non–trisomy 12 cases (median MFI, 4,971 vs 8,138; \( P = .038 \)), but none showed much dimmer expression than did normal peripheral blood B cells. No other cytogenetic subgroups showed significant differences in CD200 expression, although CLL cases with deletions involving the \( ATM \) locus showed a trend toward slightly dimmer expression.

CD200 Expression in MCL

Fifty-eight (95%) of 61 cases of MCL were positive or dim for CD200 (36 negative and 22 dim; Figure 1). The difference in CD200 expression levels between CLL (median MFI, 6,444) and all MCL cases (median MFI, 40) was highly statistically significant (\( P < .0001 \)).

However, three cases of MCL showed moderately bright CD200 expression, overlapping with CLL cells and/or normal peripheral blood B cells, with MFI values of 5,535 (case 1), 2,335 (case 2), and 827 (case 3). These three cases all showed cyclin D1 overexpression by IHC. Cases 2 and 3 also had t(11;14)(q13;q32) shown by FISH and conventional cytogenetics. Case 1 was negative for t(11;14)(q13;q32) by both methods but showed del(14q31q32) in three of 20 metaphases, with confirmed loss of one \( IGH \) locus by FISH. Flow cytometry immunophenotyping in all three cases showed an atypical pattern of CD23 expression, positive on most lymphoma cells.

CD200 Expression in FL

FLs overall showed a spectrum of CD200 expression across the cases in this study, ranging from negative to moderate (Figure 1). There was no apparent difference in the expression levels of CD200 between low-grade (grades 1-2) and high-grade (grade 3) FLs (\( P = .49 \)), although the number of high-grade lymphomas was small (n = 5).

FLs showed differential CD200 expression in different sites of involvement, with markedly higher levels in most PB and BM specimens than in most tissue biopsy specimens. The difference in the median CD200 MFI between BM and tissue biopsy specimens was highly significant (867 vs 138; \( P = .0005 \)). Six patients had paired specimens with FL involving both BM and lymph nodes. In each patient, CD200 was significantly brighter in BM than in lymph nodes (median MFI, 1,608 vs 138; \( P = .013 \)).
Expression of CD200 levels in benign and neoplastic B-cell populations (shown are B cells in A-G and I and total lymphocytes in H). A, Normal bone marrow with benign B-lineage cells showing moderately bright CD200 expression (green, hematogones; blue, mature B cells). B, Lymph node with reactive follicular hyperplasia (green, follicle center B cells, dim for CD200; blue, extrafollicular B cells, moderately bright). C, Chronic lymphocytic leukemia involving lymph node (red, neoplastic B cells, very bright for CD200; blue, normal B cells, moderately bright). D, Mantle cell lymphoma involving lymph node, dim for CD200 (colors as in C). E, Mantle cell lymphoma with atypically bright CD200 involving bone marrow (red, neoplastic B cells; green, hematogones). F, Follicular lymphoma in bone marrow, typical pattern with a subset dim for CD200 (colors as in C). G, Follicular lymphoma in lymph node, showing atypically bright CD200 (colors as in C). H-I, Marginal zone lymphoma with partial CD5 expression and dim CD200 (gray, T cells in H; red, lymphoma cells).

In comparison to normal B-cell populations in the same sites, a subset of FL cases showed aberrant patterns of CD200 expression. Taken as a group, FL cases involving BM showed significantly dimmer CD200 expression than did CD10+ hematogones (median MFI, 867 vs 1,590; \( P = .0005 \)). In many of these cases, there was a large subset of CD10+ lymphoma cells with dim CD200 expression, which could distinguish them from hematogones (Image 1F) (compared with Image 1A).

In lymphoid tissues, most FL cases showed dim CD200 expression, similar to reactive CD10+ follicle center B cells (overall median MFI, 138 vs 211; \( P = .55 \)) and lower than in CD10– extra–follicle center B cells (138 vs 1,089; \( P < .0001 \)). A subset of FL cases in tissues did show atypically increased CD200 expression for this site, with a subset of CD10+ lymphoma cells showing an overlap in CD200 levels with CD10– benign extrafollicular cells (Image 1G) (compared with Image 1B).
CD200 Expression in Other B-Cell Neoplasms

Among the other low-grade B-cell lymphomas and leukemias (Figure 1), uniformly bright CD200 expression was observed in HCL, with moderate expression seen in LPL (in the limited number of cases tested). A spectrum of CD200 expression, from negative to bright, was seen in MZL.

MZLs showed differences in CD200 expression by subtype Figure 2C. Nodal MZLs were few in this series but showed significantly brighter CD200 expression compared with mucosa-associated lymphoid tissue (MALT) lymphomas and splenic MZLs (P = .011 and P = .042, respectively). MALT lymphomas showed a spectrum of expression for CD200, while most splenic MZLs were dim. There were no significant differences in CD200 expression for MZLs whether they involved PB, BM, or tissue biopsy specimens (data not shown).

There was a highly significant difference in CD200 expression between CLL and MZL cases taken as a group (median MFI, 6,444 vs 168; P < .0001). However, nine (35%) of 26 cases of MZL, mostly nodal or MALT types, showed moderate to bright CD200 expression that overlapped with the range of expression in CLLs. The few MZL cases with partial expression of CD5 were dim to negative for CD200 Figure 1H and Figure 1I.

Among high-grade B-cell neoplasms (Figure 1), LBCLs showed a spectrum of CD200 expression. Most cases were brighter than BLs, which were uniformly dim to negative. Taken as a group, CD10+ LBCLs showed significantly dimmer CD200 expression than did CD10– LBCLs (median MFI, 88 vs 960; P = .0031), although the ranges were overlapping between these subsets (Figure 2C).
Discussion

In this study, we assessed CD200 expression by flow cytometry in a large number of B-cell neoplasms in blood, lymphoid tissue, and BM in comparison to normal B-cell subsets in these sites. This extensive data set allowed us to add to the previous literature by characterizing multiple subsets of the common leukemia and lymphoma subtypes and by better defining their range of CD200 expression levels, including clinically significant outliers.

In normal B cells, CD200 was moderately bright in peripheral blood B cells and in BM hematogones and mature B cells. In reactive lymph nodes, extra–follicle center B cells were nearly as bright as bone marrow B cells, in contrast to CD10+ follicle center B cells, which were dim. Among B-cell lymphomas, CD200 was bright in HCL and moderate to bright in CLL, including all CLL subtypes examined. Most cases of CD10– LBCL and most FLs in BM showed moderate or slightly dim CD200 expression. There was a range of CD200 expression in MZLs, with most splenic cases showing dim expression. CD200 was also dim in most CD10+ LBCLs and FLs in lymph nodes. Expression was very dim or negative in BL and nearly all cases of MCL, although rare MCL cases (about 5%) showed moderate or bright expression.

To our knowledge, this study is the first to assess the CD200 expression levels in CLL with regard to cytogenetic abnormalities and somatic hypermutation status. Cases with trisomy 12 comprised the only CLL subset with a significant difference in CD200 expression. However, even though these were on average dimmer than other CLLs, CD200 expression was still as bright as or brighter than in normal peripheral blood B cells and most other B-cell neoplasms. Cases with trisomy 12 often show an atypical phenotype for CLL, with brighter expression of surface immunoglobulins and pan–B-cell markers. Bright CD200 expression can assist in differentiating trisomy 12 cases, as well as other CLL cases with an atypical phenotype, from other B-cell neoplasms. Overall, our findings are consistent with previous reports showing that virtually all CLL cases show moderate to bright expression of CD200.12,15-18,26,27

As has been shown by others, CD200 expression is useful for distinguishing CLL from MCL,12,15-18,27 since almost all reported MCL cases were CD200 dim or negative. However, our MCL study group included three (5%) of 61 cases of MCL with moderate to bright CD200 expression, with most lymphoma cells comparable to normal peripheral blood B cells or CLL cases, and at least 90% of lymphoma cells positive compared with background staining. This appears to be a novel finding. A previous report by Palumbo and colleagues12 did describe three (21%) of 14 MCL cases with some CD200 expression; however, in those cases, the staining was dim, and the fraction of positive cells was only 4% to 16%. We note that the CD200+ MCL cases in both our series and that of Palumbo et al12 showed atypically bright expression of CD23. Thus, in a CD5+ B-cell neoplasm, dim or absent CD200 expression virtually excludes a CLL diagnosis, but bright expression of CD200 does not completely exclude MCL.

Our findings for FL involving lymph nodes are in line with previous reports,17,18,27 with most cases showing dim or absent CD200 expression, similar to benign reactive CD10+ follicle center B cells. However, analysis of FL in BM led to an unexpected finding, since most cases were significantly brighter for CD200 than FL in lymph nodes, even in cases with involvement by lymphoma in both sites in the same patient. The biological significance of this differential expression is uncertain. Recognition of this phenomenon could be important for detection of FL in the BM, where CD200 expression on lymphoma cells may be brighter than expected, in comparison to a previously characterized nodal site. In addition, we would suggest that triage for possible anti-CD200 therapy in FL should not be based on expression levels in the BM but rather on the levels assessed in a tissue site.

Hematogones show a highly reproducible pattern of moderately bright uniform CD200 expression, and this can help distinguish them from CD10+ B-lineage neoplasms involving BM specimens. Although relatively bright for CD200 in many cases, FL cells in BM in most cases show dimmer expression than hematogones in at least a subset of lymphoma cells. Cases of BL may show a CD10+, CD38 very bright+, CD43+ phenotype and thus mimic many features of hematogones; however, their dim to negative levels of CD200 clearly mark them as aberrant in the BM. Our study did not include any cases of B-acute lymphoblastic leukemia (B-ALL), but others have suggested that decreased CD200 levels on aberrant lymphoblasts may help identify residual B-ALL cells after therapy.15

Earlier studies have reported dim or absent expression of CD200 in MZL16,17 or a spectrum including moderate expression in a few cases,18,27 but previous reports have not compared nodal, extranodal, and splenic subtypes in detail. In this study, we found that nodal MZLs showed bright CD200 expression, while most splenic MZLs had dim or absent expression, and cases of extranodal MZLs showed a spectrum of expression levels. Previously, using immunohistochemical (IHC) studies, Dorfman and Shalasaefai19 reported that CD200 was negative in splenic and extranodal MZL. It appears that flow cytometry may detect CD200 expression at lower levels than IHC, as seen in MZL cases studied by flow cytometry in a subsequent series by the same group17 and in this study.

Splenic MZL in a PB or BM specimen may be difficult to distinguish from CLL with an atypical phenotype. This study helps confirm that CD200 should be useful in this differential diagnosis in most cases, since bright expression is unusual in splenic MZL and dim expression appears to be very rare.
even in atypical CLL. A subset of MZL cases may show CD5 expression (often partial). A CD5+, CD200+ MZL could potentially be difficult to distinguish from CLL. However, such cases appear to be rare, and we did not observe such a case in our series.

All cases of HCL in this study showed very bright expression of CD200, as others have shown in larger series. We confirm that MZL cases of all subtypes show much dimmer CD200 expression than HCL cases, and this finding could be useful in the differential diagnosis of these CD5–, CD10– B-cell neoplasms in the PB or BM.

In conclusion, our results suggest that CD200 is a useful addition to a flow cytometry marker panel for analysis of B cells in lymphoid tissue biopsy specimens, BM aspirates, and PB. CD200 is particularly useful in distinguishing atypical CLL/small lymphocytic lymphoma (SLL) from other CD5+B-cell neoplasms, which usually show lower expression levels than CLL/SLL. It is, however, important to recognize exceptions to this rule, such as rare MCLs and a subset of nodal MZLs and MALT lymphomas, which may be relatively bright for CD200. CD200 staining can also assist in detecting CD10+ neoplasms such as FL, BL, and B-ALL in the BM, since they show dimmer expression than background benign hematogones. Last, CD200 could be a potential therapeutic target, particularly for patients with B-cell neoplasms that brightly express CD200, such as CLL and HCL.

References


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