Role for expression of CD200 in multiparameter flow cytometric differential diagnosis of mature B-cell neoplasms

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Introduction

This issue of the journal includes an important study by Sandes *et al.* (1), which explores whether inclusion of analysis of CD200 expression can contribute to improved classification of mature B cell neoplasms (MBN). Currently, the most frequent subgroupings used for classification purposes are (2,3): CD5+CD10− [chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL)]; CD5−CD10+ [Follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), and Burkitt's lymphoma]; and CD5−CD10− [marginal zone lymphoma (MZL), lymphoplasmacytic lymphoma, and lymphomas with hairy/villous cells including splenic marginal zone lymphoma (SMZL), and hairy cell leukemia (HCL)].

Further differentiation of CD5+CD10− cells into CLL and MCL is based upon CD23 expression analysis, with the former being CD23+, while most cases of MCL are CD23− (4). Unfortunately this delineation is far from perfect, and seems to break down completing in cases of atypical CLL, where substantial overlap with the heterogeneous group of MCL occurs. Poor distinction using these criteria is also seen amongst other MBN subgroups, including FL and DLCBL, and HCL and SMZL. Such difficulties with the current classification systems highlight the need for additional immunophenotypic markers which might, in combination with those above, improve the overall utility of multiparameter flow cytometry (MFC) in differential diagnosis. Analysis of CD200 expression seems to fulfill just this need, as indicated in previous reports of its expression in typical CLL and MCL (5-8). The study by Sandes *et al.* confirmed this analysis in other cases of MBN, and includes atypical CLL cases for comparison also. In all samples studied the gold standard criteria used to discriminate amongst the MBNs was based upon WHO classification using clinical, morphologic, immunophenotypic and genetic criteria. A revised Matutes score for immunophenotypic markers including CD5, CD23, FMC7 and weak/null expression of surface immunoglobulin and CD79b was used to classify CLL (atypical CLL scored <4 and/or lacked CD23); while immunohistochemical detection of cyclin D1, and FISH analysis of t (11:14) (q13:q32), absence of IGH/CCND1 rearrangement or CLL-related cytogenetic abnormalities were confirmed in CD5+NHL non-CLL, non-MCL.

All 45 cases of typical CLL, and all 11 atypical CLL, showed expression of CD200, though in atypical CLL the intensity of staining was more variable, and weaker, than in typical CLL cases. As noted above CD23 expression was often absent in atypical CLL, and CD79b often present. All MCL cases were negative for staining with CD200, although 4/14 stained for CD23. Amongst cases of CD10+ MBNs, expression of CD200 was absent or low (DLBCL) and absent [3]/low[6] in 9/11 FL cases. For CD5−CD10− B cell lymphomas no CD200 expression was seen in ~30% of cases, with the remainder showing highly heterogeneous expression. In B cell lymphomas with hairy/villous lymphocytes, all HCL stained the highest of all MBNs for CD200, with SMZL having significantly lower expression

than HCL and less even that typical CLL.

The authors propose a diagnostic algorithm for classification of CD5+ MBNs based upon the combined expression of CD200, CD11c, heavy chain immunoglobulins and the Matutes score, in which in cases with score ≤3, CD200 becomes a key feature in which positivity indicates either atypical CLL or NHL. Samples lacking CD200 and CD11c with high expression of IgM and IgD are likely MCL. The low/absent CD200 expression in CD10– MBNs was obviously less useful for classification of FL *vs.* SLBCL, and again the heterogeneous pattern of expression in the CD5–CD10– subgroup resulted in CD200 expression having little role to play in classification of these B cell neoplasms, other than for lymphocytes with hairy/villous cells.

Discussion

Beyond this analysis of the usefulness of assessing CD200 expression for diagnostic classification purposes, it is worthwhile asking are there other reasons to investigate CD200 expression? Does the molecule serve a function in disease? If so, what is it, and how might this be modified if that is an issue?

CD200 itself is a Type-1 membrane glycoprotein of the immunoglobulin supergene family, expressed by, amongst others, neuronal cells, B cell precursors and mature B cells, subsets of T cells, and dendritic cells (9). It has been shown previously to be expressed in distinct hematologic malignancies, including acute leukemia (10), multiple myeloma (11), and both B and T cell lymphomas (12-14). There is a growing body of evidence indicating increased expression of this molecule also on cells in solid tumors, including melanoma and ovarian tumors (15) as well as breast cancer (16,17), where its expression seems to impact on both growth and metastasis of the tumor (18). Its co-expression has been deemed to occur on cancer cells with stem cell properties (19). Expression of CD200 on mesenchymal stem cells in marrow seems to control normal osteoblastogenesis (20-22) while there are also clear cut reports of an important role for CD200 expression on normal brain differentiation and function (23).

In terms of its importance in B cell malignancies, there are numerous reports indicating an effect of CD200 expression on human CLL growth in animal models (14), along with evidence that levels of a soluble (serum-expressed) form of CD200 are related to prognosis in CLL patients (24). It is now thought that CD200 can be released

from CD200+ malignant cells by ectodomain shedding, likely associated with expression of key ADAMs in the leukemic population (25,26). Most importantly, the levels of serum sCD200 in CLL patients have been associated with disease progression/prognosis but those levels are not simply a function of the expression levels on the CLL cells themselves, but presumably also a function of the expression of those sheddases responsible for release of the sCD200 (24-26). Thus, exploration of levels of both membrane-bound and released (serum) CD200 are implicated in providing information of both diagnostic/prognostic value in CLL. It remains to be determined if this is the case in other B cell malignancies, but given the discriminatory information afforded by measuring membrane-bound CD200, as pointed out above, in different MBCs, this becomes an intriguing possibility.

Both cell bound and soluble forms of CD200 expression have been linked with engagement of inhibitory receptors, in this case CD200R, which are implicated in enhanced tumor growth (27,28), likely through attenuation of inflammatory and immune reactivity, and/or augmented induction/activation of regulatory cells in the tumor environment (29,30). In association with augmented growth of CLL cells in NOD.SCID mice following infusions of exogenous CD200 we have reported that the local tumor environment is infiltrated with CD200R+ cells, implying again an important role for CD200:CD200R interactions in augmenting CLL growth in this xenogeneic model [Wong-personal communication-see also (24)]. These data indicating a role for CD200 expression in favouring growth of tumors in a variety of models have formed the basis for the notion that targeting CD200 expression itself may represent a novel approach to immunotherapy of CD200+ tumors (30,31). In a breast cancer model, we have shown that manipulation of CD200:CD200R interactions can be used to cure mice both of local tumor growth and distant metastases (28). Preliminary reports in patients have also suggested that anti-CD200 therapy may play a role in regulating lymphoma growth (32).

Summary

More recently we have also found that in the very act of shedding CD200, CD200+ CLL cells can translocate a cytoplasmic domain of the molecule to the nucleus where it can act to alter transcription of genes already implicated in growth of leukemic cells (Chen *et al.*: submitted, 2017). This opens up the possibility that tumor CD200 expression

can act to augment tumor growth not merely by influencing the local inflammatory/immune environment (through engagement of CD200R), but also can act internally in CD200+ cells to alter their growth and development. Whether this represents another key mechanism whereby CD200 expression bodes an unfavourable prognosis for patients with hematologic malignancies remains to be determined. Nevertheless, and as the data in the Sandes study implies, it seems that including measurements of CD200 expression on MBCs, as well as measuring sCD200 levels in serum (see discussion above) will prove useful not merely, as suggested by these authors, in terms of diagnostic classification, but also, we predict, in terms of prognostication and even for considering unique CD200-directed adjunct therapy.

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Footnote

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