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Original Contribution

Increased CD200 expression in post-transplant lymphoproliferative disorders correlates with an increased frequency of FoxP3(+) regulatory T cells

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ABSTRACT

CD200 is a membrane protein with immunosuppressive function and is expressed in many hematopoietic neoplasms, including acute myeloid leukemia (AML), plasma cell myeloma (PCM), and B-cell lymphoproliferative disorders, but is mostly negative in diffuse large cell lymphoma (DLBCL). CD200 has been shown to be a poor prognostic marker in AML and PCM; in AML, its immunomodulatory effect was linked to its ability to induce FoxP3(+) regulatory T cells (Tregs). Post-transplant lymphoproliferative disorders (PTLDs) arise in the setting of immune dysregulation, and tumor-infiltrating T cells, including Tregs, have been shown to correlate with outcome in these disorders. Because there is no literature data and CD200 is a potentially useful diagnostic and prognostic marker, we studied the expression of CD200 in a series of 38 PTLDs by immunohistochemistry (ICH), and found that 23.7% PTLDs were CD200(+) and showed strong membrane and cytoplasmic positivity in the neoplastic cells. All CD200(+) monomorphic PTLDs were DLBCLs and the median FoxP3(+) Treg count/hpf was higher in CD200(+) than in CD200(−) PTLDs: 22.6 vs. 0.30 ($p < 0.001$). These results indicated that almost a quarter of PTLDs in our series are CD200(+) by IHC, and CD200 expression correlates with the frequency of immunosuppressive Tregs. This is novel data and supports a pathophysiologic link between CD200 activity and Tregs. In our series, the 5-year overall survival was shorter in CD200(+) PTLDs, compared to CD200(−) patients, although this difference did not reach statistical significance. In addition, we find a higher proportion of CD200(+) monomorphic PTLD-DLBCLs (31.0%), as compared to de novo DLBCLs (7–8%, as found here and in other studies). This may indicate differential expression of CD200 in B-cell lymphomas arising in the setting of immune dysregulation, and raises the possibility of anti-CD200 immunotherapy for these cases.

1. Introduction

CD200 is a member of the type-1 immunoglobulin superfamily that is highly expressed in the central nervous system, dendritic cells, and lymphocytes [1,2]. CD200 is functionally involved in an immunosuppressive signaling pathway, via interaction with its receptor, CD200R, with downstream effects of macrophage inhibition, induction of regulatory T cells, and inhibition of tumor-specific T cells [3]. Besides being expressed in normal tissues, CD200 has also been demonstrated in solid tumors and hematologic malignancies, such as plasma cell myeloma (PCM), acute myeloid leukemia (AML), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), hairy cell leukemia (HCL), primary mediastinal large B-cell lymphoma (PMLBCL), classic Hodgkin lymphoma (cHL), and angioimmunoblastic T-cell, but is mostly negative in diffuse large cell lymphoma (DLBCL) [4-9]. CD200 has been shown to represent a poor prognostic marker in PCM and in AML; in AML, its immunomodulatory effect was linked to several mechanisms, including suppression of NK cell function, and to its ability to upregulate FoxP3(+) regulatory T cells (Tregs) [4,5,8,9].

Post-transplant lymphoproliferative disorders (PTLDs) occur in a minority (less than 5%) of solid organ or bone marrow transplant recipients and are often associated with Epstein-Barr virus (EBV) infection. In some patients, reduction of immunosuppression may suffice for clinical management, while in others, successful treatment requires chemotherapeutic regimens [10,11]. PTLDs arise in the setting of immune dysregulation, and tumor-infiltrating T cells, including Tregs, have been shown to correlate with outcome in these disorders, as well as in similar, de novo occurring lymphomas, such as DLBCL [12,13].

To date, CD200 expression and Treg infiltration have not been studied simultaneously in any PTLDs. Because there is no literature data

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and CD200 is a potentially useful diagnostic and prognostic marker, we explored CD200 expression by immunohistochemistry (IHC) and Treg infiltration (as defined by FoxP3 IHC positivity) in PTLDs and correlated with clinicopathologic features. We also compared our findings to previous reports of tumor CD200 and/or host Treg frequency in PTLDs and de novo DLBCL to further investigate the relationship of CD200 expression and Treg infiltration in PTLD.

2. Materials and methods

2.1. Patients

We searched our pathology archives to identify cases diagnosed as "post-transplant lymphoproliferative disorder" between 1994 and 2017. All cases were reviewed and the diagnosis was confirmed by two of the authors (HO and JV). Cases were classified according to the 2016 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues [14]. Ten cases without adequate formalin-fixed, paraffin-embedded tissue (FFPE) were excluded. Clinical information was collected from the electronic medical record, and included: age at diagnosis, gender, anatomic site of PTLD presentation, transplant type, time to transplant, and follow-up (alive vs. dead). Overall survival (OS) was calculated from the day of PTLD diagnosis to either death or the last known follow-up. A separate cohort of 110 de novo DLBCLs served as controls. This study received institutional review board approval.

2.2. Immunohistochemistry

Immunohistochemistry was performed on 4-micron sections from formalin-fixed, paraffin embedded tissue blocks. Staining was performed on a Dako (Carpinteria, CA) Autostainer Plus using the Dako EnVision™ FLEX, high pH detection Kit (K81010), according to manufacturer's recommendations, with the following antibodies: CD3 (polyclonal rabbit, Dako #IS503, ready-to-use), CD20 (monoclonal mouse clone L26, Dako #IS648, ready-to-use), FoxP3 (monoclonal mouse clone 236A/E7, Abcam #ab20034, 1:100), CD10 (monoclonal mouse clone 56C6, Dako #IS648, ready-to-use), BCL-6 (monoclonal mouse clone PG-B6p, Dako #IS625, ready-to-use), and MUM-1 (monoclonal mouse clone MUM1p, Dako #IS644, ready-to-use), with appropriate positive and negative controls. CD200 immunohistochemistry was performed with anti-human CD200 goat monoclonal antibody (R&D Systems, Minneapolis, MN), at 1:100 dilution, using antigen heat retrieval in 10 mmol/L citrate buffer, pH 6.0, and a SuperPicTure Polymer Detection Kit (Invitrogen, Carlsbad, CA; #87-9363), according to manufacturer's recommendation. CD200 expression was considered positive when present in > 20% lymphoma cells. Total T cells were enumerated by CD3 positivity and Tregs were by FoxP3 nuclear positivity (see below). The Hans criteria [15] were used to establish cell of origin (COO) phenotype in the subset of 29 PTLD-DLBCL cases. EBV status was determined by in situ hybridization (Dako). Cases were considered positive when EBER was expressed in lymphoma cells beyond the occasional rare positive small B cells typically seen in immunocompetent patients with prior exposure to EBV infection.

2.3. Flow cytometry

Specimens were processed and stained using a routine lyse/wash/stain procedure, with the following monoclonal antibodies: CD5, CD10, CD19, CD20, CD22, CD23, CD38, CD45, CD200, and kappa and lambda immunoglobulin light chains. All fluorochrome-conjugated antibodies were from BD Biosciences (San Jose, CA). Events were acquired on a FACSCanto or FACSLyric flow cytometer (BD Biosciences). Expression of CD200 was assessed in the clonal B cells based on internal control populations, and defined as positive when at least 20% of events exceeded a negative control threshold.

2.4. Data analysis

CD3 and FoxP3 T-cell counts were performed at 400× total magnification using a Miller disc. Positive cells within the grid were counted. In cases where manual counts for the entire grid area were not feasible (as with CD3 staining) the number of cells within the grid were calculated/estimated by multiplying the number of cells intersected by the outer grid lines on perpendicular axis. Ten intratumoral high power fields (hpfs) were counted with data reported as a range, mean, and median per hpf.

2.5. Statistical analysis

Statistical analyses were carried out in GraphPad Prism, version 6.07 (GraphPad Software, San Diego, CA). Variables between groups were compared by Fisher's exact and non-parametric Mann-Whitney tests, and comparison of survival curves was done using the log-rank (Mantel-Cox) test. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

We identified 38 PTLD cases: 32 (84.2%) monomorphic PTLDs (29 DLBCL and 3 Burkitt lymphoma) and 6 (15.8%) polymorphic PTLDs. No non-destructive, monomorphic T/NK-cell, or classic Hodgkin lymphoma PTLDs were represented in this series. The clinicopathologic features are presented according to status of CD200 expression in Table 1. The median age of the patients at PTLD diagnosis was 47.5 years, with a range of 20 to 77 years. The male:female ratio was 2.2:1. Transplant types included 28 solid organ transplants (17 kidney, 6 liver, 3 lung, and 2 heart) and 10 bone marrow transplants. The majority of PTLDs (23/38, 60.5%) presented greater than one year after transplant, with a median time from transplant to PTLD diagnosis of 26 months (range: 1–158 months). Primary extranodal disease was common; anatomic sites of the PTLDs included 23 based in lymphoid tissue (lymph node, spleen, or tonsil) with or without additional organ involvement, and 17 at extra-nodal sites (9 in the gastrointestinal tract, 4 in the lung, and 4 in the central nervous system). On hematoxylin and eosin (H&E) stained slides, necrosis was noted in 31/38 cases (81.6%). By EBER in situ hybridization, 27/38 cases (71.1%) were positive for EBV. At the time of this retrospective review (median follow-up: 55 months, range: 1–188 months), 24 patients were alive, 9 were deceased, and 5 were lost to follow-up with no known mortality. The deceased patients had a median time from PTLD diagnosis to death of 50 months (range: 1–75 months). Of the 24 living patients with follow-up information available, the median time from diagnosis to last follow-up was 55 months (range: 14–188 months). The 5-year overall survival (OS) for monomorphic PTLDs was 68.9%.

3.2. CD200 expression in PTLD cases

CD200 expression was positive in 9/38 (23.7%) of PTLDs and 9/29 (31.0%) of monomorphic PTLD-DLBCLs. All CD200 (+) cases showed a strong membranous and cytoplasmic staining patterns in > 75% of the neoplastic cells (7/9 cases; 78%) or in 50–75% of the neoplastic cells (2/9 cases, 22%), respectively (Fig. 1). All polymorphic PTLDs and the three BL monomorphic PTLDs were negative for CD200, with no tumor cells staining. The 5-year OS was shorter for CD200 (+) PTLDs, compared to CD200 (−) PTLDs: 29.6% vs. 80.0%; however, the difference was not statistically significant ($p = 0.197$). Similarly, there were no other statistically significant differences, including age (median, 50 vs. 47 years; $p = 0.676$), gender, time from transplant to PTLD diagnosis (19.6 vs. 10 months, $p = 0.928$), bone marrow vs. solid organ transplant, anatomic site, monomorphic vs. polymorphic PTLD, EBV status,

Table 1
Clinicopathologic features of post-transplant lymphoproliferative disorder (PTLD) cases.

Case #	CD200	Age at Dx	Sex	PTLD type	Transplant type	Dx to death (days)	Site of PTLD	Necrosis	EBER
1	Positive	26	M	PTLD-DLBCL	Kidney	NA (alive)	LN, abdominal	Yes	Positive
2	Positive	63	M	PTLD-DLBCL	BMT	NA (alive)	LN, cervical	Yes	Negative
3	Positive	66	M	PTLD-DLBCL	Heart	NA (alive)	LN, cervical	Yes	Positive
4	Positive	57	M	PTLD-DLBCL	Heart	1660	Lung	Yes	Positive
5	Positive	54	F	PTLD-DLBCL	BMT	45	LN, cervical	No	Positive
6	Positive	33	M	PTLD-DLBCL	Kidney	Lost to f/u	Lung	Yes	Negative
7	Positive	50	F	PTLD-DLBCL	BMT	NA (alive)	CNS and paravertebral soft tissue	Yes	Positive
8	Positive	46	F	PTLD-DLBCL	Lung	NA (alive)	LN, cervical	No	Negative
9	Positive	37	M	PTLD-DLBCL	BMT	Lost to f/u	LN, cervical	Yes	Positive
10	Negative	62	M	PTLD-DLBCL	Liver	NA (alive)	Tonsil	Yes	Negative
11	Negative	34	M	PTLD-DLBCL	Liver	NA (alive)	LN, inguinal	Yes	Negative
12	Negative	34	M	PTLD-DLBCL	BMT	NA (alive)	Tonsil	Yes	Positive
13	Negative	56	F	PTLD-DLBCL	Kidney	162	Lung	Yes	Negative
14	Negative	65	F	PTLD-DLBCL	Lung	Lost to f/u	Colon	Yes	Positive
15	Negative	38	M	PTLD-DLBCL	Kidney, pancreas	Lost to f/u	Small bowel and mesenteric LN	Yes	Negative
16	Negative	54	F	PTLD-DLBCL	BMT	NA (alive)	LN, cervical	Yes	Positive
17	Negative	35	M	PTLD-DLBCL	BMT	72	LN, cervical	Yes	Positive
18	Negative	36	M	PTLD-DLBCL	Kidney	Lost to f/u	Small bowel	Yes	Negative
19	Negative	52	M	PTLD-DLBCL	Kidney	2279	Small bowel and mesenteric LN	Yes	Positive
20	Negative	30	F	PTLD-DLBCL	Kidney	NA (alive)	LN, thoracic	No	Positive
21	Negative	54	M	PTLD-DLBCL	Kidney	Lost to f/u	CNS	Yes	Positive
22	Negative	37	M	PTLD-DLBCL	BMT	Lost to f/u	LN, cervical	Yes	Positive
23	Negative	50	F	PTLD-DLBCL	Kidney	NA (alive)	LN, cervical	Yes	Negative
24	Negative	37	M	PTLD-DLBCL	BMT	Lost to f/u	Lung	Yes	Positive
25	Negative	33	M	PTLD-DLBCL	Kidney	NA (alive)	CNS	Yes	Positive
26	Negative	59	M	PTLD-DLBCL	Kidney	1243	CNS	Yes	Positive
27	Negative	22	M	PTLD-DLBCL	Kidney	NA (alive)	Colon and mesenteric LN	No	Positive
28	Negative	77	F	PTLD-DLBCL	Liver	NA (alive)	Sinus	Yes	Positive
29	Negative	34	M	PTLD-DLBCL	Liver	NA (alive)	Colon, rectum	Yes	Positive
30	Negative	52	M	PTLD-BL	Kidney	NA (alive)	Small bowel	Yes	Positive
31	Negative	67	M	PTLD-BL	Kidney	NA (alive)	Liver, mesenteric LN	Yes	Positive
32	Negative	56	M	PTLD-BL	Liver	27	Duodenum	Yes	Negative
33	Negative	48	F	P-PTLD	Lung	NA (alive)	LN, thoracic	Yes	Negative
34	Negative	20	M	P-PTLD	Kidney	NA (alive)	Tonsil	Yes	Positive
35	Negative	47	M	P-PTLD	BMT	138	Small bowel	Yes	Positive
36	Negative	36	F	P-PTLD	Kidney	NA (alive)	LN, cervical	Yes	Positive
37	Negative	32	M	P-PTLD	Kidney	NA (alive)	Spleen, perihilar LN, native kidneys	No	Positive
38	Negative	68	F	P-PTLD	Liver	NA (alive)	LN, cervical	No	Positive

DLBCL = diffuse large B cell lymphoma; P-PTLD = polymorphic PTLD; BL = Burkitt lymphoma; BMT = bone marrow transplant; LN = lymph node(s); LAD = lymphadenopathy; EBER = Epstein-Barr-encoding region in situ hybridization; NA-not applicable.

and presence of necrosis between CD200 (+) and CD200 (−) cases. Of note, the 5-year OS was shorter for PTLDs developed after bone marrow transplant, compared to those occurring in solid organ transplant recipients: 48.5% vs. 77.7%; however, the difference was not statistically significant ($p = 0.069$).

3.3. CD200 expression, cell of origin, and EBV status in monomorphic PTLD, DLBCL cases

Of the 29 monomorphic PTLD, DLBCLs, 14 (48.2%) were sub-typed as germinal center (GC) cell of origin, and 15 (51.7%) as non-GC type. The proportion of CD200 (+) non-GC type PTLD-DLBCLs was similar to that seen in GC type cases (Table 2). The 5-year OS was shorter for CD200 (+) vs. CD200 (−) PTLD-DLBCLs: 29.6% vs. 78.8%, albeit not statistically significant ($p = 0.190$). However, the 5-year OS was longer

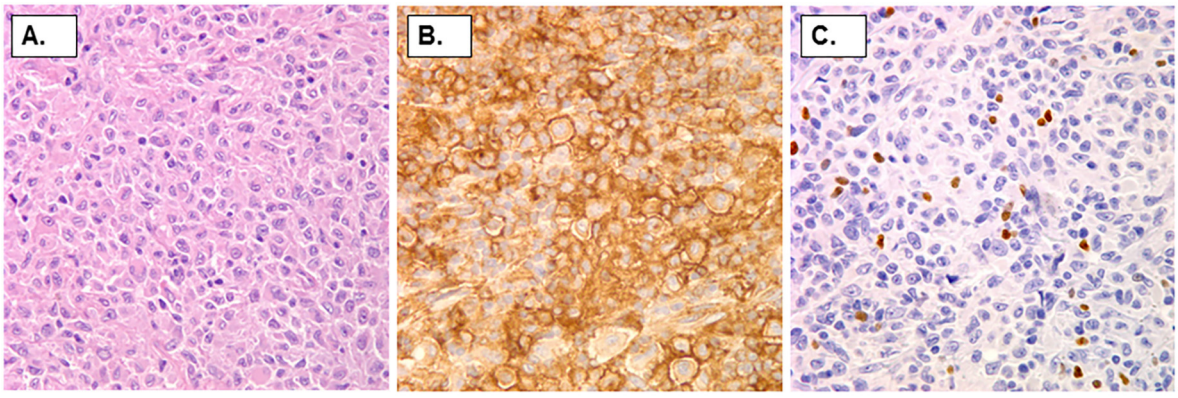

Fig. 1. (A) Hematoxylin and eosin, (B) CD200 immunohistochemistry, and (C) FoxP3 immunohistochemistry (all at 500× magnification) in a CD200 (+), EBV (+) monomorphic post-transplant lymphoproliferative disorder-diffuse large B-cell lymphoma.

Table 2
Comparison of clinicopathologic features of monomorphic post-transplant lymphoproliferative disorder, diffuse large B cell lymphoma (PTLD-DLBCL) cases based on CD200 expression.

	Monomorphic PTLD-DLBCLs (n = 29)		p-Value
	CD200(+) (n = 9)	CD200(−) (n = 20)	
Age (median, range)	50 (26–66)	44 (33–77)	0.865
Sex (M:F)	2:1	1.9:1	1.000
Time from transplant to PTLD, median (months)	19.6	10	0.887
Transplant type, BMT	4/9 (44.4%)	5/20 (25%)	0.396
Alive at latest f/u	6/9 (33.3%)	11/20 (50.5%)	0.694
EBV (+)	8/9 (88.9%)	12/20 (60%)	0.201
Necrosis present	7/9 (77.8%)	18/20 (90.0%)	0.568
CD3(+) cells, median/hpf (range)	161 (102–185)	72 (9–110)	0.008
FoxP3(+) cells, median/hpf (range)	22.6 (9.1–35.1)	0.45 (0–13)	< 0.001
FoxP3/CD3 ×100, median	14.85	0.54	0.001
GC phenotype	3/9 (33%)	11/20 (55%)	0.427

in GC type PTLD-DLBCLs compared to non-GC cases: 75.6% vs. 23.4%, p = 0.041. PTLD-DLBCLs were EBER(+) in 20/29 cases (69%), and the proportion of positive cases was similar in CD200 (+) tumors compared to CD200 (−) PTLD-DLBCLs: 88.9% vs. 60%, p = 0.201 (Table 2). The 5-year OS was similar for EBV (+) and EBV (−) PTLD-DLBCLs: 58.9% vs. 85.7%, p = 0.296.

For comparison, we evaluated CD200 expression by flow cytometry in a series of de novo DLBCLs, and revealed positivity in 9 (8.2%) of 110 cases, using similar criteria for CD200 expression by IHC in the PTLD cohort. The proportion of CD200-positive de novo DLBCLs is similar (6.7%) to that reported in a prior study.

3.4. Treg infiltration in PTLD cases

In the 38 PTLD cases studied for CD3 and FoxP3 by IHC (Fig. 1), the median CD3 (+) T-cell and FoxP3 (+) Treg counts/hpf were 96 (range, 9–509) and 1.2 (range, 0–35.1), respectively. Median FoxP3 counts were higher in monomorphic: 7.6 (range, 0–35.1) vs. polymorphic PTLDs: 0.55 (range, 0–1.7), although not statistically significant (p = 0.130). The median CD3 (+) T-cell and FoxP3 (+) Treg count/hpf was higher in CD200 (+) than in CD200 (−) PTLDs, 161 vs. 51 (p = 0.003) and 22.6 vs. 0.3 (p < 0.001), respectively.

FoxP3 and T cell subset data for the 29 PTLD-DLBCLs are summarized in Table 2. The median CD3 (+) T-cell and FoxP3 (+) Treg count/hpf was higher in CD200 (+) than in CD200 (−) PTLD-DLBCLs, 161 vs. 72 (p = 0.008) and 22.6 vs. 0.45 (p < 0.001), respectively.

4. Discussion

CD200 is a membrane glycoprotein with relatively frequent expression on hematopoietic cells, including lymphoid (B and T), myeloid, and plasma cells, in both normal tissues and hematopoietic neoplasms. As such, CD200 expression assessed by either immunohistochemistry or flow cytometry has demonstrated added value in diagnostic hematopathology. In normal tissues, CD200 expression by immunohistochemistry and/or flow cytometry has been demonstrated in follicular dendritic cells associated with germinal centers of secondary lymphoid follicles, and weak staining in primary follicles and mantle zones (B cells); in addition, weak staining/expression intensity was also reported in thymocytes [6,16]. In cases of nodular lymphocyte predominant Hodgkin lymphoma, CD200 expression was shown in T cells that form characteristic rosettes at the periphery of the neoplastic T cells. These cells have been shown to display a follicular helper T-cell immunophenotype, including antigen expression such as CXCL13 and PD-1 [6]. Furthermore, CD200 was shown to be expressed in follicular helper T cells to a greater extent than in Th1 and Th2 central memory cells, or effector memory T cells [17]. In contrast, Tregs have not been reported to show significant CD200 and/or CD200R expression, and their involvement in immunosuppressive mechanisms occurs by interacting with other cell types expressing these molecules, as detailed later in the discussion.

Of the other B-lineage neoplasm, CD200 immune reactivity is primarily seen in mature lymphoproliferative disorders, such as CLL/SLL, HCL, cHL, and PMLBCL, but also in immature processes (B-lymphoblastic leukemia/lymphoma) [6,7]. CD200 is useful in the differential diagnosis of CLL/SLL vs. mantle cell lymphoma, by flow cytometry or immunohistochemistry [6,18] and may also help in the differential diagnosis of large B-cell neoplasms of the mediastinum; the latter is based on the observation that 94% of PMLBCL cases exhibited immunostaining for CD200, in a series of 35 patients, compared to 6.7% cases of de novo DLBCL [7].

Given the paucity of data regarding CD200 in PTLD, we performed an IHC study in a cohort of 38 patients, and found that CD200 is expressed in a significant proportion of PTLDs, with an overall positivity of 23.7%. We observed CD200 expression to be restricted to the subset of monomorphic PTLDs, DLBCL. None of the polymorphic PTLDs and monomorphic PTLDs, BL demonstrated CD200 expression by IHC. While the findings of CD200 negativity in BL arising in the post-transplant setting matches that reported in the literature for de novo BL, the proportion of CD200-positive monomorphic PTLD, DLBCLs (31% in our cohort) occurs at an 4–5-fold higher rate than in de novo cases, as seen in comparison with our series of 110 de novo DLBCL cases, and which showed a similar rate of CD200 positivity by flow cytometry as to the one reported in a prior study by immunohistochemistry [7]. Furthermore, although we used a relatively conservative cut-off of designating CD200 positivity in PTLD tissue biopsies (20% or more of the neoplastic cells), and similar to other studies assessing this marker in B-cell neoplasms, all cases showed expression in more than half of the lymphoma cells.

This observation has potential biological implications for the pathophysiology and treatment of PTLDs. For cases expressing CD200, it raises the possibility of anti-CD200 therapy with monoclonal antibodies targeting this molecule, as shown to be effective in animal models and clinical trials for CLL/SLL and PCM [19-21]. The tumor growth inhibitory effect of such immunotherapeutic agents is thought to be due to blocking of CD200 receptor-ligand interaction, as well as by reducing Treg frequency, which in turn enhances immune-mediated antitumor efficacy. It is not known whether the percentage of CD200 (+) cells would necessarily correlate with response to treatment. However, the neoplasms that are being currently investigated or have been proposed for anti-CD200 immunotherapy (CLL/SLL, PCM, and AML) [9,22], typically show a high proportion of abnormal cells with uniform expression of CD200 and as such, a higher proportion of CD200 (+) tumor cells may be therapeutically advantageous when blocking CD200.

Recently, CD200 expression has been used to define a subgroup of PCM and AML (approximately 75% and 43% of cases, respectively) with unfavorable prognosis [4,5,23]. There is no literature data on prognostic information of CD200 expression in high-grade mature B-cell neoplasms, such as de novo DLBCL or monomorphic PTLD, DLBCL. In our patient cohort, we observed a shorter 5-year OS in CD200 (+) PTLDs, compared to CD200 (−) cases, but the difference was not statistically significant. We observed a similar trend in the 5-year OS of the CD200 (+) DLBCL subset of monomorphic PTLDs, and no differential expression of CD200 when compared to other prognostic parameters, such as cell of origin (COO) and EBV status. This is an important observation, as the 5-year OS was better in GC-type monomorphic PTLD, DLBCL cases, compared to non-GC, as shown in other studies [24]. Given that there was an almost equal proportion of GC and non-GC type DLBCLs in our patient group, any difference in survival related to

CD200 status would be likely attributable to other parameters.

In a prior study, we have observed the prognostic effect of another marker, CD30, on immune activation and anti-tumor response in PTLD [25]. Similar to that antigen, CD200 appears to be more frequently found in PTLD-derived DLBCLs, compared to de novo cases, which raises the possibility of a potentially unique immune environment controlling lymphomagenesis in the immunosuppressive post-transplant setting. In AML, CD200 induces proliferation of Tregs and promotes their suppression function, and this subset of CD4/FoxP3-positive immunosuppressive T cells was associated with a poor prognosis [8,23]. Based on that observation, we expected to find greater Treg infiltration in CD200 (+) vs. CD200 (−) PTLDs. We found a statistically significant positive correlation with PTLD CD200 expression and median Treg infiltration ($p < 0.001$), and this correlation held true in the PTLD-DLBCL subset, as well. We saw a similar difference in the total number of infiltrating T cells between CD200 (+) and CD200 (−) cases, which may indicate that the difference in FoxP3(+) Tregs is simply a reflection of the differential number of T cells. However, we argue against this potential explanation by pointing out that the ratio of CD3/FoxP3 T cells is not proportional between CD200 (+) and CD200 (−) PTLDs. Our study is the first to explore CD200 positivity and Tregs in PTLDs, and this novel data suggests a pathophysiologic link between CD200 expression and Treg infiltration. Furthermore, while we did not observe a statistically significant difference in the OS between CD200 (+) and CD200 (−) cases, there was a trend toward a less favorable outcome in CD200 (+) PTLD-derived DLBCLs, reminiscent of the unfavorable prognosis of CD200 positivity observed in patients with AML and PCM.

There are several mechanisms that have been proposed for the interplay between CD200/CD200R and Tregs in abnormal pathophysiology. CD200 bind to its receptor, CD200R1, on macrophages and dendritic cells, resulting in the regulation of cytokine production (such as IL-2, IL66, TNF-alpha, and IFN-gamma), inflammatory immune responses, and maintenance of immune homeostasis. In addition, the CD200/CD200R1 interaction was shown to be implicated in the development of tolerogenic dendritic cells that preferentially induce Tregs capable of diminishing and preventing immune responses [26]. While this mechanism has obvious implications in the pathogenesis of autoimmune disease, similar observations were made in hematolymphoid malignancies, such as CLL/SLL, where the upregulation of CD200 in the neoplastic B cells has been associated with downregulation of a Th1 immune response, including cytokines such as IL-2 and IFN-gamma, and in the induction of Tregs, which are postulated to dampen a tumor-specific T-cell immunity [27]. This hypothesis was demonstrated by showing that abrogation of the CD200/CD200R1 interaction with an anti-CD200 antibody significantly decreases the number or Tregs, and therefore may provide therapeutic benefits in patients with CLL/SLL, by augmenting an antigen-specific T-cell response with suppression of Tregs. Furthermore, it showed that downregulation of Tregs though CD200 blockade supports previous observations that engagement of the CD200 receptor promotes bone marrow stem cells to differentiate toward suppressive antigen-presenting cells, that can in turn induce Tregs [27,28]. Similar results were observed in patients with AML, where increased CD200 expression on blasts was linked to both increased number of Tregs and a more potent suppression of tumor-specific T-cell proliferation in these patients. Also, CD200-induced Tregs are linked to Th1-mediated immunosuppression, as shown by levels of Th1-associated cytokines (IL-2, TNF-alpha, and IFN-gamma) measured in AML patients with high levels of CD200 expression [8,9].

There may be several factors responsible for the lack of a definitive link between outcome and CD200 status in PTLD. One of the limitations of our study is that the patients were accrued over a long time period, with variations in immunosuppressive regimens and/or lymphoma therapies used. The high proportion of patients still alive (24 of 38) and the incomplete follow-up data in a subset of cases also limits our ability to assess long-term outcomes. Larger studies aggregating more PTLD cases and longer follow-up times with detailed treatment data might better address the prognostic significance of CD200 expression in PTLDs.

In summary, we confirm that a significant minority of PTLDs expresses CD200, and we report the novel finding that significantly more Treg infiltration is present in CD200 (+) PTLDs. We define a distinct phenotype of monomorphic PTLD-DLBCL, compared to de novo DLBCL, with significantly higher frequency of CD200 expression and more reminiscent of PMLBCL. To our knowledge, this is the first study to compare data on CD200 expression of de novo DLBCL and monomorphic PTLD-DLBCL. Our findings, combined with the few other studies addressing CD200 in high-grade mature B-cell lymphomas, suggest differential expression of CD200 in B-cell lymphomas arising in the setting of immune dysregulation.

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