

Alteration of Cell Surface Markers CD38 and CD138 in Lymphoproliferative Disorders in the Ocular Adnexa

YUKA SUIMON¹, SATORU KASE¹, ICHIRO MIURA², KAN ISHIJIMA¹ and SUSUMU ISHIDA¹

¹*Department of Ophthalmology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan;*

²*Department of Diagnostic Pathology, Social Welfare Corporation Hokkaido Institutional Society Obihiro Hospital, Obihiro, Japan*

Abstract. *Background/Aim:* CD38 is a cell surface marker commonly present in plasma cells and activated T cells, while CD138 is a representative plasma cell marker. The aim of this study was to describe the expression of cell surface markers including CD38 and CD138, in the tumors of patients with IgG4-related ophthalmic disease (IgG4-ROD) and extranodal marginal zone B-cell lymphoma (EMZL) of the ocular adnexa. *Materials and Methods:* Twenty-four consecutive patients of whom 12 had IgG4-ROD and 12 EMZL were enrolled in this study. Medical records were reviewed for flow cytometry (FCM) results on conventional T-cell markers, B-cell markers, CD38 and CD138. *Results:* Positive rates of T-cell markers, CD38 and CD138 were significantly higher in IgG4-ROD than in EMZL ($p < 0.01$ and $p < 0.05$, respectively). *Conclusion:* Our FCM results on CD38 and CD138 showed that the lymphocyte populations were different between IgG4-ROD and EMZL, which may reflect the different pathophysiology of the two diseases.

Extranodal marginal zone B-cell lymphoma (EMZL) of mucosa-associated lymphoid tissue often arises from the ocular adnexa. While, Immunoglobulin (Ig) G4-related ophthalmic disease (IgG4-ROD) is a chronic inflammatory condition characterized by infiltration of lymphocytes and IgG4-positive plasma cells, and by fibrosis in the orbital tissues (1). The most frequent disease among orbital lymphoproliferative disorders in Japan is EMZL (40%), followed by IgG4-ROD (22%) (2). Since it is likely that EMZL simulates, coexists with, and arises from IgG4-ROD (3, 4), the differentiation of the two diseases is important for the ophthalmologists to initiate appropriate

treatments. To make a correct diagnosis, histopathological evaluations, including *in situ* hybridization, are useful (4), but those might not be enough to determine B-cell monoclonality. Therefore, it is critical to add other ancillary tests for monoclonal Ig heavy chain (IgH) gene rearrangement using Southern blot or polymerase chain reaction, as well as Ig light chain (IgL) kappa/lambda deviation using flow cytometry (FCM) or immunohistochemistry, both of which may confirm B-cell monoclonality.

FCM is a method to assess the presence of cell surface markers on various leukocyte populations. Recently, FCM has been reported as a useful examination for the diagnosis of ocular lymphoproliferative diseases. We and others have previously reported the profiles of several cell surface markers, showing that the rates of CD2, CD3, CD4, CD7, CD10 and CD23 positive cells were higher, and that CD19, CD20 and CD25 were lower in surgically excised tissues of IgG4-ROD compared to EMZL (5, 6).

CD38 is detectable in various cells of the immune system such as activated T cells, activated B cells (plasma cells) and NK cells (7). In contrast, CD138, or syndecan 1, is a heparan sulfate-rich integral membrane proteoglycan, which functions as a matrix receptor by binding to interstitial collagens, fibronectin, and thrombospondin (8). In the case of B-cell markers, both CD38 and CD138 are present on pre-B cells and activated B cells but absent on circulating and peripheral B lymphocytes (8, 9). IgG4-ROD presents inflammatory infiltration of tissue by T and B lymphocytes and IgG4-positive plasma cells (1, 10, 11). EMZL can manifest plasmacytic differentiation (12, 13). Based on their leukocyte features, we asked the question whether the populations of CD38 and CD138 detected by FCM are different between the IgG4-ROD and EMZL. In this study, we examined the presence of CD38 and CD138, in IgG4-ROD and EMZL tissues using FCM.

Materials and Methods

Materials. This is a retrospective observational study. The institutional review board in Hokkaido University Hospital approved this study (IRB number: 18-184). The current study

Correspondence to: Satoru Kase, Department of Ophthalmology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan. Tel: +81 117065944, Fax: +81 117065948, e-mail: kaseron@med.hokudai.ac.jp

Key Words: Flow cytometry, IgG4-related ophthalmic disease, lymphoma.

adhered to Declaration of Helsinki. Patients who were diagnosed between January 2015 and July 2018 were eligible. All the patients having ocular adnexal tumors underwent surgical resection of the tumors. The excised tumor tissues were immediately submitted for FCM without fixation. This study enrolled 24 consecutive patients of whom 12 had IgG4-ROD and 12 EMZL and for which the results of FCM were available. Diagnosis of EMZL was based on histological, monoclonal IgH gene rearrangement, and restricted IgL surface expression analysis. In this study, plasmacytoma and other types of lymphoma arising from the ocular adnexa such as diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma and NK/T cell lymphoma were excluded. Patients with IgG4-ROD were diagnosed based on the diagnostic criteria published in 2015 (1). Briefly, there were 3 characteristic findings including enlargement of ocular adnexa tissues, elevated serum IgG4 levels (>135 mg/dl) and marked IgG4-positive plasma cell infiltration in the tissues (1).

FCM. Cells were stained using fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies against T-cell markers (CD2, CD3, CD4, CD5, CD7, CD8), B-cell markers (CD10, CD19, CD20, CD21, CD22, CD23), natural killer (NK) cell marker (CD56) and activated T cell, plasma cell and NK cell marker (CD38), and plasma cell marker (CD138). Cells were suspended in phosphate-buffered saline supplemented with 1% bovine serum albumin and antibody reactions were performed at 25°C for 15 min. After a washing step, the cells were resuspended, and then five-color FCM was performed using a flow cytometer (Beckman Coulter Cytomics FC 500, Miami, FL, USA) within the next 2 h. Multi-parameter analysis of different cell populations was performed based on the morphological properties and FITC/PE intensity of the pan-leukocyte antigen CD45. Relative percentages of various cell subsets in each case were subjected to statistical analysis.

Statistical analysis. The patients' age and the percentages of the cell populations expressing each cell surface marker were compared between IgG4-ROD and EMZL using the Mann-Whitney *U*-test. The frequency of gender between IgG4-ROD and EMZL patients was evaluated using Chi-square test. A *p*-value of less than 0.05 was considered statistically significant. Correlation between CD38 and CD138 percentage was assessed using Spearman correlation coefficient test. All analyses were carried out using MS Excel 2011 and XLSTAT software.

Results

The patients' mean age was 56.2±9.2 years and 62.5±18.5 years in IgG4-ROD and EMZL groups, respectively. There was not a significant difference in the patients' age between the two groups. The IgG4-ROD group included 4 males and 8 females, while the EMZL group included 9 males and 3 females, and the number of male patients was significantly greater in the EMZL group than the IgG4-ROD group (*p*=0.04).

The percentages of each cell surface marker are summarized in Tables I and II. The positive rates of T cell markers, including CD2, CD3, CD4, CD5, CD7 and CD8, were significantly higher in the IgG4-ROD group than in the EMZL group (Table I). In contrast, the rates of B cell

Table I. *T-cell and B-cell lineage population (%) in IgG4-ROD and EMZL.*

	IgG4-ROD (N=12)	EMZL (N=12)	<i>p</i> -Value
T cell lineage			
CD2	48.5±6.0	23.1±16.2	0.0002
CD3	45.9±9.0	21.1±16.1	0.0002
CD4	37.3±4.4	14.7±13.3	0.0002
CD5	52.0±5.7	29.4±16.9	0.0031
CD7	42.5±5.7	20.0±14.1	0.0002
CD8	9.5±3.7	6.3±3.4	0.0251
B cell lineage			
CD10	5.0±3.6	1.0±1.0	<0.0001
CD19	58.9±7.8	79.9±16.8	0.0003
CD20	54.5±7.3	75.1±17.3	0.0004
CD21	53.1±8.1	49.9±25.7	ns
CD22	53.9±8.3	56.1±24.7	ns
CD23	43.3±10.0	14.8±11.5	<0.0001

IgG4-ROD: IgG4-related ophthalmic disease; EMZL: extranodal marginal zone B-cell lymphoma.

Table II. *Other cell lineages population (%) in IgG4-ROD and EMZL.*

	IgG4-ROD (N=12)	EMZL (N=12)	<i>p</i> -Value
CD56	0.5±0.3	0.2±0.1	0.0016
CD38	73.7±12.6	31.6±23.1	<0.0001
CD138	2.5±1.5	1.3±1.3	0.0317

IgG4-ROD: IgG4-related ophthalmic disease; EMZL: extranodal marginal zone B-cell lymphoma.

markers, CD10 and CD23, were significantly higher in the IgG4-ROD group, whereas the CD19 and CD20 percentages were significantly greater in the EMZL group (Table I). These results are consistent with the previous reports (5, 6).

The population of cells expressing other cell surface markers, CD56, CD38 and CD138 were significantly greater in the IgG4-ROD group than in the EMZL group (Table II). The rate of the expression of the NK cell marker CD56 was higher in IgG4-ROD compared to EMZL (0.5±0.3% and 0.2±0.1%, respectively; *p*<0.05). The population of CD38-positive cells was significantly greater in IgG4-ROD than EMZL (73.7±12.6% and 31.6±23.1%, respectively; *p*<0.05). Although the CD138 percentages were overall low in both IgG4-ROD and EMZL, CD138 was statistically significantly more commonly present in IgG4-ROD compared to EMZL (2.5±1.5% and 1.3±1.3%, respectively; *p*<0.01). The CD38 and CD138 percentages were strongly correlated in EMZL tissues (*r*_s=0.78, *p*=0.001) (Figure 1A), whereas the correlation was absent in IgG4-ROD (*r*_s=-0.03, *p*=0.93) (Figure 1B). In all the cases of EMZL and IgG4-ROD, the

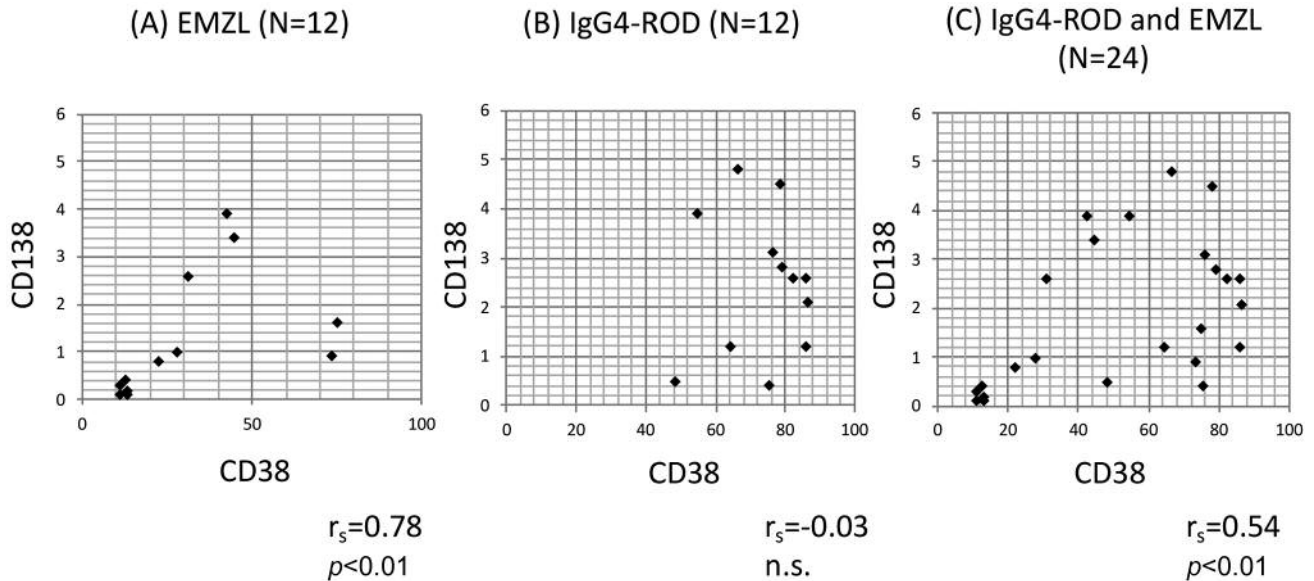


Figure 1. Correlations between CD38 and CD138 percentages. The percentages of CD38- and CD138-positive cells calculated using FCM analysis had a strong positive correlation in EMZL tissues ($r_s=0.78$, $p=0.001$) (A), but not in IgG4-ROD ($r_s=-0.03$, $p=0.93$) (B). In all the cases of EMZL and IgG4-ROD, CD38 and CD138 percentages had a moderate positive correlation ($r_s=0.54$, $p=0.007$) (C).

CD38 and CD138 percentages had a moderate positive correlation ($r_s=0.54$, $p=0.007$) (Figure 1C). Double-staining FCM analyses of CD38 and CD5 was performed in 2 patients with IgG4-ROD and disclosed that the percentage of CD38 and CD5 double positive cells were 45% in one case and 20% in the other (Figure 2A and B).

Discussion

IgG4-ROD is a chronic inflammatory condition characterized by enlargement of orbital tissues such as lacrimal glands, extraocular muscles and infraorbital nerves. Histologically, IgG4-ROD demonstrates tissue infiltration by T and B lymphocytes, as well as IgG4-secreting plasma cells with fibrosis (1). It has been hypothesized that IgG4-ROD is triggered by autoimmunity and infectious agents (10). Th2-dominant helper T cells and regulatory T cells release various cytokines, which subsequently activate various inflammatory cells such as B lymphocytes, fibroblasts and eosinophils (10). Activated B lymphocytes turn into plasma cells, secreting IgG4. Activated fibroblasts play a critical role in fibrosis formation in IgG4-ROD tissues (10).

In contrast, EMZL is a low-grade B-cell lymphoma, derived from memory B cells in the marginal zone of lymphoid organs. Histological findings in EMZL tissues revealed infiltration of centrocyte-like and monocytoid tumor cells in the marginal zone surrounding reactive follicles. The fact that tumor cells often manifest plasmacytic differentiation is of morphological importance. Coupland *et al.* have reported

that 57 (42%) of the 136 EMZL tissues of the ocular adnexa showed a plasmacytic differentiation (13).

This is the first study investigating the difference in CD38 and CD138-positive rates between IgG4-ROD and EMZL tissues. We found that although the rates of CD138-positive cells were low in both IgG4-ROD and EMZL, the number of both CD38- and CD138-positive cells were greater in IgG4-ROD than in EMZL. CD38 is detectable throughout the cells of the immune system: activated T cells, activated B cells (plasma cells) and NK cells (7). In addition, both CD38 and CD138 are present on pre-B-cells and plasma cells but they are absent in circulating and peripheral B lymphocytes (8, 9). In this study, among the IgG4-ROD cases, there was no positive correlation between the rates of CD38 and CD138 (Figure 1A). Double-staining FCM demonstrated that several CD38-positive cells were positive for CD5, a T-cell marker, in two available IgG4-ROD cases (Figure 2). Therefore, the higher rate of CD38 in IgG4-ROD may possibly reflect the infiltration of activated T cells in the tissue.

As described above, CD38 is expressed not only in plasma cells and activated T cells but also in NK cells. In this study, the population of CD56-positive cells, a common marker for NK cells, was significantly higher in IgG4-ROD than in EMZL, although the number of NK cells was considered low compared to CD38-positive cells. Therefore, the CD38 population detected in IgG4-ROD tissues may be partially correlated with NK cells; however, there is no evidence that NK cells play a role in the pathogenesis of IgG4-ROD. Further studies are needed to clarify the role of NK cells in IgG4-ROD.

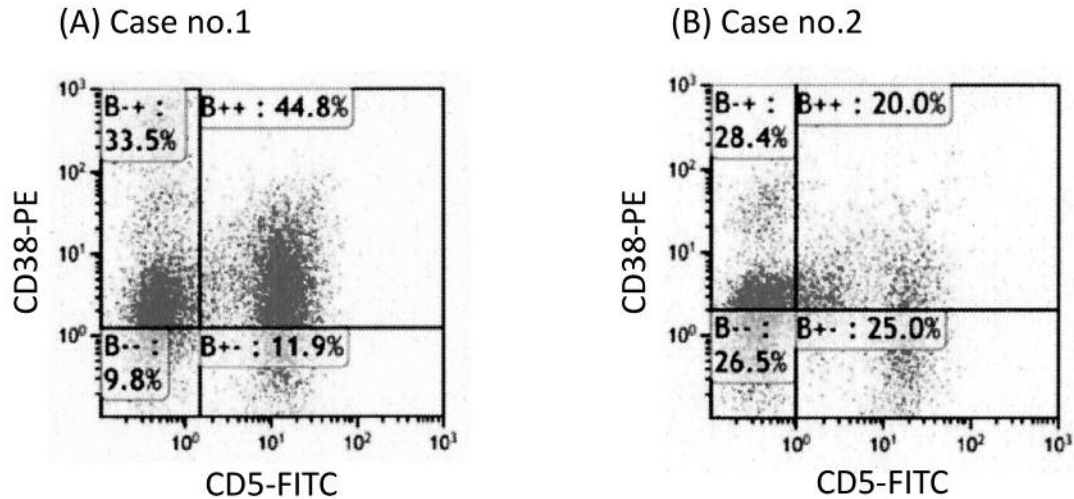


Figure 2. Double-staining FCM analysis for CD38, a marker for activated T cells, plasma cells and NK cells, and CD5, a representative T cell marker, in 2 cases with IgG4-ROD. In one case, the rate of CD38 and CD5 double positive cells was 45% (A). In the other case, it was 20% (B).

Concerning EMZL, a previous study has demonstrated that almost half of ocular adnexal EMZL tissues showed histologically a plasmacytic differentiation, in which most of plasmacytoid cells were positive for CD38 (89%) and CD138 (91%) (13). In this study, among the EMZL cases, there was a positive correlation between CD38 and CD138 (Figure 1B), suggesting that CD38-positive cells detected by FCM may be associated with the plasmacytic differentiation.

There are limitations in this study. First, there is relatively a small number of patients examined in this study. Next, although plasma cell infiltration is one of the characteristic pathological findings in IgG4-ROD, the rates of CD138-positive cells were low in both IgG4-ROD and EMZL ($2.5 \pm 1.5\%$ and $1.3 \pm 1.3\%$, respectively). These results might not reflect the true population of CD138-positive cells in the tissues of each disease owing to the decreased sensitivity of the antibody used or unknown problems during the process of FCM procedure. Further studies are required to clarify the correlation between the percentages of FCM-based and histological CD138-positive cells. Third, we applied double staining for CD38 and CD5 with FCM in only 2 cases that were available. Further examinations are needed to clarify the origin of high CD38 population in IgG4-ROD. Finally, this study did not examine orbital tissues from patients with plasmacytoma and IgG4-producing EMZL, in which populations of CD38 and CD138 might also be altered. Therefore, further enrollment of patients that can provide such tissue and FCM analysis would provide additional insights.

In conclusion, FCM-based analysis of the difference in the rates of CD38- and CD138-positive cells between IgG4-ROD and EMZL, due to different lymphocyte populations, may

lead to better understanding of the pathogenesis of the two diseases often hardly distinguishable from each other.

Conflicts of Interest

The Authors declare no conflicts of interest and no funding associated with this study.

Authors' Contributions

YS corrected and analyzed the data, and wrote this article; SK supervised this study, surgically corrected the tissues, confirmed the results and revised this article; IM gave advice about the interpretation of the results and revised this article; KI and SI did critical revision on this article.

References

- Goto H, Takahira M, Azumi A and Japanese Study Group for IgG4-Related Ophthalmic Disease: Diagnostic criteria for IgG4-related ophthalmic disease. *Jpn J Ophthalmol* 59(1): 1-7, 2015. PMID: 25392273. DOI: 10.1007/s10384-014-0352-2
- Japanese study group of IgG4-related ophthalmic disease: A prevalence study of IgG4-related ophthalmic disease in Japan. *Jpn J Ophthalmol* 57(6): 573-579, 2013. PMID: 23917985. DOI: 10.1007/s10384-013-0266-4
- Kase S, Noda M, Ishijima K, Yamamoto T, Hatanaka K and Ishida S: IgG4-related inflammation of the orbit simulating malignant lymphoma. *Anticancer Res* 33(6): 2779-2783, 2013. PMID: 23749941.
- Sato Y, Ohshima K, Takata K, Huang X, Cui W, Ohno K and Yoshino T: Ocular adnexal IgG4-producing mucosa-associated lymphoid tissue lymphoma mimicking IgG4-related disease. *J Clin Exp Hematopathol* 52(1): 51-55. 2012. PMID: 22706531. DOI: 10.3960/jslrt.52.51

- 5 Ueda S, Usui Y, Nagai T, Diaz-Aguilar D, Nagao T and Goto H: Immunophenotypic profiles for distinguishing orbital mucosa-associated lymphoid tissue lymphoma from benign lymphoproliferative tumors. *Jpn J Ophthalmol* 61(4): 354-360, 2017. PMID: 28421369. DOI: 10.1007/s10384-017-0513-1
- 6 Kase, S, Ishijima K, Uraki T, Suimon Y, Suzuki Y, Kase M and Ishida S: Usefulness of flow cytometry in diagnosis of IgG4-related ophthalmic disease and extranodal marginal zone B-cell lymphoma of the ocular adnexa. *Anticancer Res* 37(9): 5001-5004, 2017. PMID: 28870925. DOI: 10.21873/anticancer.11913
- 7 Quarona V, Zaccarello G, Chillemi A, Brunetti E, Singh VK, Ferrero E, Funaro A, Horenstein AL and Malavasi F: CD38 and CD157: a long journey from activation markers to multifunctional molecules. *Cytometry B Clin Cytom* 84(4): 207-217, 2013. PMID: 23576305. DOI: 10.1002/cyto.b.21092
- 8 Sanderson RD, Lalor P and Bernfield M: B lymphocytes express and lose syndecan at specific stages of differentiation. *Cell Regul* 1(1): 27-35, 1989. PMID: 2519615. DOI: 10.1091/mbc.1.1.27
- 9 Mehta K, Shahid U and Malavasi F: Human CD38, a cell-surface protein with multiple functions. *FASEB J* 10(12): 1408-1417, 1996. PMID: 8903511. DOI: 10.1096/fasebj.10.12.8903511
- 10 Stone JH, Zen Y and Deshpande V: IgG4-related disease. *N Engl J Med* 366(6): 539-551, 2012. PMID: 22316447. DOI: 10.1056/NEJMra1104650
- 11 Suimon Y, Kase S, Ishijima K, Kanno-Okada H and Ishida S: A clinicopathological study on IgG4-related ophthalmic disease. *Int J Ophthalmol* 11(9): 1539-1544, 2018. PMID: 30225231. DOI: 10.18240/ijo.2018.09.18
- 12 Schreuder MI, van den Brand M, Hebeda KM, Groenen PJTA, van Krieken JH and Scheijen B: Novel developments in the pathogenesis and diagnosis of extranodal marginal zone lymphoma. *J Hematop* 10(3-4): 91-107, 2017. PMID: 29225710. DOI: 10.1007/s12308-017-0302-2
- 13 Coupland SE, Hellmich M, Auw-Haedrich C, Lee WR, Anagnostopoulos I and Stein H: Plasmacellular differentiation in extranodal marginal zone B cell lymphomas of the ocular adnexa: an analysis of the neoplastic plasma cell phenotype and its prognostic significance in 136 cases. *Br J Ophthalmol* 89(3): 352-359, 2005. PMID: 15722318. DOI: 10.1136/bjo.2004.047092

Received February 23, 2020

Revised March 6, 2020

Accepted March 9, 2020