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Original Article

Profiles of peripheral B cell subsets in a cohort of primary Sjögren's syndrome patients and their potential clinical significance

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KEYWORDS
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Flow cytometry

Abstract *Background/purpose:* Primary Sjögren's syndrome is a prototypical autoimmune disease, with B cell dysfunction as a dominant feature. Further insights into distribution of B cell subsets in primary Sjögren's syndrome are urgently required to identify the most appropriate target subpopulation. We aimed to evaluate the profiles of B lymphocyte subpopulations in primary Sjögren's syndrome patients and to investigate their clinical significance.
Materials and methods: Thirty primary Sjögren's syndrome patients and 15 age-and sex-matched healthy controls were recruited. Peripheral B cell subsets were analyzed by flow cytometry.
Results: Compared to healthy controls, circulating $CD19^+$ B cells, $CD19^+CD20^-$ B cells, $CD19^+CD27^-IgD^+$ naïve B cells, $CD19^+IgD^+CD38^{high}$ plasmablasts, $CD19^+CD24^{high}CD38^{high}$ transitional B cells and $CD19^+CD20^-CD27^+CD38^+$ plasma cells were elevated in patients with primary Sjögren's syndrome, whereas $CD19^+CD27^+$ memory B cells, $CD19^+CD27^-IgD^-$ double negative B cells and $CD19^+CD24^{hi}CD27^+$ Bregs were decreased. Furthermore, the percentage of circulating $CD19^+CD20^-CD27^+CD38^+$ plasma cells was positively correlated with serum IgG levels and the proportional area of lymphocytic infiltration of labial gland.
Conclusion: We identified a comprehensive B lymphocyte subset distribution profile in primary Sjögren's syndrome. Moreover, we detected a clinical significance of $CD19^+CD20^-CD27^+CD38^+$ plasma cells, suggesting that these cells might play a key role in disease pathology and represent potential therapeutic targets.

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Introduction

Primary Sjögren's syndrome (pSS) is a chronic systemic autoimmune disorder with a specific predisposition for causing inflammation of the exocrine glands. Salivary and lacrimal glands are predominantly affected, resulting in characteristic dryness of the oral and ocular mucosa.[1,2] Dryness of the mouth and eyes, fatigue, and joint pain are present in >80 % of the patients with this disease,[1] and systemic complications develop in one-third of patients. With a population prevalence of 0.3–3 per 1000 of the general population and a female-to-male predominance of 9:1, pSS is associated with a high burden of illness.[3]

Although the underlying pathogenesis of pSS is unclear, a number of lines of evidence support the involvement of B cells.[4] B cell dysfunction is a dominant feature of pSS, resulting in serum polyclonal hypergammaglobulinemia, multiple autoantibodies, anti-Sjögren-syndrome-related antigens A and B (SSA and SSB; also known as Ro and La, respectively) antibodies, rheumatoid factor, cryoglobulins. Futhermore, in the patients of pSS, peripheral blood and salivary gland B cell subsets distribution are altered, leading to the formation of ectopic germinal centers in the exocrine glands where autoreactive clones may escape tolerance checkpoints. Moreover, compared with other rheumatic diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), patients with pSS have higher risk of developing B cell lymphoma.[5] Thus, targeting B lymphocytes directly and/or indirectly has become a cornerstone of developing therapeutic strategies for pSS.[6] CD20 is most extensively investigated B cell-related therapeutic target and rituximab (RTX) is the only anti-CD20 monoclonal antibody (mAb) approved for treating pSS. Retrospective analyses, open-label studies and randomized double-blind placebo-controlled trials have shown the efficacy of the RTX for at least 6–9 months in patients with active primary SS; however, the two large RCTs did not meet their composite of primary endpoints (pain, fatigue, sicca and global improvement).[7,8] The clinical efficacy of RTX-induced B cell depletion therapy in pSS still remains controversial. In addition, the anti-BAFF mAb, belimumab, which is approved for the treatment of SLE, has also shown promising efficacy and safety in open-label trials for the treatment of pSS.[9,10] However, all these B cell depletion therapies are associated with adverse effects and more precise therapies targeting specific and pathogenic B cell subsets are required.

Presence of pathogenic autoantibodies is not only the hallmark of pSS and the clue for diagnosis, but associated with more severe clinical manifestations of disease —— longer disease duration, more severe dysfunction of the exocrine glands, recurrent parotid gland enlargement and higher intensity of the lymphocytic infiltrates in the minor salivary glands,[11] indicating the crucial role of the precursor of immunoglobulin (Ig) secreting cell, namely plasma cell in the pathological of pSS.

Besides plasma cells that producing various autoantibodies, growing evidence indicates the functional diversities of B cell subsets in both immunity and autoimmune pathogenesis. $CD19^{+}CD24^{hi}CD38^{hi}$ transitional B cells (TBs) significantly inhibit the differentiation of proinflammatory cytokine expressing $CD4^{+}$ T cells.[12] These B-cell subsets suppress the differentiation of T helper (Th)1 cells in an IL-10 dependent manner, and altered distribution of this transitional B-cell subsets highlights different regulatory defects in patients with different autoimmune diseases including pSS.[13] Besides $CD19^{+}CD24^{hi}CD38^{hi}$ transitional B cells, $CD19^{+}CD24^{hi}CD27^{+}$ B cells (human equivalent of murine B10 cells) are another IL-10-producing regulatory B cells (Bregs) and more efficient than TBs at suppressing $CD4^{+}$ T cell proliferation and IFN-g/IL-17 expression.[14] However, whether these Bregs are altered in SS is still unclear.

Abnormalities of memory B cells seem to be closely involved in the pathogenesis of pSS. Reduction of peripheral memory $CD27^{+}$ B cells may indicate a lack of appropriate censoring mechanisms and incomplete differentiation processes within the ectopic lymphoid tissues in pSS. Recently, double negative (DN) ($CD19^{+}CD27^{-}IgD^{-}$) B cells are B cells that undergo class switching but lack expression of immunoglobulin D and the memory marker CD27. These cells have been suggested to contribute to the pathophysiology of several autoimmune disorders such as SLE and seem to serve different functions in the context of different diseases.[15] Detecting the frequency of DN B cells in patients may be useful for patient stratification and monitoring as well as provide a biomarker for clinical intervention studies. Their potential pathological role in pSS warrants further investigations.

Actually, there are several studies detecting the peripheral B-lymphocyte subsets in pSS. However, the results seem controversial and each study focused on limited or specific B cell subsets, lacking the comparability to describe the B subgroups in pSS uniformly. Therefore, relatively comprehensive new data about B cell subsets in SS will be of significance and clinically important. Detecting these cells in patients may be useful for patient stratification and monitoring as well as provide a biomarker for clinical intervention studies. All in all, the paradigm based on B cell involvement, coupled with the ever-growing variety of B cell subsets, present controversial results and poor comparability among available studies raise the issue as to which of the above B subsets underpins the autoimmune features characteristic of pSS. And the present study aimed to determine B lymphocyte subset distribution profile in pSS and their association with the features and severity of the disease.

Materials and methods

Study population

Thirty patients fulfilling the 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for pSS were enrolled in the study. Only patients without any medical treatment were included. Sex- and age-matched healthy individuals (n = 15) were studied in parallel as healthy controls (HCs).

Ethical approval

The study was approved by the Institutional Review Board of Peking University School and Hospital of Stomatology (KUSSIRB-201631139). Patients and healthy volunteers were recruited after obtaining informed consent and all methods were performed in accordance with the relevant guidelines and regulations.

Isolation of human peripheral blood mononuclear cells (PBMCs)

Blood samples were collected into EDTA-containing tubes and PBMCs were isolated by Ficoll-Hypaque (TBD Science, Tianjin, China) density gradient centrifugation. Briefly, blood was layered carefully onto an equal volume of Ficoll in a centrifuge tube before centrifugation at 500×*g* for 20 min. The band of mononuclear cells at the interface was harvested and washed twice by sterile phosphate buffer saline (PBS) followed by centrifugation at 300×*g* for 10 min at room temperature.

Flow cytometry

Immunofluorescence staining for flow cytometric analysis was performed by incubating PBMCs (10^7/mL) with anti-CD19, anti-CD20, anti-IgD, anti-CD38, anti-CD27 and anti-CD24 mAbs (5 μl per million cells in 100 μl staining volume) (BioLegend, San Diego, CA, USA) for 30 min. After washing with PBS, the cells were then analyzed by flow cytometry (Beckman Coulter, Brea, CA, USA).

Histopathology and measurement of lymphocytic foci size

The labial glands of SS patients were collected and fixed in 4 % paraformalclehyde overnight, processed and embedded in paraffin. The tissue sections were stained with hematoxylin and eosin (H&E). The ratio of lymphocyte infiltration area to the total section area were determined in 5—8 nonconsecutive sections per gland. The percentage of the area occupied by a focus was calculated as: (∑area of individual focus in a given section) × 100 ÷ total section area. The slides were masked and two individuals performed the histologic analysis as a blindfolded experiment.

Statistical analysis

Descriptive analyses of clinical data are presented as mean ± standard deviation (SD) or median and interquartile range (IQR) when continuous and as frequency and proportion (%) when categorical. Differences between continuous data were analyzed using the Mann—Whitney *U* test or Kruskal—Wallis comparison test. The Pearson's normally distributed correlation coefficient for linear data and Spearman's non-normally distributed correlation for nonlinear data were reported. Statistical analyses were performed using SPSS version 19.0 and Prism version 9. $P < 0.05$ was set as the threshold for statistical significance.

Results

Patient characteristics

Thirty pSS patients were included; all were women. Nineteen pSS patients (63.3 %) presented anti-SSA antibodies (SSA^+ SS), while 11 (36.7 %) were anti-SSA-negative (SSA^- pSS). The HC group comprised 15 age-matched women. The demographic, clinical, and immunological characteristics of the patients are shown in Table 1. SSA^+ pSS tended to be diagnosed earlier than SSA^- pSS (48.05 ± 3.099 y vs. 57.55 ± 4.237 y; $P = 0.0782$). Serum IgG levels were evaluated in 73.68 % of SSA^+pSS, which was more frequent than in SSA^- pSS (54.54 %). ANA titers ≥1:320 were also more frequent in SSA^+ pSS (73.68 %) compared to SSA^- pSS (27.27 %) (Table 1).

B lymphocyte characterization

Peripheral blood B cell subsets classified according to CD19 and CD20 expression

CD19 is one of the B-cell antigen receptor coreceptors and it is the most broadly used B-cell marker for flow-cytometric immunophenotyping of lymphocytes.[16] Compared with HCs, the proportion of $CD19^+$ B cells was elevated in the peripheral blood of pSS patients (2.809 ± 0.3911 vs. 6.060 ± 0.7410; $P = 0.0047$) (Fig. 1A). Although there was no significant difference in proportion of $CD19^+$ B cells in SSA^+ SS compared to SSA^- pSS, the

Table 1 Patients' characteristics.

	Total pSS (n = 30)	SSA^+ pSS (n = 19)
Sex, F/M	30/0	19/0
Age at diagnosis (y), mean ± SD	51.53 ± 2.601	48.05 ± 3.099
Ocular symptoms, n (%)	17 (56.67)	12 (63.16)
Oral symptoms, n (%)	27 (90)	18 (94.74)
Decreased salivary flow, n (%)	23 (76.67)	16 (84.21)
Salivary gland swelling, n (%)	2 (6.67)	2 (10.53)
ANA ≥1/320, n (%)	16 (53.34)	14 (73.68)
Elevated IgG levels	20 (66.67)	16 (84.21)

Patient characteristics are represented as number of occurrences (n) and percentages (%). pSS, primary Sjögren's syndrome; F, female; M, male; y, years; SSA, anti-Sjögren-syndrome-related antigen A; ANA, antinuclear antibody.

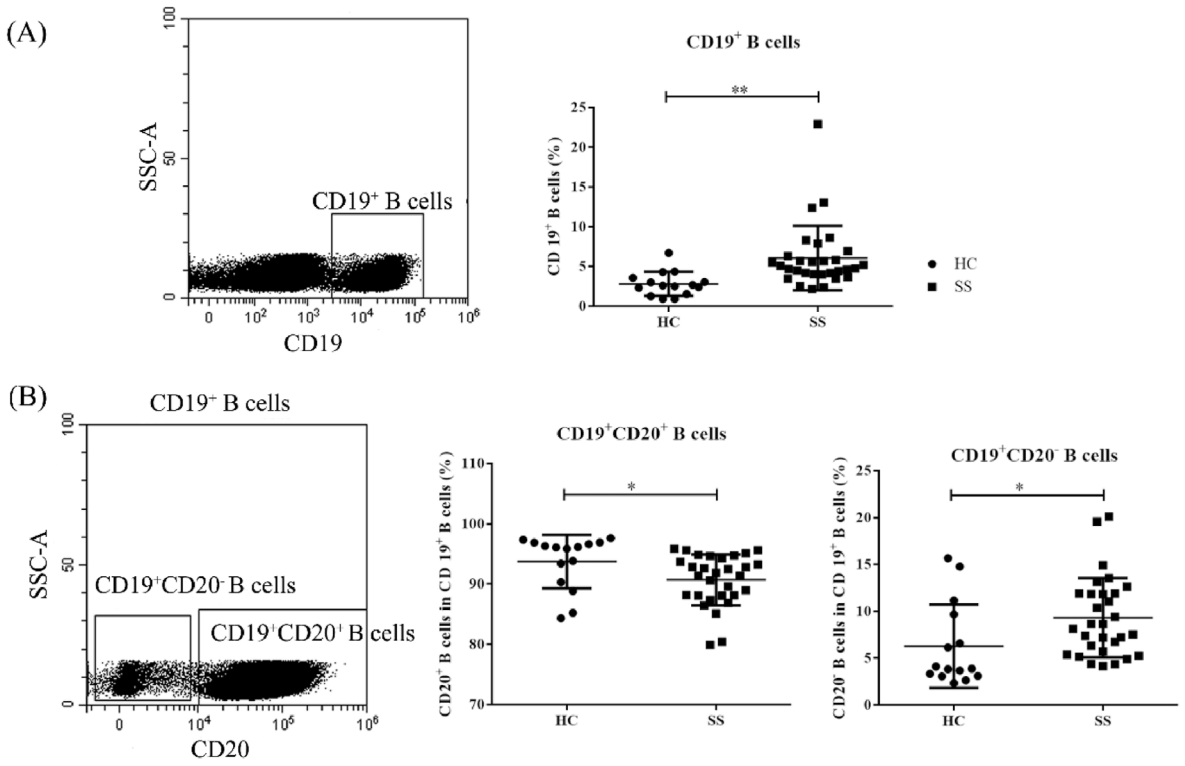

Figure 1 Peripheral blood B cell subsets classified according to CD19 and CD20 expression. (A) Gating strategy for the identification of $CD19^+$ B cells and scatter dot plots showing the distribution of $CD19^+$ B cells as a percentage of the total B cells; (B) Gating strategy for the identification of $CD19^+CD20^-$ and $CD19^+CD20^+$ B cells and scatter dot plots showing the distribution of these cells as a percentage of the total B cells. *$P < 0.05$, **$P < 0.01$.

proportion of $CD19^+$ B cells was slightly elevated in SSA^+ SS ($P = 0.0886$).

CD20 is a general B-cell marker expressed by the majority of B cells but not expressed in terminally differentiated plasmablasts and plasma cells. We classified $CD19^+$ B cells according to the expression of the stage-specific B cell surface antigen CD20, with the $CD19^+CD20^+$ and $CD19^+CD20^-$ phenotypes defining early- and late-stage B cells, respectively. Compared to HCs, the proportion of $CD19^+CD20^+$ B cells was decreased in peripheral blood of pSS patients ($P = 0.0306$) whereas the proportion of $CD19^+CD20^-$ B cells was increased ($P = 0.0306$) (Fig. 1B).

Peripheral blood B cell subsets classified according to CD27 and IgD expression

Human memory B cells can be identified by the expression of CD27. Together with IgD, CD27 can determine four relatively well-characterized $CD19^+$ cell subtypes in the human peripheral blood (Fig. 2A). $CD19^+CD27^+$ cells are considered as total memory B cells, switched memory B cells (SwM) are $CD19^+IgD^-CD27^+$ and unswitched memory B cells (UnSwM) are $CD19^+IgD^+CD27^+$. $CD19^+CD27^-IgD^+$ B cells are naïve B cells and $CD27^-IgD^-$ B cells are referred to as double-negative B cells (DN). We detected significant differences in the proportion of all memory populations between pSS and HCs. The proportions of total memory ($P = 0.0003$), SwM ($P = 0.0013$) and UnSwM ($P = 0.0169$) and DN ($P < 0.0001$) B cells were all decreased in peripheral blood of pSS patients compared with HCs, whereas $CD19^+CD27^-IgD^+$ naïve B cells were increased ($P < 0.0001$) (Fig. 2B).

Peripheral blood B-cell subsets according to Bm1–Bm5 classification

Membrane proteins IgD and CD38 were usually used to identify Bm1-Bm5 subsets[17] as follows: naïve Bm1 (IgD^+, $CD38^-$), Bm2 cells (IgD^+, $CD38^+$), Bm2' cells (IgD^+, $CD38^{++}$), Bm3 and Bm4 cells (IgD^-, $CD38^{++}$), early memory Bm5 cells (eBm5, IgD^-, $CD38^+$) and memory Bm5 cells (IgD^-, $CD38^-$) (Fig. 3A). Although no significant differences were found regarding each Bm subset, the percentage of Bm2+Bm2' cells was higher in pSS patients ($P < 0.0001$) while the percentage of Bm5+eBm5 cells was lower compared to HCs ($P = 0.0018$) (Fig. 3B). The proportion of plasmablasts ($CD19^+IgD^+CD38^{high}$) was also elevated in pSS ($P = 0.0109$) (Fig. 3C).

Peripheral blood B-cell subsets classified according to CD24 and CD27 or CD38 expression

Transitional B cells represent a crucial link between immature B cells in the bone marrow and mature peripheral B cells. Human TB cells were first described in detail in 2005, and are often characterized by a $CD24^{hi}CD38^{hi}$ phenotype.[18] Patients with pSS had higher proportions of

transitional B cells ($CD19^+CD24^{hi}CD38^{hi}$) than HCs ($P = 0.0062$). Breg subset in humans, characterized by the expression of $CD24^{hi}CD27^+$, have been described an abnormal number or function in specific autoimmune diseases. Patients with pSS had lower proportions of Breg cells ($CD19^+CD24^{hi}CD27^+$) compared to HCs ($P = 0.0002$) (Fig. 4B).

Peripheral blood $CD19^+CD20^-$ B-cell subsets classified according to CD27 and CD38 expression

CD20 is lost in terminally differentiated plasma cells, while CD27 and CD38 are positive expressed in plasma cells. Patients with pSS had higher proportions of $CD19^+CD20^-CD27^+CD38^+$ plasma cells (terminally differentiated B cells) than HCs ($P = 0.0025$) (Fig. 4C).

All in all, Peripheral blood B cell subsets characteristics of pSS patients and HCs were showed in Supplementary Table 1 (Table S1).

Relevance of B cell subset profile to the clinical features of pSS

Association between B cell subsets and serum anti-SSA Abs

There were no significant differences in the proportion of B cells subsets between SSA^+ pSS and SSA^- pSS (data were not shown), with the exception of $CD19^+CD20^-CD27^+CD38^+$ plasma cells, which were significantly higher in SSA^+ pSS ($P = 0.0181$) (Fig. 5A).

Correlation between B cell subsets and serum IgG levels

There were no significant correlations between B cell subsets and IgG levels, with the exception of $CD19^+CD20^-CD27^+CD38^+$ plasma cells, which correlated positively with serum IgG levels ($P < 0.01$, $r = 0.656$) (Fig. 5B).

Correlation between B cell subsets and saliva flow rate

No significant correlations between saliva flow rate (mL/min) and percentages of B cell subsets were identified. However, when pSS patients were divided into saliva flow rate subgroups, there was a marginally significant difference: a higher percentage of $CD19^+CD20^-CD27^+CD38^+$ plasma cells in the <0.1 mL/1 min subgroup ($P = 0.05462$) compared with that in the ≥ 0.1mL/1min subgroup (data were not shown).

Correlation between B cell subsets and lymphocytes infiltration of the labial salivary gland

In the present study of 30 patients, nine had a biopsy of the labial salivary gland (the representative histopathological picture is shown in Fig. S1). The focus score (FS) and area of lymphocytic infiltrates were recorded. FS was not related to circulating B cell subsets (Fig. S2), whereas the area of lymphocytic infiltrates was positively correlated with the percentage of circulating $CD19^+CD20^-CD27^+CD38^+$ plasma cells ($P < 0.01$, $r = 0.817$) (Fig. 5C).

All in all, heatmap of percentages of B cell subsets and clinical features of pSS patients is showed in Supplementary Fig. 3 (Fig. S3).

Discussion

In the present study, we conducted a comprehensive investigation of the distribution of circulating B cell subsets in pSS and analyzed their association with the features and severity of the disease. Overall, compared with HCs, $CD19^+$ B cells, $CD19^+CD20^-$ B cells, $CD19^+CD27^-IgD^+$ naïve B cells, $CD19^+IgD^+CD38^{high}$ plasmablasts, $CD19^+CD24^{high}CD38^{high}$ transitional B cells and $CD19^+CD20^-CD27^+CD38^+$ plasma cells were elevated in the peripheral blood of pSS patients, whereas $CD19^+CD27^+$ total memory B cells, $CD19^+CD27^-IgD^-$ DN B cells and $CD19^+CD24^{hi}CD27^+$ Bregs were decreased (Fig. S4). These results are consistent with some previous reports, but inconsistent with others.[19–22] We speculate that there are some reasons why it is difficult to draw a unified conclusion: 1) it is difficult to carry out the large sample study; 2) The baseline data of patients in different studies were inconsistent; 3) There is no "golden" criterion for the classification of B cell subsets; 4) Other health status of subjects (e.g., catch cold, vaccination, etc.) may affect the percentages of immune cell subsets. The present results provide new insights into the circulating numbers of $CD19^+CD24^{hi}CD27^+$ Bregs and $CD19^+CD20^-CD27^+CD38^+$ plasma cells in pSS. In addition to an abnormal proportion in circulating blood of patients with pSS, the percentage of circulating $CD19^+CD20^-CD27^+CD38^+$ plasma cells correlated positively with serum IgG levels and the area of lymphocytic infiltration of the labial salivary glands. In the current study, we also identified some striking differences in peripheral blood B cell subsets of pSS patients compared with HCs as well as an association between $CD19^+CD20^-CD27^+CD38^+$ plasma cells and the immunological and pathological features of the disease.

CD20 is a B cell-specific differentiation antigen that regulates proliferation and activation. CD20 is expressed on mature B lymphocytes, but not on early B cell progenitors or late mature plasma cells.[23] Studies have shown that the proportions of $CD19^+CD20^-$ B cells were increased in patients with SLE, corelated with clinical parameters and had the ability to proliferate and secrete anti-dsDNA and IgG.[24] In the present study, we showed that the percentage of $CD19^+CD20^-$ B cells was also elevated in pSS patients. The anti-CD20 chimeric mAb RTX can be used to deplete CD20-positive B cells. Although RTX has been shown to have beneficial effects on pSS in small-scale studies, two recent large randomized controlled trials did not meet their primary end-points[25]; thus, the benefits of RTX-mediated B cell depletion therapy in treating pSS remain to be verified. It can be speculated that the unsatisfactory results obtained are due the ability of RTX to eliminate mature $CD19^+CD20^+$ B cells, but not pathogenic $CD19^+CD20^-$ plasma cells.

Our analysis of the distribution and possible role of $CD19^+CD20^-CD27^+CD38^+$ plasma cells in pSS revealed a significantly higher proportion of this B cell subpopulation in the peripheral blood of pSS patients and a positive correlation with serum IgG levels and the area of lymphocytic infiltration of the labial salivary gland. Thus, these plasma cells might play a vital role in the pathogenesis of this disease and indicate the potential therapeutic importance

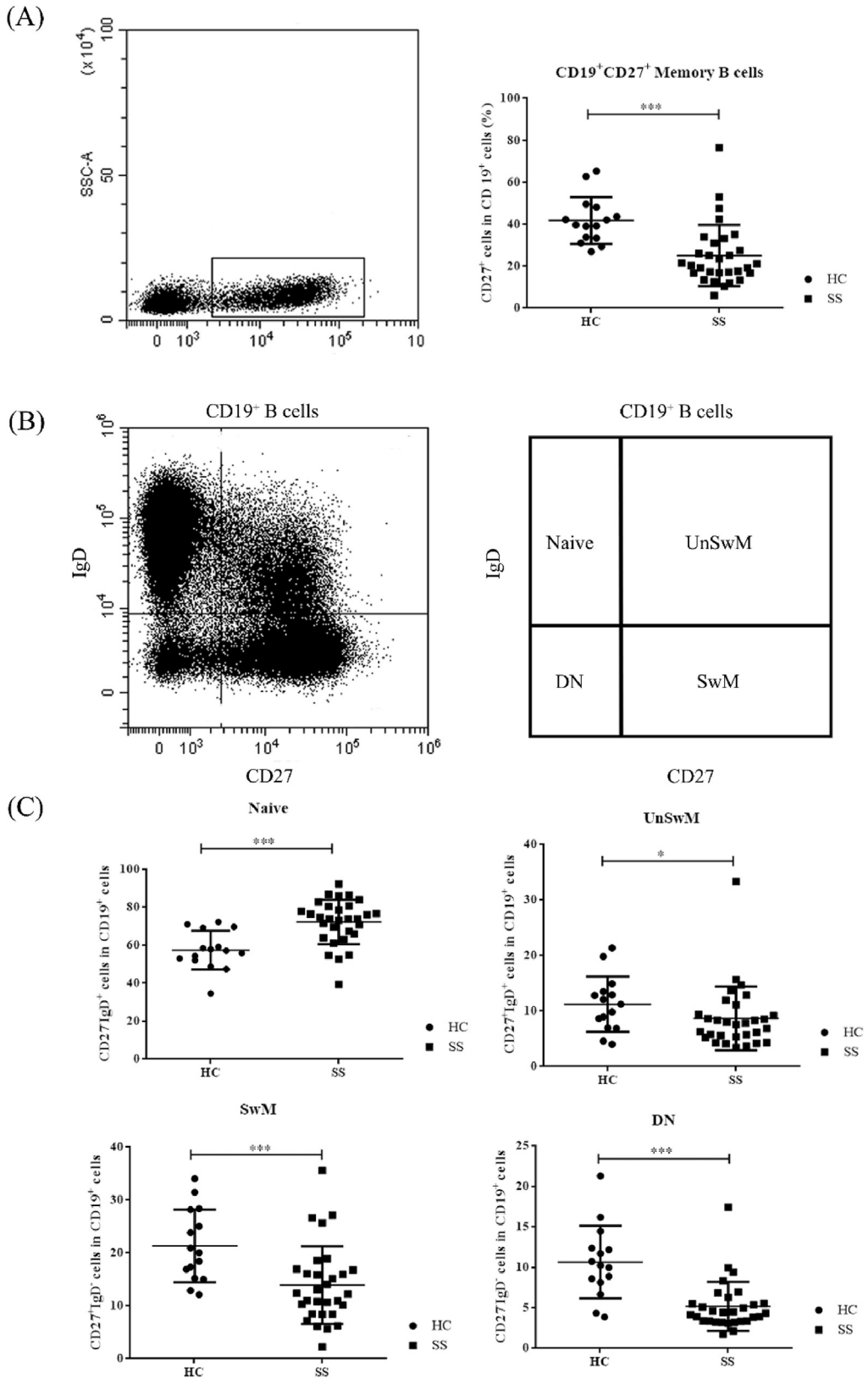

Figure 2 Peripheral blood B cell subsets classified according to CD27 and IgD expression. (A) Gating strategy for the identification of $CD19^{+}CD27^{+}$ memory B cells and scatter dot plots with the distribution of these cells as a percentage of the total B cells; (B) Gating strategy for the identification of $CD19^{+}CD27^{-}IgD^{+}$ naïve B cells, $CD19^{+}IgD^{-}CD27^{+}$ switched memory B cells, $CD19^{+}IgD^{-}CD27^{+}$ unswitched memory B cells and $CD27^{-}IgD^{-}$ DN B cells. (C) Scatter dot plots showing the distribution of these cells as a percentage of the total B cells. $^{*}P < 0.05$, $^{***}P < 0.001$.

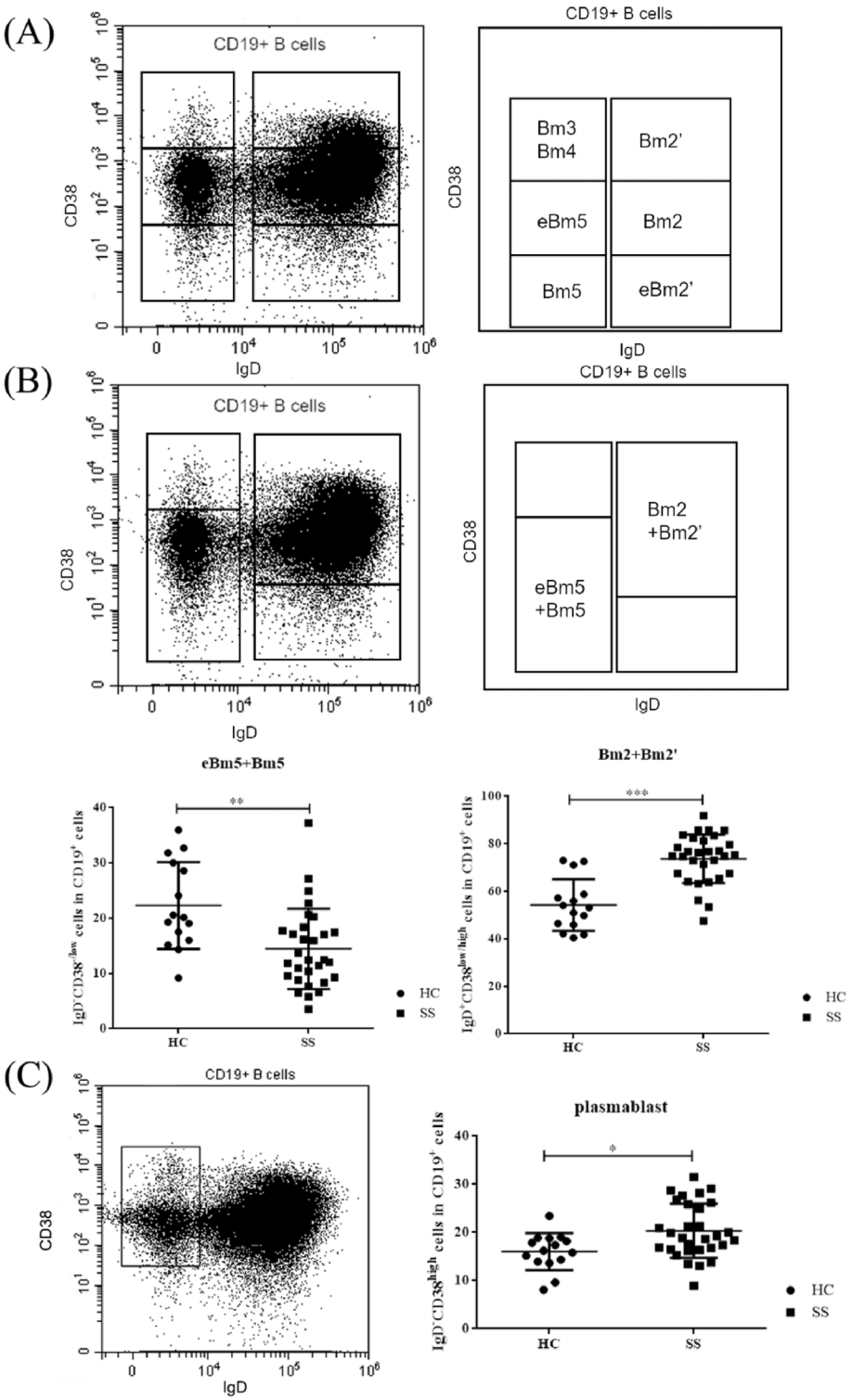

Figure 3 Peripheral blood B cell subsets classified according to CD38 and IgD expression. (A) Gating strategy for the identification of Bm cells; (B) Gating strategy for the identification of eBm5+Bm5 and Bm2+Bm2′ and scatter dot plots showing the distribution of these cells as a percentage of the total B cells; (C) Gating strategy for the identification of $CD19^{+}IgD^{+}CD38^{high}$ plasmablasts and scatter dot plots showing the distribution these cells as a percentage of the plasmablasts. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

of plasma cell depletion in pSS. Among such treatments, proteasome inhibitors are already under investigation in SLE[26] and anti-CD38 daratumumab monotherapy has shown promise in multiple myeloma.[27] Thus, plasma cell depletion or suppression of their activation are implicated as novel therapeutic strategies in patients with pSS.[4]

TBs cells represent a crucial link between immature B cells in the bone marrow and mature peripheral B cells.

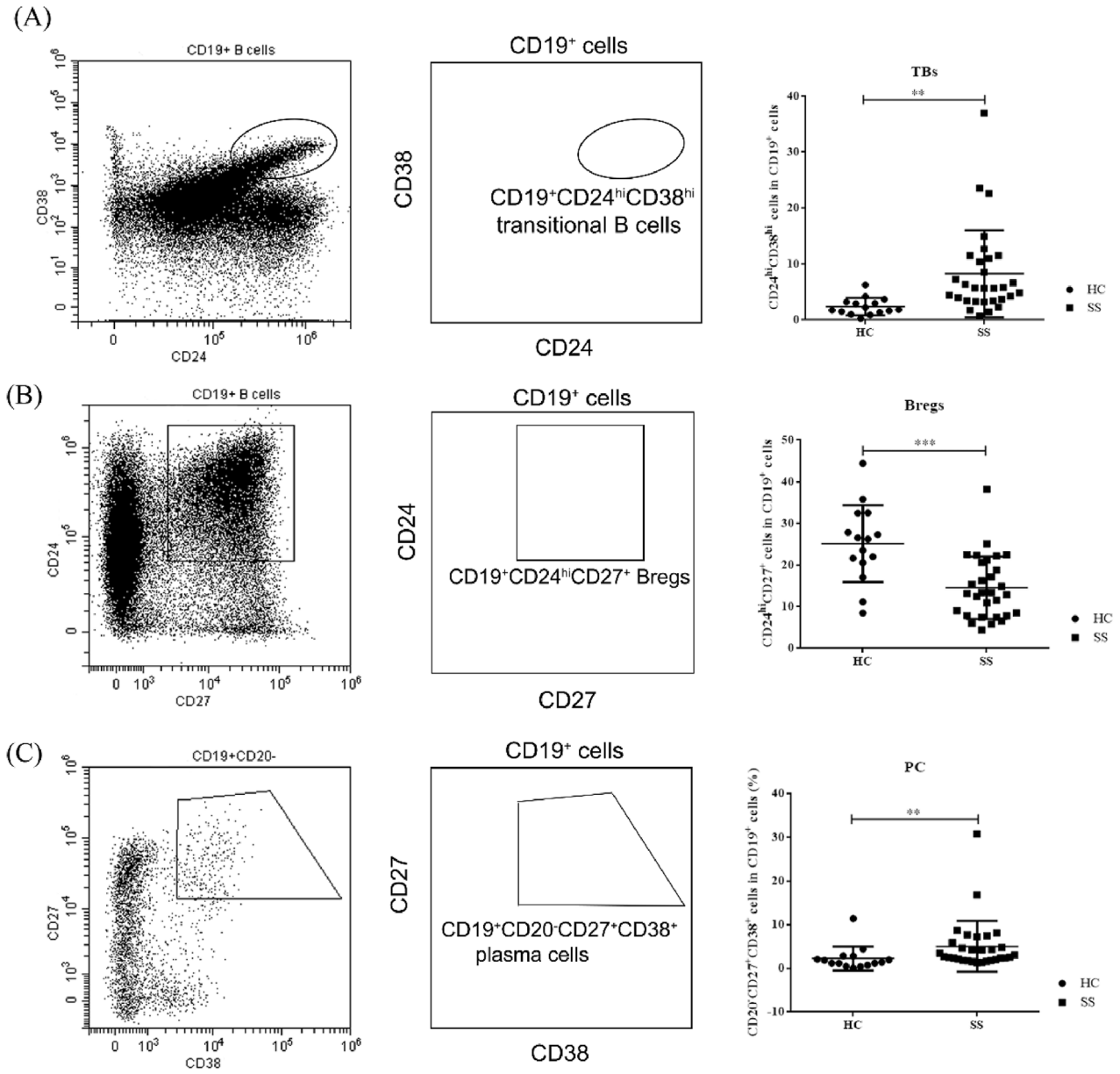

Figure 4 Peripheral blood B-cell subsets classified according to CD24 and CD27 or CD38 expression. (A) Gating strategy for the identification of $CD19^{+}CD24^{high}CD38^{high}$ transitional B cells and scatter dot plots showing the distribution of these cells as a percentage of the total B cells; (B) Gating strategy for the identification of $CD19^{+}CD24^{hi}CD27^{+}$ Bregs and scatter dot plots showing the distribution of these cells as a percentage of the total B cells; (C) Gating strategy for the identification of $CD19^{+}CD20^{-}CD27^{+}CD38^{+}$ plasma cells and scatter dot plots showing the distribution of these cells as a percentage of the total B cells. $^{**}P < 0.01$, $^{***}P < 0.001$.

Although TBs represent one of the regulatory B cell subpopulations in healthy individuals, the frequency of these cells in the circulation may be elevated in individuals with autoimmune diseases.[18] In accordance with previous studies, we demonstrated that this population of cells is expanded in pSS patients.[22] Given that TBs isolated from patients with SLE fail to inhibit IFN-γ and TNF-α production, the increased proportion of TBs in pSS indicates the importance of further investigation of the function of TBs isolated from pSS patients. $CD19^{+}CD24^{hi}CD27^{+}$ Bregs suppress T cell activity and maintain immune homeostasis mainly by secreting IL-10. In the present study, we found that Bregs were decreased in the peripheral blood of patients with pSS. Previous studies have shown that $CD19^{+}CD24^{hi}CD27^{+}$ Bregs were decreased in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV)[28,29] and patients with systemic sclerosis[30] compared to HCs; however, this has not yet been reported in patients with pSS. The decreased numbers of memory Bregs support the involvement of B cell autoimmunity in the pathology of pSS, and indicate the potential of promoting this population numerically or functionally as a therapeutic strategy for pSS.

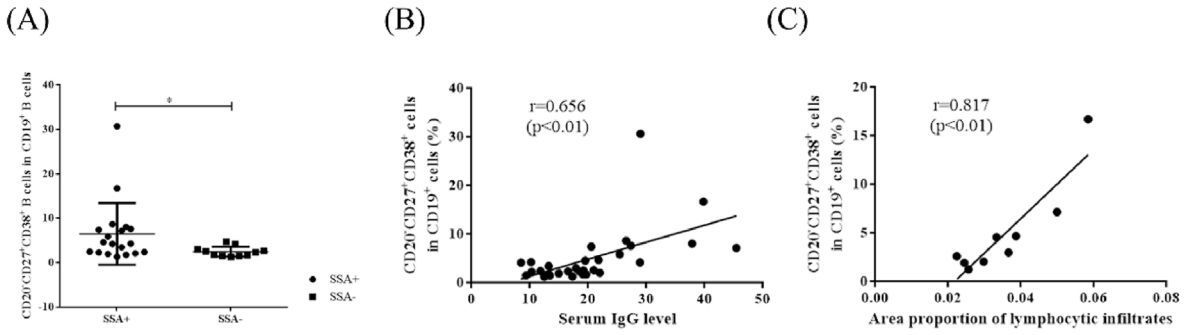

Figure 5 Correlation between B cell subsets and clinical parameters. (A) $CD19^{+}CD20^{-}CD27^{+}CD38^{+}$ plasma cells in pSS patients correlate positively with serum IgG levels; (B) $CD19^{+}CD20^{-}CD27^{+}CD38^{+}$ plasma cells in pSS patients correlate positively with the proportional area of lymphocytic infiltrates.

In this study, we showed that the proportion of peripheral $CD19^{+}IgD^{-}CD27^{-}$ DN B cells was significantly reduced in pSS patients compared with HCs, which is consistent with a previous study.[20] However, these cells are normally expanded in patients with autoimmune diseases and have been implicated in the pathophysiology of conditions such as neuromyelitis optica spectrum disorder, SLE, RA and Hashimoto's thyroiditis.[15] This disparity in the pattern of changes in the $CD19^{+}IgD^{-}CD27^{-}$ DN B cell population between pSS and other rheumatic diseases warrants further investigation.

Although pSS is a systemic disease, the typical local sicca symptom is dryness of the mucosal surfaces, principally in the mouth and eyes. Xerostomia caused by pSS can result in rampant caries, periodontal disease or fungal infections.[31] Therefore, understanding the pathogenesis of pSS and identifying new strategies for targeted therapy are also urgently required by oral practitioners.

In summary, abnormalities in the number and function of B cells might play a crucial role in the pathogenesis of pSS. In this study, we identified a distinct B lymphocyte subset distribution profile in pSS patients and suggested the role of $CD19^{+}CD20^{-}CD27^{+}CD38^{+}$ plasma cells in the pathogenesis of pSS. These findings provide a platform for further studies to identify the specific role of these cells in pSS and indicating that new therapeutic strategies might target these plasma cells.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2023.12.024.

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