

Regulatory B Cell Imbalance Correlates With TFH Expansion in Systemic Sclerosis.

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Abstract

Objective. Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis, microangiopathy and immune dysfunction. B cell abnormalities characterized by autoantibody production and polyclonal B cell activation play an important role in the pathogenesis of SSc. We previously identified an expansion of functional and activated circulating T follicular helper (cTfh) cells in SSc patients. The aim of this study was to analyze the frequency of regulatory B (Breg) cell subsets and the correlation with Tfh in SSc patients.

Methods. Circulating Breg cells CD24^{hi}CD38^{hi} and CD27⁺CD24^{hi} levels and cTfh cells CD4⁺CXCR5⁺PD1⁺ were determined by cytometry in 50 SSc patients and 32 healthy subjects.

Results. The frequency of Breg cells CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ was significantly reduced in patients with SSc as compared to controls ($p=0.02$ and $p<0.001$, respectively). In contrast, when examining the CD21^{low} B cell subset, the frequency was significantly increased in SSc patients compared to healthy controls, ($p<0.001$). There was no difference in Breg cell levels in patients with diffuse SSc and limited SSc. However, CD24^{hi}CD27⁺ Breg cell frequency was significantly decreased in SSc patients with pulmonary arterial hypertension ($p=0.014$), but not in patients with interstitial lung disease ($p=0.058$). Furthermore, we observed a negative correlation between cTfh and CD24^{hi}CD27⁺ Breg cell levels in SSc patients but not in healthy controls ($p=0.02$).

Conclusions: These results suggest that Breg cell subsets may participate in the regulation of cTfh and disease severity. Decreased CD24^{hi}CD27⁺ Breg cell frequency may contribute to the development of SSc.

Highlights

The Breg cell subpopulations CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ are decreased in patients with SSc in comparison to healthy controls.

The regulatory B cell subpopulations decrease is more pronounced in SSc with PAH and ILD impairments.

B regulatory cells decrease is balanced by the CD21^{low} B cells were increased in SSc patients as compared to healthy controls.

Regulatory B cells were negatively correlated with cTFH and could participate to the pathogeny of these cells in autoimmune diseases.

Introduction

Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis, microangiopathy and immune dysfunction. The exact pathophysiology of SSc is not well established, and mechanisms leading to dysimmunity remain to be established (1). B lymphocytes are involved in the pathophysiology of SSc

and several autoantibodies can be detected in the sera of SSc patients including anti-DNA topoisomerase I (anti-topo I) and anti-centromere antibodies (2). It has been previously reported that B cell homeostasis is disturbed in SSc patients, with increased naïve and reduced numbers of memory B lymphocytes (3, 4); as well as an increased level of the B cell activating factor (BAFF) cytokine in the blood of SSc patients (5, 6). In addition, B lymphocytes in patients with SSc secrete high levels of IL-6 and TGF- β and are able to promote skin fibrosis (7). The beneficial effect of B cell depletion with rituximab in SSc patients further supports the involvement of B cells in disease development and/or progression (8).

The maintenance of immune tolerance and prevention of autoimmune induction is exerted by various regulatory B cell subpopulations which in humans, include CD24^{hi}CD38^{hi} transitional B cells and CD24^{hi}CD27⁺ B cells, the human equivalent of murine B10 cells (9, 10). Another subset of B lymphocytes, the CD21^{ow} B cells (CD19⁺CD27⁻CD21^{ow}) has been found to be expanded in several autoimmune diseases such as lupus erythematosus, Sjogren's syndrome and SSc, and was involved in the development of chronic graft-versus-host disease (4, 11–14). We recently reported that circulating T follicular helper (cTfh) cells were increased in SSc patients and they presented an activated phenotype with the expression of BCL6 and HLA-DR and could also activate B cell plasmablasts to secrete immunoglobulins (4, 15). As human Breg cells could control Tfh development (16), we hypothesized that an imbalance in Breg cell subpopulations in patients could affect cTfh expansion and function. The aim of this study was to investigate frequencies of Breg cell subsets including CD21^{ow} B cells, CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ in the peripheral blood of SSc patients and to correlate them with cTfh expansion and clinical manifestations of the disease.

Patients And Methods

Patients

In this prospective study we included 50 SSc patients and 32 healthy controls. All patients fulfilled the 2013 American College of Rheumatology (17)/European League Against Rheumatism(18) criteria for SSc (19). Patients with a concomitant infectious disease, active neoplasm or an associated autoimmune disease were excluded. SSc patients had not received any immunomodulatory treatment at the time of the analysis. All SSc patients enrolled in this study were followed at Saint-Antoine Hospital and had given written informed consent. This study was approved by the Ethic committee of "Kremlin Bicêtre University" (N° ID-RCB 2017-AO3380-53).

For each patient, the following clinical data were analyzed: age, gender, disease duration (from the date of the first non-Raynaud's phenomenon), type of SSc (diffuse dSSc or limited lSSc), presence of active digital ulcers, presence of joint, heart, gastrointestinal and lung involvement, modified Rodnan skin score (mRSS), presence of pulmonary arterial hypertension (PAH). The lung involvement was defined as the presence of interstitial lung disease (ILD) on high resolution computed tomography (CT) scan. Laboratory data included C-reactive protein level, total lymphocyte count, plasma creatinine, urea, creatinine

phosphokinase enzymes and antinuclear autoantibodies (anti-centromeres, anti-topoisomerase I, anti-PM-Scl, anti-RNA polymerase III autoantibodies). Healthy controls were obtained from "l'Etablissement francais du sang" (median age 54 years [range, 23–64], 44% female).

Biological samples

Venous blood samples were collected in EDTA tubes (BD Biosciences, Le Pont de Claix, France). Peripheral blood mononuclear cells (PBMCs) were isolated with a standard gradient centrifugation procedure on a lymphocyte separation medium (Lymphosep separation media, Dutscher, Issy-les-Moulineaux, France).

Flow cytometry analysis

For B lymphocyte analysis, PBMCs were stained with the following fluorochrome-conjugated antibodies: IgD FITC, CD45 BV510 (both from BD Biosciences), and CD19 PC7, CD27 PE, CD24 ECD, CD38 PECY5.5, CD21 Pacific Blue (all from Beckman Coulter, Villepinte, France). Cells were analyzed on a Cytoflex flow cytometer (Beckman Coulter) using Kaluza 5.1 software (Beckman Coulter).

For Tfh analysis, PBMCs were stained with the following fluorochrome-conjugated antibodies: CXCR5 (CD185) PE (eBioscience, ThermoFisher, Villebon, France); CD45RA ECD, PD-1 (CD279) PECY5.5, ICOS (CD278) APC, CD3 AA750 (Beckman Coulter); CD45 BV510, CD4 BV650, HLA-DR BV786 (BD Biosciences).

Statistical analysis

Data are expressed as means \pm SD, medians with ranges, and frequencies with percentages. Qualitative values were compared with the parametric chi-square test or Fischer's exact test according to distribution, and continuous quantitative variables with the Student's t-test or Mann-Whitney test. The Pearson test was used to determine the correlation between variables. The analyses were done using Graphpad Prism 5.0 (GraphPad Software, San Diego, CA) and a $p < 0.05$ value was considered as significant.

Results

Increase of CD21^{low} B cells is balanced by decreased Breg CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ subsets in patients with systemic sclerosis

SSc patient characteristics are depicted in Table 1. The median age was 61 years, 80% were female and 32% had dSSc.

Table 1
SSc patient characteristics.

Characteristics	SSc patients (n = 50)
Age (years)	61 (32–81)
Age at first non-Raynaud symptom (years)	48 (26–72)
Female sex n (%)	40 (80)
Disease duration (years)	12 (0–19)
European ethnicity n (%)	30 (60)
Skin involvement	
Diffuse systemic sclerosis n (%)	16 (32)
Limited systemic sclerosis n (%)	33 (66)
Rodnan score	10 (2–37)
Active digital ulcers n (%)	8 (16)
Pulmonary involvement	
Interstitial lung disease n (%)	19 (38)
Pulmonary arterial hypertension n (%)	5 (10)
FVC (% of the predicted value)	105 (37–154)
DLCO (% of the predicted value)	61 (14–98)
Other organ involvement	
Joint involvement n (%)	6 (12)
Kidney sclerosis crisis n (%)	1 (2)
Heart impairment n (%)	1 (2)
Laboratory data	
BNP (mg/L)	33.5 (10–300)
CRP (mg/L)	1 (1–50)
Autoantibodies	
Anti-centromere n (%)	25 (50)

Values are medians with ranges and frequencies with percentages.

FVC, Forced vital capacity; DLCO, Diffusing capacity for carbon monoxide; BNP, Brain natriuretic peptide; CRP, C-reactive protein; Anti-RNAPol3, anti-RNA polymerase 3 antibody.

Characteristics	SSc patients (n = 50)
Anti-topoisomerase I n (%)	11 (22)
Anti-RNAPol3 n (%)	6 (12)
Values are medians with ranges and frequencies with percentages.	
FVC, Forced vital capacity; DLCO, Diffusing capacity for carbon monoxide; BNP, Brain natriuretic peptide; CRP, C-reactive protein; Anti-RNAPol3, anti-RNA polymerase 3 antibody.	

The gating strategy to identify the various B cell subpopulations is shown in Fig. 1A. Among B cell subsets, the frequencies and the absolute numbers of memory B cells (CD19⁺CD27⁺) (mean 27.4 ± 15.0% vs 34.2 ± 9.9%; p = 0.008) and (57.4 ± 7.7 cells/μL vs 68.9 ± 8.1 cells/μL; p = 0.04), respectively and of pre-germinal center B cells (CD19⁺CD27⁺ IgD⁺CD38⁺) (0.34 ± 0.27% and 0.45 ± 0.26%; p = 0.03) were significantly decreased in SSc compared to healthy controls (Fig. 1B-D). The absolute numbers and frequencies of plasmablast B cells were similar in both groups (Fig. 1D).

When examining the CD21^{low} B cell subset, we observed that the frequency and absolute numbers were significantly increased in SSc patients compared to healthy controls: 5.3 ± 4.3% versus 2.3 ± 1.3% (p < 0.001) and 10.50 ± 8.9 cells/μL versus 3.7 ± 1.8 cells/μL (p < 0.001), respectively (Fig. 1E-F). By contrast, the frequency of Breg cells CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ was significantly reduced in patients with SSc as compared to controls: 0.25 ± 0.22% versus 0.34 ± 0.23% (p = 0.02); and 19.0 ± 12.6% versus 29.0 ± 8.9% (p < 0.001), respectively (Fig. 1G-H).

Correlation Of Breg Cell Levels With Clinical Severity

Among the 50 SSc patients, there were 16 with dSSc and 34 with ISSc. The median mRSS was significantly higher in patients with dSSc, as expected (data not shown). There was no difference in total B cells, CD21^{low}, CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ Breg proportions in patients with dSSc and ISSc (data not shown).

We then analyzed the frequencies of CD21^{low} B cells and CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ subsets according to SSc severity. The CD24^{hi}CD27⁺ Breg cell frequency was significantly decreased in SSc patients with PAH (8.6 ± 4% versus 20.6 ± 13%; p = 0.014), but not in patients with ILD (15 ± 10% versus 23 ± 14%; p = 0.058) (Fig. 2). Breg CD24^{hi}CD27⁺ frequency was not different in SSc patients with active digital ulcers and did not correlate with the mRSS scale or BNP levels.

No difference was observed for CD24^{hi}CD38^{hi} and CD21^{low} B cell frequencies with respect to the presence of ILD, of active digital ulcers, of arterial hypertension, and no correlation was detected with mRRS or BNP levels (data not shown).

Decrease in Breg cells correlates with the expansion of cTfh in SSc patients

In a previous study analyzing cTfh in SSc patients, we observed an expansion of these cells, notably in dSSc patients. As Breg cells could be involved in the regulation of Tfh, we evaluated the correlation of cTfh frequency with Breg cell subsets. Interestingly, we observed a negative correlation between cTfh and CD24^{hi}CD27⁺ Breg cells in SSc patients but not in healthy controls (Fig. 3A-B). We did not observe any correlation between cTfh and CD24^{hi}CD38^{hi} B cells or between cTfh and CD21^{low} B cells (Fig. 3C-D).

Discussion

In this study, we report the decrease of Breg cell subpopulations CD24^{hi}CD38^{hi} and CD24^{hi}CD27⁺ in patients with SSc in comparison to healthy controls, with a more pronounced decrease of Breg lymphocytes in SSc with PAH and ILD impairments. In contrast, the CD21^{low} B cells were increased in SSc patients as compared to healthy controls. These CD21^{low} B cells have already been described in different autoimmune diseases such as rheumatoid arthritis (20) Sjogren's syndrome (SS) (11) and systemic lupus erythematosus (12, 21). This particular B cell population is predominantly composed of memory B cells (22) and expresses high levels of activation markers, inhibitory receptors and a peculiar pattern of homing receptors. These cells are usually considered as having features of anergic and exhausted cells, as characterized by increased apoptosis and decreased proliferation after stimulation (11). These CD21^{low} B cells highly express autoreactive antibodies and thus can be enriched by autoreactive B cell clones that may have been selected by self-antigens.

In our study, we did not detect differences in CD21^{low} B cells according to clinical severity as previously reported (4). Marrapodi et al. reported that CD21^{low} B cells in SSc were increased in comparison to healthy controls with a higher prevalence of PAH in those with more than 10% of CD21^{low} B cells (14, 23).

Regulatory B cells is a relatively newly recognized subset of B cells which have an immunoregulatory role by suppressing excessive inflammatory responses through the inhibition of T CD4⁺ Th1 and Th17 cell proliferation and the capacity to express inflammatory cytokines and the induction of regulatory T lymphocytes (Tregs) (24). Several studies have demonstrated decreased Breg cells in various autoimmune diseases, arguing for the potential involvement of these B cell subsets in the regulation of autoimmune diseases. Data about Breg cell impairment in SSc are still scarce but show decreased frequencies of several Breg cell subpopulations in various subtypes of SSc. Thus, Breg cell numbers could be lower in patients with severe and extensive SSc, as found in dSSc, in patients with PAH and ILD impairments (25) (26). Few studies have analyzed the functional impairment of regulatory B cells in SSc and have mainly showed decreased numbers of IL-10 positive B cells (25, 27). The TIM-1 + IL-10 expressing B cells are reduced in SSc patients and have reduced ability to suppress CD4⁺ T cell production of inflammatory cytokines (27). The results reported by Matsushita et al. showing that Breg cell levels correlated negatively with the titre of anti-topo I antibody (Ab) and anti-centromere Ab in SSc patients and are in line with our observations of a negative correlation between CD24^{hi}CD27⁺ Breg cells and cTfh (25). We did not detect a correlation between the CD24^{hi}CD38⁺ Breg cell subset and cTfh, suggesting that these two subsets display different functional characteristics as previously reported (28).

It remains to be investigated whether these mechanisms are involved in the direct regulation of Tfh, which are likely IL-10 independent because both of these two subsets express IL-10 (28).

It is interesting to highlight that BAFF inhibition therapies have been found to promote Breg cell numbers, by selectively depleting B effector cells producing IL-6 while sparing Breg cells (23). In addition, immunosuppressive therapies such as autologous hematopoietic stem-cell transplantation increased regulatory T and CD24^{hi}CD38^{hi} B cells at 6- and 12 months at higher levels in responders than in non-responders (29). Moreover, BAFF has been shown to regulate Tfh cells and promote their accumulation (30). Thus, a selective B cell depletion sparing Breg cells in SSc could be a potent therapeutic strategy (23).

Taken together, increased numbers of CD21^{low} B cells in the blood of SSc patients instead of being eliminated, and impaired regulatory B cell function could be part of a favorable environment to break tolerance leading to the development and/or extension of SSc. Further studies are necessary to determine the functional profile of Breg cell subsets and their ability to regulate cTfh and disease severity.

Conclusions

These results suggest that Breg cell subsets may participate in the regulation of cTfh and disease severity. Decreased CD24^{hi}CD27⁺ Breg cell frequency may contribute to the development of SSc.

Abbreviations

B cell activating factor: BAFF

Circulating T follicular helper: cTfh

computed tomography : CT

IL-6 Interleukin 6

Regulatory B cells: Breg

Rodnan skin score: mRSS

Systemic sclerosis:SSc

Declarations

Ethics approval and consent to participate:

All SSc patients enrolled in this study were followed at Saint-Antoine Hospital and had given written informed consent. This study was approved by the Ethic committee of “Kremlin Bicêtre University” (N° ID-RCB 2017-A03380-53).

Consent for publication:

All SSc patients enrolled in this study were followed at Saint-Antoine Hospital and had given written informed consent. This study was approved by the Ethic committee of “Kremlin Bicêtre University” (N° ID-RCB 2017-AO3380-53).

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Author contributions and Conflict of Interest Statements

All authors listed on the manuscript have substantially contributed to this work. LR, BG and AM designed the experimental research, perform experiments, interpreted data and wrote the manuscript; FM, MM, SR and OF interpreted data and participated in the manuscript writing.

Mohamad Mohty reports grants and/or lecture honoraria from Janssen, Sanofi, Maat Pharma, JAZZ pharmaceutical, Celgene, Amgen, BMS, Takeda, Pfizer, Novartis, and Roche, all outside the submitted work.

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The other authors declare no conflicts of interest.

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References

1. Gabrielli A, Avvedimento EV, Krieg T, Scleroderma. *N Engl J Med.* 2009;360(19):1989–2003.
2. Kraaij MD, van Laar JM. The role of B cells in systemic sclerosis. *Biologics.* 2008;2(3):389–95.
3. Sato S, Fujimoto M, Hasegawa M, Takehara K. Altered blood B lymphocyte homeostasis in systemic sclerosis: expanded naive B cells and diminished but activated memory B cells. *Arthritis Rheum.* 2004;50(6):1918–27.
4. Forestier A, Guerrier T, Jouvray M, Giovannelli J, Lefevre G, Sobanski V, et al. Altered B lymphocyte homeostasis and functions in systemic sclerosis. *Autoimmun Rev.* 2018;17(3):244–55.

5. Matsushita T, Hasegawa M, Yanaba K, Koderu M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum.* 2006;54(1):192–201.
6. Wutte N, Kovacs G, Berghold A, Reiter H, Aberer W, Aberer E. CXCL13 and B-cell activating factor as putative biomarkers in systemic sclerosis. *Br J Dermatol.* 2013;169(3):723–5.
7. Dumoitier N, Chaigne B, Regent A, Lofek S, Mhibik M, Dorfmueller P, et al. Scleroderma peripheral B lymphocytes secrete interleukin-6 and TGF-beta and activate fibroblasts. *Arthritis Rheumatol.* 2016.
8. Elhai M, Boubaya M, Distler O, Smith V, Matucci-Cerinic M, Alegre Sancho JJ, et al. Outcomes of patients with systemic sclerosis treated with rituximab in contemporary practice: a prospective cohort study. *Ann Rheum Dis.* 2019;78(7):979–87.
9. Matsushita T, Horikawa M, Iwata Y, Tedder TF. Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. *J Immunol.* 2010;185(4):2240–52.
10. Matsumoto M, Baba A, Yokota T, Nishikawa H, Ohkawa Y, Kayama H, et al. Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. *Immunity.* 2014;41(6):1040–51.
11. Saadoun D, Terrier B, Bannock J, Vazquez T, Massad C, Kang I, et al. Expansion of autoreactive unresponsive CD21⁻/low B cells in Sjogren's syndrome-associated lymphoproliferation. *Arthritis Rheum.* 2013;65(4):1085–96.
12. Wehr C, Eibel H, Masilamani M, Illges H, Schlesier M, Peter HH, et al. A new CD21^{low} B cell population in the peripheral blood of patients with SLE. *Clin Immunol.* 2004;113(2):161–71.
13. Greinix HT, Kuzmina Z, Weigl R, Kormoczi U, Rottal A, Wolff D, et al. CD19 + CD21^{low} B cells and CD4 + CD45RA + CD31 + T cells correlate with first diagnosis of chronic graft-versus-host disease. *Biol Blood Marrow Transplant.* 2015;21(2):250–8.
14. Marrapodi R, Pellicano C, Radicchio G, Leodori G, Colantuono S, Iacolare A, et al. CD21^(low) B cells in systemic sclerosis: A possible marker of vascular complications. *Clin Immunol.* 2020;213:108364.
15. Ricard L, Jachiet V, Malard F, Ye Y, Stocker N, Riviere S, et al. Circulating follicular helper T cells are increased in systemic sclerosis and promote plasmablast differentiation through the IL-21 pathway which can be inhibited by ruxolitinib. *Ann Rheum Dis.* 2019;78(4):539–50.
16. Achour A, Simon Q, Mohr A, Seite JF, Youinou P, Bendaoud B, et al. Human regulatory B cells control the TFH cell response. *J Allergy Clin Immunol.* 2017;140(1):215–22.
17. Rodriguez-Pinto I, Moitinho M, Santacreu I, Shoenfeld Y, Erkan D, Espinosa G, et al. Catastrophic antiphospholipid syndrome (CAPS): Descriptive analysis of 500 patients from the International CAPS Registry. *Autoimmun Rev.* 2016;15(12):1120–4.
18. van Laar JM, Farge D, Sont JK, Naraghi K, Marjanovic Z, Larghero J, et al. Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. *JAMA.* 2014;311(24):2490–8.

19. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum.* 2013;65(11):2737–47.
20. Thorarinsdottir K, Camponeschi A, Jonsson C, Granhagen Onnheim K, Nilsson J, Forslind K, et al. CD21(-/low) B cells associate with joint damage in rheumatoid arthritis patients. *Scand J Immunol.* 2019;90(2):e12792.
21. Thorarinsdottir K, Camponeschi A, Gjertsson I, Martensson IL. CD21 -/low B cells: A Snapshot of a Unique B Cell Subset in Health and Disease. *Scand J Immunol.* 2015;82(3):254–61.
22. Thorarinsdottir K, Camponeschi A, Cavallini N, Grimsholm O, Jacobsson L, Gjertsson I, et al. CD21(-/low) B cells in human blood are memory cells. *Clin Exp Immunol.* 2016;185(2):252–62.
23. Matsushita T, Kobayashi T, Mizumaki K, Kano M, Sawada T, Tennichi M, et al. BAFF inhibition attenuates fibrosis in scleroderma by modulating the regulatory and effector B cell balance. *Sci Adv.* 2018;4(7):eaas9944.
24. Sakkas LI, Daoussis D, Mavropoulos A, Liossis SN, Bogdanos DP. Regulatory B cells: New players in inflammatory and autoimmune rheumatic diseases. *Semin Arthritis Rheum.* 2019;48(6):1133–41.
25. Matsushita T, Hamaguchi Y, Hasegawa M, Takehara K, Fujimoto M. Decreased levels of regulatory B cells in patients with systemic sclerosis: association with autoantibody production and disease activity. *Rheumatology.* 2016;55(2):263–7.
26. Mavropoulos A, Simopoulou T, Varna A, Liaskos C, Katsiari CG, Bogdanos DP, et al. Breg Cells Are Numerically Decreased and Functionally Impaired in Patients With Systemic Sclerosis. *Arthritis Rheumatol.* 2016;68(2):494–504.
27. Aravena O, Ferrier A, Menon M, Mauri C, Aguillon JC, Soto L, et al. TIM-1 defines a human regulatory B cell population that is altered in frequency and function in systemic sclerosis patients. 2017;19(1):8.
28. Hasan MM, Thompson-Snipes L, Klintmalm G, Demetris AJ, O'Leary J, Oh S, et al. CD24(hi)CD38(hi) and CD24(hi)CD27(+) Human Regulatory B Cells Display Common and Distinct Functional Characteristics. *J Immunol.* 2019;203(8):2110–20.
29. Arruda LCM, Malmegrim KCR. Immune rebound associates with a favorable clinical response to autologous HSCT in systemic sclerosis patients. 2018;2(2):126–41.
30. Coquery CM, Loo WM, Wade NS, Bederman AG, Tung KS, Lewis JE, et al. BAFF regulates follicular helper t cells and affects their accumulation and interferon-gamma production in autoimmunity. *Arthritis Rheumatol.* 2015;67(3):773–84.
31. Taylor PV, Campbell JM, Scott JS. Presence of autoantibodies in women with unexplained infertility. *Am J Obstet Gynecol.* 1989;161(2):377–9.

Figures

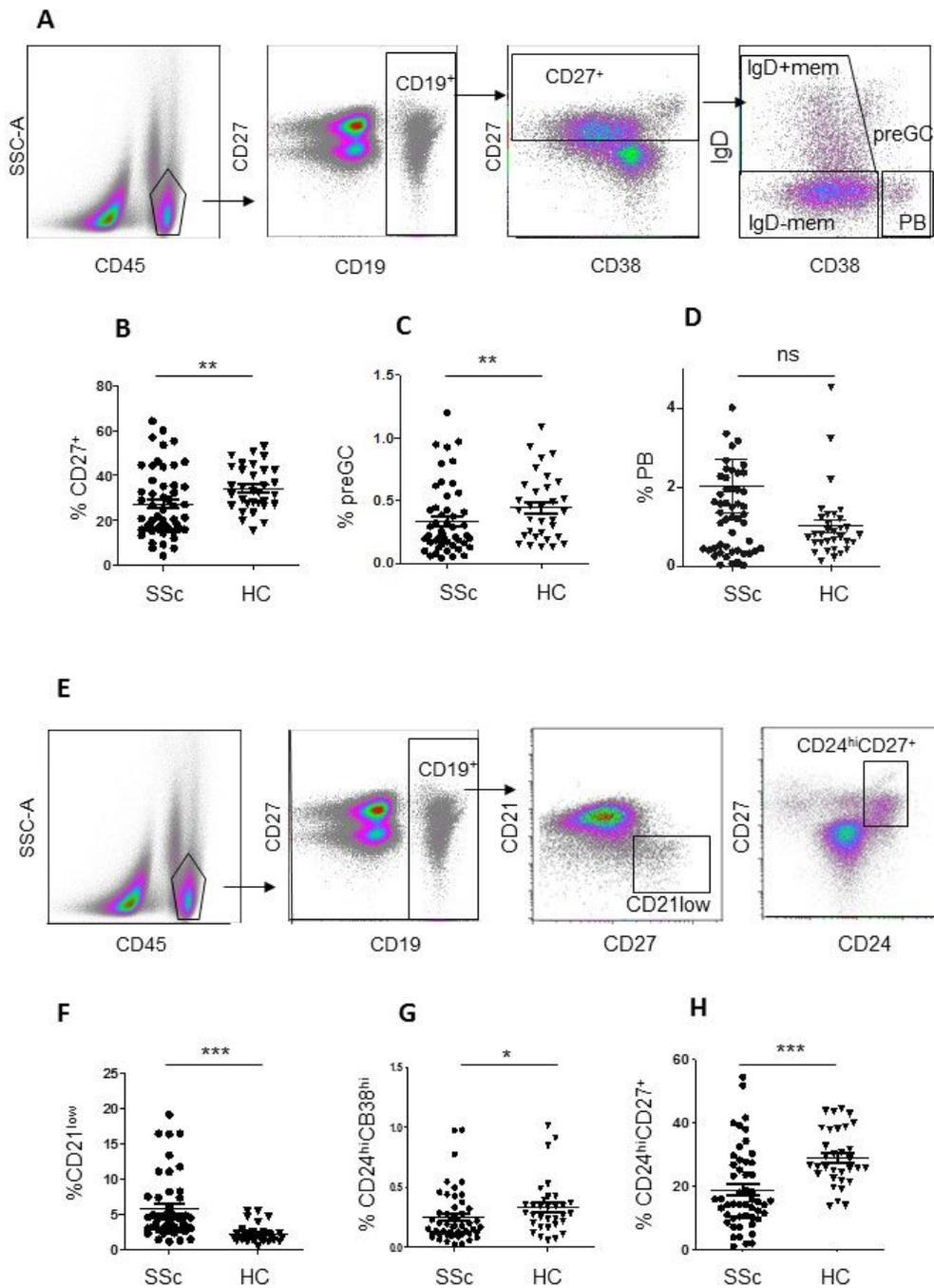


Figure 1

Figure 1

B cell subpopulations frequencies in SSc patients. (A) Gating strategy to identify the B lymphocyte subpopulations by flow cytometry; Frequencies of memory B CD27⁺ B cells (B), pre-germinal center B cells (preGC)(C) and plasmablast cells (D) within total CD19⁺B cells in SSc patients (n=50) and healthy controls (n=32); (E) Gating strategy to identify CD21^{low} and CD24^{hi}CD27⁺ B cells by flow cytometry;Frequencies of CD21^{low} B cells (F), CD27⁺24^{hi}CB38^{hi}(G) and CD24^{hi}CD27⁺(H) Breg cells

within CD19+ cells in SSc patients (n=50) and healthy controls (n=32). Data expressed as means with standard deviation; *P<0.05, **P<0.01, ***P<0.001 by Mann-Whitney test.

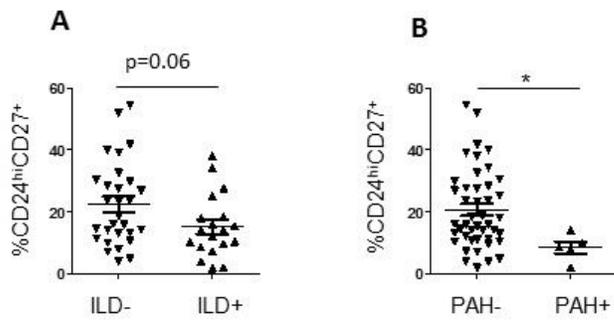


Figure 2

Figure 2

Frequencies of Breg subsets according to disease severity. Frequencies of CD24^{hi}CD27⁺Breg cells in SSc patients with or without pulmonary arterial hypertension (PAH) (A) and with or without interstitial lung

disease(ILD) (B). Data expressed as means with standard deviation; *P<0.05, **P<0.01, ***P<0.001 by Mann-Whitney test.

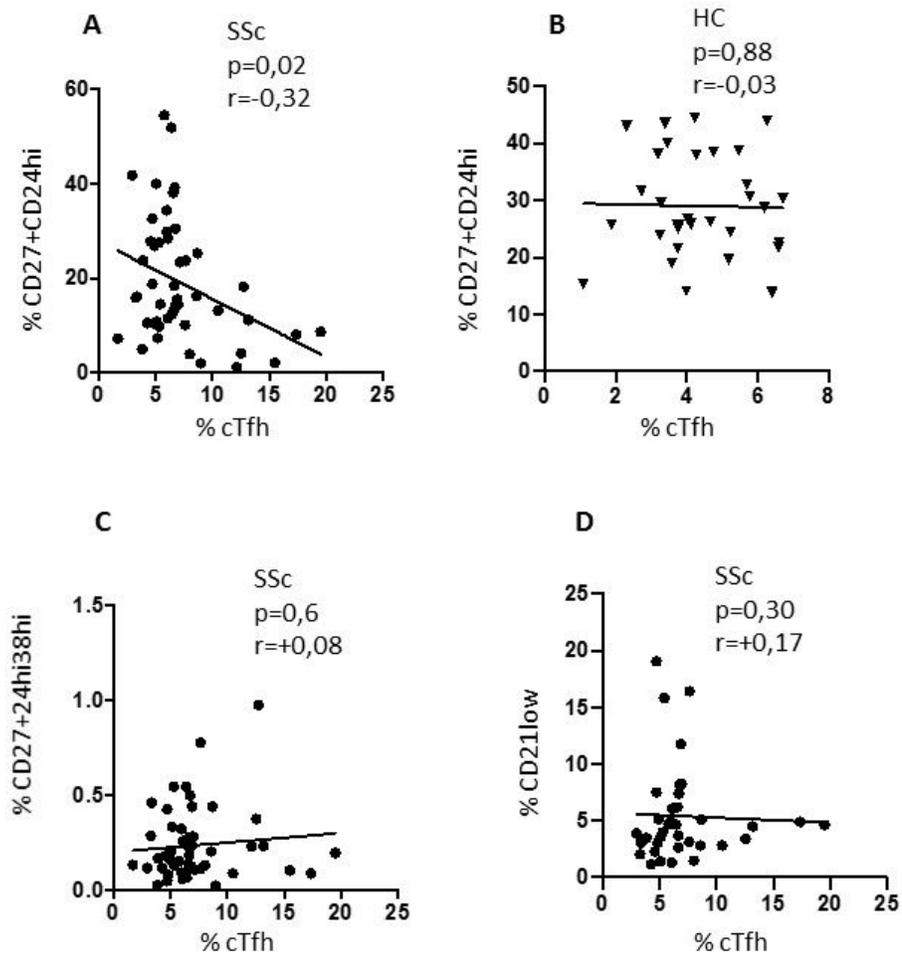


Figure 3

Figure 3

Decrease of CD24hiCD27+ inversely correlates with cTfh in SSc patients. Inverse correlation between CD24hiCD27+B cells and CD4+CXCR5+PD1+ cTfh in SSc patients (A) or healthy controls (B). No

correlation between CD27+CD24hiCD38hi B cells and cTfh (C) or CD21low B cells and cTfh (D). A-D, Spearman test.