CD19$^+$CD24^{high}CD27$^+$ B cell and interleukin 35 as potential biomarkers of disease activity in systemic lupus erythematosus patients

Hui Xiong[1,2], Zengqi Tang[1], Ying Xu[3], Zhenrui Shi[1], Zhixuan Guo[4], Xiuting Liu[1], Guozhen Tan[1], Xuechen Ai[5*] and Qing Guo[1*]

Abstract

Background: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that associates with aberrant activation of B lymphocytes and excessive autoantibodies. Interleukin 10 (IL-10)/interleukin 35 (IL-35) and IL-10/IL-35-producing regulatory B cells have been demonstrated to possess immunosuppressive functions during systemic lupus erythematosus. Here, we detected the proportion of CD19$^+$CD24^{high}CD27$^+$ B cells as well as IL-10 and IL-35 levels in peripheral blood of SLE patients and healthy individuals, and investigated their relations with clinical features of SLE.

Methods: 41 SLE patients and 25 healthy controls were recruited. The patients were divided into groups based on SLEDAI score, anti-dsDNA antibody, rash, nephritis and hematological disorder. Flow cytometry was used to detect the proportion of CD24^{hi}CD27$^+$ B cells. ELISA was used to detect serum levels of IL-10 and IL-35.

Results: Our results showed that the CD19$^+$CD24^{high}CD27$^+$ B population was decreased in active SLE patients, and anti-correlated with the disease activity. Of note, we found significant increase of IL-10 and decrease of IL-35 in SLE patients with disease activity score > 4, lupus nephritis or hematological disorders compared to those without related clinical features.

Conclusions: Reduced CD19$^+$CD24^{high}CD27$^+$ B cells expression may be involved in the pathogenesis of SLE. Moreover, we supposed that IL-35 instead of IL-10 played a crucial role in immune regulation during SLE disease.

Keywords: Systemic lupus erythematosus, Regulatory B cells, Interleukin 35, Interleukin 10

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that characterized by autoantibody production and associated with a wide range of harmful clinical feature in various tissues including skin, joints, lung, brain, kidney, heart and hematological system [1].

Although excessive activation and function of B lymphocytes play a pivotal role in promoting the development of SLE, the pathophysiology of SLE remains elusive [2].

Recently, a B cell subtype, namely, regulatory B cells (Bregs), have been identified as a regulatory population that contributes to immune tolerance [3]. Indeed, Bregs can alleviate inflammatory responses in varying mouse models of autoimmune disease, including Type I diabetes, collagen-induced arthritis and contact hypersensitivity [4, 5]. Furthermore, Bregs are capable to produce substantial anti-inflammatory cytokines such as TGF-β, IL-10 and IL-35 to restrain tissue

*Correspondence: aixch@mail2.sysu.edu.cn; guoqingzsy@163.com

[1] Department of Dermatology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong, China
[5] Department of Dermatology, The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen 518033, Guangdong, China
Full list of author information is available at the end of the article

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inflammation [6, 7]. IL-10 on the one hand arrests the antigen-presenting process in dendritic cells and macrophages, which is critical for effector T cell response. On the other hand, IL-10 directly inhibits T cell activation and represses the expression of proinflammatory cytokines in effector T cells, leading to an immunoregulatory role in the immune system [8, 9]. Additionally, IL-35 is involved in the regulatory function of Breg cells and contributes to the resolution of inflammation: mice lacking IL-35 subunits, p35 or EBi3 fail to recover from EAE (a mouse model for multiple sclerosis in human), and reveal an elevated activation of effector B cells [10]. Moreover, the regulatory function of IL-10$^+$ Bregs is found to be in IL-35 [11]. Of interest, IL-35 is sufficient to differentiate T and B cells with regulatory phenotypes, suggesting an essential role of IL-35 in Breg immunity.

Since Iwata et al. have verified that CD19$^+$IL-10$^+$ Bregs can be further characterized as CD24^{high}CD27$^+$ B cells in human peripheral blood [12], CD19$^+$CD24^{high}CD27$^+$ as a convincing marker has been widely used in Bregs field. It was reported that the percentage of CD19$^+$CD24^{high}CD27$^+$ B cells was decreased in Henoch-Schönlein purpura nephritis children patients with hematuria and proteinuria or massive proteinuria [13]. In addition, the dysfunction of CD19$^+$CD24^{high}CD27$^+$ B regulatory cells in patients results in bullous pemphigoid [14]. Emerging evidence suggests the impaired CD19$^+$CD24^{high}CD27$^+$ B cells often lead to autoimmune diseases such as Graves' Disease [15], Crohn's Disease [16] and rheumatoid arthritis (RA) [17]. In light of the predominant regulatory function of CD19$^+$CD24^{high}CD27$^+$ B cells, it has been an attractive biomarker for the response to biologic therapies in rheumatoid arthritis patients under biologic drug treatment [18].

Although a recent study has investigated the relevance between disease activity and CD19$^+$CD24^{high}CD27$^+$ B cells in new-onset SLE patients, their inclusion criteria were limited [19]. Data of refractory SLE patients and the correlation of CD19$^+$CD24^{high}CD27$^+$ B cells with related cytokines remain unclear. Here, we utilized CD19$^+$CD24^{high}CD27$^+$ as surface markers of Bregs in this study. Furthermore, we compared the percentage of CD19$^+$CD24^{high}CD27$^+$ B cells and related cytokines level in serum from SLE patients with those of healthy group. In addition, we analyzed the associations between the proportion of CD19$^+$CD24^{high}CD27$^+$ B cells and the major clinical features of SLE patients. We aimed to explore the role of regulatory B cells and related cytokines in the pathogenesis of SLE.

Table 1 Demographic and clinical characteristics of SLE patients. Continuous variables are represented as mean ± standard deviation (SD)

Characteristics	SLE patients (n = 41)
Demographic characteristics	
Female/male	37/4
Age (year)	31.6 ± 13.2
Clinical features	
Disease duration (year)	6.0 ± 7.0
SLEDAI	8.2 ± 6.0
Inactive patients (n) (%)	14 (34.1)
Active patients (n) (%)	27 (65.9)
Clinical manifestations (n) (%)	
Rash	24 (58.5)
Oral ulcers	3 (7.3)
Alopecia	18 (43.9)
Arthritis	16 (39.0)
Pleuritis	2 (4.9)
Nephritis	24 (58.5)
Hematological disorders	19 (46.2)
Neurologic disorders	1 (2.4)
Pericarditis	0
Fever	0
Serological features	
Serum complement C3 (mg/L)	668.4 ± 351.6
Serum complement C4 (mg/L)	133.9 ± 149.8
ESR (mm/h)	51.0 ± 34.4
Total bilirubin (μmol/L)	8.9 ± 4.9
Serum creatinine (μmol/L)	79.7 ± 39.5
Urine protein (g/24 h)	2.39 ± 4.55
Anti-dsDNA positive (n) (%)	11 (26.8)
Anti-Sm positive (n) (%)	11 (26.8)
Anti-SSA positive (n) (%)	26 (63.4)
Anti-nucleosome positive (n) (%)	15 (36.6)
Current therapy (n) (%)	
Glucocorticoids	31 (75.6)
Immunosuppressants	10 (24.4)

SLEDAI systemic lupus erythematosus disease activity index. ESR erythrocyte sedimentation rate

Methods
Patients and controls
Forty-one SLE patients were enrolled from dermatological department in Sun Yat-sen Memorial Hospital, Sun Yat-sen University (Guangzhou, China) during the year from 2017 to 2018 on the basis of SLE diagnosis criteria of American college of Rheumatology (ACR) in 2009 [20]. In addition, a group of 25 age and gender-matched healthy individuals (HC) without any evidence of autoimmune disease or infection were also recruited as controls. The demographic characteristics of these patients

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are shown in Table 1. To further investigate the relation of Bregs to T lymphocyte subpopulations, we conducted an additional trial enrolling another 5 SLE patients and 5 healthy individuals with diagnosis criteria as above.

Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was used to assess the disease activity. Patients with a SLEDAI score of ≥ 5 were classified as active state, as inactive groups referred to the patients with the score of ≤ 4 [21]. Individual patient data concerning demographic information and clinical characteristics were obtained from medical charts. In addition, the result of laboratory detection in routine blood and urine levels, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), liver function(AST,ALT,γ-GGT), serum creatine (Cr), blood urea nitrogen (BUN), serum complement C3 and C4 levels, immunoglobulin levels (IgG, IgM and IgA) autoantibody detection and 24 h urinary protein, were also recorded. The blood samples were stored in heparin sodium anticoagulant. Sera were collected and stored at − 80 °C until further analysis.

This study was approved by the Clinical Research Ethics Committee of Sun Yat-sen University and conducted in accordance with the Declaration of Helsinki guidelines.

Analysis of Breg and T cell subpopulations by flow cytometry

B and T lymphocyte phenotyping of fresh blood samples from SLE patients and healthy controls were analyzed by flow cytometry (Beckman Coulter FC500/Beckman Coulter Navios, USA). The monoclonal immunofluorescent antibodies were used for B and T cell phenotyping as follows: CD19-APCH7 (allophycocyanin-H7), CD24-PE (phyco-ertyhrin), CD27-APC, CD45-PerCP

(peridinin-chlorophyll-protein), CD4-FITC (fluorescein isothiocyanate), CD25-PC5, CD3-PC5, CD127-PE. All antibodies are obtained from Beckman Coulter. At least 2000 cells were analyzed for each sample. The gating strategy for Breg cells was based on markers expression including CD19, CD24 and CD27 as shown in Fig. 1.

Cytokine analysis in serum by ELISA
Serum samples from each participant were collected and stored at − 80 °C. The levels of interleukin-10 (IL-10) (KHC0101, Thermo Fisher Scientific) and interleukin-35 (IL-35) (SEC008Hu, USCN) in serum were measured using respective ELISA kits. The analysis was performed according to the instruction of manufacturers. Each sample was tested in duplicate.

Statistical analysis
Statistical analysis was performed by SPSS 23.0. For continuous variables, results are expressed as mean ± SD; for discontinuous variables, results are expressed as number (in percentage). Difference between groups was analyzed by the Kruskal–Wallis or Mann–Whitney U test as indicated. Correlation was determined by Spearman's rank correlation coefficient. A P-value < 0.05 was regarded statistically significant.

Results
Patient characteristics
Demographic information and clinical characteristics of SLE patients are assessed and summarized in Table 1. A total of 41 SLE patients (37 females and 4 males; mean age 31.6 ± 13.2 years, ranging from 10 to 65 years) and 25 healthy controls (23 females and 2 males; mean age
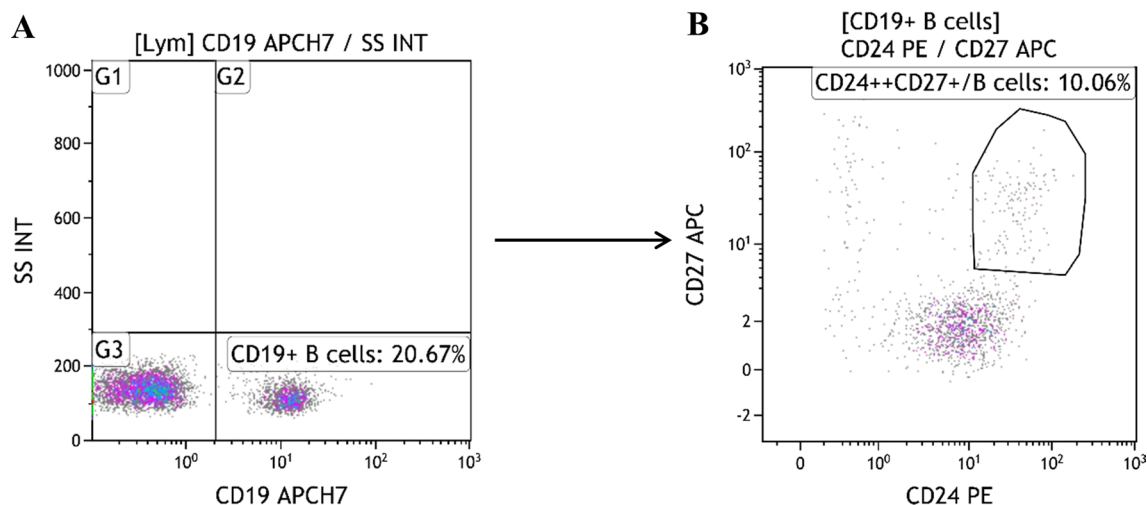

Fig. 1 Gating strategy for B lymphocytes by flow cytometry. **A** expressed as a single parameter of CD19 and scatter, **B** expressed as CD24^high^CD27^+^ B cells

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36.9 ± 18.3 years, ranging from 13 to 73 years) were enrolled in this study. There was no significant difference in age and gender between SLE patients and healthy controls ($p = 0.367$ and $p = 0.977$, relatively). The average disease course of the SLE patients was $6.0 ± 7.0$ years, ranging from 0 to 25 years. The mean SLEDAI score was $8.2 ± 6.0$, ranging from 0 to 18. Of the 41 SLE patients, the most common disorders were rash and nephritis (58.5%), followed by hematological disorders (46.3%), alopecia (43.9%), arthritis (39%), oral ulcers (7.3%), pleuritis (4.9%), neurological disorders (2.4%). In addition, 75.6% of the SLE patients were taking glucocorticoids, and 24.4% were taking immunosuppressants. Regarding major laboratory parameters, the mean serum concentrations of complements C3 and C4 were $668.4 ± 354.6$ and $133.9 ± 149.8$ pg/ml, respectively. Moreover, anti-dsDNA antibody was detected in 26.8% of the SLE patients.

Association of CD19$^+$CD24^{high}CD27$^+$ B lymphocytes with disease activity

To explore the potential involvement of Bregs during SLE progression, the current study examined the level of CD19$^+$CD24^{high}CD27$^+$ B cells in the peripheral blood of SLE. SLE patients group was separated into active and inactive groups according to the disease activity scores. In comparison with HCs, the absolute account of CD19$^+$CD24^{high}CD27$^+$ B cells was remarkably reduced in active SLE patients ($51.49 ± 26.79 × 10^6$/L vs. $23.28 ± 23.12 × 10^6$/L, $p < 0.01$) and inactive patients ($51.49 ± 26.79 × 10^6$/L vs. $21.31 ± 27.79 × 10^6$/L, $p < 0.001$), although the numbers were comparable between the disease sub-groups (Fig. 2A). Further analysis on the clinical relevance of Bregs indicated that the proportion of CD19$^+$CD24^{high}CD27$^+$ B cells was negatively correlated with SLEDAI score of SLE patients ($r = -0.379$, $p = 0.015$, Fig. 2B). In addition, we observed a considerably lower percentage of CD19$^+$CD24^{high}CD27$^+$ B cells among circulating B lymphocytes from active SLE patients ($10.94 ± 12.16$%, $n = 27$) compared to healthy individuals ($16.65 ± 8.29$%, $n = 25$, $p < 0.01$) or inactive SLE patients ($16.68 ± 10.14$%, $n = 14$, $p < 0.05$), however, there was no significant difference between inactive SLE patients and healthy controls (data not shown). Representative cases of a healthy control, an inactive SLE patient and an active SLE patient are shown in Fig. 2C–E. Additional experiments exploring the relation of CD24^{high}CD27$^+$ B lymphocytes to T cell subsets in SLE and healthy individuals revealed a negative correlation between the proportion of CD19$^+$CD24^{high}CD27$^+$ B cells with the percentage of CD3$^+$IL-17$^+$ Th17 cells among T
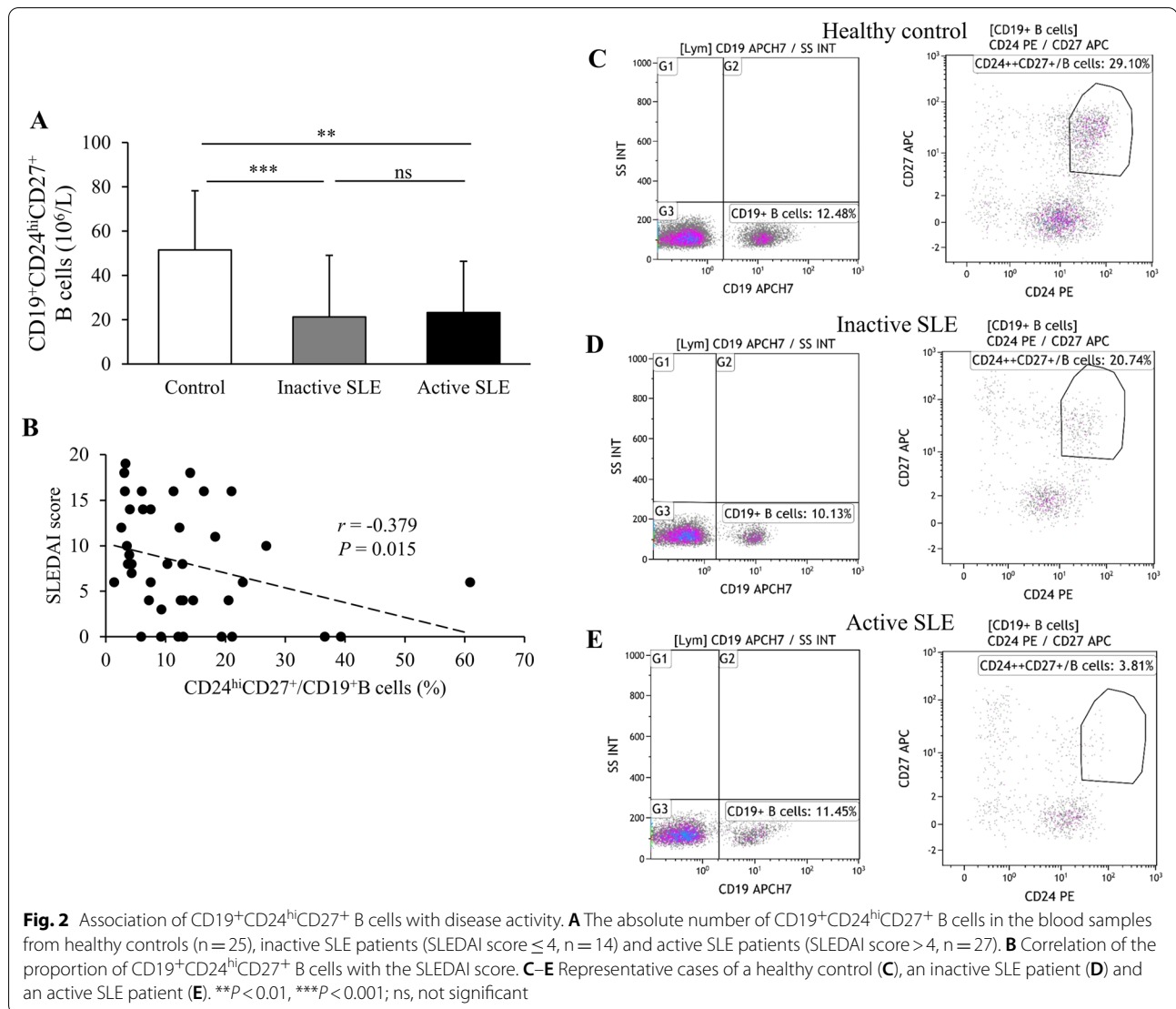
lymphocytes ($r = -0.673$, $p = 0.033$, $n = 10$) (Additional file 1: Fig. S1a). Moreover, we observed lower levels of CD4$^+$CD25^{high}CD127$^-$ regulatory T cells (Tregs) and higher ratios of Th17/Tregs in SLE patients than healthy controls, though the differences were not statistically significant ($p = 0.095$ and $p = 0.056$, respectively) (Additional file 1: Fig. S1b–c). These data showed less expression of CD24^{high}CD27$^+$ B lymphocytes in SLE patients' peripheral blood, which provided evidence for the potential involvement of Bregs in disease development.

Association of CD19$^+$CD24^{high}CD27$^+$ B cells with SLE-related features

The forementioned finding prompted us to investigate the potential correlation of Bregs with SLE clinical features. The percentage of CD19$^+$CD24^{high}CD27$^+$ B cells is positively correlated with level of serum complement C3 and C4 in SLE patients ($r = 0.501$, $p < 0.01$; $r = 0.482$, $p < 0.01$; respectively) (Fig. 3A, B). However, no correlation was found in those of ESR (Fig. 3C), Cr, BUN, and 24 h urinary protein level (Table 2). Interestingly, the total number of CD19$^+$CD24^{high}CD27$^+$ B cells was positively associated with the number of monocytes ($r = 0.432$, $p = 0.006$) (Fig. 3D). SLE patients were further divided into two groups separately according to anti-dsDNA, rash, hematological disorders, lupus nephritis, respectively. The proportion of CD19$^+$CD24^{high}CD27$^+$ B cells was much lower in patients with anti-dsDNA positive ($5.59 ± 4.04$% vs. $15.58 ± 12.49$%, $p < 0.001$, $n = 11$) or rash ($8.50 ± 5.74$% vs. $19.11 ± 15.02$%, $p < 0.01$, $n = 24$) than those with anti-dsDNA negative or without rash (Fig. 3E, F). Nonetheless, no differences were observed in lupus nephritis (LN) patients and patients with hematological disorder comparisons (Fig. 3G, H).

Association between serum IL-10 expression and SLE-related parameters

The function of Bregs is characterized by the production of the immunosuppressive cytokine IL-10, which signals through a receptor complex including IL-10R1 and IL-10R2 on target cells [22]. The relations between IL-10 and SLE-related clinical parameters, including organ involvement, the disease activity and laboratory variables were further explored. Interestingly, serum IL-10 levels were significantly increased in active SLE patients ($n = 14$) compared with inactive patients ($n = 10$) or healthy subjects ($n = 16$) ($4.18 ± 3.16$ pg/ml vs. $1.30 ± 1.54$ pg/ml, $p < 0.01$; $4.18 ± 3.16$ pg/ml vs. $1.55 ± 1.69$ pg/ml, $p < 0.05$; respectively) (Fig. 4A). In addition, IL-10 concentration was also positively correlated with SLEDAI score ($r = 0.602$, $p = 0.002$) (Fig. 4B).

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Fig. 2 Association of CD19+CD24hiCD27+ B cells with disease activity. **A** The absolute number of CD19+CD24hiCD27+ B cells in the blood samples from healthy controls (n = 25), inactive SLE patients (SLEDAI score ≤ 4, n = 14) and active SLE patients (SLEDAI score > 4, n = 27). **B** Correlation of the proportion of CD19+CD24hiCD27+ B cells with the SLEDAI score. **C**–**E** Representative cases of a healthy control (**C**), an inactive SLE patient (**D**) and an active SLE patient (**E**). **P < 0.01, ***P < 0.001; ns, not significant

Of note, serum IL-10 levels are significantly higher in the anti-dsDNA positive group (n = 7, 5.42 ± 3.54 pg/ml vs. 1.98 ± 2.03 pg/ml, $p < 0.01$), lupus nephritis group (n = 14, 4.07 ± 3.23 pg/ml vs. 1.47 ± 1.65 pg/ml, $p < 0.05$), or SLE patients with hematological disorders (n = 11, 4.36 ± 3.45 pg/ml vs. 1.82 ± 1.88 pg/ml, $p < 0.05$), compared with their negative counterparts (Fig. 4C, E, F). However, no difference was observed between the rash (n = 11) and no-rash groups (Fig. 4D). Moreover, serum IL-10 levels are negatively related with levels of complement C3 and C4 levels in serum ($r = -0.622$, $p = 0.002$; $r = -0.528$, $p = 0.011$; respectively), positively correlated with ESR and 24 h urinary protein ($r = 0.528$, $p = 0.014$; $r = 0.491$, $p = 0.015$; respectively) in SLE patients (Fig. 4G–I, Table 3). The

levels of serum IL-10 and blood urea nitrogen or serum creatine are irrelevant (both $p > 0.05$, Table 3). In conclusion, increased serum IL-10 concentration was significantly related to disease progression, as well as kidney and hematological involvement in SLE patients.

Association between serum IL-35 level and SLE-related parameters

IL-35 is another immunosuppressive cytokine produced by Breg cells. We further explored the relations between IL-35 protein level and SLE-related data. The IL-35 level in serum was significantly decreased in active SLE patients compared to inactive patients and healthy subjects as well (60.91 ± 15.81 pg/ml vs. 88.73 ± 17.08 pg/ml, $p < 0.01$; 60.91 ± 15.81 pg/ml vs. 90.05 ± 15.86 pg/

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Fig. 3 Association of the proportion of CD19$^+$CD24^{hi}CD27$^+$ B cells with SLE-related parameters. **A**–**D** Correlation of the proportion of CD19$^+$CD24^{hi}CD27$^+$ B cells with level of complement C3, C4, ESR and monocyte number in peripheral blood. **E** The proportion of CD19$^+$CD24^{hi}CD27$^+$ B cells from anti-dsDNA negative patients (n = 30) and anti-dsDNA positive patients (n = 11). **F**–**H** The proportion of CD19$^+$CD24^{hi}CD27$^+$ B cells in SLE patients with rash (n = 24) and without rash (n = 17); with hematological disorders (n = 19) and without hematological disorders (n = 22); with nephritis (n = 24) and without nephritis (n = 17). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant
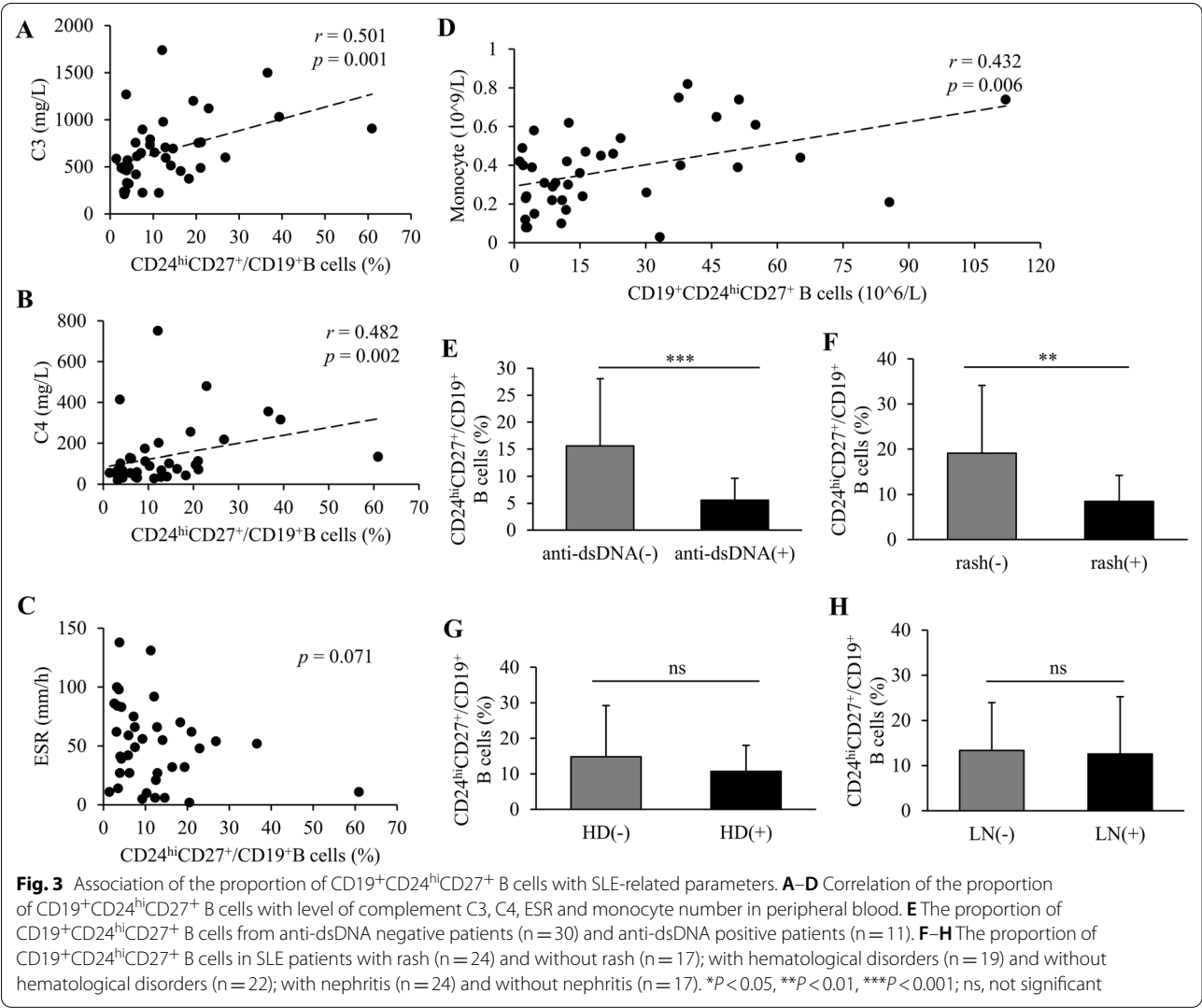
Table 2 Correlation between the proportion of CD24^{hi}CD27$^+$CD19$^+$ B cells with serum creatine (Cr), blood urea nitrogen (BUN), and 24 h urinary protein

	CD24^{hi}CD27$^+$/CD19$^+$ B cells (%)	
	r Value	**P value**
Serum creatine (μmol/L)	− 0.037	0.819
Blood urea nitrogen (mmol/L)	0.062	0.699
24 h urinary protein (g/24 h)	− 0.011	0.944

P-value was determined by Spearman's rank correlation coefficients

ml, $p < 0.001$; respectively) (Fig. 5A), and were negatively correlated with SLEDAI score (r = − 0.560, $p = 0.004$) (Fig. 5B). Additionally, as shown in Fig. 5C–F, patients with anti-dsDNA positive, nephritis or hematological

disorders showed significantly decreased levels of serum IL-35 compared to those without clinical characteristics (56.54 ± 20.79 pg/ml vs. 79.08 ± 18.16 pg/ml, $p < 0.05$; 63.84 ± 16.23 pg/ml vs. 84.63 ± 22.22 pg/ml, $p < 0.05$; 59.07 ± 16.40 pg/ml vs. 83.87 ± 18.33 pg/ml, $p < 0.01$; respectively). No difference was found in patients with or without rash. Moreover, the IL-35 level in serum was positively correlated with the levels of complement C3 and C4 (r = 0.601, $p = 0.003$; r = 0.544, $p = 0.009$; respectively), but negatively correlated with ESR, blood urea nitrogen and serum creatine (r = − 0.711, $p < 0.001$; r = − 0.614, $p = 0.001$; r = − 0.447, $p = 0.029$; respectively) (Fig. 5G–I, Table 3). No significant association was analysed between serum IL-35 level and 24 h urinary protein ($p > 0.05$, Table 3). The data above indicated that decreased serum IL-35 levels may have an impact on the clinical picture of SLE patients.
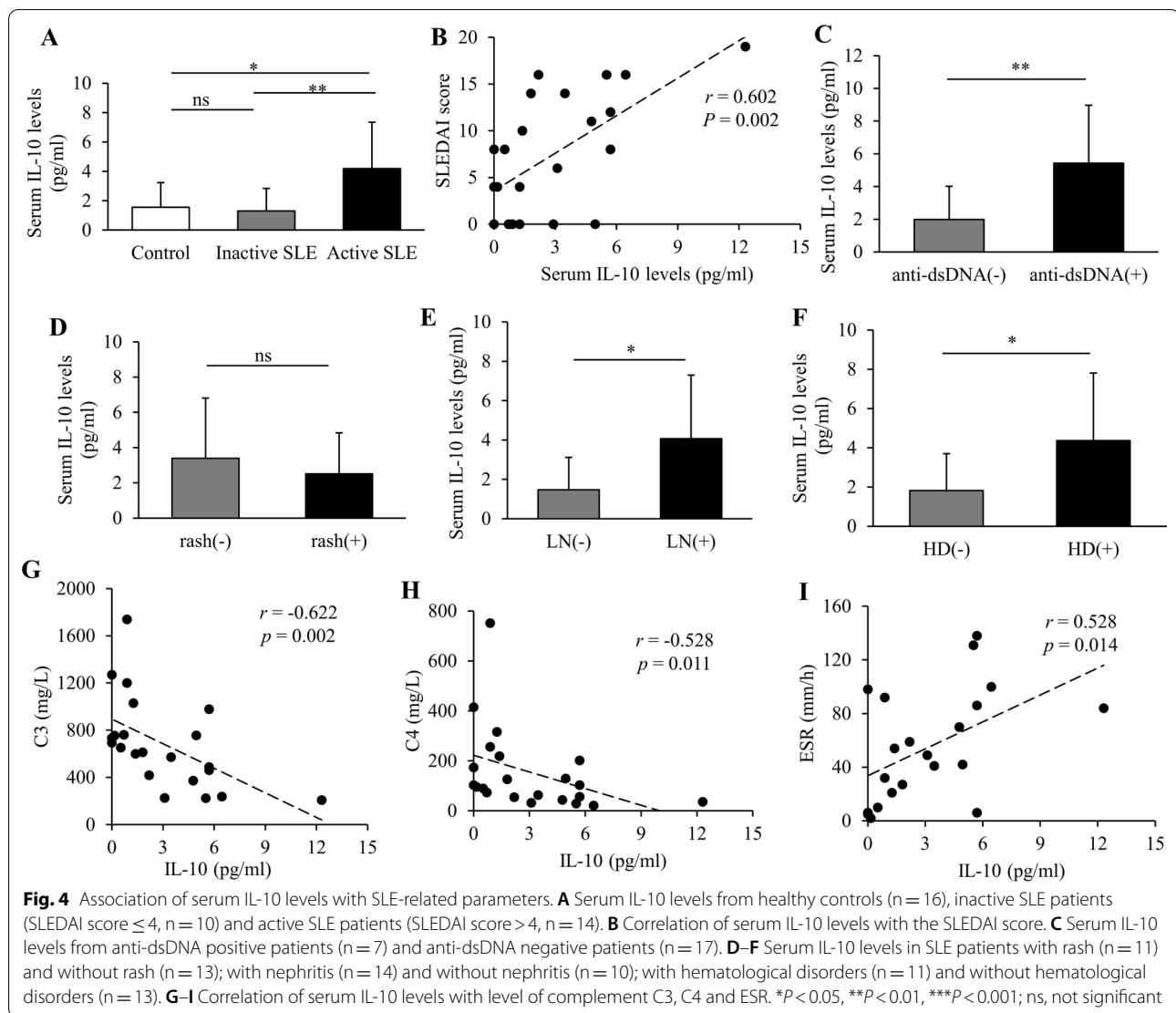
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Fig. 4 Association of serum IL-10 levels with SLE-related parameters. **A** Serum IL-10 levels from healthy controls (n = 16), inactive SLE patients (SLEDAI score ≤ 4, n = 10) and active SLE patients (SLEDAI score > 4, n = 14). **B** Correlation of serum IL-10 levels with the SLEDAI score. **C** Serum IL-10 levels from anti-dsDNA positive patients (n = 7) and anti-dsDNA negative patients (n = 17). **D**–**F** Serum IL-10 levels in SLE patients with rash (n = 11) and without rash (n = 13); with nephritis (n = 14) and without nephritis (n = 10); with hematological disorders (n = 11) and without hematological disorders (n = 13). **G**–**I** Correlation of serum IL-10 levels with level of complement C3, C4 and ESR. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant

Table 3 Correlation between serum IL-35 and IL-10 level with serum creatine (Cr), blood urea nitrogen (BUN), and 24 h urinary protein

	IL-35 (pg/ml)		IL-10 (pg/ml)	
	r value	P value	r value	P value
Serum creatine (μmol/L)	− 0.447	0.029*	0.164	0.443
Blood urea nitrogen (mmol/L)	− 0.614	0.001**	0.311	0.140
24 h urinary protein (g/24 h)	− 0.341	0.102	0.491	0.015*

P-value was determined by Spearman's rank correlation coefficients. *$P < 0.05$; **$P < 0.01$

Association of serum cytokines with CD24^{high}CD27$^+$ B cells in SLE patients

Intrigued by the above findings, we further determined the association between serum cytokines expression and CD19$^+$CD24^{high}CD27$^+$ B cells. Our results in Table 4 showed that the percentage of CD19$^+$CD24^{high}CD27$^+$ B cells was negatively related with serum IL-10 level (r = − 0.496, $p = 0.014$), but positively correlated with IL-35 (r = 0.412, $p = 0.045$). No relation was found between the account of CD19$^+$CD24^{high}CD27$^+$ B cells and serum cytokines.

Discussion

SLE is a complex autoimmune disorder mainly induced by the disruption of self-tolerance. Several subtypes of immune cells, including Th1, Th2 cells and effector B cells, are involved in the progress of SLE and the production of SLE autoantibodies [23]. Since its discovery, Bregs has aroused great concern from the field of autoimmune disease [24–26]. Previous studies have suggested that there
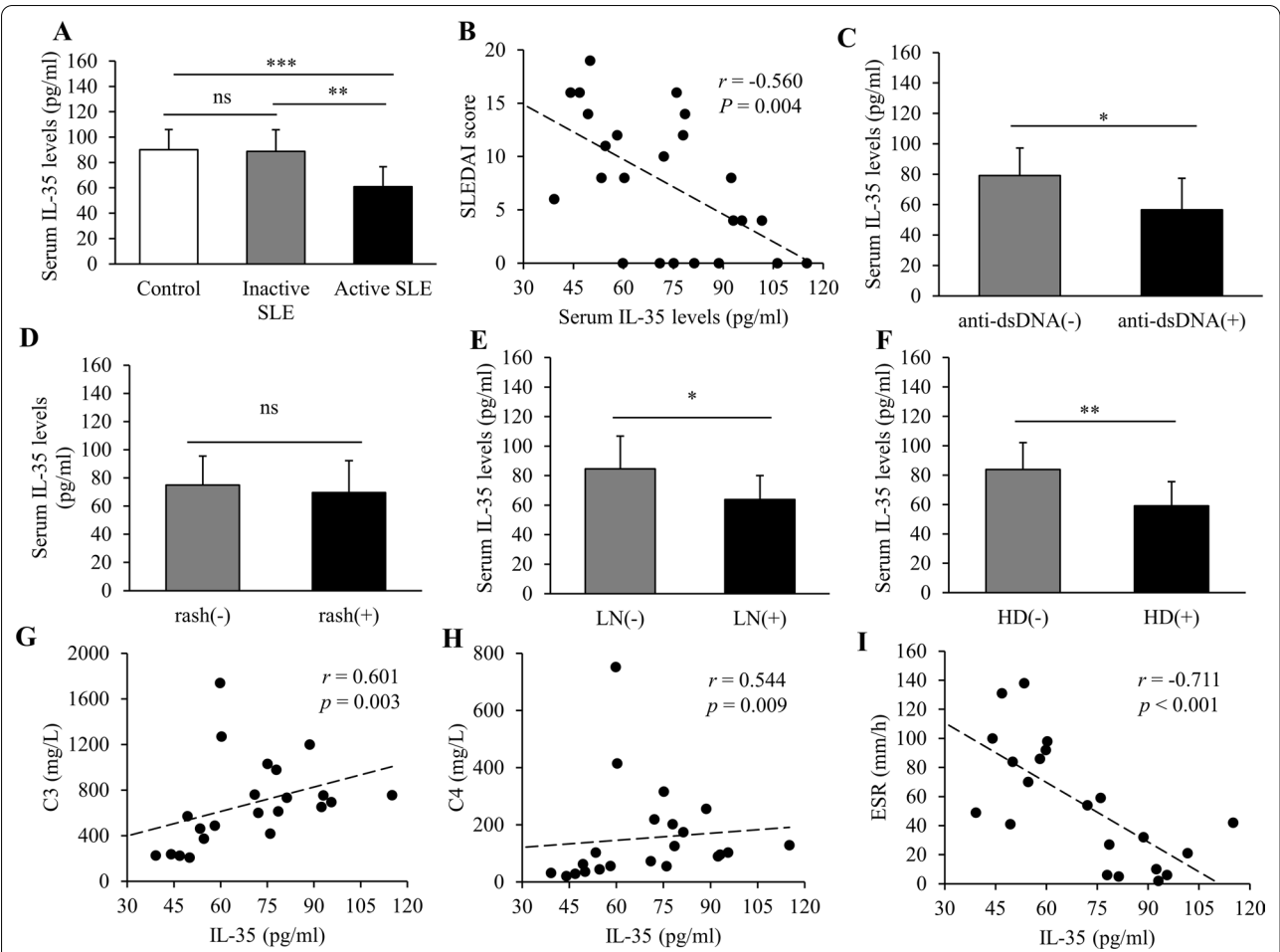
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Fig. 5 Association of serum IL-35 levels with SLE-related parameters. **A** Serum IL-35 levels from healthy controls (n = 16), inactive SLE patients (SLEDAI score ≤ 4, n = 10) and active SLE patients (SLEDAI score > 4, n = 14). **B** Correlation of serum IL-35 levels with the SLEDAI score. **C** Serum IL-35 levels from anti-dsDNA positive patients (n = 7) and anti-dsDNA negative patients (n = 17). **D**–**F** Serum IL-35 levels in SLE patients with rash (n = 11) and without rash (n = 13); with nephritis (n = 14) and without nephritis (n = 10); with hematological disorders (n = 11) and without hematological disorders (n = 13). **G**–**I** Correlation of serum IL-35 levels with level of complement C3, C4 and ESR. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant

Table 4 Correlation between the proportion and absolute number of CD24^{hi}CD27$^+$CD19$^+$ B cells with serum IL-35 and IL-10 level

	IL-10 (pg/ml)		IL-35 (pg/ml)	
	r value	P value	r value	P value
CD24^{hi}CD27$^+$/CD19$^+$ B cells (%)	− 0.496*	0.014*	0.412*	0.045*
CD19$^+$CD24^{hi}CD27$^+$ B cells (*10^6/L)	− 0.060	0.782	0.269	0.204

P-value was determined by Spearman's rank correlation coefficients. *$P < 0.05$

was a strong relationship between Bregs dysfunction and the occurrence of autoimmune disease progression in animal models [27], and altered distribution of Breg subpopulations might participate in the pathogenesis

of multiple autoimmune diseases, such as rheumatoid arthritis [26], pemphigus [24], juvenile idiopathic arthritis [28], type I autoimmune pancreatitis [29] and systemic sclerosis [25]. Although there is no consensus on a unique surface marker to identify Bregs, several subtypes of human Bregs, including CD24^{high}CD38^{high}CD19$^+$ [30] and CD19$^+$CD24^{high}CD27$^+$ [12] were identified and used in different studies. We have evaluated the frequency of CD19$^+$CD24^{high}CD38^{high} Bregs in peripheral blood from SLE patients, and discovered no correlation between CD24^{high}CD38^{high} Bregs and SLEDAI score (data not shown), similar to a recent study [31]. However, the relationship between CD19$^+$CD24^{high}CD27$^+$ B cells and SLE is rarely studied.

In this study, we observe significantly decreased proportion of CD19$^+$CD24^{high}CD27$^+$ B cells in active SLE

patients compared to inactive patients and healthy individuals. Moreover, our results indicate that the proportion of $CD19^+CD24^{high}CD27^+$ B cells is negatively correlated with SLEDAI score and Th17 cells, in agreement with previous research [19], while the inclusion criteria are broader in our work. As illustrated in recent studies, $CD19^+CD24^{high}CD27^+$ B cells play a vital part in Tregs differentiation and inhibit the production of pro-inflammatory cytokines such as TNF-α and IL-17 that are critical drivers for SLE progress [32]. Our results suggested that altered distribution of B cell subpopulations occurred in the peripheral blood cells of active SLE patients, and decreased $CD19^+CD24^{hi}CD27^+$ Bregs might contribute to the imbalance of T cell subpopulations, as well as the onset and pathogenesis of SLE. Except for Bregs, natural $FoxP3^+CD4^+CD25^{high}CD127^-$ regulatory T cells have also been discovered to suppress immune response [33]. Our results showed that the absolute amount of Tregs and Tregs/Th17 ratio tended to reduce in peripheral blood from SLE patients, while the lack of statistical significance is possibly due to a small sample size.

Furthermore, we explored the role of Bregs in SLE clinical features. We note that $CD19^+CD24^{high}CD27^+$ B cells are significantly less in patients with positive anti-dsDNA antibody or rash, compared to those without these clinical manifestations. Similarly, the relationship of Bregs to autoantibodies and clinical features has also been noticed in other autoimmune disorders such as rheumatoid arthritis and ANCA-associated vasculitis [34]. Therefore, $CD19^+CD24^{high}CD27^+$ B cells could be a useful tool to assess the disease activity and progression, as well as the efficacy of therapy in clinical practice. In addition, raised autoantibody titers and decreased levels of complement are strongly related to the activation of effector B cells in the process of SLE [35]. Collectively, the results above suggest that there is a tendency of naïve B cells to differentiate into immune-promoting subsets rather than immunosuppressive types among active SLE patients, the underlying mechanism remains unclear.

According to recent reports, the suppressive function of Bregs is highly depended on the output of anti-inflammatory cytokines such as IL-10, IL-35 and TGF-β [32, 36]. Since previous data have shown comparable levels of serum TGF-β between SLE patients and healthy individuals [31, 37], $IL-10^+$ Bregs were mostly researched over the last decade. As a traditional immunosuppressive agent, IL-10 showed the ability to prevent antigen-presenting process and inhibit the secretion of inflammatory cytokines by dendritic cells and macrophages [9, 38]. Besides, IL-10 could directly inhibit the activation of Th cells and promote T cell differentiation into Tregs [39].

Interestingly, our results show that serum IL-10 levels are increased in active SLE patients and positively related to disease activity, but are inversely correlated with the proportion of $CD19^+CD24^{high}CD27^+$ B cells in SLE patients, which is opposite to our initial hypothesis.

Although IL-10 was commonly regarded as an immunosuppressive cytokine in most studies, it can also be produced by effector B cells, monocytes and T cells in lupus patients [40]. In addition, the production of IL-10 in Bregs could be induced by inflammatory cytokines such as IL-21 [41]. In vitro research demonstrated that the suppressive effect of IL-10 on inflammatory cytokines production was indeed impaired in SLE monocytes compared to those from healthy donors, possibly due to the existence of immune complexes [42]. Furthermore, the expression of IL-10R1 on T cells was downregulated in lupus nephritis patients [22]. Based on these findings, we posit that there is an abnormal response of monocytes and T cells to IL-10 stimulation in active SLE patients. On the other side, the function of IL-10 in inducing the proliferation and activation of B cells [43] could potentially promote the progression of SLE. Here, we also detected significant increase of IL-10 levels in patients with positive anti-dsDNA, lupus nephritis and hematological disorders compared with those without related characteristics. The data therefore suggest that the elevated IL-10 level may be involved in the pathogenesis of renal and hematological damage in SLE.

IL-35, comprised of two different subunits p35 and Ebi3, was considered as a newly emerging anti-inflammatory factor produced by both Tregs and Bregs in recent studies [44]. Subsequent reports have revealed abnormal expression of IL-35 in autoimmune diseases and cancer [45, 46]. Additionally, IL-35 could suppress the function of dendritic cells, inhibit inflammatory cytokine production and T cell generation during autoimmune diseases [47].

In our study, we observe a significantly decrease of IL-35 levels in active SLE patients compared to the other two groups. Moreover, IL-35 levels are in a negative correlation with SLEDAI score, similar to previous research [48]. We further investigated that lower IL-35 concentrations are related to higher blood urea nitrogen and serum creatine, as well as nephritis and hematological disorders in SLE patients.

Recently, the p35 subunit of IL-35 was found to be able to induce the proliferation of $IL-10^+$ and $IL-35^+$ Bregs and ameliorate autoimmune uveitis in mice [49]. What's more, IL-35 could induce human or mouse T cells to differentiate into regulatory populations [50]. In support of these findings, our data reveal a positive correlation between IL-35 levels and the rate of $CD19^+CD24^{high}CD27^+$ B cells in SLE, suggesting that

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the decrease of CD19$^+$CD24^{high}CD27$^+$ B cell proportion in SLE patients may be owing to reduced IL-35 levels. Besides, IL-33/IL-31 axis is active player in SLE pathogenesis based on several studies of SLE patients and lupus mouse [51]. Mechanically, IL-33 induces IL-31 expression. Augment expression of IL-33 induced by cell death causes the induction of other cytokines, including IL-31. In particular, IL-4 induces the gene expression and release on IL-31 from Th2 cell, and IL-33 further enhances the IL-4-induced IL-31 release [52]. The IL-31 and IL-33 pathways are linked to each other, and there is a significant positive correlation between their expression and disease severity [53]. Once expression of one of them increases, it could induce the other one's expression, leading to multiplying inflammation and disease progression. What's more, it is reported that IL-35 could modify IL33/IL31 axis. MH Shamji et al. demonstrated that the presence of IL-35 inhibited the pro-inflammatory function of IL-33, and suppressed the release of downstream cytokines such as IL-5 and IL-13, thus induced immune tolerance in allergic disease [54, 55]. In addition, as highlighted by Nie et al. [56], IL-35 can suppress type 2 inflammation-induced cytokines. More specifically, IL-35 inhibited the production of IL-33, and IL-35-treated allergic rhinitis mice appeared with decreased level of IL-33 in nasal lavage fluid. Taking together, these results propose that IL-35 acts as a crucial mediator in the immune regulation of SLE patients.

However, there are several limitations in our study. First, the sample size of the study is too small. Besides, further analysis of mRNA levels, signal pathway and imbalance between regulatory and effective T cells are not conducted. Therefore, the outcome of this study is not functional enough to support a definitive conclusion.

Conclusion

Overall, our study demonstrates that CD19$^+$CD24^{high}CD27$^+$ B cells are decreased in SLE patients and negatively correlated with the disease activity, which may be involved in the pathogenesis of SLE. We observe significant increase of IL-10 and decrease of IL-35 in active SLE patients, and the diverse expression of IL-10 and IL-35 may contribute to the oncome of lupus nephritis and hematological disorders. Furthermore, our data suggests that IL-35, but not IL-10, plays a crucial role in immune regulation during SLE disease. Our study reinforces the significance of Breg cells and IL-35 production in regulating SLE and provide therapeutic targets to treat SLE disease.

Supplementary Information

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> **Additional file 1: Figure S1** Analysis of T lymphocyte subpopulations in SLE patients and healthy controls. **a** Correlation of the proportion of CD19$^+$CD24^{hi}CD27$^+$ B cells with the percentage of Th17 cells in SLE and healthy individuals (n = 10). **b** The absolute number of CD4$^+$CD25^{hi}CD127$^-$ regulatory T cells in the blood samples from SLE patients (n = 5) and healthy controls (n = 5). **c** The ratio of Th17/Treg in SLE patients (n = 5) and healthy controls (n = 5). ns, not significant.

Availability of data and materials
The datasets used during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by ethics committee of Sun Yat-sen Memorial hospital (No. SYSEC-KY-KS-2018-083).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

Author details
[1]Department of Dermatology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong, China. [2]Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetic and Gene Regulation, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong, China. [3]Department of Clinical Laboratory, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong, China. [4]Department of Dermatology, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen 518040, Guangdong, China. [5]Department of Dermatology, The Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen 518033, Guangdong, China.

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