Expression of CD19⁺CD24^{high}CD38^{high} B cells, IL-10 and IL-10R in peripheral blood from patients with systemic lupus erythematosus

TAO WANG^{1,2}, ZHIJUN LI², XINGFU LI¹, LINJIE CHEN², HAO ZHAO², CHAO JIANG² and LIJUN SONG¹

¹Department of Rheumatology, Qilu Hospital of Shandong University, Jinan, Shandong 250012; ²Department of Rheumatology, The First Affiliated Hospital of Bengbu Medical College, Bengbu, Anhui 233004, P.R. China

Received April 28, 2016; Accepted March 23, 2017

DOI: 10.3892/mmr.2017.7381

Abstract. The present study aimed to examine the status and clinical significance of cluster of differentiation (CD) 19⁺CD24^{high}CD38^{high} regulatory B cells (Bregs), serum interleukin (IL)-10, serum transforming growth factor (TGF)- β and IL-10 receptor (IL-10R) expression in peripheral blood from patients with systemic lupus erythematosus (SLE). A total of 56 patients with SLE and 35 healthy individuals were recruited to the present study. The SLE disease activity index (SLEDAI) was calculated, and other laboratory parameters were measured. Peripheral blood was collected from all participants. The frequency of CD19⁺CD24^{high}CD38^{high} Bregs and IL-10R⁺ expression on circulating lymphocytes was examined by flow cytometry. The serum levels of IL-10 and TGF- β were measured using enzyme-linked immunosorbent assay. The associations between these measurements and SLEDAI or other laboratory parameters were analyzed by correlation analysis. The percentage of CD19⁺CD24^{high}CD38^{high} Bregs and the serum levels of IL-10 were significantly increased, whereas the expression of IL-10R on circulating lymphocytes was markedly reduced in patients with SLE compared with in healthy controls. The serum levels of TGF- β 1 were not markedly different between the groups. In addition, these factors were correlated with other SLE laboratory parameters, and inter-correlations were presented with different degrees of significance. The percentage of CD19⁺CD24^{high}CD38^{high} Bregs was positively correlated with the percentage of IL-10R⁺ lymphocytes, mean fluorescence intensity (MFI) of IL-10R⁺ lymphocytes and serum IL-10 levels. In addition, the percentage of IL-10R⁺ lymphocytes was positively correlated with its expression level (MFI), whereas serum TGF- β 1 levels were negatively correlated with serum IL-10 levels. The present results indicated that expansion

of CD19⁺CD24^{high}CD38^{high} Bregs, upregulation of IL-10 and deficient lymphocyte-associated IL-10R may serve as novel SLE biomarkers. It may be hypothesized that deficient IL-10R expression results in compensatory enhanced IL-10 expression, expansion of Bregs, and/or compromised Breg and IL-10 functions, thus contributing to SLE development. Therefore, targeting the 'Bregs/IL-10/IL-10R' system may provide a novel therapeutic approach for the treatment of SLE.

Introduction

Systemic lupus erythematosus (SLE) is a chronic, systemic autoimmune inflammatory disease characterized by the production of numerous autoantibodies, particularly anti-nuclear antibodies (ANA), increased immune complex deposition, and multiple organ damage (1). To quantitatively assess SLE disease activity, the SLE disease activity index (SLEDAI) was developed by weighting organ involvement; this index includes a list of 24 items, of which 16 pertain to clinical manifestations associated with various organ systems and eight refer to laboratory test results (2). In addition, other laboratory findings specific for SLE may facilitate the diagnosis of SLE, including elevations in double-stranded DNA (dsDNA) antibodies, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and reductions in complement C3 and C4 levels, and lymphocyte count (3).

Genetic and environmental factors may contribute to the development of SLE; however, the precise etiology of SLE is not fully understood. As a central player in SLE development, the immune system, including T and B cells, contributes to the pathogenesis of human SLE (4). Historically, B cells are considered to be positive regulators of humoral immune responses, due to their ability to terminally differentiate into plasma cells and produce antigen-specific antibodies (5). However, specific B cell subsets negatively regulate immune responses and have been termed regulatory B cells (Bregs) (6-8). Bregs are associated with the cluster of differentiation (CD) 19⁺CD24^{high}CD38^{high} phenotype (9), and are characterized by production of the immunoregulatory cytokines interleukin (IL)-10 and transforming growth factor (TGF)- β (10-13). Through the secretion of these immunosuppressive cytokines, Bregs suppress other immune cells and have been studied extensively for their potential role in the treatment of various autoimmune diseases (13).

Correspondence to: Dr Xingfu Li, Department of Rheumatology, Qilu Hospital of Shandong University, 44 Wenhua Xi Road, Jinan, Shandong 250012, P.R. China
E-mail: xingfu_lee888@126.com

Key words: regulatory B cells, interleukin-10, interleukin-10 receptor, systemic lupus erythematosus, transforming growth factor- β

Previous studies have indicated that Bregs are functionally impaired in patients with SLE, thus suggesting their potential involvement in the pathogenesis of lupus (9,14). However, to the best of our knowledge, no studies have systematically characterized the status of Bregs, the serum levels of IL-10 and TGF- β , or IL-10 signaling in SLE. Furthermore, their associations with the SLEDAI and other laboratory parameters in patients with SLE have yet to be examined. Therefore, the present study aimed to address these issues, in order to significantly improve understanding regarding the pathogenesis of SLE, and to justify targeting Bregs as a therapeutic approach for the treatment of SLE and potentially other autoimmune diseases.

Materials and methods

Patients and controls. The present study was approved by the Ethics Committee of Bengbu Medical College (Bengbu, China), and written informed consent was obtained from each participant. A total of 56 patients (female, $n=53$; male, $n=3$; age, 35.38 ± 12.22) with SLE and 35 healthy individuals (controls; female, $n=33$; male, $n=2$; age, 32.77 ± 7.36) were enrolled into the present study. The patients and control participants were recruited between November 2014 and June 2015, and were matched for general demographic characteristics, including age, sex, lifestyle and geographical lineage. The diagnosis of SLE was established according to the SLE Classification Criteria proposed by the American College of Rheumatology in 1997 (15). Lupus disease activities were assessed using the SLEDAI, as described previously (2). According to the presence of lupus nephritis (LN), the patients with SLE were divided into two groups: Those with lupus nephritis (LN group; $n=24$) and those without (SLE group; $n=32$). The diagnosis of LN was based on persistence of proteinuria (>0.5 g/24 h), the presence of cellular casts and persistent hematuria, or biopsy evidence of LN. Whole blood was collected from all participants; blood samples were split into three portions: One portion for serum isolation via centrifugation ($1,000 \times g$ for 5 min at 4°C), which was stored at -70°C until further use, one portion for flow cytometric analysis, and one portion for the performance of laboratory tests.

Performance of laboratory tests. Routine laboratory tests were performed on one portion of the blood samples. These tests included the following:

Erythrocyte sedimentation rate (ESR) determination. ESR was measured using the modified Westergren method and the InteRRliner ESR Analyzer automated system (Sysmex America, Inc., Lincolnshire, IL, USA).

Peripheral blood parameter analysis. The peripheral complete blood cell parameters, red blood cell (RBC) count and lymphocyte count, were determined using the LH780 hematology auto-analyzer and the associated software version 2D3 (Beckman Coulter, Inc., Brea, CA, USA) according to the manufacturer's instructions.

Serum immunological index detection. The concentrations of serum CRP, immunoglobulin (Ig) G, IgM, IgA, and

complement C3 and C4 were measured by rate turbidimetry and nephelometry. The 6 reagent kits (IgG, cat. no. OSR61172; IgA, cat. no. OSR61171; IgM, cat. no. OSR61173; CRP, cat. no. 447280; C3, cat. no. 988462; C4, cat. no. 988471) were purchased from the Beckman Coulter, Inc. and measurements were taken using the IMMAGE 800 Immunochemistry System (Beckman Coulter, Inc.), according to the manufacturer's instructions. An indirect immunofluorescence assay was performed for the ANA titer (cat. no. FA1510) and the anti-dsDNA antibody titer (cat. no. FA1572) according to the manufacturer's instructions (both kits were purchased from the EUROIMMUN, Lübeck, Germany).

Liver function tests for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) determination. Liver function tests were performed using reagent kits for AST, ALP, ALT and indirect bilirubin levels purchased from Beckman Coulter, Inc. The levels of all four were measured on the Beckman Coulter AU5800 automatic analyzer (Beckman Coulter, Inc.), according to the manufacturer's instructions.

Urinary protein analysis. Specimens were obtained from the 56 patients with SLE. Urine was collected from each patient over a 24 h period; each patient collected all urine produced during this period in one container to generate one complete specimen. Urine specimens were analyzed using the Beckman Coulter Urine Protein Calibrator (Beckman Coulter, Inc.), according to the manufacturer's instructions.

Immunostaining and flow cytometric analysis. For flow cytometric analysis, RBC lysis was performed on whole blood samples, using the BD Multitest™ IMK kit (cat. no. 340503; BD Biosciences, San Jose, CA, USA), according to the manufacturer's instructions. The resulting cellular components were washed with phosphate-buffered saline twice and stained with fluorophore-conjugated antibodies at 4°C in the dark for 30 min. The following antibodies were purchased from BD Biosciences (the manufacturer's working dilution was used for all antibodies): Phycoerythrin (PE)-conjugated anti-CD19 (cat. no. 340364), fluorescein isothiocyanate-conjugated anti-CD24 (cat. no. 555427), allophycocyanin-conjugated anti-CD38 (cat. no. 340439) and PE-conjugated anti-CD210 (IL-10R; cat. no. 308804). Flow cytometry (16) was performed on a BD FACSCalibur cytometer (BD Biosciences), and data were analyzed using WinMDI 2.8 software (Scripps Research Institute, La Jolla, CA, USA).

Measurement of cytokine production. The serum levels of TGF- β 1 (cat. no. BMS249/4) and IL-10 (cat. no. BMS215/2) from all participants were measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, CA, USA), according to the manufacturer's protocols.

Statistical analysis. Statistical analysis was performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA) software. Data distribution was checked for normality using the Kolmogorov-Smirnov test. Normally distributed data are expressed as the mean $\pm$ standard deviation, and the differences were examined using two-tailed t-tests. Correlation

coefficients and their significance were calculated by two-tailed Pearson correlation. For nonparametric data, the results are presented as medians (25-75th percentile). The Mann-Whitney U-test was used to compare the data between the patient and control groups. To examine the correlations among the parameters measured in the present study, multivariate analysis was performed. Correlation coefficients and their significance were calculated by two-tailed Spearman's Rho correlation. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

CD19⁺CD24^{high}CD38^{high} Breg cells are enriched in the circulating lymphocytes of patients with SLE. To determine the potential involvement of Bregs in SLE development, the present study examined the frequency of CD19⁺CD24^{high}CD38^{high} Bregs in patients with SLE and compared it with that in healthy controls. As shown in Fig. 1, there was a higher percentage of CD19⁺CD24^{high}CD38^{high} Bregs among circulating lymphocytes from patients with SLE compared with in healthy individuals (39.83 ± 21.39 vs. $8.74 \pm 3.97\%$; $P < 0.001$).

Further analysis on the clinical significance of Bregs indicated that the frequency of CD19⁺CD24^{high}CD38^{high} Bregs was not significantly correlated with the SLEDAI score ($r = -0.111$, $P = 0.416$; Fig. 2A) or the presence of LN ($P > 0.05$, data not shown) in patients with SLE; however, it was negatively correlated with blood complement C3 ($r = -0.432$, $P = 0.002$; Fig. 2B), complement C4 ($r = -0.497$, $P < 0.001$; Fig. 2C) and serum indirect bilirubin ($r = -0.335$, $P = 0.035$; Fig. 2D) levels.

IL-10R expression in lymphocytes from patients with SLE is significantly lower compared with in healthy controls. Bregs are characterized by production of the immunosuppressive cytokine IL-10 (17), which signals through a receptor complex consisting of two IL-10R1 and two IL-10R2 molecules on target cells (18). To determine whether aberrant IL-10R expression exists in patients with SLE, the expression of IL-10R was detected on lymphocytes from patients with SLE and healthy controls by flow cytometry. As shown in Fig. 3A, the percentage of IL-10R⁺ cells among circulating lymphocytes was markedly lower in patients with SLE compared with in healthy individuals (23.76 ± 0.62 vs. $51.01 \pm 16.03\%$; $P < 0.001$). Furthermore, the expression levels of IL-10R, based on the mean fluorescence intensity (MFI) of these cells, were also significantly lower in patients with SLE compared with in healthy individuals (20.18 ± 3.88 vs. 23.23 ± 3.66 ; $P < 0.001$; Fig. 3B).

Further correlation analysis was conducted (Fig. 3C-F). The results indicated that the MFI of IL10-R1⁺ lymphocytes was not significantly correlated with the presence of LN in patients with SLE ($P > 0.05$, data not shown), the SLEDAI ($r = 0.009$, $P = 0.951$; Fig. 3C), serum ALT ($r = 0.254$, $P = 0.075$; Fig. 3E) or serum ALP ($r = 0.252$, $P = 0.077$; Fig. 3F); however, it was negatively correlated with ESR ($r = -0.389$, $P = 0.016$; Fig. 3D).

Serum IL-10 levels are elevated in patients with SLE. To examine whether the increased number of circulating CD19⁺CD24^{high}CD38^{high} Bregs led to elevated IL-10 production, the present study examined the serum IL-10 levels using

Figure 1. CD19⁺CD24^{high}CD38^{high} regulatory B cells are enriched in the circulating lymphocytes of patients with SLE compared with in HCs. Following red blood cell lysis, cells within the peripheral blood were stained for the expression of CD19, CD24 and CD38, and were analyzed by flow cytometry. (A) Gating on FSC-SSC plot (R1) was set to include lymphocytes for further analysis. (B) R2 gating was set for CD19⁺CD24^{high} cells for the next analysis. (C and D) Further gating on the CD24^{high}CD38^{high} quadrant from a representative sample of (C) patients with SLE and (D) HCs. The percentages indicate the percentage of CD19⁺CD24^{high}CD38^{high} cells/CD19⁺ cells. (E) Quantification of flow cytometric analysis of CD19⁺CD24^{high}CD38^{high} cells from patients with SLE vs. HCs. CD, cluster of differentiation; FSC, forward scatter; HCs, healthy controls; SLE, systemic lupus erythematosus; SSC, side scatter.

ELISA. The results demonstrated that serum IL-10 concentration was significantly higher in patients with SLE compared with in healthy controls [3.65 (2.22 - 9.17) pg/ml in patients with SLE vs. 1.04 (0.43 - 1.46) pg/ml in healthy controls; $P < 0.001$; data not show].

Correlation analysis indicated that serum IL-10 levels were not correlated with the presence of LN in patients with SLE ($P > 0.05$, data not shown) or the SLEDAI; however, it was positively correlated with ESR, ANA titer, IgG, IgM and AST, and was negatively correlated with complement C3 levels, as well as RBC and lymphocyte counts (Table I).

Serum TGF- β 1 levels are significantly lower in the LN group compared with in the healthy control group. In addition to serum IL-10 levels, the present study measured TGF- β 1 serum

Figure 2. Correlation analysis between the frequency of CD19⁺CD24^{high}CD38^{high} regulatory B cells with clinical parameters: (A) SLEDAI, (B) complement C3 levels, (C) complement C4 levels and (D) IBIL levels. CD, cluster of differentiation; IBIL, indirect bilirubin; SLEDAI, systemic lupus erythematosus disease activity index.

Figure 3. IL-10R expression in lymphocytes was significantly lower in patients with SLE compared with in HCs. (A) Percentage of IL-10R⁺ lymphocytes was compared between the patients with SLE and the HCs. (B) MFI of IL-10R on circulating lymphocytes was compared between the patients with SLE and the HCs. Correlation analysis between the MFI of IL-10R⁺ lymphocytes and (C) SLEDAI, (D) blood ESR, (E) ALT levels and (F) ALP levels. ALP, alkaline phosphatase; ALT, alanine transaminase; CD, cluster of differentiation; ESR, erythrocyte sedimentation rate; HCs, healthy controls; IL-10R, interleukin-10 receptor; MFI, mean fluorescent intensity; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index.

Table I. Correlation between serum IL-10 levels and clinical parameters.

Variable	SLEDAI	ESR	ANA	ds-DNA	IgG	IgM	IgA	C3	Lym	RBC	AST
r	0.160	0.411	0.401	0.228	0.376	0.334	0.045	-0.466	-0.385	-0.337	0.367
P-value	0.240	0.008	0.014	0.188	0.007	0.017	0.752	0.002	0.005	0.015	0.007

SLEDAI, systemic lupus erythematosus disease activity index; ANA, antinuclear antibody; ds-DNA, double-stranded DNA; Ig, immunoglobulin; Lym, lymphocyte count; RBC, red blood cell count; AST, aspartate aminotransferase level. Correlation coefficients and their significance were calculated by two-tailed Pearson correlation or Spearman's Rho correlation.

Figure 4. Serum TGF-β1 levels were not significantly different between patients with SLE and HCs. (A) Serum TGF-β1 levels were compared between patients with SLE and HCs. (B) Serum TGF-β1 levels were compared between patients with SLE and LN, and HCs. (C) Serum TGF-β1 levels were compared between patients with SLE with or without LN. TGF-β1 levels in 4 of the 35 HCs were recorded as 0 pg/ml due to limitations of the kits used, thus, these 4 statistical anomalies in the HC group were removed from the dataset. HCs, healthy controls; LN, lupus nephritis; SLE, systemic lupus erythematosus; TGF-β1, transforming growth factor-β1.

levels. As presented in Fig. 4A, serum TGF-β1 levels were not significantly different between patients with SLE and healthy controls ($11,262.02 \pm 7,784.17$ vs. $13,143.27 \pm 5,559.21$ pg/ml; $P=0.239$). However, serum TGF-β1 levels in patients with SLE and LN were significantly lower compared with in healthy controls ($9,382.07 \pm 7,558.16$ vs. $13,143.27 \pm 5,559.21$ pg/ml; $P=0.038$; Fig. 4B). Conversely, there was no significant difference in serum TGF-β1 levels between the patients with SLE with or without LN ($9,382.07 \pm 7,558.16$ vs. $12,671.98 \pm 7,767.39$ pg/ml; $P=0.118$; Fig. 4C).

Correlation analysis indicated that serum TGF-β1 levels were not significantly correlated with the SLEDAI score; however, they were positively correlated with RBC count, and negatively correlated with globulin, 24-h urine protein, and IgM and AST levels (Table II).

Correlations among various measurements. To examine the correlations among the parameters measured in the present study, a multivariate analysis was performed. As shown in Table III, the percentage of CD19⁺CD24^{high}CD38^{high} Bregs was positively correlated with the percentage of IL-10R⁺

lymphocytes, the MFI of IL-10R⁺ lymphocytes, and serum IL-10 levels. In addition, the percentage of IL-10R⁺ lymphocytes was positively correlated with its expression level (MFI), whereas serum TGF-β1 levels were negatively correlated with serum IL-10 levels.

Discussion

The present study demonstrated that CD19⁺CD24^{high}CD38^{high} Bregs were enriched in the circulating lymphocytes of patients with SLE compared with in healthy individuals. The increase in CD19⁺CD24^{high}CD38^{high} Bregs was associated with elevated serum IL-10 levels, no significant difference in serum TGF-β1 levels, and a significant reduction in IL-10R expression on circulating lymphocytes.

The main function of B cells is to serve as antigen-presenting cells, which leads to optimal expansion of antigen-specific CD4⁺ T cells, memory formation and cytokine production (19-21). Furthermore, B cells positively regulate CD8⁺ T cell responses in murine models of autoimmune diseases (22,23). Therefore, in addition to producing

Table II. Correlation between serum TGF- β 1 levels and clinical parameters.

Variable	SLEDAI	Globulin	24-h up	IgM	RBC	AST	ds-DNA	C3	ESR	CRP
r	0.190	-0.300	-0.413	-0.312	0.342	-0.321	0.106	-0.028	-0.080	0.037
P-value	0.160	0.032	0.015	0.026	0.013	0.020	0.544	0.843	0.624	0.806

SLEDAI, systemic lupus erythematosus disease activity index; 24-h up, 24-h urine protein; Ig, immunoglobulin; RBC, red blood cell count; AST, aspartate aminotransferase level; ds-DNA, double-stranded DNA; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein level. Correlation coefficients and their significance were calculated by two-tailed Pearson correlation or Spearman's Rho correlation.

Table III. Multivariate correlation analysis.

Variable	CD210%	CD210-MFI	IL-10 serum levels (pg/ml)	TGF- β 1 serum levels (pg/ml)
CD19 ⁺ CD24 ^{high} CD38 ^{high} Bregs (%)				
r	0.364	0.312	0.265	0.069
P-value	0.006	0.022	0.048	0.612
CD210+ lymphocytes (%)				
r		0.397	-0.097	0.118
P-value		0.003	0.479	0.388
MFI of CD210 on lymphocytes				
r			0.111	-0.002
P-value			0.424	0.990
Serum IL-10 level (pg/ml)				
r				-0.306
P-value				0.022

Bregs, regulatory B cells; CD, cluster of differentiation; IL, interleukin; MFI, mean fluorescence intensity; TGF, transforming growth factor.

antibodies, B cells are able to positively regulate cellular immune responses. However, specific B cell subsets negatively regulate immune responses and have been termed Bregs (24). At present, IL-10-producing Bregs are the most extensively studied, and these cells are characterized by the surface expression profile CD19⁺CD24^{high}CD38^{high} (24). The present study demonstrated that CD19⁺CD24^{high}CD38^{high} Bregs were highly enriched in patients with SLE. For SLE, which is an autoimmune disease characterized by the persistent activation of autoreactive B cells, the expansion of immunosuppressive Bregs seems paradoxical to the well-accepted pathogenesis of the disease. However, to the best of our knowledge, the present study is not the first to report Breg expansion in SLE. Sims *et al* (25) detected significantly higher percentages of CD19⁺CD38^{high}CD24^{high} B cells in the peripheral blood mononuclear cells of patients with SLE compared with in healthy individuals. However, a more recent study reported that a similar number of CD19⁺CD38^{high}CD24^{high} B cells were detected in patients with SLE and healthy individuals, whereas the CD19⁺CD38^{high}CD24^{high} B cells in patients with SLE were functionally impaired (9). The differences in circulating Breg levels between these studies may reflect variations in patient geographic lineages, genetic background, clinical characteristics or received therapies. The biological significance and the underlying mechanisms for Breg expansion in SLE, if

present, still remain to be elucidated. The negative correlations between the percentage of Bregs within circulation and other clinical parameters, including serum C3, C4 and indirect bilirubin levels, suggest that the Breg level reflects patient characteristics and disease progression.

Similar to alterations in the percentage of Bregs, the present study observed an elevation in serum IL-10 levels in patients with SLE compared with in healthy individuals. Although not the only source of IL-10, IL-10 is the key functional cytokine generated by CD19⁺CD24^{high}CD38^{high} Bregs. It has previously been reported that the serum levels of IL-10 are markedly increased in patients with SLE, and are correlated with the SLEDAI or the production of antibodies (anti-DNA) (26). However, in the present study, a significant association was not detected between serum IL-10 levels and SLEDAI or anti-dsDNA antibody levels. Conversely, a significant correlation was determined between serum IL-10 levels and other clinical parameters that reflect disease activity, including ESR, ANA titer, IgG, IgM and C3, thus suggesting the significance of serum IL-10 levels in reflecting disease status. Given the immunosuppressive activities of Bregs and IL-10, the upregulation of these two factors indicates the body's efforts to inhibit autoimmunity within patients with SLE, but with no success. The failed efforts of elevated Bregs and serum IL-10 levels suggest potential defects in downstream signaling from Bregs

and IL-10. A previous study demonstrated that in patients with SLE, the capacity of IL-10 to suppress the production of inflammatory cytokines is attenuated and that defects in IL-10 homeostatic function contribute to SLE pathogenesis (27). Furthermore, IL-10 may have a deleterious effect on humoral-based autoimmune diseases, such as SLE, since excessive IL-10 promotes B cell differentiation and autoantibody production, eventually contributing to SLE development. IL-10 may also suppress the ability of T cells, monocytes and natural killer cells to produce TGF- β , thus promoting the development of SLE (28). Notably, these diverse activities may well explain the phenomenon that IL-10 appears to serve a protective role in some diseases, but is harmful in SLE and other autoimmune diseases.

IL-10 signals through the IL-10R, which is a complex composed of two IL-10R1 and two IL-10R2 molecules, and subsequently through Jak-signal transducer and activator of transcription signaling. Cairns *et al* (29) demonstrated that the expression of IL-10R on leukocytes is not significantly different between patients with SLE and controls. Conversely, Li *et al* (30) reported that the mRNA expression of IL-10R on peripheral blood mononuclear cells is increased in patients with active SLE. Furthermore, other studies have suggested that the expression of IL-10R on dendritic cells from patients with SLE is significantly lower compared with in controls (31,32). The present study demonstrated that the expression of IL-10R on circulating lymphocytes was significantly reduced in patients with SLE compared with in healthy controls. The lower expression was not only determined based on the percentage of IL-10R⁺ lymphocytes but also on the MFI of IL-10R. Functionally, the reduced IL-10R expression was negatively correlated with ESR, a type of disease activity marker. However, no significant correlation was detected between IL-10R and the SLEDAI, ALT or ALP. These data provided a novel mechanism for defective Breg function in patients with SLE, that is, deficient IL-10R expression and subsequent IL-10/IL-10R signaling. It is also possible that Breg expansion and upregulation of serum IL-10 levels occur as a compensatory mechanism for deficient IL-10R expression. For further mechanistic studies, it is important to understand the molecular control of IL-10R expression in lymphocytes and how such control may be altered in SLE.

TGF- β is another characteristic suppressive cytokine produced by Bregs. A previous study revealed no significant differences in the serum levels of bioactive TGF- β between healthy control subjects and patients with inactive and active SLE (33). However, a more recent study reported that reduced serum TGF- β 1 levels may be the most consistent cytokine abnormality in SLE, correlating with disease activity, a reduction in CD4⁺, CD8⁺ and natural killer cell numbers, and severe organ damage in active SLE (34). Numerous factors may contribute to the reduced serum levels of TGF- β in patients with SLE. One of these factors may be the reduced level of IL-2 in patients with SLE (35). IL-2 serves an essential role in the development of induced forkhead box P3⁺ regulatory T cells, and can induce natural mouse and human CD4⁺CD25⁺, and TGF- β -induced CD4⁺CD25⁺ Treg cells to produce and express mature TGF- β on the cell surface (35). Another potential factor is IL-10. IL-10 may suppress the ability of T cells, monocytes and natural killer cells to produce TGF- β , thus

promoting the development of SLE. Consistently, the present study identified a negative correlation between serum TGF- β 1 levels and IL-10 levels. However, when considering absolute serum levels, although a trend was detected for reduced serum TGF- β 1 levels in patients with SLE compared with in healthy individuals, no statistical significance was noted. Further analysis indicated that TGF- β 1 levels in were significantly lower in patients with LN compared with in healthy individuals. Consistently, TGF- β 1 levels were positively correlated with the RBC count, but were negatively correlated with globulin, 24-h urinary protein excretion, and IgM and AST levels, thus suggesting the potential involvement of TGF- β in the development of nephritis among patients with SLE.

Approximately 25-50% of patients with SLE may develop abnormal liver function (36). In various liver diseases, significant correlations have been reported between IL-10 or TGF- β and liver function (37-39). Therefore, the present study examined the correlations between circulating Bregs, serum IL-10, serum TGF- β 1 or lymphocyte-associated IL-10R expression, and various parameters representing liver function, including indirect bilirubin, CRP, AST, ALT and ALP. The results demonstrated a significant negative correlation between the number of Bregs and indirect bilirubin levels, as well as between serum TGF- β 1 and AST levels. In addition, a positive correlation was observed between serum IL-10 and AST levels, thus suggesting that these immune regulators may modulate liver function during SLE development. However, due to the relatively small sample size, it is difficult to draw a definite conclusion between the immune regulators and liver function, as well as other clinicopathological parameters. Further studies including a larger number of patients are required to verify the data from the present study, and to explore the underlying molecular mechanisms.

In conclusion, the present study demonstrated that in the patient population examined, the percentage of circulating CD19⁺CD24^{high}CD38^{high} Bregs and serum IL-10 levels were significantly increased, whereas the expression of IL-10R in lymphocytes was markedly decreased, in patients with SLE compared with in healthy individuals. Conversely, serum TGF- β 1 levels were only significantly different between patients with SLE and LN, and healthy individuals. In addition, these four factors all exhibited correlations with other laboratory parameters reflecting SLE activities. These findings indicated that the 'Bregs/IL-10/IL-10R' system may serve an important role in the pathogenesis of SLE. Therefore, deficient IL-10R expression on lymphocytes may provide a novel mechanism for the impaired function of Bregs and IL-10. These factors may not only serve as markers for SLE progression but may also provide future therapeutic targets for SLE and other autoimmune disorders.

References

1. Cojocaru M, Cojocaru IM, Silosi I and Vrabie CD: Manifestations of systemic lupus erythematosus. *Maedica (Buchar)* 6: 330-336, 2011.
2. Bombardier C, Gladman DD, Urowitz MB, Caron D and Chang CH: Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 35: 630-640, 1992.
3. Manson JJ and Rahman A: Systemic lupus erythematosus. *Orphanet J Rare Dis* 1: 6, 2006.

4. Lipsky PE: Systemic lupus erythematosus: An autoimmune disease of B cell hyperactivity. *Nat Immunol* 2: 764-766, 2001.
5. LeBien TW and Tedder TF: B lymphocytes: how they develop and function. *Blood* 112: 1570-1580, 2008.
6. DiLillo DJ, Matsushita T and Tedder TF: B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity and cancer. *Ann N Y Acad Sci* 1183: 38-57, 2010.
7. Fillatreau S: Novel regulatory functions for Toll-like receptor-activated B cells during intracellular bacterial infection. *Immunol Rev* 240: 52-71, 2011.
8. Mauri C: Regulation of immunity and autoimmunity by B cells. *Curr Opin Immunol* 22: 761-767, 2010.
9. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR and Mauri C: CD19 (+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity* 32: 129-140, 2010.
10. Yanaba K, Bouaziz JD, Matsushita T, Magro CM, St Clair EW and Tedder TF: B-lymphocyte contributions to human autoimmune disease. *Immunol Rev* 223: 284-299, 2008.
11. Mauri C and Bosma A: Immune regulatory function of B cells. *Annu Rev Immunol* 30: 221-241, 2012.
12. Pistoia V: Production of cytokines by human B cells in health and disease. *Immunol Today* 18: 343-350, 1997.
13. Yang M, Rui K, Wang S and Lu L: Regulatory B cells in autoimmune diseases. *Cell Mol Immunol* 10: 122-132, 2013.
14. Pillai S, Mattoo H and Cariappa A: B cells and autoimmunity. *Curr Opin Immunol* 23: 721-731, 2011.
15. Hochberg MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 40: 1725, 1997.
16. Davies R, Vogelsang P, Jonsson R and Appel S: An optimized multiplex flow cytometry protocol for the analysis of intracellular signaling in peripheral blood mononuclear cells. *J Immunol Methods* 436: 58-63, 2016.
17. Candando KM, Lykken JM and Tedder TF: B10 cell regulation of health and disease. *Immunol Rev* 259: 259-272, 2014.
18. Mosser DM and Zhang X: Interleukin-10: New perspectives on an old cytokine. *Immunol Rev* 226: 205-218, 2008.
19. Linton PJ, Harbertson J and Bradley LM: A critical role for B cells in the development of memory CD4 cells. *J Immunol* 165: 5558-5565, 2000.
20. Crawford A, Macleod M, Schumacher T, Corlett L and Gray D: Primary T cell expansion and differentiation in vivo requires antigen presentation by B cells. *J Immunol* 176: 3498-3506, 2006.
21. Bouaziz JD, Yanaba K, Venturi GM, Wang Y, Tisch RM, Poe JC and Tedder TF: Therapeutic B cell depletion impairs adaptive and autoreactive CD4⁺ T cell activation in mice. *Proc Natl Acad Sci USA* 104: 20878-20883, 2007.
22. Homann D, Tishon A, Berger DP, Weigle WO, von Herrath MG and Oldstone MB: Evidence for an underlying CD4 helper and CD8 T-cell defect in B-cell-deficient mice: Failure to clear persistent virus infection after adoptive immunotherapy with virus-specific memory cells from muMT/muMT mice. *J Virol* 72: 9208-9216, 1998.
23. Bergmann CC, Ramakrishna C, Kornacki M and Stohlman SA: Impaired T cell immunity in B cell-deficient mice following viral central nervous system infection. *J Immunol* 167: 1575-1583, 2001.
24. Miyagaki T, Fujimoto M and Sato S: Regulatory B cells in human inflammatory and autoimmune diseases: From mouse models to clinical research. *Int Immunol* 27: 495-504, 2015.
25. Sims GP, Ettinger R, Shirota Y, Yarboro CH, Illei GG and Lipsky PE: Identification and characterization of circulating human transitional B cells. *Blood* 105: 4390-4398, 2005.
26. Peng H, Wang W, Zhou M, Li R, Pan HF and Ye DQ: Role of interleukin-10 and interleukin-10 receptor in systemic lupus erythematosus. *Clin Rheumatol* 32: 1255-1266, 2013.
27. Yuan W, DiMartino SJ, Redecha PB, Ivashkiv LB and Salmon JE: Systemic lupus erythematosus monocytes are less responsive to interleukin-10 in the presence of immune complexes. *Arthritis Rheum* 63: 212-218, 2011.
28. Zheng SG: Transforming growth factor-beta level: Indicator for severity of disease and organ damage in patients with systemic lupus erythematosus. *J Rheumatol* 37: 1983-1985, 2010.
29. Cairns AP, Crockard AD and Bell AL: Interleukin-10 receptor expression in systemic lupus erythematosus and rheumatoid arthritis. *Clin Exp Rheumatol* 21: 83-86, 2003.
30. Li YM, Chen ZQ, Yao X, Yang AZ, Li AS, Liu DM and Gong JQ: mRNA expression of chemokine receptors on peripheral blood mononuclear cells and correlation with clinical features in systemic lupus erythematosus patients. *Chin Med Sci J* 25: 162-168, 2010.
31. Cui HD, Qi ZM, Yang LL, Qi L, Zhang N, Zhang XL, Du SY and Jiang Y: Interleukin-10 receptor expression and signalling were down-regulated in CD4 (+) T cells of lupus nephritis patients. *Clin Exp Immunol* 165: 163-171, 2011.
32. Figueroa-Vega N, Galindo-Rodriguez G, Bajaña S, Portales-Pérez D, Abud-Mendoza C, Sánchez-Torres C and González-Amaro R: Phenotypic analysis of IL-10-treated, monocyte-derived dendritic cells in patients with systemic lupus erythematosus. *Scand J Immunol* 64: 668-676, 2006.
33. Bennett AL, Chao CC, Hu S, Buchwald D, Fagioli LR, Schur PH, Peterson PK and Komaroff AL: Elevation of bioactive transforming growth factor-beta in serum from patients with chronic fatigue syndrome. *J Clin Immunol* 17: 160-166, 1997.
34. Becker-Merok A, Eilertsen GØ and Nossent JC: Levels of transforming growth factor-beta are low in systemic lupus erythematosus patients with active disease. *J Rheumatol* 37: 2039-2045, 2010.
35. Zheng SG, Wang J, Wang P, Gray JD and Horwitz DA: IL-2 is essential for TGF-beta to convert naive CD4⁺CD25⁻ cells to CD25⁺Foxp3⁺ regulatory T cells and for expansion of these cells. *J Immunol* 178: 2018-2027, 2007.
36. van Hoek B: The spectrum of liver disease in systemic lupus erythematosus. *Neth J Med* 48: 244-253, 1996.
37. El-Emshaty HM, Nasif WA and Mohamed IE: Serum Cytokine of IL-10 and IL-12 in Chronic Liver Disease: The Immune and Inflammatory Response. *Dis Markers* 2015: 707254, 2015.
38. Taylor A, Verhagen J, Blaser K, Akdis M and Akdis CA: Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: The role of T regulatory cells. *Immunology* 117: 433-442, 2006.
39. el-Houseini ME, Mohammed MS, Elshemey WM, Hussein TD, Desouky OS and Elsayed AA: Enhanced detection of hepatocellular carcinoma. *Cancer Control* 12: 248-253, 2005.