

# Elevated Production of B Cell Chemokine CXCL13 is Correlated with Systemic Lupus Erythematosus Disease Activity

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## Abstract

**Introduction** B lymphocyte chemoattractant (BLC/CXCL13), a CXC chemokine, is involved in B1 and B2 cell trafficking for the activation of autoreactive T helper (Th) cells and autoantibody production in target organs during the development of lupus. CXCL13 can induce the trafficking of CXCR5+ T lymphocyte subset designated as follicular helper T lymphocytes (T<sub>FH</sub>) which are specifically involved in autoantibody production.

**Materials and Methods** We herein measured the plasma concentrations of CXCL13, B-cell-activating factor of the TNF family (BAFF), and T<sub>FH</sub>-cells-related cytokine IL-21 and cell surface expression of T<sub>FH</sub>-related receptor CXCR5 and IL-21R on CD4+Th and CD19+B cells in 35 systemic lupus erythematosus (SLE) patients and 23 sex- and age-matched control subjects (NC) using enzyme-linked immunosorbent assay and flow cytometry, respectively.

**Results and Discussion** Plasma CXCL13, BAFF, and IL-21 concentrations were significantly higher in SLE patients than NC group (all  $p < 0.0001$ ). Increase in CXCL13 concentration correlated positively and significantly with SLEDAI score in SLE patients ( $r = 0.399$ ,  $p = 0.032$ ). Cell surface expression of

CXCR5 on Th and B cells and IL-21R on B cells was however significantly lower in SLE patients than controls (both  $p < 0.01$ ). It may indicate that most differentiated T<sub>FH</sub> cells migrate out from circulation into lymphoid organ upon activation during the disease development of SLE.

**Conclusions** The above results suggest that the elevated production of CXCL13, BAFF, and IL-21 may be associated with the function of T<sub>FH</sub> for the immunopathogenesis in SLE, and CXCL13 may serve as a potential disease marker of SLE.

**Keywords** B cells · B-cell-activating factor of the TNF family · chemokines · CXCL13 · follicular helper T lymphocytes · systemic lupus erythematosus

## Introduction

Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease characterized by various immunological abnormalities, including dysregulated activation of both T and B lymphocytes with overt production of autoreactive antibodies [1, 2]. Aberrant production and imbalance of T helper (Th) cell cytokines have been implicated in the pathogenesis of autoimmunity [3]. However, the cytokines and chemokines for the activation and migration of B cells have not been well investigated. CXCL13/B lymphocyte chemoattractant (BLC) is a small cytokine belonging to the CXC chemokine family that is produced by cells in the omentum, peritoneal macrophages, and dendritic cells [4, 5]. CXCL13 is selectively chemotactic for B cells including both the B1 and B2 subsets by interacting with specific chemokine receptor CXCR5 [4, 6]. CXCL13 and CXCR5 together control the organization of

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B cells within follicles of lymphoid tissues [7] and is highly expressed in the liver, spleen, lymph nodes, and gut of humans [6]. Previous studies have revealed that CXCL13 can induce the trafficking of distinct CXCR5<sup>+</sup> T cells designated as follicular helper T cells (T<sub>FH</sub>) which are specifically involved in high-affinity IgG production in germinal centers developed within B cell follicles of secondary lymphoid tissues including lymph nodes, spleen, and tonsils [8–11]. CD4<sup>+</sup>T<sub>FH</sub> cells, located at B cell follicles, provide a T helper function to B cells and represents one of the most numerous and important subsets of effector T cells in lymphoid tissue [12, 13]. Zinc finger transcription factor BCL-6 may play certain role for driving naïve Th cells to the T<sub>FH</sub> cell lineage [12]. IL-21 is a pleiotropic cytokine produced by CD4<sup>+</sup>Th cells that modulate the differentiation and function of T, B, natural killer (NK), and dendritic cells by binding to the receptor composing of the IL-21 receptor- $\alpha$  (IL-21R $\alpha$ ) and the common  $\gamma$  chain [14, 15]. Recent study has intimated that IL-21 can mediate the differentiation and generation of T<sub>FH</sub> cells [10, 15]. Nevertheless, autocrine production of IL-21 from T<sub>FH</sub> cells can potently stimulate the differentiation of B cells into antibody-forming cells through IL-21R [12]. As a result, dysregulation of T<sub>FH</sub> cell function may relate to the pathogenesis of SLE. IL-21 has been shown to contribute to the development of autoimmune diseases in different animal models of SLE, experimental autoimmune encephalomyelitis, and rheumatoid arthritis [14]. Although genetic association of IL-21 polymorphisms has been demonstrated in SLE [16], the detailed mechanisms of the subsequent B cells activation in SLE patients requires further characterization.

B1 cells have different origin and function from the major recirculating conventional B (B2) cells by their unique tissue distribution, cell surface phenotype, and function [17]. Cell surface expression of CXCR5 on CD5<sup>+</sup>B1 cells was significantly higher than B2 cells. The accumulation of B1 cells in the peritoneal cavity and spleen are responsible for the body cavity immunity and the production of autoantibody for the development of autoimmune disease in the murine model [4, 17, 18]. Elevated levels of B1 cells have been documented in patients with autoimmune disorders such as Sjogren's syndrome and rheumatoid arthritis [19, 20]. Previous studies using murine model of SLE showed that CXCL13 is highly produced by CD11b<sup>+</sup>CD11c<sup>+</sup> dendritic cells (DC) in the target organs including thymus and kidney for the chemoattraction of B1 cells into target organ [18, 21–23]. Therefore, the elevated expression of CXCL13 by myeloid DC in the target organs may play a crucial role in breaking the immune tolerance in the thymus leading to the activation of self-reactive CD4<sup>+</sup>Th cells and the recruitment of autoantibody producing B cells in the development of murine lupus [18, 22, 23]. Tumor necrosis factor (TNF)- $\alpha$  expression is increased in the SLE murine

model and plays crucial role in the recruitment and maturation of DC into CXCL13 producing DC during disease development [23]. Apart from CXCL13, B cell activation factor of the TNF family (BAFF) is a TNF family member that binds to B cells and potentiates B cell receptor-mediated proliferation [24]. BAFF was reported to increase the in vitro chemotactic response of primary human B cells to CXCL13 [25]. BAFF actually processes more potent effect on memory B cells to CXCL13 than that of naive B cells [25]. Transgenic mice over-expressing the BAFF exhibited impaired B cell tolerance and altered T cell differentiation for the development of an autoimmune disorder similar to SLE [26]. In order to confirm the immunopathological roles of CXCL13 and characterize the T<sub>FH</sub> dysregulation in SLE, the plasma concentrations of CXCL13, BAFF, T<sub>FH</sub>-cells-related cytokine IL-21, and cell surface expression of T<sub>FH</sub>-related receptor CXCR5 and IL-21R on CD4<sup>+</sup>Th and CD19<sup>+</sup> B cells were investigated in the present study.

## Materials and Methods

### SLE Patients, Control Subjects, and Blood Samples

Thirty-five Chinese SLE patients (31 females and four males) were recruited at the Rheumatology Out-patient Clinic of the Prince of Wales Hospital, Hong Kong. Diagnosis of SLE was established according to the 1982 revised American Rheumatism Association criteria [27], and disease activity was evaluated by the SLE disease activity index (SLEDAI) score [28]. Twenty-three sex- and age-matched healthy Chinese volunteers were recruited as controls (NC group). Twelve milliliters of EDTA venous peripheral blood were collected from each patient and control subject. The above protocol was approved by the Clinical Research Ethics Committee of The Chinese University of Hong Kong New Territories East Cluster Hospitals, and informed consent was obtained from all participants according to the Declaration of Helsinki.

### Assay of Human CXCL13, BAFF, and IL-21

Concentrations of CXCL13 and BAFF, and IL-21 in plasma were measured by enzyme-linked immunosorbent assay (ELISA) using Quantikine colorimetric reagent kit (R&D Systems, MN, USA) and Bender MedSystems GmbH, Vienna, Austria, respectively.

### Quantitative PCR of BCL-6 Gene Expression

Total RNA of peripheral blood mononuclear cells (PBMC) was extracted by RNeasy Mini Kit (Qiagen Inc., Canada), following manufacturer's instruction. All specimens were

pre-treated with deoxyribonuclease I (Invitrogen Corp., CA, USA) and then stored at  $-70^{\circ}\text{C}$ . For each reaction, approximately 0.5  $\mu\text{g}$  of total RNA was reversely transcribed to complementary DNA (cDNA) with TaqMan Reverse Transcription Reagents (Applied Biosystems Inc., CA, USA). The mRNA expression of BCL-6 and  $\beta$ -actin (endogenous control) in SLE patients' PBMC was quantified by real-time polymerase chain reaction (PCR) with the use of Applied Biosystems 48-well StepOne™ Real Time PCR System. The primers and probes for human BCL-6 and  $\beta$ -actin were purchased from Applied Biosystems for TaqMan gene expression assay. Real-time PCR was performed in a 20- $\mu\text{L}$  reaction mixture containing TaqMan Fast Universal PCR Master Mix (Applied Biosystems), TaqMan  $\beta$ -actin/BCL-6 Detection Reagent and cDNA sample. The real-time PCR reaction was performed at fast mode:  $95^{\circ}\text{C}$  for 1 s to denature cDNA and  $60^{\circ}\text{C}$  for 20 s to allow the TaqMan probe and primers to anneal to the denatured cDNA. The cycles were repeated 30 times after an initial 20 s denaturation at  $95^{\circ}\text{C}$ . A relative BCL-6 mRNA expression was obtained by comparing the relative expression of BCL-6 and  $\beta$ -actin.

Multiparametric Flow Cytometric Analysis of CXCR5 and IL-21R on CD4+Th and CD19+B Cells

Peripheral blood mononuclear cells were prepared by the centrifugation of blood using a density gradient (Ficoll-Paque Plus; GE Healthcare Corp., NJ, USA). Cells were incubated with fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CXCR5 monoclonal antibody (mAb; R&D Systems) or unconjugated mouse anti-human IL-21R mAb (R&D Systems) followed with secondary FITC-conjugated goat anti-mouse immunoglobulin (Ig) antibody (Invitrogen). The immunophenotyping of CD4+Th cells and CD19+B cells was labeled with phycoerythrin (PE)-conjugated anti-CD4 and allophycocyanin (APC)-conjugated anti-CD19 antibody (Becton Dickinson Pharmingen Corp., CA, USA). PE, FITC, or APC-conjugated mouse IgG (BD Pharmingen) was used as isotypic control accordingly. Cell surface expression of CXCR5 and IL-21R on CD4+Th and CD19+B lymphocytes was analyzed by flow cytometry as presented in mean fluorescence intensity (MFI) on 5,000 viable cells (BD FACSCalibur).

Statistical Analysis

Numerical data were expressed as median (interquartile range, IQR) if they were not in Gaussian distribution. Difference in plasma concentration among groups was compared with Mann–Whitney rank sum test. Spearman's rank correlation test was used to assess the correlation of plasma chemokines and cytokine with SLEDAI score.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, Version 16.0 (SPSS Inc, IL, USA). Probability values ( $p$ ) < 0.05 were considered as significant.

Results

SLE Patients and Control Subjects

The age, sex, SLEDAI score, duration of diagnosis, and drug treatment of the study groups are summarized in Table 1.

Elevated Plasma Concentrations of CXCL13, BAFF, and IL-21 in SLE Patients

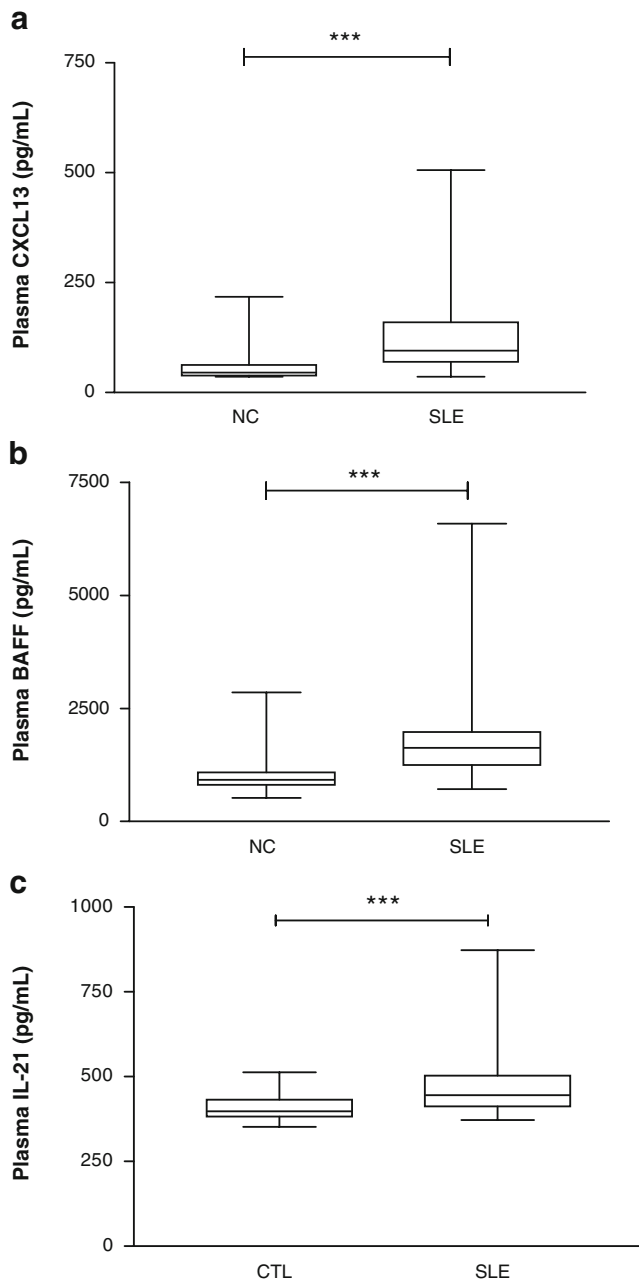
As shown in Fig. 1a and b, plasma CXCL13 [median (interquartile range), SLE, 95.3 (69.9–159.4) vs control, 45.3 (38.3–63.1) pg/mL,  $p < 0.0001$ ] and BAFF [SLE, 1,627 (1,248–1,981) vs control, 923 (809.5–1,087) pg/mL,  $p < 0.0001$ ] concentration was significantly higher in SLE patients than in control subjects. Similarly, plasma  $T_{\text{FH}}$  cytokine IL-21 concentration was also significantly higher

Table 1 Characteristics of SLE Patients and Control Subjects

	SLE	NC
Number	35	23
Sex (female/male)	31/4	23/0
Age, year, mean $\pm$ SD	41 $\pm$ 10	46 $\pm$ 7
Range	21–65	25–57
Duration of diagnosis, year, mean $\pm$ SD	12 $\pm$ 7	NA
Range	0.49–30	
SLEDAI score, mean $\pm$ SD	3.1 $\pm$ 3	NA
Range	0–12	
Anti-dsDNA titer, mean $\pm$ SD	391.7 $\pm$ 327.3	NA
Range	0–1,280	
Treatment with prednisolone		
Patients, no. (%)	33 (94.3)	
Daily dose, mg, mean $\pm$ SD	14.1 $\pm$ 55.6	NA
Range	2.5–500	
Treatment with hydroxychloroquine		
Patients, no. (%)	11 (31.4)	
Daily dose, mg, mean $\pm$ SD	217.3 $\pm$ 56.8	NA
Range	20–300	
Treatment with azathioprine		
Patients, no. (%)	12 (34.3)	
Daily dose, mg, mean $\pm$ SD	63.9 $\pm$ 27.2	NA
Range	25–100	

Values in mean  $\pm$  SD (range)

NA not applicable



**Fig. 1** Plasma CXCL13, BAFF, and IL-21 concentrations of SLE and normal control subjects. The Mann–Whitney rank sum test was used to assess the differences of concentration of **a** CXCL13, **b** BAFF, and **c** IL-21 between the SLE and NC groups. Results are presented as *box-and-whisker plots* showing the median (IQR). Whitney rank sum test was used to assess the differences of concentration between patients and control subjects. \*\*\* $p<0.001$

in SLE patients than in control subjects [SLE, 445.4 (411.8–503.1) vs control, 398.0 (382.2–432.2) pg/mL;  $p<0.001$ ] (Fig. 1c). Table 2 shows that there was a significant and positive correlation between CXCL13 concentration and SLEDAI score in SLE patients ( $r=0.399$ ,  $p=0.032$ ). However, there were no significant correlations between plasma

**Table 2** Correlation Between Plasma Concentrations of CXCL13, BAFF, and IL-21 and SLEDAI Score

	<i>r</i> value	<i>p</i> value
CXCL13	0.399	0.032*
BAFF	–0.236	0.279
IL-21	0.234	0.190

Spearman’s rank correlation test was used to assess the correlations.  $r$  = Spearman’s correlation coefficient

\* $p<0.05$

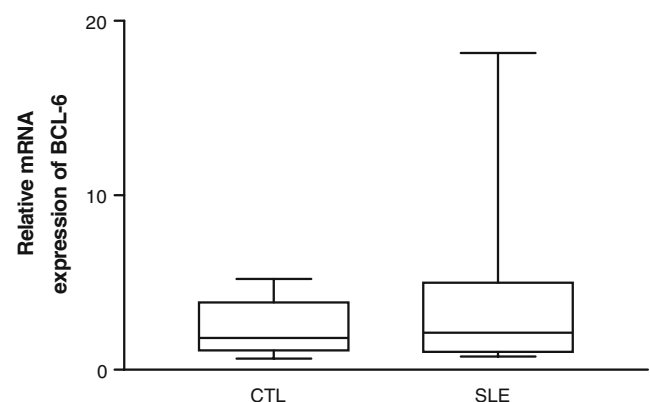
BAFF or IL-21 concentrations with SLEDAI score in SLE patients (Table 2, both  $p>0.05$ ).

#### No Difference of mRNA Expression of BCL-6 in PBMC

Since BCL-6 is the transcription factor for  $T_{FH}$  cells development [12], we used TaqMan quantitative PCR method for the assay of the mRNA expression of BCL-6 in PBMC from SLE patients and control subjects. As shown in Fig. 2, there was no significant difference of BCL-6 gene expression between SLE subjects and control subjects ( $p>0.05$ ).

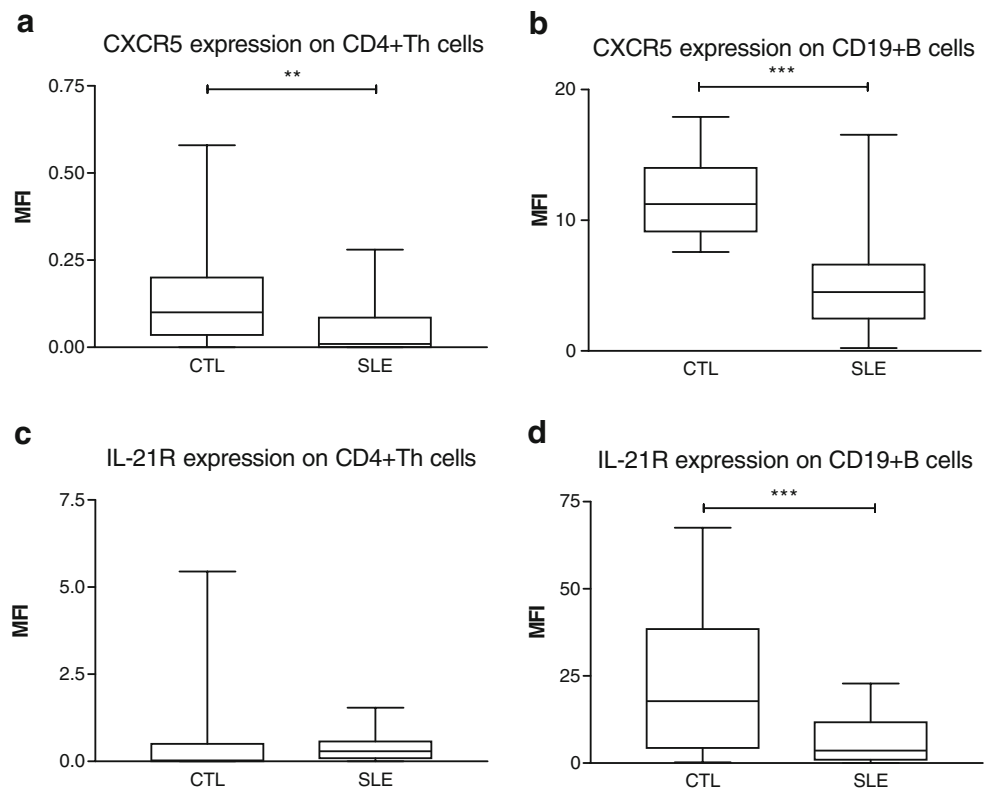
#### Down-Regulated Cell Surface Expression of CXCR5 and IL-21R on Th Cells and B Cells

Since we found that B cell chemokine CXCL13 and  $T_{FH}$  cytokine IL-21 was significantly higher in SLE than controls, it prompts us to evaluate the expression of receptors for CXCL13 and IL-21 on their target cells. As shown in Fig. 3, the cell surface expression of CXCR5 on



**Fig. 2** BCL-6 expression in SLE patients and control subjects. Total RNA was extracted from PBMC and subjected for the quantitative PCR assay of the relative mRNA expression of BCL-6. Results are presented as *box-and-whisker plot* showing the median (IQR). Whitney rank sum test was used to assess the differences of concentration between patients and control subjects

**Fig. 3** Expression of CXCR5 and IL-21R on CD4+Th cells and B cells in SLE patients and control subjects. The expression of CXCR5 and IL-21R are shown as MFI subtracting corresponding isotypic controls with *box-and-whisker plot* showing the median (IQR). Mann–Whitney rank sum test was used to assess the differences of concentration between patients and control subjects. \*\* $p < 0.01$ , \*\*\* $p < 0.001$



Th cells and B cells and IL-21R on B cells was significantly down-regulated in SLE patients while comparing with controls [median (interquartile range), CXCR5 on Th cells, SLE, 0.01 (0.00–0.09) vs controls, 0.1 (0.04–0.20); CXCR5 on B cells, SLE, 4.5 (2.48–6.61) vs controls, 11.2 (9.25–14.02); IL-21R on B cells, SLE, 3.6 (0.98–11.71) vs controls, 17.8 (4.33–38.44), all  $p < 0.01$ ]. However, the expression of IL-21R on Th cells did not have any significant difference between SLE and control subjects [SLE, 0.29 (0.09–0.57) vs controls, 0.03 (0.0–0.5),  $p > 0.05$ ].

## Discussion

SLE has been postulated to be an autoantibody, immune complex, and Th cytokine-mediated disease [29]. Previous studies demonstrated that B cell chemokine CXCL13 is ectopically and highly expressed in thymus and kidney in murine model for SLE [23]. In the present study, we have confirmed the significant increase in plasma concentration of CXCL13 in SLE patients and the elevated plasma concentration of CXCL13 correlated significantly with SLE disease severity (Fig. 1 and Table 2). The results are in concordance with the recent publication of the elevation of serum CXCL13 levels in SLE patients with different ethnic group [30]. Apart from SLE, previous study has demonstrated the elevation of CXCL13 in active rheuma-

toid arthritis (RA) patients comparing with quiescent disease and healthy controls, correlating with Disease Activity Score in 28 joints [31]. Anti-TNF treatment was found to reduce the plasma level of CXCL13 in RA patients [31]. Therefore, CXCL13 can act as a disease activity marker for both RA and SLE patients. Production of CXCL13 is not higher in other immunological disorders such as allergic asthma and psoriasis; this indicates the specific role of the elevated CXCL13 concentration in autoimmune disease [32].

Our present study also confirmed that the expression of BAFF increases in SLE, thereby suggesting the pathogenic role of BAFF in SLE by stimulating T-cell-dependent B cell production of autoantibodies [33, 34]. BAFF is actually a fundamental survival factor for transitional and mature B cells and its overexpression leads to an expanded B cell compartment and autoimmunity in mice, and elevated serum concentration of BAFF can be found in autoimmune patients with lupus and rheumatoid arthritis [34]. Since BAFF can increase the chemotactic response of primary and memory human B cells to CXCL13 [25], the synergy between the elevated CXCL13 and BAFF produced by stromal cells and follicular dendritic cells can have important implications for B cell activation in SLE [25]. Elevated plasma IL-21 in SLE is produced by  $T_{FH}$  cells for the germinal centers formation [10]. The central role of IL-21 in germinal centers formation actually related to its

effects on  $T_{FH}$  cell generation [10]. Recent animal study has revealed that elevated production of IL-21,  $T_{FH}$  dysfunction within germinal centers and aberrant positive selection of germinal center B cells are required for the production of autoantibodies and systemic autoimmunity [35, 36]. Therefore, our results of the elevated CXCL13, BAFF, and IL-21 may relate with the immunopathogenesis mediated by the function of  $T_{FH}$  cells in autoimmune disease. As also observed by us, the plasma CXCL13, BAFF, and IL-21 concentrations in SLE patients did not show any correlation with the dosages of prednisolone, hydroxychloroquine, and azathioprine. Although we could not find the correlation between the plasma CXCL13, BAFF, and IL-21 concentrations and serum autoantibody titers, the concentration of autoantibody could be highly produced at lymphoid tissues in SLE patients with elevated plasma CXCL13 concentration [12, 13]. Nevertheless, Schiffer et al. have recently reported that serum CXCL13 concentration correlated with dsDNA titer in SLE patients with active disease [30].

Since our present study cohorts only recruited SLE patients with relatively inactive disease (SLEDAI mean  $\pm$  SD,  $3.1 \pm 3$ ) without end-organ manifestation, the correlation of CXCL13 levels with end-organ disease manifestations such as renal or central nervous system (CNS) lupus were not studied. However, previous animal studies have indicated that CXCL13 is increased in aged kidney of SLE mouse model [22]. Moreover, aberrant CXCL13 can induce the formation of ectopic lymphoid tissues in non-lymphoid organs and related to the accumulation of inflammatory cells in kidneys in SLE [22, 37]. Recent clinical study also confirmed that CXCL13 concentrations were higher in SLE patients with nephritis than SLE patients without nephritis [30]. Although CXCL13 plays a role in experimental autoimmune encephalomyelitis of animal model of multiple sclerosis [38], the correlation of CXCL13 level and CNS lupus activity in patient study has not been reported.

Tfh cells retain intense expression of CXCR5, which directs these cells toward CXCL13 localized areas within germinal centers [9]. We observed that the cell surface expression of  $T_{FH}$ -related CXCR5 and IL-21R was significantly down-regulated on Th and B cells in SLE patients, it may indicate that most differentiated  $T_{FH}$  cells can migrate out from the circulation and localize in the lymphoid organ upon activation during the disease development of SLE. Elevated lymphoid tissue homing chemokine CXCL13 and B cell survival factor BAFF actually synergistically enhance the chemotactic response of B cells and CD4+ CXCR5+ T cells to the lymphoid follicles and CXCL13 can be produced by follicular dendritic cells at germinal center [39, 40]. Activated  $T_{FH}$  cells upon migration to the B cell

follicles can interact with antigen-primed B cells that have specifically migrated outward. This physical interaction then facilitates CD40-CD40L-dependent B cell activation [12]. As a result, it may account for the decreased circulating number of CXCR5+IL-21R+ $T_{FH}$  cells in SLE in our study. We could not detect any significant difference of transcription factor BCL-6 gene expression in PBMC between SLE and control subjects. Although BCL-6 is preferentially expressed by  $T_{FH}$  cells but not Th1 or Th2 cells for the development of  $T_{FH}$  cells [12], only 10–15% of CD4+T cells in germinal center at follicles appear to express BCL-6 protein [41]. This may account for the non-significant difference of BCL-6 expression in PBMC between SLE and control subjects.

In view of the recent development and clinical trials of monoclonal antibody such as belimumab against BAFF that effectively deplete pathogenic B cells and target pathways essential for B-cell-dependent autoantibody-mediated autoimmune response, B-cell-directed therapies represent promising treatments for autoimmune disorders [42, 43]. Using murine model, neutralization of IL-21 by IL-21R-Fc fusion protein treatment can minimally attenuate SLE disease progression [44], and anti-CXCL13 treatment inhibit the development of collagen-induced arthritis and reduce follicular response in both lymphoid and non-lymphoid tissues [45]. In conjunction with above studies, our results of the pathological role of elevated production of  $T_{FH}$ -related CXCL13, BAFF, and IL-21 in SLE therefore provides further evidences that the human pathological  $T_{FH}$  and B cell responses can be suppressed by targeted approaches including the anti-B cell activating factor (BAFF and CXCL13) or anti-IL-21 for the treatment of SLE and other autoimmune diseases. Moreover, circulating concentration of CXCL13, the signature molecule of  $T_{FH}$  cells, may act as a surrogate disease marker of SLE.

**Conflicts of Interest** No conflict of interest has been declared by the authors.

## References

1. Heinlen LD, McClain MT, Merrill J, et al. Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. *Arthritis Rheum.* 2007;56:2344–51.
2. Kotzin BL. Systemic lupus erythematosus. *Cell.* 1996;85:303–6.
3. Viillard JF, Pellegrin JL, Ranchin V, et al. Th1 (IL-2, interferon-gamma (IFN-gamma)) and Th2 (IL-10, IL-4) cytokine production by peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol.* 1999; 115:189–95.

4. Ansel KM, Harris RBS, Cyster JG. CXCL13 is required for B1 cell homing, natural antibody production, and body cavity immunity. *Immunity*. 2002;16:67–76.
5. Visser JL, Hartgers FC, Lindhout E, Figdor CG, Adema GJ. BLC (CXCL13) is expressed by different dendritic cell subsets in vitro and in vivo. *Eur J Immunol*. 2001;31:1544–9.
6. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med*. 1998;187:655–60.
7. Ansel KM, Ngo VN, Hyman PL, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature*. 2000;406:309–14.
8. Schaerli P, Willmann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med*. 2000;192:1553–62.
9. Breitfeld DL, Ohl L, Kremmer EK, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med*. 2000;192:1545–51.
10. Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. *Immunity*. 2008;29:127–37.
11. Fazilleau N, Mark L, McHeyzer-Williams LJ, McHeyzer-Williams MG. Follicular helper T cells: lineage and location. *Immunity*. 2009;30:324–35.
12. King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. *Annu Rev Immunol*. 2008;26:741–66.
13. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. *Nat Rev Immunol*. 2005;5:853–65.
14. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu Rev Immunol*. 2007;26:57–79.
15. Nurieva RI, Chung Y, Hwang D, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity*. 2008;29:138–49.
16. Sawalha AH, Kaufman KM, Kelly JA, et al. Genetic association of interleukin-21 polymorphisms with systemic lupus erythematosus. *Ann Rheum Dis*. 2008;67:458–61.
17. Hayakawa K, Hardy RR. Development and function of B-1 cells. *Curr Opin Immunol*. 2000;12:346–53.
18. Sato T, Ishikawa S, Akadegawa K, et al. Aberrant B1 cell migration into the thymus results in activation of CD4 T cells through its potent antigen-presenting activity in the development of murine lupus. *Eur J Immunol*. 2004;34:3346–58.
19. Dauphinee M, Tovar Z, Talal N. B cells expressing CD5 are increased in Sjogren's syndrome. *Arthritis Rheum*. 1988;31:642–7.
20. Plater-Zyberk C, Maini RN, Lam K, Kennedy TD, Janosy G. A rheumatoid arthritis B cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. *Arthritis Rheum*. 1985;28:971–6.
21. Ito T, Ishikawa S, Sato T, et al. Defective B1 cell homing to the peritoneal cavity and preferential recruitment of B1 cells in the target organs in a murine model for systemic lupus erythematosus. *J Immunol*. 2004;172:3628–34.
22. Ishikawa S, Sato T, Abe M, et al. Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) by C11b+CD11c+ dendritic cells in murine lupus and preferential chemotaxis of B1 cells towards BLC. *J Exp Med*. 2001;193:1393–402.
23. Ishikawa S, Matsushima K. Aberrant B1 cell trafficking in a murine model for lupus. *Front Biosci*. 2007;12:1790–803.
24. Batten M, Groom J, Cachero TG, et al. BAFF mediates survival of peripheral immature B lymphocytes. *J Exp Med*. 2000;192:1453–66.
25. Badr G, Borhis G, Lefevre EA, et al. BAFF enhances chemotaxis of primary human B cells: a particular synergy between BAFF and CXCL13 on memory B cells. *Blood*. 2008;111:2744–54.
26. Groom JR, Fletcher CA, Walters SN, et al. BAFF and MyD88 signals promote a lupuslike disease independent of T cells. *J Exp Med*. 2007;204:1959–71.
27. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1982;25:1271–7.
28. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. The Committee on Prognosis studies in SLE: Derivation of the SLEDAI: a disease activity index for lupus patients. *Arthritis Rheum*. 1992;35:630–40.
29. Mohan C, Adams S, Stanik V, Datta SK. Nucleosome: a major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J Exp Med*. 1993;177:1367–81.
30. Schiffer L, Kumpers P, Davalos-Misslitz AM et al. B-cell-attracting chemokine CXCL13 as a marker of disease activity and renal involvement in systemic lupus erythematosus (SLE). *Nephrol Dial Transplant*. 2009 doi:10.1093/ndt/gfp343
31. Rioja I, Hughes FJ, Sharp CH, et al. Potential novel biomarkers of disease activity in rheumatoid arthritis patients: CXCL13, CCL23, transforming growth factor alpha, tumor necrosis factor receptor superfamily member 9, and macrophage colony-stimulating factor. *Arthritis Rheum*. 2008;58:2257–67.
32. Fulkerson PC, Zimmermann N, Hassman LM, Finkelman FD, Rothenberg ME. Pulmonary chemokine expression is coordinately regulated by STAT1, STAT6, and IFN-gamma. *J Immunol*. 2004;173:7565–74.
33. Morimoto S, Nakano S, Watanabe T, et al. Expression of B-cell activating factor of the tumour necrosis factor family (BAFF) in T cells in active systemic lupus erythematosus: the role of BAFF in T cell-dependent B cell pathogenic autoantibody production. *Rheumatology*. 2007;46:1083–6.
34. Mackay F, Schneider P, Rennert P, Browning J. BAFF AND APRIL: a tutorial on B cell survival. *Annu Rev Immunol*. 2003;21:231–64.
35. Bubier JA, Sproule TJ, Foreman O, et al. A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSb-Yaa mice. *Proc Natl Acad Sci U S A*. 2009;106:1518–23.
36. Linterman MA, Rigby RJ, Wong RK, et al. Follicular helper T cells are required for systemic autoimmunity. *J Exp Med*. 2009;206:561–76.
37. Adalid-Peralta L, Mathian A, Tran T, Delbos L, Durand-Gasselini I, Berrebi D, et al. Leukocytes and the kidney contribute to interstitial inflammation in lupus nephritis. *Kidney Int*. 2008;73:172–80.
38. Bagaeva LV, Rao P, Powers JM, Segal BM. CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis. *J Immunol*. 2006;176:7676–85.
39. Shi K, Hayashida K, Kaneko M, et al. Lymphoid chemokine B cell-attracting chemokine-1 (CXCL13) is expressed in germinal center of ectopic lymphoid follicles within the synovium of chronic arthritis patients. *J Immunol*. 2001;166:650–5.
40. Magliozzi R, Columba-Cabezas S, Serafini B, Aloisi F. Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 2004;148:11–23.
41. Ree HJ, Kadin ME, Kikuchi M, Ko YH, Suzumiya J, Go JH. Bcl-6 expression in reactive follicular hyperplasia, follicular lymphoma, and angioimmunoblastic T-cell lymphoma with hyperplastic germinal centers: heterogeneity of intrafollicular T-cells and their

- altered distribution in the pathogenesis of angioimmunoblastic T-cell lymphoma. *Hum Pathol.* 1999;30: 403–11.
42. Sabahi R, Anolik JH. B-cell-targeted therapy for systemic lupus erythematosus. *Drugs.* 2006;66:1933–48.
  43. Sun J, Li Z, Feng J, Li Y, Shen B. BAFF-targeting therapy, a promising strategy for treating autoimmune diseases. *Eur J Pharmacol.* 2008;597:1–5.
  44. Bubier JA, Bennett SM, Sproule TJ, et al. Treatment of BXSB-Yaa mice with IL-21R-Fc fusion protein minimally attenuates systemic lupus erythematosus. *Ann N Y Acad Sci.* 2007;1110: 590–601.
  45. Zheng B, Ozen Z, Zhang X, et al. CXCL13 neutralization reduces the severity of collagen-induced arthritis. *Arthritis Rheum.* 2005; 52:620–6.