Interleukin 21 Correlates with T Cell and B Cell Subset Alterations in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by alterations of the B cell subset, global regulatory T cell (Treg) depletion, and an increase in Th17 cells. Interleukin 21 (IL-21) plays a critical role in T cell and B cell homeostasis. Our objective was to determine the implication of IL-21 and IL-21-producing CD4+ T cells in the pathogenesis of SLE.

Methods. Twenty-five patients with SLE and 25 healthy donor controls were included. Analysis of CD4+ T cells producing IL-21, Th1, Th2, Th17, Treg, follicular helper T (TFH) cells, and B cells was performed in peripheral blood, and levels of cytokines were measured in culture supernatants.

Results. Circulating CD4+ T cells producing IL-21 were markedly expanded in patients with SLE compared to controls and were correlated with increased Th17, decreased Treg, and increased memory B cells. CD4+ T cells producing IL-21 were composed of CXCR5+ and CXCR5–CD4+ T cell subsets. Both IL-21-producing CXCR5+CD4+ T cells and CXCR5–CD4+ T cells were increased in patients with SLE, the CXCR5–CD4+ subset correlating with Th17 cells and Treg, while the CXCR5+CD4+ subset was correlated with alterations of the B cell subset. The CXCR5+CD4+ subset comprised mainly circulating Bcl6+CXCR5+CD4+ TFH cells that were markedly expanded in patients with SLE and were correlated with increased circulating Bcl6+CXCR5+ germinal center B cells.

Conclusion. These findings suggest that IL-21, produced by distinct cellular CD4+ T cells, correlates with alterations of T cell and B cell subsets in SLE, and that targeting IL-21 could provide beneficial effects on both T cell and B cell alterations. (First Release Aug 1 2012; J Rheumatol 2012;39:1819–28; doi:10.3899/jrheum.120468)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS INTERLEUKIN 21 REGULATORY T CELLS
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Systemic lupus erythematosus (SLE) is a disorder characterized by involvement of skin, joints, serositis, central nervous system, and kidney. Therapeutic management is based on the type and severity of organ involvement and includes nonsteroidal antiinflammatory drugs, hydroxychloroquine, corticosteroids, and immunosuppressive agents[1]. However, longterm corticosteroids and/or immunosuppressive agents remain associated with morbidity and mortality[2]. SLE is a T and B cell-dependent autoimmune disease characterized by the appearance of a variety of autoantibodies, some of which are pathogenic[1,3]. T cells are needed to initiate and sustain the secretion of antibodies, in particular to histones and double-stranded DNA[4]. SLE is also associated with major alterations of blood B cell subsets[5,6], global depletion of T regulatory cells (Treg)[7], an increase in Th lymphocytes producing interleukin 17 (Th17 cells)[8,9], and increased expression of interferon (IFN)-inducible genes[10]. Although studies have contributed to understanding of the pathophysiology of SLE, data are lacking on cytokines and/or cellular subpopulations that could drive these T cell and B cell subset alterations and could represent potential targets for novel therapy.

IL-21, the most recently identified member of the type 1

cytokine family[11], was suggested to be involved in the pathogenesis of SLE. IL-21 is produced mainly by activated CD4+ T cells but targets a much broader range of cells[12]. IL-21 was shown to potently induce Th17 differentiation and suppress FoxP3 expression[13,14,15], supporting the damaging effect of IL-21 on Th17 cell and Treg balance. Besides its role on T cells, IL-21 was shown to stimulate the differentiation of human B cells, and to take part in promoting B cell activation and expansion, class-switch recombination, plasma cell differentiation, and immunoglobulin (Ig) production during CD4+ T cell-dependent B cell responses[16,17]. Thus, IL-21 could represent a key cytokine modulating T cell and B cell responses in SLE.

We observed that IL-21 produced by distinct cellular CD4+ T cells correlates with alterations of T cell and B cell subsets in SLE. Circulating CD4+ T cells producing IL-21 are markedly expanded in patients with SLE compared to controls, and correlate with Th17, Treg, and IgG class-switched memory B cells. Both follicular helper CD4+ T (TFH) cells and non-TFH CD4+ T cells producing IL-21 are increased in patients with SLE. The expansion of CD4+ TFH cells correlates with the expansion of circulating Bcl6+ germinal center B cells, while the expansion of non-TFH CD4+ T cells correlates with increase of Th17. These findings suggest that targeting IL-21 could provide beneficial effects on perturbations of both T cell and B cell homeostasis.

MATERIALS AND METHODS

Study population. The study population consisted of 25 SLE patients fulfilling at least 4 of the 1997 American College of Rheumatology criteria for SLE[18]. Blood samples from 25 healthy donor controls were obtained from Etablissement Français du Sang (Hôpital Pitié-Salpêtrière, Paris). The study was approved by our institutional ethics review board and was performed according to the Declaration of Helsinki. All donors gave informed consent.

For each patient, demographic, clinical, and biological characteristics were recorded. Disease activity was assessed using the Safety of Estrogens in Lupus Erythematosus National Assessment — Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI)[19,20]. Routine measures were used to determine antinuclear antibodies by indirect immunofluorescence on HEp2 cells (Immunoconcepts, Sacramento, CA, USA), titers of anti-dsDNA and anti-nucleosome antibodies by ELISA, and complement C3 and C4 levels.

Analysis of cytokine production. Peripheral blood mononuclear cells (PBMC) from patients with SLE and controls were stimulated 4 h with 50 ng/ml phorbol myristate acetate and 1 mM ionomycin (Sigma-Aldrich, Lyon, France) in the presence or absence of brefeldin A (BD Pharmingen, Le Pont de Claix, France). Cells cultured in the presence of brefeldin A were stained for cell-surface markers then permeabilized with Cytofix/Cytoperm buffer (BD Pharmingen) and stained with IFN-γ-FITC, IL-4-FITC (BD Pharmingen), IL-17A-Alexa Fluor 647 (eBiosciences, Paris, France), and IL-21-Alexa Fluor 647 (Biolegend, Saint-Quentin en Yvelines, France). Data were acquired using a Navios flow cytometer and analyzed with the Navios software (Beckman Coulter, Villepinte, France). Culture supernatants in the absence of brefeldin A were harvested and immediately frozen at −80°C. Quantitative determination of IL-21 (Biolegend) was performed by ELISA.

Flow cytometric analysis. Counts of PBMC subsets (cells/μl) were established from fresh blood samples using Cyto-Stat tetraChrome kits with Flowcount fluorescents beads as internal standard and tetra CXP software with a Navios cytometer according to manufacturer's instructions (Beckman Coulter). PBMC were also stained with the following conjugated monoclonal antibodies, at predetermined optimal dilutions, for 30 min at 4°C: CD3-ECD, CD4-PCy7, CD4-ECD, CD8-PCy7, CD8-APC, CD10-APC, CD16-FITC, CD19-ECD, CD27-PE, CD28-FITC, CD45RO-FITC, CD45RA-APC, CD56-PE, HLA-DR-PCy7 (Beckman Coulter), CD25-PE, CD27-APC, CD38-PCy7, CD56-FITC, CD62L-FITC, CD95-FITC, CD95-APC, IgD-FITC, IgG-APC (BD Pharmingen), CCR7-PE, and IL-21R-APC (R&D Systems), CD127-FITC (eBioscience), and CXCR5-PCy5.5 (Biolegend). Intracellular detection of FoxP3 (eBioscience) and Bcl-6 (R&D Systems) was performed on fixed and permeabilized cells using appropriate buffer (eBioscience). Cell acquisition and analysis by flow cytometry were performed using a Navios cytometer (Beckman Coulter). Data were analyzed with CXP software and Kaluza software (Beckman Coulter).

Statistical analyses. Data are presented as mean (SEM) for continuous variables and percentage for qualitative variables. Fisher's exact test was used to compare qualitative variables, and nonparametric Mann–Whitney test to compare continuous variables as appropriate. Correlations were evaluated with the Spearman rank coefficient. A p value < 0.05 was considered significant. Statistical analyses were performed using GraphPad Prism version 4.0 and Instat version 3.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Characteristics of patients with SLE. Patients' baseline characteristics are listed in Table 1. Patients had a median SELENA-SLEDAI of 2 (range 0–8), indicating low disease activity.

CD4+ T cells producing IL-21 are increased in SLE and correlate with increased Th17 cells and decreased Treg. We first assessed the intracellular production of IL-21 by CD4+ T cells using flow cytometry. CD4+ T cells producing IL-21 were markedly expanded in peripheral blood of patients compared to controls (5.6% ± 1.5% vs 1.9% ± 1.0% of CD4+ T cells, respectively; $p < 0.0001$; Figure 1A). This

Table 1. Characteristics of the patients.

Characteristics	n = 25
Age, mean, yrs (SD)	31 ± 8
Female, n (%)	23 (92)
Previous SLE manifestations	
Skin, n (%)	14 (56)
Joints, n (%)	12 (48)
Serous, n (%)	4 (16)
Kidney, n (%)	12 (48)
Central nervous system, n (%)	1 (4)
SELENA-SLEDAI at Day 0, mean (range)	2 (0–8)
Associated treatments at time of study	
Prednisone, n (%)	17 (68)
Prednisone, median, mg/day (range)	5 (0–15)
Hydroxychloroquine, n (%)	21 (84)
Azathioprine, n (%)	2 (8)
Mycophenolate mofetil, n (%)	2 (8)

SLE: systemic lupus erythematoaus; SELENA-SLEDAI: Safety of Estrogens in Lupus Erythematosus National Assessment - Systemic Lupus Erythematosus Disease Activity Index.

expansion of CD4+ T cells producing IL-21 was positively correlated with Th17 cells ($r^2 = 0.17$, $p = 0.04$; Figure 1B) and Th2 cells ($r^2 = 0.34$, $p = 0.002$; Figure 1C), but not with Th1 cells ($r^2 = 0.06$, $p = 0.25$). Conversely, CD4+ T cells producing IL-21 were negatively correlated with CD4+CD25^{hi}CD127−FoxP3+ Treg ($r^2 = 0.14$, $p = 0.06$) and in particular the CD4+CD25+++CD45RA− activated memory Treg subset (amTreg; $r^2 = 0.20$, $p = 0.02$; Figure 1D), while no correlation was found with the CD4+CD25++ CD45RA+ resting Treg subset (rTreg). IL-21-producing CD4+ T cells did not produce significant levels of IL-17A or IFN-γ and displayed the phenotype of the central memory T cell, as indicated by the expression of CD45RO and CD27 (Figure 2). Together, these findings indicated that IL-21 production correlated with perturbations in the Th17/Treg balance.

We next assessed the levels of IL-21 in culture supernatants of PBMC. We found increased levels of IL-21 in

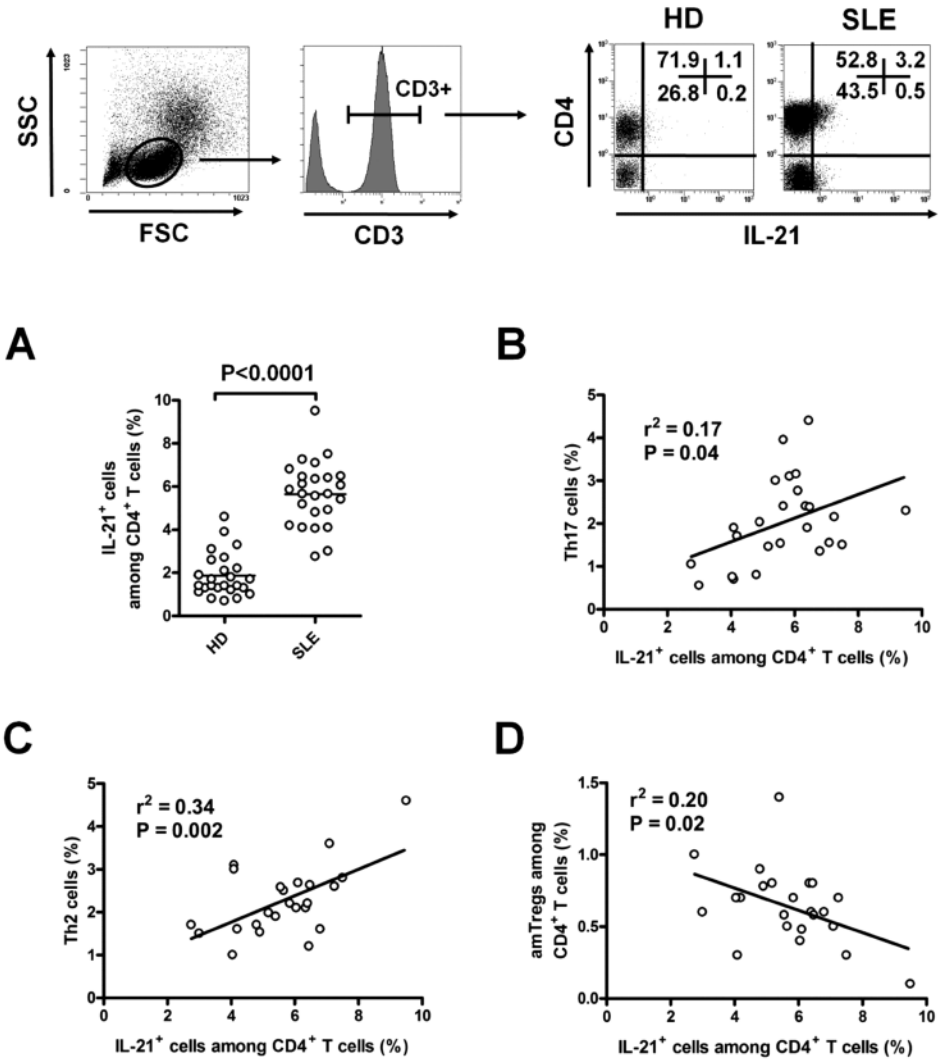

Figure 1. CD4+ T cells producing interleukin 21 (IL-21) are increased in systemic lupus erythematosus (SLE) and correlate with Th17 cells, Th2 cells, and activated memory regulatory T cells (Treg). Peripheral blood mononuclear cells were stimulated for 4 h with phorbol myristate acetate and ionomycin. After gating on CD3+ T cells, frequencies of CD4+ T cells producing IL-21 were analyzed. A. Frequencies of CD4+ T cells producing IL-21 from patients with SLE and controls. A marked enrichment in CD4+ T cells producing IL-21 was noted in patients with SLE compared to controls. B, C, D. Correlations between CD4+ T cells producing IL-21 and CD4+ T cells producing IL-17A (Th17 cells) (B); CD4+ T cells producing IL-4 (Th2 cells) (C); and CD4+CD25+++CD45RA− activated memory Treg (amTreg; D) in patients with SLE. CD4+ T cells producing IL-21 correlate positively with Th17 and Th2 cells and negatively with activated memory Treg. All population percentages shown here represent averages from analysis of 25 patients with SLE and 25 controls. SSC: side scatter data; FSC: forward scatter data; HD: healthy donor controls.

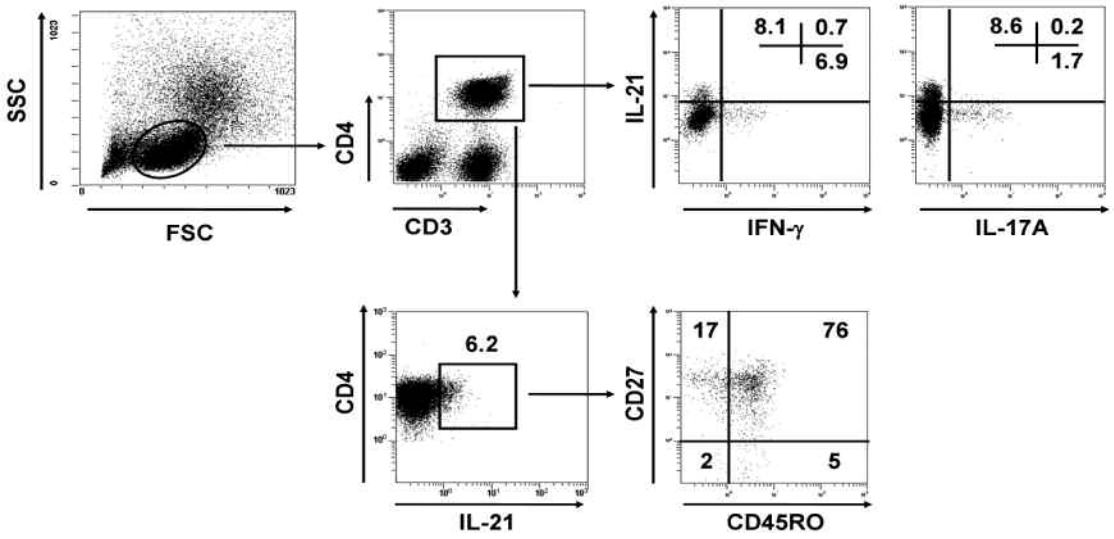

Figure 2. CD4+ T cells producing interleukin 21 (IL-21) did not produce significant levels of IL-17A or interferon-γ (IFN-γ) and displayed phenotype of central memory T cells. Peripheral blood mononuclear cells were stimulated for 4 h with phorbol myristate acetate and ionomycin. After gating on CD3+CD4+ T cells, surface CD27 and CD45RA stainings and intracellular production of IL-21, IL-17A, and IFN-γ were analyzed. Most of the CD4+ T cells producing IL-21 did not produce IL-17A and IFN-γ, and displayed the phenotype of CD27+CD45RO+ central memory T cells. SSC: side scatter data; FSC: forward scatter data.

SLE patients compared to controls (57 ± 64 vs 19 ± 11 pg/ml; p = 0.002).

CD4+ T cells producing IL-21 correlate with increased effector memory and activated CD8+ T cells. CD4+ T cells producing IL-21 tended to be positively correlated with the expansion of the CD62L−CD45RA− effector memory subset in CD8+ T cells ($r^2 = 0.15$, p = 0.06) and were significantly positively correlated with the expression of the HLA-DR activation marker ($r^2 = 0.23$, p = 0.02) in CD8+ T cells. Conversely, CD4+ T cells producing IL-21 tended to be negatively correlated with the CD62L+CD45RA+ naive subset ($r^2 = 0.15$, p = 0.06) in CD8+ T cells.

Expansion of CD4+ T cells producing IL-21 correlates with increased memory B cells. The expansion of CD4+ T cells producing IL-21 was positively correlated with IgG+ class-switched memory CD19+ B cells ($r^2 = 0.19$, p = 0.03). We also assessed correlations between CD4+ T cells producing IL-21 and B cell differentiation using IgD and CD38 expression (Figure 3A). CD4+ T cells producing IL-21 were correlated with circulating Bm2 (IgD+CD38+) + Bm2' (IgD+CD38++) naive B cells ($r^2 = 0.17$, p = 0.04; Figure 3B), early Bm5 (eBm5, IgD-CD38+) + Bm5 (IgD−CD38−) memory B cells ($r^2 = 0.37$, p = 0.001; Figure 3C), and Bm2+Bm2'/eBm5+Bm5 ratio ($r^2 = 0.26$, p = 0.01; Figure 3D). Together, these findings indicated that IL-21 production correlated with B cell subset alterations observed in SLE.

CD4+ T cells producing IL-21 are composed of 2 phenotypically distinct subpopulations according to CXCR5 expression. Because IL-21 was previously shown to be expressed by distinct CD4+ T cell subsets, i.e., CXCR5+CD4+ T cells and CXCR5-CD4+ cells, we analyzed CXCR5 expression on CD4+ T cells from patients with SLE. We observed that 42.0% ± 9.0% of CD4+ T cells producing IL-21 from SLE were CXCR5+CD4+ T cells, while 58.0% ± 9.0% were CXCR5−CD4+ T cells. Compared to controls, both IL-21-producing CXCR5−CD4+ T cells (3.3% ± 1.0% vs 1.7% ± 0.5%; p < 0.0001; Figure 4A) and IL-21-producing CXCR5+CD4+ T cells (2.4% ± 0.9% vs 0.4% ± 0.2%; p < 0.0001; Figure 4B) were increased in patients with SLE, supporting different cellular sources of IL-21 in patients with SLE that may exhibit different functions.

The expansion of IL-21-producing CXCR5−CD4+ T cells was positively correlated with Th17 cells ($r^2 = 0.26$, p = 0.009; Figure 4C) and negatively with CD4+CD25^{hi} CD127−FoxP3+ Treg ($r^2 = 0.20$, p = 0.02; data not shown). In contrast, no correlation was found between IL-21-producing CXCR5+CD4+ T cells and Th17 cells and Treg (data not shown). Conversely, IL-21-producing CXCR5+CD4+ T cells were positively correlated with eBm5+Bm5 memory B cells ($r^2 = 0.36$, p = 0.001; Figure 4D) and negatively with Bm2+Bm2' naive B cells ($r^2 = 0.23$, p = 0.01) and the Bm2+Bm2'/eBm5+Bm5 ratio ($r^2 = 0.21$, p = 0.02; data not shown). In contrast, no correlation was found between IL-21-producing CXCR5−CD4+ T cells and B cell subset alterations (data not shown). These findings showed that the IL-21-producing CXCR5−CD4+ T cell subset correlated with Th17 cells and Treg, while the IL-21-producing

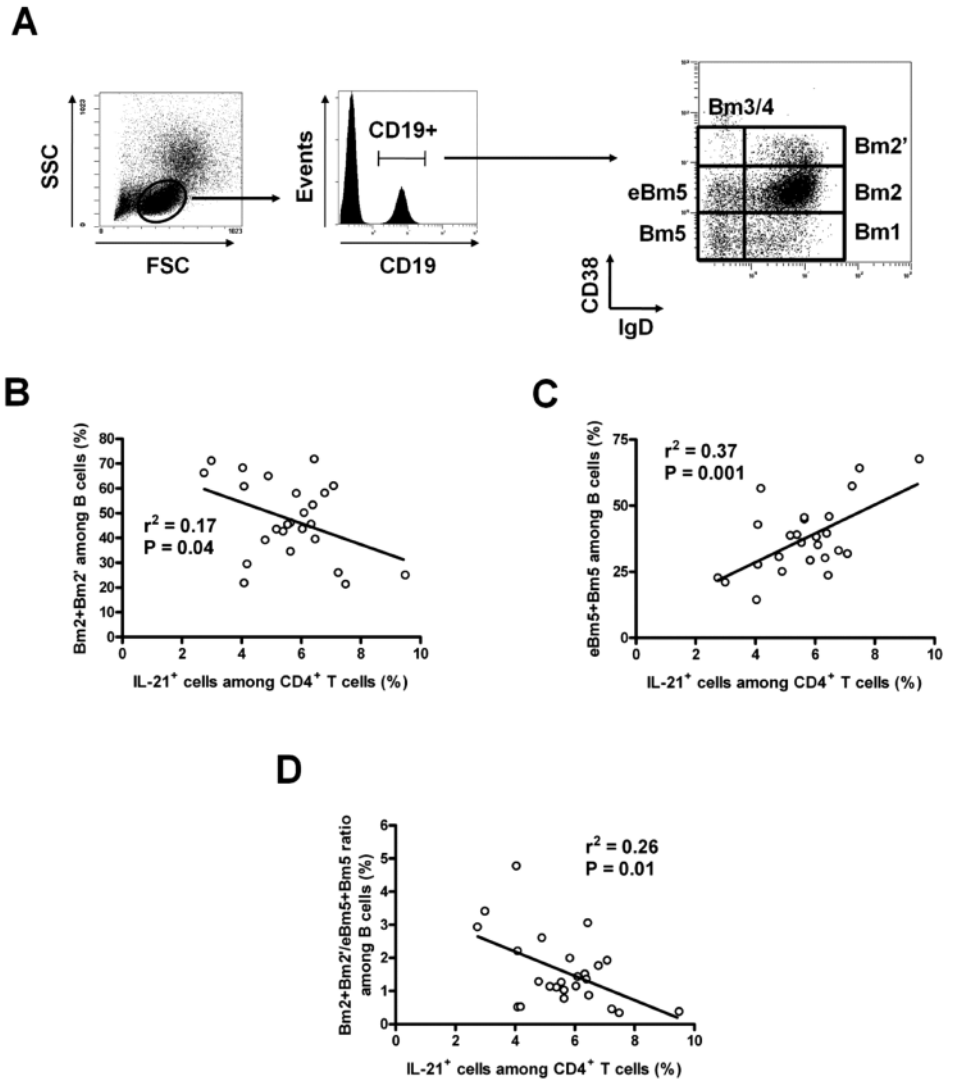

Figure 3. CD4+ T cells producing interleukin 21 (IL-21) correlated with increased memory B cells. Fresh peripheral blood mononuclear cells were stained with anti-CD19, anti-IgD, anti-CD27, anti-CD38, and anti-CD95 (A). After gating on CD19+ T cells, frequencies of Bm2 (IgD+CD38+), Bm2' (IgD+CD38++), early Bm5 (eBm5, IgD-CD38+) and Bm5 (IgD–CD38–) CD19+ B cells were analyzed. Correlations between CD4+ T cells producing IL-21 and Bm2+Bm2' naive B cells (B), eBm5+Bm5 memory B cells (C), and Bm2+Bm2'/eBm5+Bm5 ratio (D) are shown. CD4+ T cells producing IL-21 correlate positively with eBm5+Bm5 memory B cells, and negatively with Bm2+Bm2' naive B cells and Bm2+Bm2'/eBm5+Bm5 ratio. SSC: side scatter data; FSC: forward scatter data; IgD: immunoglobulin D.

CXCR5+CD4+ T cell subset correlated with B cell subset alterations.

Expansion of circulating CXCR5+Bcl6+CD4+ TFH cells correlates with increased circulating germinal center B cells in SLE. TFH are classically defined as CXCR5+Bcl6+ CD4+ T cells, and expressed high levels of IL-21[21]. Because we identified increased IL-21-producing CXCR5+CD4+ T cells in peripheral blood of patients with SLE, we more precisely assessed the circulating CD4+ TFH cell subset in SLE through the characterization of CXCR5 and Bcl6 expression, Bcl6 being the master regulator of TFH differentiation (Figure 5A). We found an increase in proportion (6.4% ± 2.8% vs 2.5% ± 1.0%; $p < 0.0001$) and in absolute number (28.4 ± 19.3 vs 12.6 ± 5.8 cells/μl; $p = 0.0003$) of CXCR5+Bcl6+CD4+ TFH cells in patients with SLE compared to controls (Figures 5A, 5B, 5C). Circulating CXCR5+Bcl6+CD4+ TFH cells also expressed high levels of IL-21 receptor (IL-21R; Figure 5D). The expansion of circulating CXCR5+Bcl6+CD4+ TFH cells was not correlated with disease activity, C3 complement fraction, and anti-dsDNA antibodies (data not shown).

Because CD4+ TFH cells are important for the formation

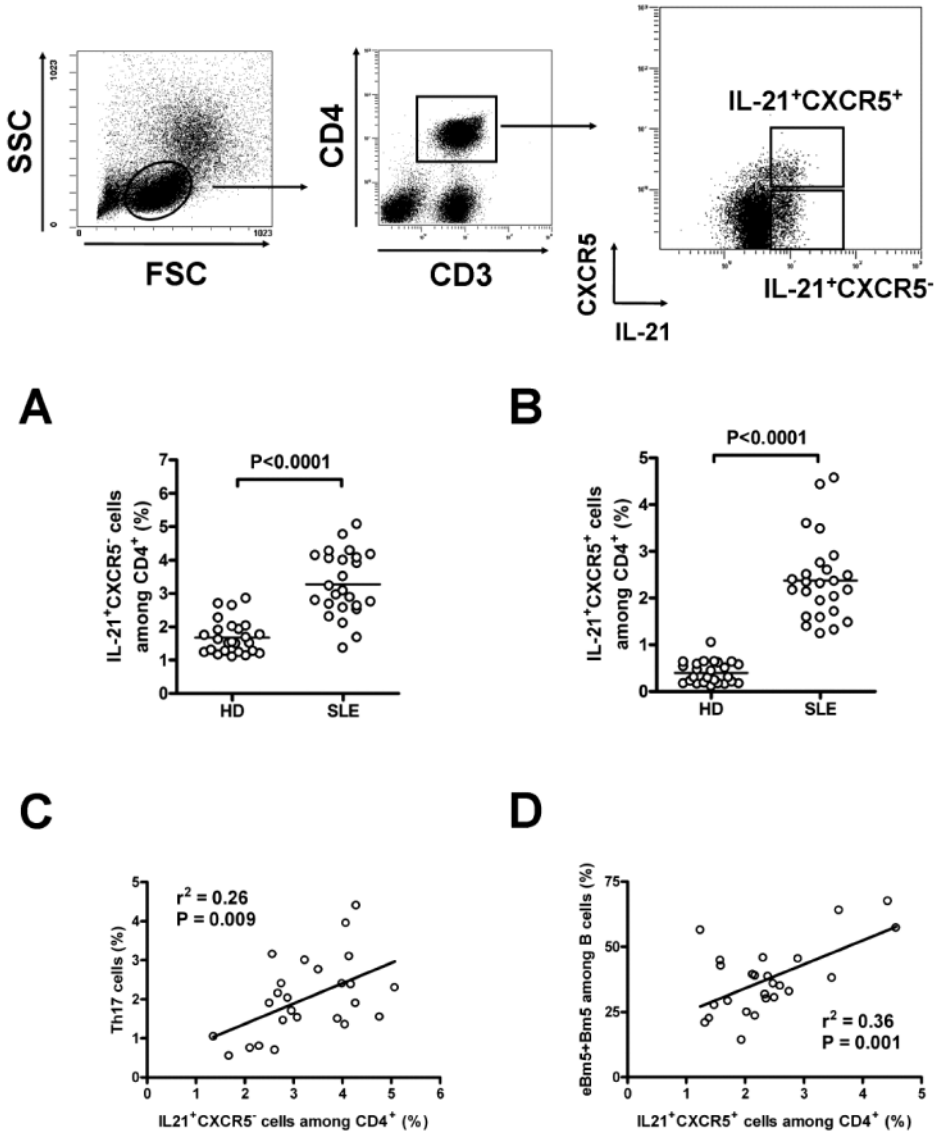

Figure 4. CD4+ T cells producing interleukin 21 (IL-21) are composed of 2 phenotypically distinct subpopulations according to CXCR5 expression in patients with systemic lupus erythematosus (SLE). Peripheral blood mononuclear cells were stimulated for 4 h with phorbol myristate acetate and ionomycin. After gating on CD3+CD4+ T cells, frequencies of CXCR5+CD4+ and CXCR5−CD4+ T cells producing IL-21 were analyzed. A, B. Frequencies of CXCR5−CD4+ (A) and CXCR5+CD4+ (B) T cells producing IL-21 from patients with SLE and controls. C, D. Correlations between CXCR5−CD4+ T cells producing IL-21 and Th17 cells (C) and CXCR5+CD4+ T cells producing IL-21 and eBm5+Bm5 memory B cells (D) in patients with SLE. IL-21-producing CXCR5−CD4+ T cell subset correlates with Th17 cells, while the IL-21-producing CXCR5+CD4+ T cell subset correlates with B cell subset alterations. SSC: side scatter data; FSC: forward scatter data; HD: healthy donor controls.

of germinal centers and are the specialized providers of B cell help for class-switch recombination, memory B cell and plasma cell differentiation, and Ig production[21,22], we assessed the presence of circulating germinal center B cells in SLE peripheral blood samples expressing high levels of CXCR5 and Bcl6 (Figure 6A)[22]. We decided to consider CXCR5++Bcl6+ B cells as germinal center B cells, because Bcl6 is a transcriptional repressor required in mature B cells during the germinal center reaction and CXCR5 is expressed by germinal center B cells and CD4+ TFH cells, allowing their migration to follicles and germinal centers. We found an increase in proportion and in absolute number of CXCR5++Bcl6+ B cells (8.8% ± 2.5% vs 4.1% ± 1.2%, $p < 0.0001$; and 12.5 ± 8.6 vs 7.2 ± 2.9 cells/μl, $p = 0.005$,

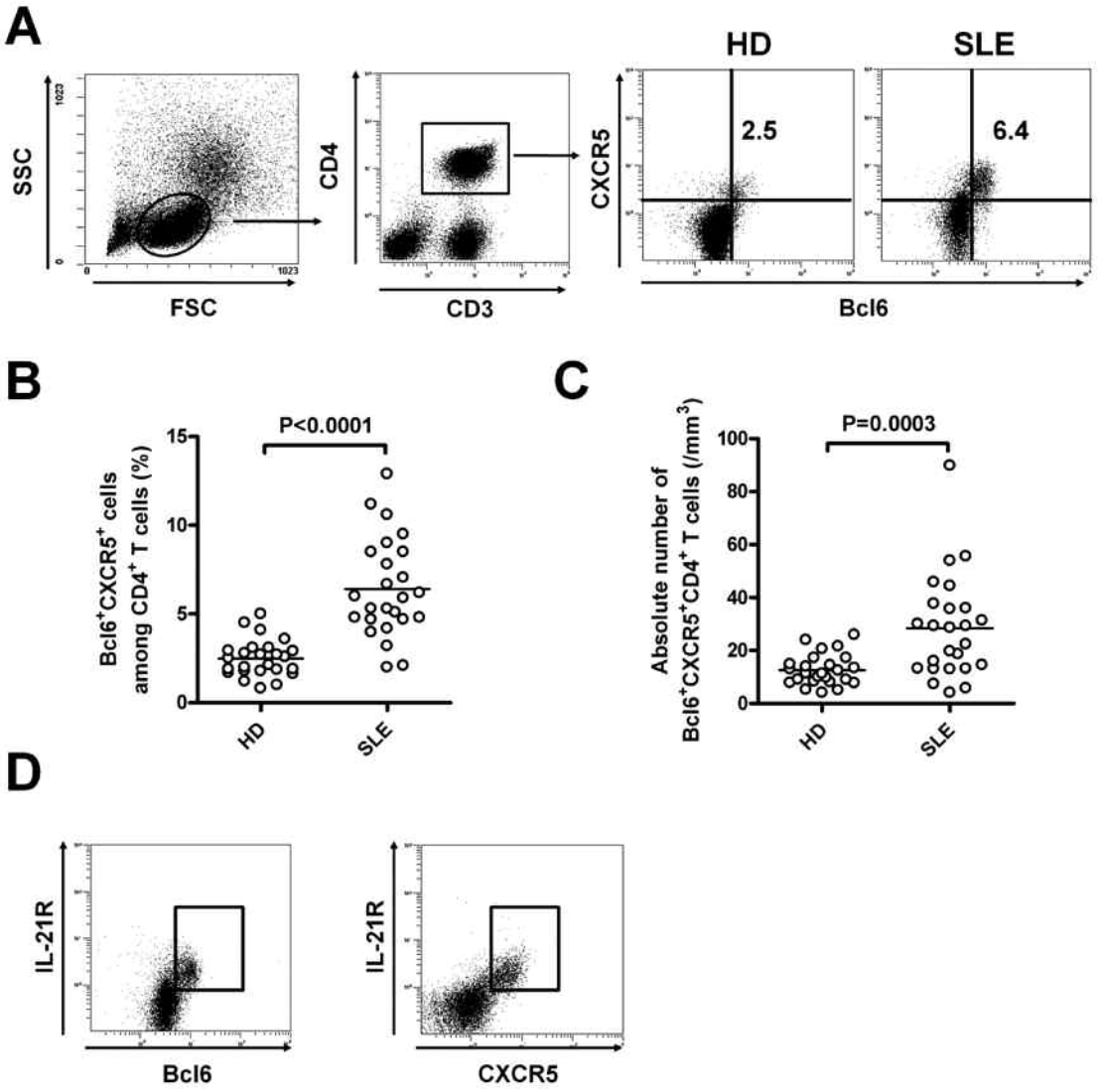

Figure 5. Expansion of circulating follicular helper CD4+ T cells in systemic lupus erythematosus (SLE). Fresh peripheral blood mononuclear cells were stained with anti-CD3, anti-CD4, anti-CXCR5, anti-IL-21R, and anti-Bcl6. A. After gating on CD3+CD4+ T cells, frequencies of CXCR5+Bcl6+ follicular helper CD4+ T cells were analyzed. B, C. Frequencies (B) and absolute numbers (C) of CXCR5+Bcl6+ follicular helper CD4+ T cells from patients with SLE and controls. Expansion of CXCR5+Bcl6+ follicular helper CD4+ T cells was noted in patients with SLE compared to controls. D. Analysis of IL-21R expression of CXCR5+Bcl6+ follicular helper CD4+ T cells in patients with SLE. All population percentages shown here represent averages from analysis of 25 patients with SLE and 25 controls. IL: interleukin; SSC: side scatter data; FSC: forward scatter data; HD: healthy donor controls.

respectively; Figures 6B, 6C) in patients with SLE compared to controls. Similarly to CD4+ TFH cells, circulating CXCR5++Bcl6+ germinal center B cells expressed IL-21R (data not shown). The expansion of circulating CXCR5++Bcl6+ germinal center B cells did not correlate with disease activity, C3 complement fraction, and anti-dsDNA and anti-nucleosome antibodies (data not shown). Finally, the expansion of circulating CXCR5++Bcl6+ germinal center B cells was positively correlated with circulating CXCR5+ Bcl6+ CD4+ TFH cells in proportion and in absolute number ($r^2 = 0.29$, $p = 0.006$; and $r^2 = 0.37$, $p = 0.002$, respectively; Figure 6D).

DISCUSSION

Previous findings on cytokines involved in disturbed Th17/Treg balance and increased memory B cell compartment prompted us to investigate the implications of IL-21 and CD4+ T cells producing IL-21 in the pathogenesis of SLE. We analyzed CD4+ T cells producing IL-21 in peripheral blood samples from patients with SLE according to CXCR5 expression in order to precisely delineate the role of these cells and their correlations with alterations of T cell and B cell subsets.

We first identified an expansion of CD4+ T cells producing IL-21 in patients with SLE. We showed that expansion

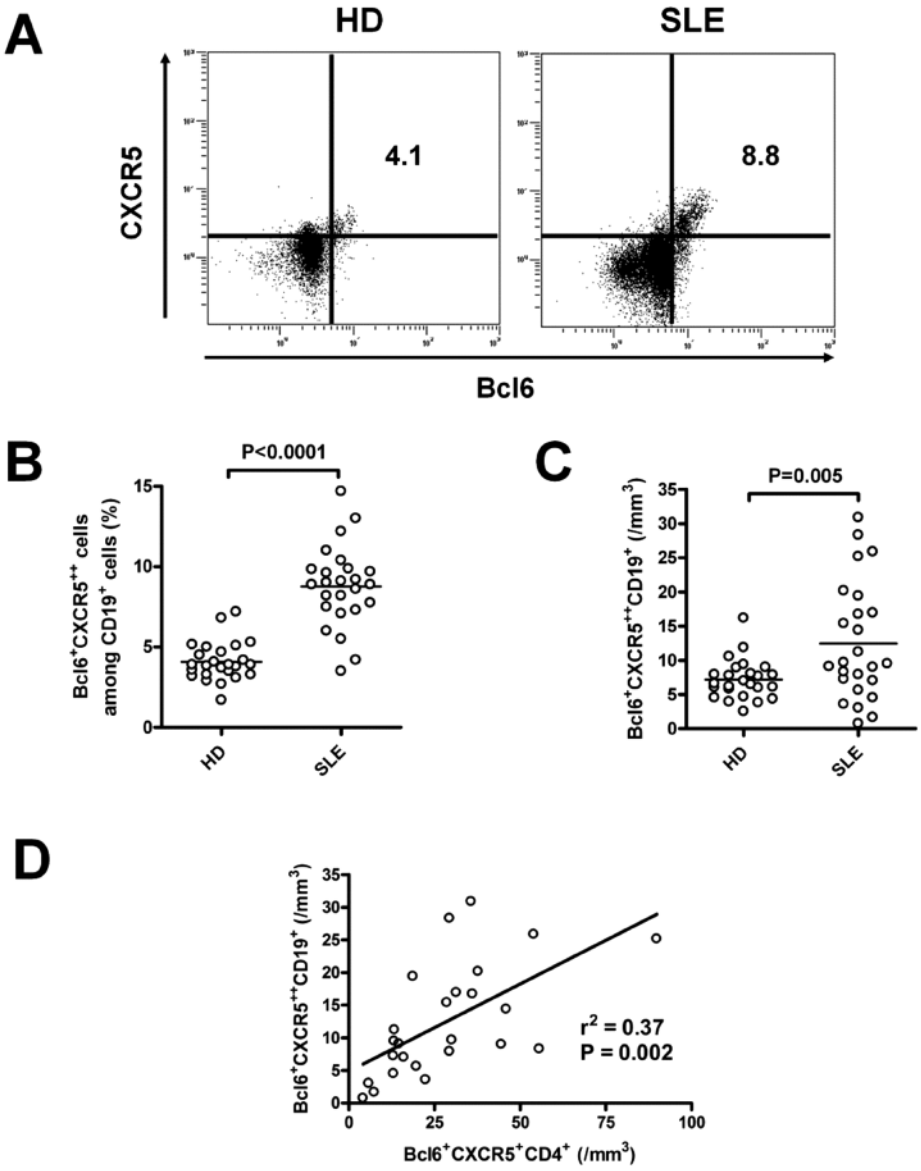

Figure 6. Expansion of circulating germinal center CD19+ B cells in systemic lupus erythematosus (SLE) correlates with follicular helper CD4+ T cells. Fresh peripheral blood mononuclear cells were stained with anti-CD19, anti-CXCR5, anti-IL-21R, and anti-Bcl6. A. After gating on CD19+ B cells, frequencies of CXCR5++Bcl6+CD19+ germinal center B cells were analyzed. B, C. Frequencies (B) and absolute numbers (C) of CXCR5++Bcl6+ germinal center B cells from patients with SLE and controls. Expansion of CXCR5++Bcl6+ germinal center B cells was noted in patients with SLE compared to controls. D. Correlations between CXCR5++Bcl6+CD4+ follicular helper T cells (TFH) and CXCR5++Bcl6+ germinal center B cells. CXCR5++Bcl6+CD4+ TFH cells correlate positively with CXCR5++Bcl6+ germinal center B cells. All population percentages shown here represent averages from analysis of 25 patients with SLE and 25 controls. SSC: side scatter data; FSC: forward scatter data; HD: healthy donor controls.

of CD4+ T cells producing IL-21 correlated with increased Th17 and decreased Treg and increased memory B cells. The critical role of IL-21 in the pathogenesis of SLE-like disease was shown in a mouse model. IL-21R-deficient mice showed none of the abnormalities characteristic of SLE in IL-21R-competent mice, including hypergammaglobulinemia, autoantibody production, renal disease, and premature morbidity[23]. In human SLE, increased plasma levels of IL-21[24] and polymorphisms of the IL-21 and IL-21R genes as genetic susceptibility factors[25,26] were demonstrated. More recently, Dolff, *et al* found an increased proportion of IL-21-producing T cells in patients with SLE correlating with the proportion of IL-17A-producing T cells[27], but did not phenotypically delineate CD4+ T cells producing IL-21 and their correlations with alterations of T cell and B cell subsets.

To better delineate CD4+ T cells producing IL-21 in SLE, we next analyzed CXCR5 expression on CD4+ T cells. We found that both IL-21-producing CXCR5−CD4+ T cells and CXCR5+CD4+ T cells were increased in patients with SLE. IL-21-producing CXCR5-CD4+ T cell subset correlated with Th17 cells and Tregs, while the IL-21-producing CXCR5+CD4+ T cell subset correlated with B cell subset alterations. These findings suggest that different sources of IL-21 may exhibit different functions according to its localization and their target cells. The chemokine receptor CXCR5 expressed by B cells is required for migration and responsiveness to CXCL13 to form follicles[28]. The high expression of CXCR5 by TFH cells producing IL-21 allows them to home to and be retained by the lymphoid follicle in response to CXCL13[29], where contact with antigen-primed B cells promotes B cell proliferation, isotype switching, and somatic mutation of the Ig repertoire[30,31]. In contrast, the absence of expression of CXCR5 by non-TFH cells producing IL-21 could allow them to recirculate in peripheral blood, where contact with naive CD4+ T cells may induce Th17 differentiation and suppress FoxP3 expression[13,14,15]. Overall, these findings suggest that the blockade of IL-21 could provide beneficial effects on T cell alterations through its action in the peripheral compartment, and on B cell alterations through its action in germinal centers, by inhibiting T–B interactions.

Finally, we observed marked expansion of circulating TFH cells and germinal center B cells in the peripheral blood of patients with SLE, using Bcl6 and CXCR5 expression. The transcription factor Bcl6 is essential for development of TFH cells and germinal center B cells. It was recently demonstrated that antigen-engaged B cells upregulated Bcl6 before clustering in germinal centers. Bcl6 upregulation in pre-germinal center B cells contributes to sustaining their interactions with helper T cells and is required for their entry to germinal center clusters[32]. These findings suggest that expansion of T cells related to the TFH program and B cells related to the germinal center program is observed in peripheral blood of patients with SLE before their entry into germinal centers, and that early intrinsic abnormalities of TFH and germinal center B cell differentiation may exist in SLE.

Our study suggests the implication of IL-21 and CD4+ T cells producing IL-21 in the pathogenesis of SLE. IL-21 produced by distinct cellular CD4+ T cell populations according to CXCR5 expression could differentially modulate the Th17/Treg balance and alterations of the B cell subset. Our findings suggest that targeting IL-21 could provide beneficial effects on alterations of both T cells and B cells in patients with SLE.

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