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Interleukin-25 is upregulated in patients with systemic lupus erythematosus and ameliorates murine lupus by inhibiting inflammatory cytokine production

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ABSTRACT

Interleukin-25 (IL-25), an anti-inflammatory member of the IL-17 family of cytokines, has been extensively investigated in multiple autoimmune and inflammatory diseases. However, its pathogenic role in systemic lupus erythematosus (SLE) remains largely unknown. This study aimed to explore the expression and clinical significance of IL-25 in patients with SLE as well as its pathogenic role in lupus-prone MRL/lpr mice. The results showed that IL-25 mRNA and serum levels were increased in patients with SLE compared with those in healthy controls. Higher IL-25 mRNA and serum levels were found in patients with an active disease. IL-25 levels were positively associated with SLEDAI, anti-dsDNA, and IgG but negatively associated with C3 and C4. Ex vivo assay showed that IL-25 could inhibit the production of the inflammatory cytokines IL-1β, IL-17, IL-6, and IFN-γ as well as TNF-α in the peripheral blood mononuclear cells in patients with SLE. In vivo studies revealed that treatment with IL-25 significantly ameliorated lupus symptoms in lupus-prone MRL/lpr mice by suppressing the production of inflammatory cytokines, including IL-1α, IL-1β, IL-6, IL-12p70, IL-17A, and IFN-β. Cumulatively, our results suggest that IL-25 levels are increased in patients with SLE and associated with disease activity; IL-25 plays a potent immunosuppressive role in the pathogenesis of SLE by suppressing the production of inflammatory cytokines. IL-25 could potentially be used as a diagnostic and therapeutic target for SLE treatment.

1. Introduction

Systemic lupus erythematosus (SLE) is a prevalent systemic autoimmune disease characterized by chronic inflammation in the joints and several organs, including the skin, heart, lungs, kidneys, and nervous system [1]. Currently, besides genetic and environmental factors, the cause of SLE remains largely unknown. It has become clear that autoantibody production, immune complex formation, and inflammatory responses mediated by multiple cytokines contribute to the pathogenesis of SLE [2]. In addition, increasing evidence has recognized that proinflammatory cytokines, including IL-1β, IL-6, IL-17, IFN-γ, and TNF-α, are key factors in the pathogenesis of SLE [3].

The interleukin (IL)-17 cytokine family includes six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (or IL-25), and IL-17F [4]. Among these, IL-17A [5–7], IL-17B [8], IL-17C [9], IL-17D [9], and IL-17F [8,10,11] have been shown to be upregulated in SLE. They can induce the expression of chemokine, cytokine, and autoantibodies, amplify the immune response, and thereby aggravate tissue inflammation and injury in SLE. However, the role of IL-25 in the pathogenesis of SLE remains unknown.

IL-25 was first reported by Fort et al. in 2001 [12]. Unlike IL-17A and other IL-17 family cytokines, IL-25 binds to the IL-17RA–IL-17RB receptor complex expressed on epithelial cells and type II T helper-2 (Th2) lymphocytes and induces the production of Th2 cytokines, including IL-4, IL-5, and IL-13 [12–14]. Moreover, IL-25 plays an anti-inflammatory role in autoimmune and inflammatory diseases, primarily through the regulation of Th1 and Th17 cell responses [15,16]. Rhinovirus-induced IL-25 has been found to drive type 2 immunity and allergic pulmonary inflammation in asthma [17]. Additionally, IL-25 attenuates rheumatoid arthritis (RA) through the destruction of Th17

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cell responses [18]. Moreover, IL-25 promotes the proliferation and proinflammatory response in keratinocytes and plays a pivotal role in psoriasis [19]. Although IL-25 has been broadly investigated in multiple autoimmune and inflammatory diseases, its role in SLE remains unclear.

In the current study, we investigated the expression of IL-25 in the serum of patients with SLE, and the relationships of serum IL-25 levels with disease activity, clinical manifestations, as well as inflammatory cytokines in patients with SLE. We also investigated the pathogenic role of IL-25 in lupus-prone MRL/lpr mice and related mechanisms.

2. Materials and methods

2.1. Patients and controls

From September 2016 to February 2018, 90 patients with SLE were recruited from the Department of Rheumatology, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University. There were 81 females and 9 males, with a mean age of (35.3 ± 12.6) years. Sixty healthy donors (50 females and 10 males; mean age 35.8 ± 11.5 years) were collected as normal controls. SLE was diagnosed according to the 1997 revised classification criteria of the ACR for SLE [20]. Lupus disease activities were quantified using the SLE Activity Index (SLEDAI) score [21]. Active lupus disease was defined as SLEDAI score ≥ 6 [22]. Lupus nephritis is defined as clinical and laboratory manifestations that meet the ACR criteria (persistent proteinuria > 0.5 g per day or > 3+ by dipstick, and/or cellular casts including red cells, hemoglobin, granular, tubular, or mixed) [23]. Clinical and laboratory data were collected from our hospital records. Briefly, the extractable nuclear antigens, including anti-double-stranded DNA (anti-dsDNA), anti-Smith, anti-anti-nucleosome antibody (AnuA), and anti-ribosomal RNP antibody (rRNP), were examined using an immunofluorescence method with a commercially available diagnostic kit (EUROIMMUN, Germany), in accordance with the manufacturer's instructions. The concentrations of serum immunoglobulin G (IgG), IgA, IgM, C3, and C4 were determined by nephelometry methods in accordance with the manufacturer's instructions (SIEMENS, Germany). Erythrocyte sedimentation rate (ESR) was determined using the ESR-30 Automatic Erythrocyte Sedimentation Rate Dynamic Analyzer (Shanghai Xunda, China), in accordance with the instructions provided by the manufacturer. Details of the demographic and clinical characteristics of the patients and healthy controls (HCs) are shown in Table 1. This study was approved by the Ethics Committee Board of The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University. Informed consent was obtained from each participant, in accordance with the committee's regulations.

2.2. mRNA extraction and RT-PCR

Peripheral blood mononuclear cells (PBMCs) were isolated from blood by Ficoll density gradient centrifugation (600 *g* for 25 min; TBDscience, China). Total RNA was extracted from PBMCs with Trizol (Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturer's instructions. cDNA was synthesized from total RNA using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with the following primers: IL-25 forward: 5′-CAGGTGGTTGCATTCTTGGC-3′ and reverse: 5′-GAGCCGG TTCAAGTCTCTGT-3′; β-catenin forward: 5′-AGCTTCCAGACACGCTAT CAT-3′ and reverse: 5′-CGGTACAACGAGCTGTTTCTAC-3′. Gene-specific amplification was performed on an ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). β-catenin was used as an internal control. Relative mRNA levels of target genes were calculated by the $2^{-\Delta\Delta ct}$ method.

Table 1
Characteristics of patients with SLE and healthy controls.

Characteristics	SLE (n = 90)	HCs (n = 60)	P value
Age (years)	35.3 ± 12.6	35.8 ± 11.5	0.869
Gender (male/female)	9/81	10/50	0.229
Disease duration (years)	3.53 ± 1.26		
Arthritis n (%)	7 (7.7)		
Renal diseases n (%)	53 (58.8)		
Fever n (%)	15 (16.6)		
Neurological disorder n (%)	2 (2.2)		
Leukopenia n (%)	14 (15.5)		
Thrombocytopenia n (%)	10 (11.1)		
Anti-dsDNA antibody n (%)	55 (61.1)		
Anti-Smith antibody n (%)	47 (52.2)		
Anti-AnuA antibody n (%)	29 (32.2)		
Anti-rRNP antibody n (%)	16 (17.7)		
ESR (mm/h)	30.8 ± 26.43		
C3 (g/l)	0.57 ± 0.30		
C4 (g/l)	0.15 ± 0.06		
IgG (g/l)	17.89 ± 7.55		
IgA (g/l)	2.15 ± 1.16		
IgM (g/l)	1.33 ± 0.53		
SLEDAI	6.4 ± 3.81 (1–14)		

Data are presented as Mean (SD). Anti-AnuA antibody, anti-nucleosome antibody; Anti-rRNP antibody, Anti-Ribosomal RNP Antibody; ESR, erythrocyte sedimentation rate; C3, complement 3; C4, complement 4; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IgA, Immunoglobulin A; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index.

2.3. Cell culture and IL-25 treatment

A total of 1×10^6/ml PBMCs were seeded in 96-well plates and stimulated with LPS (1 μg/ml; Sigma, USA) in the presence or absence of IL-25 (100 ng/ml, Cat. # 1258-IL-025; R&D Systems, MN, USA) for 24 h. Supernatants were collected for enzyme-linked immunosorbent assay (ELISA) of IL-1β, IL-6, IL-17A, IFN-γ, and TNF-α.

2.4. Mice and experimental groups

Female B6.MRL/lpr mice (12 weeks of age), and female C57BL/6 mice of the same age were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). All mice were maintained under specific pathogen-free conditions at The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University. MRL/lpr mice or control B6 mice received weekly intraperitoneal injections with rmIL-25 (1 μg per mice, Cat. # 1399-IL-025; R&D Systems, MN, USA) or PBS every 3 days from the 14th week to the 18th week. After 4 weeks of treatment, mice were anesthetized and their spleens were isolated, weighed, and photographed. Both kidneys were excised for histological analysis and immunofluorescence staining. Serum was collected for analyses of cytokines, albumin, and anti-dsDNA by ELISA at the indicated time.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Serum was obtained by centrifugation at 3000 *g* for 10 min. Serum IL-25 was quantified by using a commercial human IL-25 ELISA kit (Cat. # DY1258-05; R&D Systems, USA), in accordance with the manufacturer's protocol. All samples were examined in duplicate. Control samples and SLE samples were mixed on the plates to avoid differences caused by intra-assay variation. Detection of the levels of the cytokines IL-1β, IL-6, IL-17A, IFN-γ, and TNF-α in culture supernatants was performed using a commercial ELISA kit from MultiSciences (Hangzhou, China). Serum and urine from mice were collected every week. The concentration of urinary protein was determined with the Bradford Protein Assay Kit (Cat. # 70-PQ0041; MultiSciences, Hangzhou, China), in accordance with the manufacturer's instructions. Anti-dsDNA was

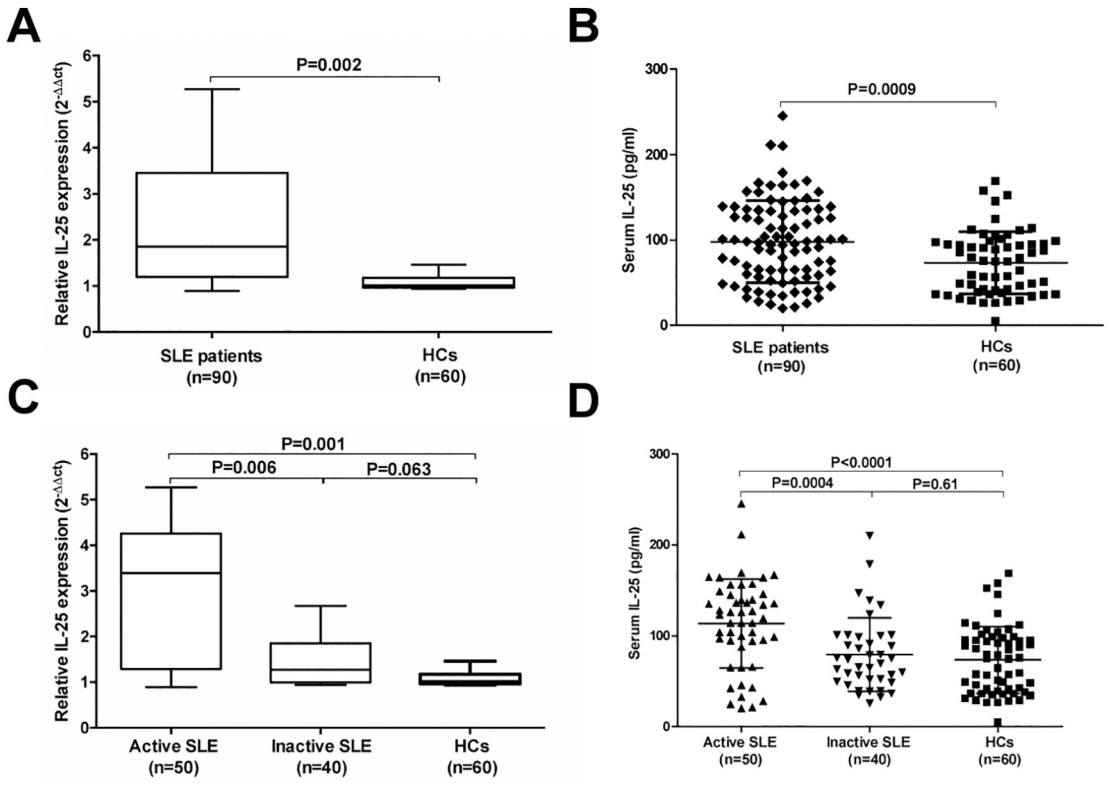

Fig. 1. The levels of IL-25 mRNA in PBMCs and protein in serum were upregulated in patients with SLE and associated with disease severity. (A) The levels of IL-25 mRNA in PBMCs from patients with SLE and HCs were measured by RT-qPCR. (B) The levels of IL-25 protein in serum from patients with SLE and HCs were measured by ELISA. Ninety patients with SLE were divided into active groups (n = 50, SLEDAI score ≥ 6) and inactive groups (n = 40, SLEDAI score < 6) according to SLEDAI. Comparison of IL-25 mRNA **(C)** and protein (D) levels among patients with active and inactive SLE were shown. Each symbol represents an individual patient with SLE or HCs. Data shown are the Mean ± SD. Differences between two groups were performed with Mann-Whitney *U* test for nonparametric data.

measured using mouse anti-double-stranded DNA antibody (IgG) ELISA kit (Cat. # CSB-E11194 m; Cusabio, Houston, TX, USA), in accordance with the manufacturer's instructions. Cytokine levels in mouse serum were measured using the LEGENDplex™ Mouse Inflammation Panel (13-plex) (Cat. # 740446; Biolegend, CA, USA), in accordance with the manufacturer's instructions.

2.6. Histological analyses

Histological analyses were performed as described previously [24]. Briefly, kidneys were isolated, cut in half laterally, and fixed in 10% formalin. Specimens were then embedded in paraffin, sectioned at a thickness of 3 μm, and stained with hematoxylin and eosin (H&E).

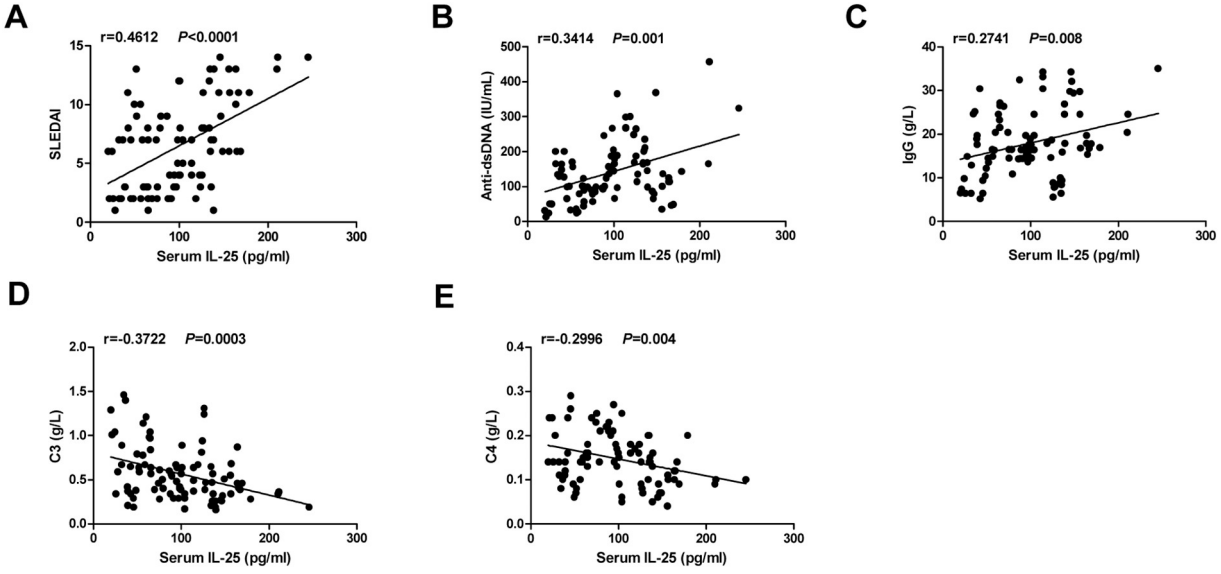

Fig. 2. Correlation between IL-25 protein levels in serum and SLEDAI as well as laboratory values. IL-25 protein levels in serum were positively correlated with SLEDAI (A), anti-dsDNA (B), and IgG (C), and negatively associated with C3 levels (D) and C4 levels (E). Each symbol represents an individual patient with SLE. The correlations were evaluated with Spearman's non-parametric test.

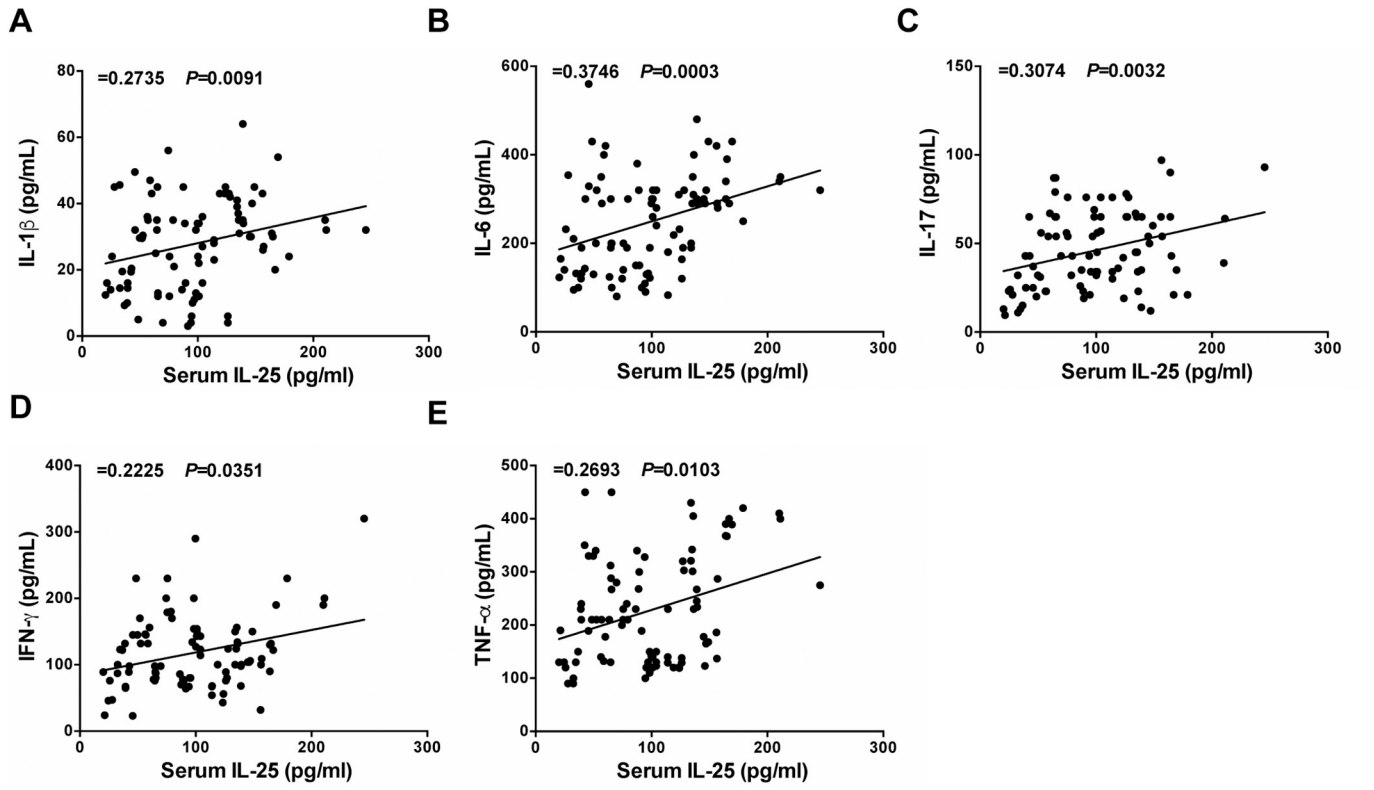

Fig. 3. IL-25 protein levels in serum are associated with inflammatory cytokines in patients with SLE. IL-25 protein levels in serum were positively correlated with IL-1β (A), IL-6 (B), IL-17 (C), IFN-γ (D) and TNF-α (E). Each symbol represents an individual patient. The correlations were evaluated with Spearman's non-parametric test.

Sections were then scored using a previously described scoring system [25].

2.7. Immunofluorescence staining

Kidneys were embedded in optimal cutting temperature embedding media, snap-frozen, and sectioned at 3 μm. After blocking with 1% BSA and washing, the sections were incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG (1:1000, Cat. # A2A28175; Invitrogen, CA, USA) overnight at 4 °C. The sections were then stained with 4,6-diamidino-2-phenylindole (DAPI) (Thermo Fisher, MA, USA) and observed under a fluorescence microscope (BX63; Olympus, Tokyo, Japan).

2.8. Statistical analysis

Statistical analysis was performed using SPSS version 21.0 statistical software (SPSS, Chicago, IL, USA). The results are expressed as mean ± standard deviation (SD). Comparisons between groups were performed using the Mann-Whitney *U* test. Spearman correlation test was used to evaluate the associations between serum IL-25 levels and different variables. One-way ANOVA was applied for statistical comparison of four groups. P value < 0.05 was considered statistically significant.

3. Results

3.1. IL-25 was upregulated in patients with SLE and associated with disease severity

As shown in Fig. 1A, the level of IL-25 mRNA in PBMCs from patients with SLE was significantly higher than that in HCs (P = 0.002). Patients with SLE had elevated serum IL-25 levels compared with HCs (98.2 ± 5.08 pg/ml vs. 73.3 ± 4.72 pg/ml, P = 0.0009; Fig. 1B). In addition, patients with active disease showed higher mRNA and serum IL-25 levels than inactive disease patients (P = 0.006 and P = 0.0004, respectively). However, no significant differences of IL-25 mRNA and serum levels between patients with inactive disease and HCs (P = 0.063 and P = 0.61, respectively; Fig. 1C and D) were found. Hence, our findings demonstrated that IL-25 might be associated with the disease activity of SLE.

3.2. Correlation between IL-25 levels and SLEDAI as well as laboratory values

As shown in Fig. 2, serum IL-25 levels were positively associated with SLEDAI (r = 0.4612, P < 0.0001; Fig. 2A), anti-dsDNA (r = 0.3414, P = 0.001; Fig. 2B), and IgG (r = 0.2741, P = 0.008; Fig. 2C), and negatively associated with C3 levels (r = −0.3722, P = 0.0003; Fig. 2D) and C4 levels (r = −0.2996, P = 0.004; Fig. 2E). However, no significant associations were observed between serum IL-25 levels and anti-Smith, anti-AnuA, ESR, IgM, and IgA (data not shown).

3.3. Serum IL-25 levels are associated with inflammatory cytokines in patients with SLE

Proinflammatory cytokines have been recognized to contribute to the pathogenesis of lupus. The data here show that serum IL-25 levels were positively correlated with the expression of IL-1β (r = 0.2735, P = 0.0091; Fig. 3A), IL-6 (r = 0.3746, P = 0.003; Fig. 3B), IL-17 (r = 0.3074, P = 0.0032; Fig. 3C), IFN-γ (r = 0.2225, P = 0.0351; Fig. 3D), as well as TNF-α (r = 0.2693, P = 0.0103; Fig. 3E), suggesting that IL-25 is significantly involved in the inflammatory response in patients with SLE.

3.4. IL-25 inhibits inflammatory cytokine production in PBMCs of patients with SLE

To examine the influence of IL-25 on the regulation of inflammatory cytokine production, PBMCs from patients with SLE or HCs were stimulated with LPS (1 μg/ml) in the presence or absence of rhIL-25 (100 ng/mL). Supernatants were collected and examined by ELISA after 24 h of culture. The results showed that the expression of the proinflammatory cytokines IL-1β, IL-6, IL-17, IFN-γ, as well as TNF-α was drastically repressed by IL-25 treatment in patients with SLE (Fig. 4A and E). However, the cytokine expression in PBMCs from HCs was not affected by IL-25 treatment (Fig. 4A–E).

3.5. IL-25 ameliorates lupus symptoms in lupus-prone MRL/lpr mice

To further explore the pathogenic role of IL-25 in SLE, we investigated the effect of treatment with rmIL-25 on the progression of lupus in lupus-prone MRL/lpr mice. MRL/lpr mice received the intraperitoneal injection of rmIL-25 (1 μg per mouse, every 3 days) from the 14th week to the 18th week. As shown in Fig. 5A, a decreased proteinuria level was observed in the rmIL-25-treated MRL/lpr mice compared with that in the vehicle-treated MRL/lpr mice. rmIL-25-treated MRL/lpr mice displayed reduced splenomegaly and spleen/weight ratio (Fig. 5B, C), and declined serum levels of anti-dsDNA antibody (Fig. 5D), compared with vehicle-treated MRL/lpr mice. H&E staining showed reduced kidney damage, which was characterized by significant reductions in glomerular hypercellularity, inflammatory cell infiltration, and renal interstitial necrosis, in mIL-25-treated MRL/lpr mice (Fig. 5E). Nephritis severity score was also significantly reduced in the rmIL-25 treatment group compared with that in the vehicle treatment group (Fig. 5F). Immunofluorescence staining of kidney sections showed that rmIL-25 treatment significantly reduced IgG deposition in the kidney of mice with lupus compared with that in vehicle treatment mice (Fig. 5G and H). These findings suggest that IL-25 could alleviate lupus symptoms in lupus-prone MRL/lpr mice.

3.6. IL-25 inhibits inflammatory cytokine production in lupus-prone MRL/lpr mice

We next examined the inflammatory cytokine levels after 4 weeks of rmIL-25 treatment in lupus-prone MRL/lpr mice by using LEGENDple Mouse Inflammation Assay, which allows the simultaneous quantification of 13 mouse cytokines, namely, IL-1α, IL-1β, IL-6, IL-10, IL-12p70, IL-17A, IL-23, IL-27, MCP-1, IFN-β, IFN-γ, TNF-α, and GM-CSF. As shown in Fig. 6A–F, among these cytokines, IL-25 treatment significantly inhibited IL-1α, IL-1β, IL-6, IL-12p70, IL-17A, and IFN-β expression in MRL/lpr mice compared with that in vehicle treatment mice. However, no significant differences of IL-10, IL-23, IL-27, MCP1, IFN-γ, TNF-α, and GM-CSF levels were observed between the two groups (Fig. S1). These findings are in accordance with some of our observations in patients with SLE, suggesting that IL-25 ameliorates lupus by suppressing inflammatory cytokine production in lupus-prone MRL/lpr mice.

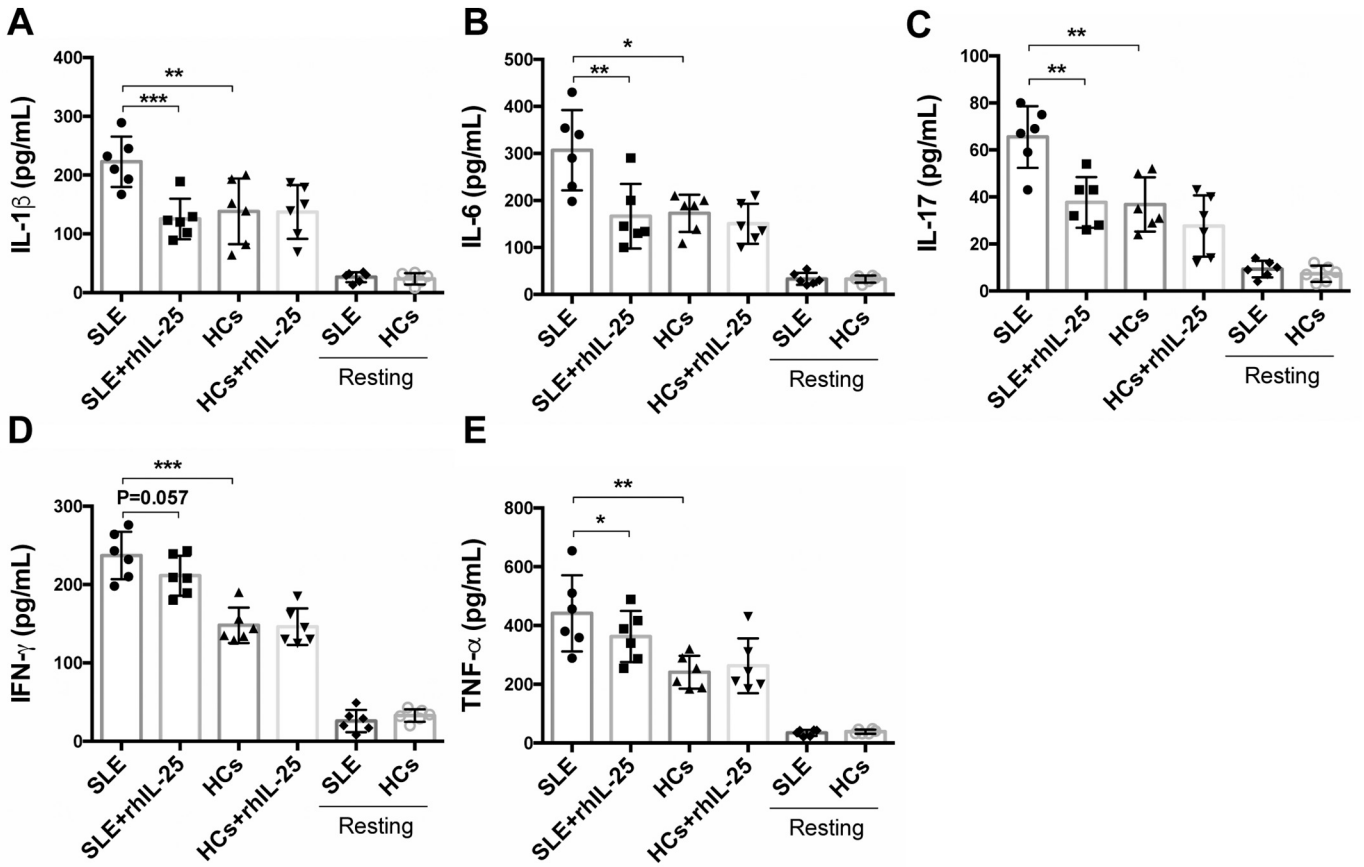

Fig. 4. IL-25 inhibits inflammatory cytokine production in PBMCs of patients with SLE. PBMCs from patients with SLE or HCs were stimulated with LPS (1 μg/ml) in the presence or absence of rhIL-25 (100 ng/mL). After 24 h of culture, supernatants were harvested and examined by ELISA. IL-1β (A), IL-6 (B), IL-17 (C), IFN-γ (D) and TNF-α (E) levels in supernatants were assessed by ELISA. Data shown are the Mean ± SD. Differences between patients with SLE with and without IL-25 treatment groups were performed with paired *t*-test. Differences between SLE and HCs were performed with Mann-Whitney U test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

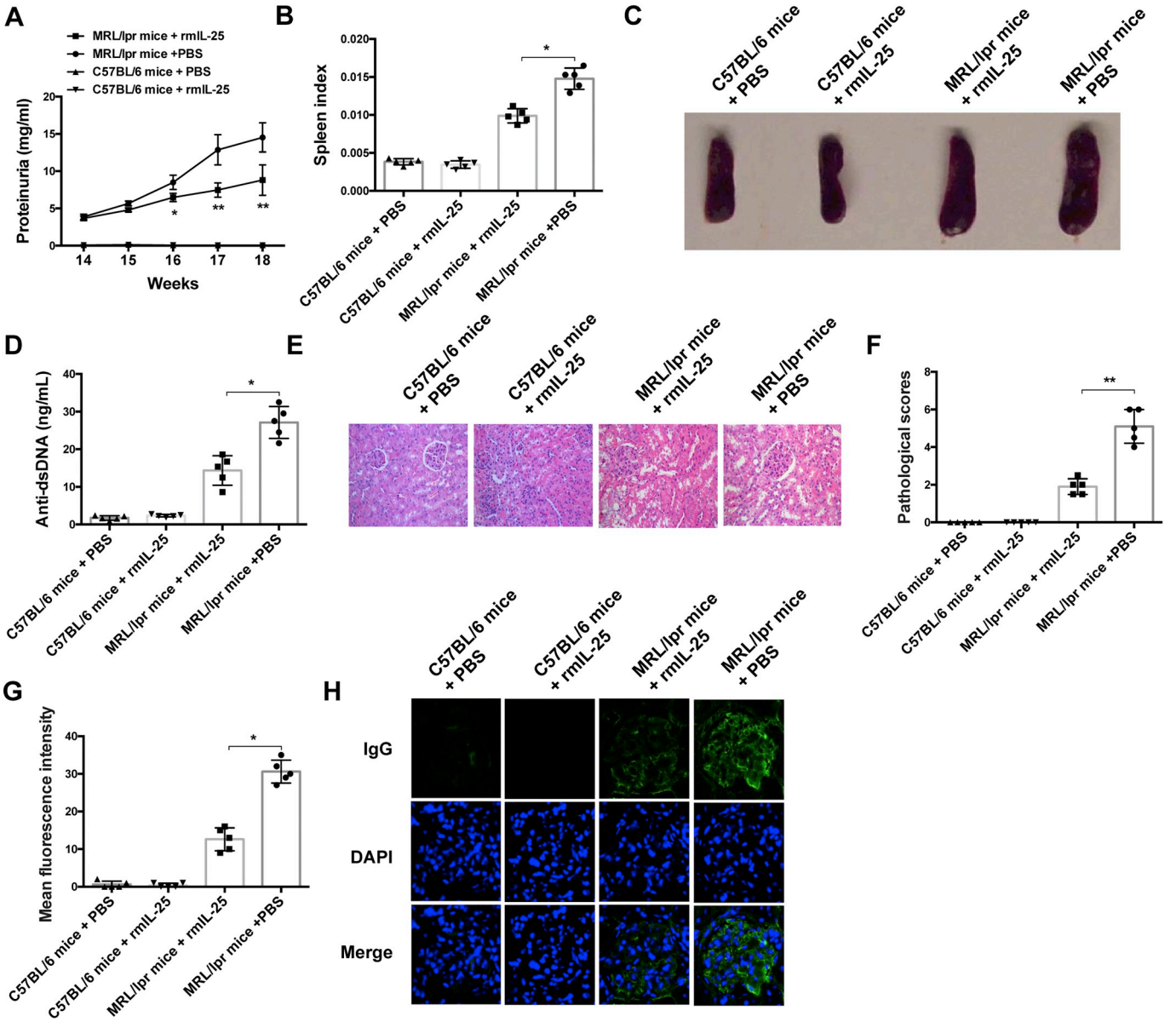

Fig. 5. IL-25 ameliorates lupus symptoms in lupus-prone MRL/lpr mice. MRL/lpr or C57BL/6 mice received intraperitoneal injection of rmIL-25 from the 14th to the 18th weeks. Mice were euthanized after 4 weeks of treatment and subjected to experiments. (A) Proteinuria was collected weekly and determined by Bradford Protein Assay Kit. (B–C) The spleen/weight ratio and representative images of marked splenomegaly in four groups of mice were shown. (D) Serum levels of anti-dsDNA antibody were determined by ELISA. (E–F) Representative kidney sections stained with H&E (magnification, ×100) and histological score were shown. (G-H) Representative immunofluorescence (magnification, ×100) of kidney sections stained with IgG, and mean immunofluorescence intensities were shown. Data shown are the Mean ± SD. Differences among four groups were performed with One-way ANOVA. *P < 0.05, **P < 0.01.

4. Discussion

Although serum IL-25 levels have been found to be upregulated in SLE as well as SLE with lupus nephritis (SLE-LN) patients [26], the clinical significance and pathogenic role of IL-25 in SLE remain largely unknown. In the current study, we provide direct evidence that interleukin-25 is upregulated in patients with SLE and ameliorates murine lupus by inhibiting inflammatory cytokine production.

In recent decades, evidence of the important roles of cytokines in the pathogenesis of SLE has been accumulating [27]. It is accepted that Th1/Th2/Th17 cytokines are predominantly involved in SLE autoimmunity [28]. The proinflammatory cytokines IL-1β, IL-6, IL-17, IFN-γ, and TNF-α are significantly elevated in the serum of patients with SLE and correlated with disease activity [3]. In our study, the levels of IL-1β, IL-6, IL-17, IFN-γ, and TNF-α in serum were significantly increased in PBMCs of patients with SLE. Blocking these proinflammatory cytokines significantly mitigated SLE development [29]. Uppal et al. reported significantly greater improvement in SLEDAI in patients with SLE who were treated with infliximab, a chimeric monoclonal antibody against TNF-α [30]. Another preliminary study showed that the blocking of IL-1 receptor with anakinra led to clinical and serological improvement in patients with severe lupus arthritis [31]. In addition, tocilizumab, an anti-human IL-6 receptor monoclonal antibody, has also shown efficacy in treating patients with SLE [32,33]. It has been shown that IL-25 could be induced or upregulated by IL-17 or TNF-α [19,34], suggesting that the unregulated proinflammatory cytokines in SLE may, in turn, stimulate the expression of the anti-inflammatory cytokine IL-25 to downregulate excessive inflammation. Therefore, it is reasonable that higher IL-25 levels are correlated with higher disease activity in SLE.

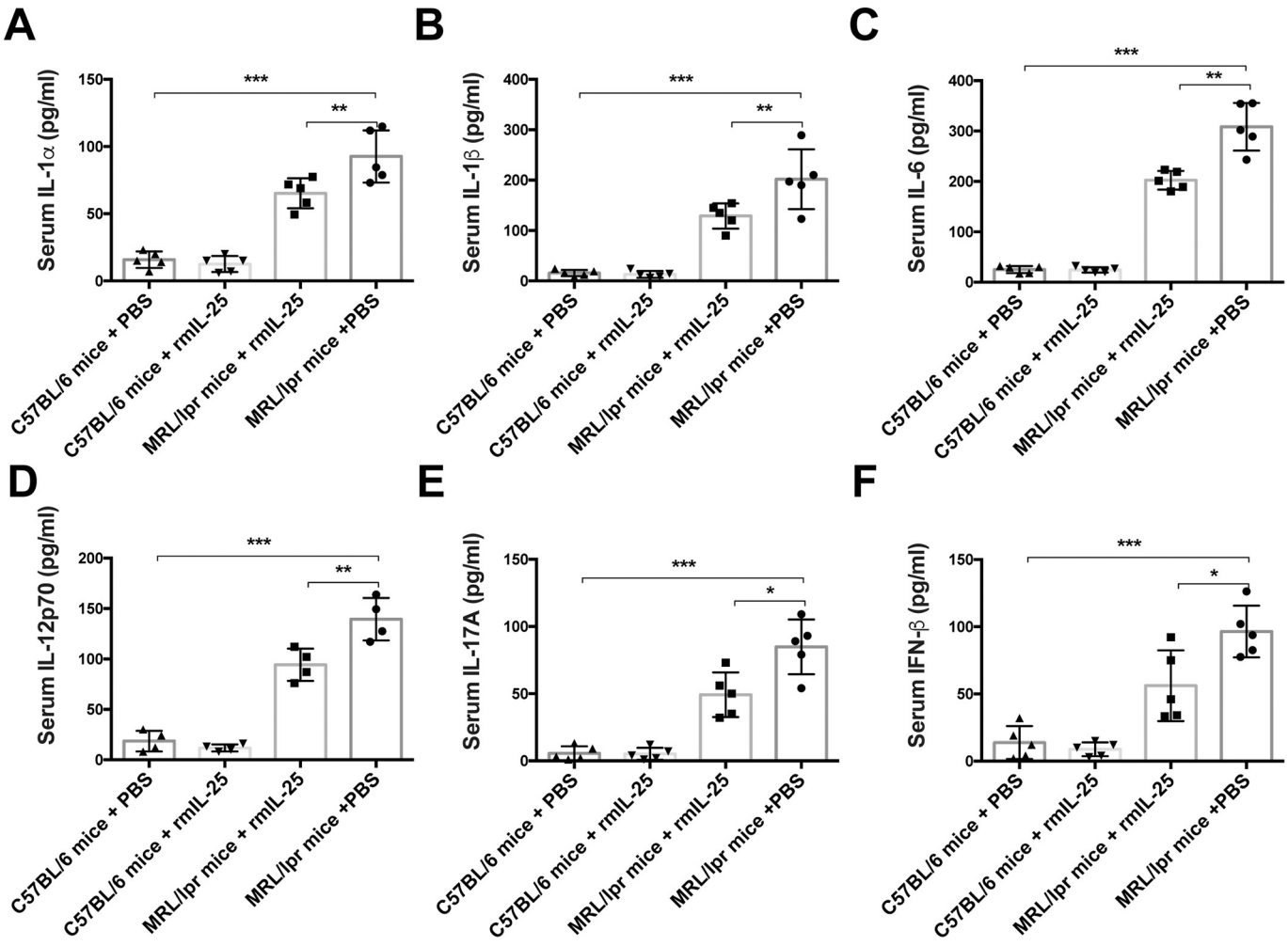

Fig. 6. IL-25 inhibits inflammatory cytokine production in Lupus-prone MRL/lpr mice. Serum levels of IL-1α, IL-1β, IL-6, IL-12p70, IL-17A and IFN-β in four groups were determined by LEGENDple Mouse Inflammation Assay. Data shown are the Mean ± SD. Differences among four groups were performed with One-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

As an anti-inflammatory cytokine that can restrict innate and adaptive immune responses [16], IL-25 is well known to regulate allergic responses and type 2 immunity, as well as being involved in autoimmune conditions such as RA and psoriasis. Liu et al. reported that IL-25 was upregulated in the serum and synovial fluid of RA patients. Higher serum IL-25 levels were associated with disease severity and inflammatory response in RA patients, and the administration of IL-25 could attenuate collagen-induced arthritis development by suppressing Th17 cell responses in a mouse model [18]. Xu et al. also found that patients with psoriasis had significantly increased IL-25 mRNA in lesional skin. However, the administration of IL-25 induced a psoriasis-like pathology, while IL-25 neutralizing antibody treatment significantly decreased the development of psoriasis in mice [19]. These inconsistent results suggest that the complex role of IL-25 in inflammatory and autoimmune diseases is context-dependent.

In this study, we did not identify the source of IL-25 in SLE. IL-25 mRNA was first found as a molecule highly expressed in polarized Th2 cells [12]. However, other cell types, including bone marrow-derived mast cells [35], macrophages [36], stem cell factor-stimulated eosinophils, and basophils [37], lung epithelial cells [38], and brain capillary endothelial cells (BCECs) [39] have been reported to produce IL-25 upon stimulation. After secretion, IL-25 binds to IL-17RA/IL17-RB receptors and activates the NF-kB and MAP kinase pathway [40,41]. IL-25 has been reported to strongly induce type 2 cytokines, such as IL-4, IL-5, IL-9, and IL-13, as well as the Th17 cytokines IL-17A and IL-23 [12,15]. Our study found that IL-25 could effectively inhibit the production of the proinflammatory cytokines IL-1β, IL-6, IL-17, IFN-γ, and TNF-α. Consistent with this, Liu et al. demonstrated a significantly positive association between serum IL-25 levels and IL-1β, IL-6, and TNF-α, but not IFN-γ, in RA.

The positive association between IL-25 and clinical factors indicates that IL-25 may be associated with disease severity and inflammatory response in patients with SLE, and implies that IL-25 may be involved in the pathogenesis of SLE. However, clinical correlation analysis did not indicate a causal relationship between IL-25 and SLE. We therefore used a mouse model to explore the role of IL-25 in SLE. MRL/lpr mouse is a much-studied model of SLE. Its causative mutation, Faslpr, promotes the survival of self-reactive lymphocytes, leading to immune proliferation, lymphadenopathy, emergence of anti-DNA antibodies, and fatal immune complex glomerulonephritis [42]. By using this mouse, we explored the pathogenic role of IL-25 in SLE by administering IL-25 from week 14 to week 18. We found that the injection of IL-25 could markedly relieve the lupus symptoms of MRL/lpr mice, with reductions of anti-dsDNA and kidney damage, as well as IgG deposition. IL-25 was found to inhibit the production of the proinflammatory cytokines IL-1β, IL-6, IL-17, IFN-γ, and TNF-α in human SLE. Consistent with this, we also observed that IL-25 treatment significantly inhibited IL-1α, IL-1β, IL-6, IL-12p70, IL-17A, and IFN-β expression in MRL/lpr mice, indicating that IL-25 may ameliorate lupus through suppressing inflammatory cytokine production in lupus-prone MRL/lpr mice.

In conclusion, our studies demonstrate that serum IL-25 levels are upregulated in patients with SLE and positively associated with disease activity; moreover, the administration of IL-25 ameliorates lupus through suppressing inflammatory cytokine production in lupus-prone MRL/lpr mice. Our findings suggest a novel role of IL-25 in SLE and that IL-25 could potentially be used as a diagnostic and therapeutic target for SLE treatment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105680.

Declaration of Competing Interest

The authors declare that they have no competing interests.

References

[1] L. Lisnevskaia, G. Murphy, D. Isenberg, Systemic lupus erythematosus, Lancet 384 (9957) (2014) 1878–1888, https://doi.org/10.1016/s0140-6736(14)60128-8.

[2] M. Wahren-Herlenius, T. Dörner, Immunopathogenic mechanisms of systemic autoimmune disease, Lancet 382 (9894) (2013) 819–831, https://doi.org/10.1016/s0140-6736(13)60954-x.

[3] S.A. Apostolidis, L.A. Lieberman, K. Kis-Toth, J.C. Crispin, G.C. Tsokos, The dysregulation of cytokine networks in systemic lupus erythematosus, J. Interf. Cytokine Res. 31 (10) (2011) 769–779, https://doi.org/10.1089/jir.2011.0029.

[4] Y. Iwakura, H. Ishigame, S. Saijo, S. Nakae, Functional specialization of interleukin-17 family members, Immunity 34 (2) (2011) 149–162, https://doi.org/10.1016/j.immuni.2011.02.012.

[5] W. Raymond, G. Ostli-Eilertsen, S. Griffiths, J. Nossent, IL-17A levels in systemic lupus erythematosus associated with inflammatory markers and lower rates of malignancy and heart damage: evidence for a dual role, Eur. J. Rheumatol. 4 (1) (2017) 29–35, https://doi.org/10.5152/eurjrheum.2017.16059.

[6] H. Wu, Y. Zhen, Z. Ma, H. Li, J. Yu, Z.G. Xu, X.Y. Wang, H. Yi, Y.G. Yang, Arginase-1-dependent promotion of TH17 differentiation and disease progression by MDSCs in systemic lupus erythematosus, Sci. Transl. Med. 8 (331) (2016) 331ra340, https://doi.org/10.1126/scitranslmed.aae0482.

[7] J.C. Martin, D.L. Baeten, R. Josien, Emerging role of IL-17 and Th17 cells in systemic lupus erythematosus, Clin. Immunol. 154 (1) (2014) 1–12, https://doi.org/10.1016/j.clim.2014.05.004.

[8] E. Robak, L. Kulczycka-Siennicka, Z. Gerlicz, M. Kierstan, A. Korycka-Wolowiec, A. Sysa-Jedrzejowska, Correlations between concentrations of interleukin (IL)-17A, IL-17B and IL-17F, and endothelial cells and proangiogenic cytokines in systemic lupus erythematosus patients, Eur. Cytokine Netw. 24 (1) (2013) 60–68, https://doi.org/10.1684/ecn.2013.0330.

[9] S. AlFadhli, A. AlFailakawi, A.A. Ghanem, Th-17 related regulatory network in the pathogenesis of Arab patients with systemic lupus erythematosus and lupus nephritis, Int. J. Rheum. Dis. (2014), https://doi.org/10.1111/1756-185x.12393.

[10] C.M. Hedrich, T. Rauen, K. Kis-Toth, V.C. Kyttaris, G.C. Tsokos, cAMP-responsive element modulator α (CREMα) suppresses IL-17F protein expression in T lymphocytes from patients with systemic lupus erythematosus (SLE), J. Biol. Chem. 287 (7) (2012) 4715–4725, https://doi.org/10.1074/jbc.M111.323261.

[11] B. Yu, M. Guan, Y. Peng, Y. Shao, C. Zhang, X. Yue, J. Zhang, et al., Copy number variations of interleukin-17F, interleukin-21, and interleukin-22 are associated with systemic lupus erythematosus, Arthritis Rheum. 63 (11) (2011) 3487–3492, https://doi.org/10.1002/art.30595.

[12] M.M. Fort, J. Cheung, D. Yen, J. Li, S.M. Zurawski, S. Lo, S. Menon, et al., IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo, Immunity 15 (6) (2001) 985–995.

[13] J. Lee, W.H. Ho, M. Maruoka, R.T. Corpuz, D.T. Baldwin, J.S. Foster, A.D. Goddard, et al., IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1, J. Biol. Chem. 276 (2) (2001) 1660–1664, https://doi.org/10.1074/jbc.M008289200.

[14] Y.H. Wang, P. Angkasekwinai, N. Lu, K.S. Voo, K. Arima, S. Hanabuchi, A. Hippe, et al., IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells, J. Exp. Med. 204 (8) (2007) 1837–1847, https://doi.org/10.1084/jem.20070406.

[15] M.A. Kleinschek, A.M. Owyang, B. Joyce-Shaikh, C.L. Langrish, Y. Chen, D.M. Gorman, W.M. Blumenschein, et al., IL-25 regulates Th17 function in autoimmune inflammation, J. Exp. Med. 204 (1) (2007) 161–170, https://doi.org/10.1084/jem.20061738.

[16] D. Saadoun, B. Terrier, P. Cacoub, Interleukin-25: key regulator of inflammatory and autoimmune diseases, Curr. Pharm. Des. 17 (34) (2011) 3781–3785.

[17] J. Beale, A. Jayaraman, D.J. Jackson, J.D. Macintyre, M.R. Edwards, R.P. Walton, J. Zhu, et al., Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation, Sci. Transl. Med. 6 (256) (2014) 256ra134, https://doi.org/10.1126/scitranslmed.3009124.

[18] D. Liu, T. Cao, N. Wang, C. Liu, N. Ma, R. Tu, X. Min, IL-25 attenuates rheumatoid arthritis through suppression of Th17 immune responses in an IL-13-dependent manner, Sci. Rep. 6 (2016) 36002, , https://doi.org/10.1038/srep36002.

[19] M. Xu, H. Lu, Y.H. Lee, Y. Wu, K. Liu, Y. Shi, H. An, et al., An interleukin-25-mediated autoregulatory circuit in keratinocytes plays a pivotal role in psoriatic skin inflammation, Immunity (2018), https://doi.org/10.1016/j.immuni.2018.03.019.

[20] Marc C. Hochberg, Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus, Arthritis Rheum. 40 (9) (2010) 1725.

[21] E. Yavuz, Ş. Tokalıoğlu, Ş. Patat, Core–shell Fe3O4 polydopamine nanoparticles as sorbent for magnetic dispersive solid-phase extraction of copper from food samples, Food Chem. 263 (2018) 232–239.

[22] D. Novick, D. Elbirt, G. Miller, C.A. Dinarello, M. Rubinstein, Z.M. Sthoeger, High circulating levels of free interleukin-18 in patients with active SLE in the presence of elevated levels of interleukin-18 binding protein, J. Autoimmun. 34 (2) (2010) 121–126.

[23] E.M. Tan, A.S. Cohen, J.F. Fries, A.T. Masi, D.J. McShane, N.F. Rothfield, J.G. Schaller, N. Talal, R.J. Winchester, The 1982 revised criteria for the classification of systemic lupus erythematosus, Arthritis Rheum. 25 (11) (1982) 1271–1277.

[24] X. Zhao, X. Tang, Q. Yan, H. Song, Z. Li, D. Wang, H. Chen, L. Sun, Triptolide ameliorates lupus via the induction of miR-125a-5p mediating Treg upregulation, Int. Immunopharmacol. 71 (2019) 14–21, https://doi.org/10.1016/j.intimp.2019.02.047.

[25] G.S. Hill, M. Delahousse, D. Nochy, E. Tomkiewicz, P. Remy, F. Mignon, J.P. Mery, A new morphologic index for the evaluation of renal biopsies in lupus nephritis, Kidney Int. 58 (3) (2000) 1160–1173, https://doi.org/10.1046/j.1523-1755.2000.00272.x.

[26] Selvaraja, M., M. Abdullah, A Md Shah, M. B. Arip, and S Amin Nordin. 2017. 314 role of cytokine il-5 and il-25 as biomarkers in systemic lupus erythematosus.

[27] D.L. Su, Z.M. Lu, M.N. Shen, X. Li, L.Y. Sun, Roles of pro- and anti-inflammatory cytokines in the pathogenesis of SLE, J Biomed Biotechnol 2012 (4) (2012) 347141.

[28] Poliana Macedo Guimarães, Bruna Miglioranza Scavuzzi, Nicole Perugini Stadtlober, Lorena Flor Da Rosa Santos Silva, Marcell Alysson Batisti Lozovoy, Tathiana Mayumi Veiga Iriyoda, Neide Tomimura Costa, Edna Maria Vissoci Reiche, Michael Maes, Isaias Dichi, Cytokines in systemic lupus erythematosus: far beyond Th1|[sol]|Th2 dualism, Immunol. Cell Biol. 95 (9) (2017).

[29] L. Rönnblom, K.B. Elkon, Cytokines as therapeutic targets in SLE, Nat. Rev. Rheumatol. 6 (6) (2010) 339–347.

[30] S.S. Uppal, S.J. Hayat, R. Raghupathy, Efficacy and safety of infliximab in active SLE: a pilot study, Lupus 18 (8) (2009) 690.

[31] B. Ostendorf, C. Iking-Konert, K. Kurz, G. Jung, O. Sander, M. Schneider, Preliminary results of safety and efficacy of the interleukin 1 receptor antagonist anakinra in patients with severe lupus arthritis, Ann. Rheum. Dis. 64 (4) (2005) 630–633.

[32] Gabor G. Illei, Yuko Shirota, Cheryl H. Yarboro, Jimmy Daruwalla, Edward Tackey, Kazuki Takada, Thomas Fleisher, James E. Balow, Peter E. Lipsky, Tocilizumab in systemic lupus erythematosus: data on safety, preliminary efficacy, and impact on circulating plasma cells from an open-label phase I dosage-escalation study, Arthritis Rheumatol. 62 (2) (2010) 542–552.

[33] V. Ocampo, D. Haaland, K. Legault, S. Mittoo, E. Aitken, Successful treatment of recurrent pleural and pericardial effusions with tocilizumab in a patient with systemic lupus erythematous, BMJ Case Rep. 2016 (2016) bcr2016215423.

[34] F. Lavocat, N. Ndongo-Thiam, P. Miossec, Interleukin-25 produced by Synoviocytes has anti-inflammatory effects by acting as a receptor antagonist for interleukin-17A function, Front. Immunol. 8 (2017) 647, , https://doi.org/10.3389/fimmu.2017.00647.

[35] K. Ikeda, H. Nakajima, K. Suzuki, S. Kagami, K. Hirose, A. Suto, Y. Saito, I. Iwamoto, Mast cells produce interleukin-25 upon fc epsilon RI-mediated activation, Blood 101 (9) (2003) 3594.

[36] C.M. Kang, A.S. Jang, M.H. Ahn, J.A. Shin, J.H. Kim, Y.S. Choi, T.Y. Rhim, C.S. Park, Interleukin-25 and interleukin-13 production by alveolar macrophages in response to particles, Am. J. Respir. Cell Mol. Biol. 33 (3) (2005) 290–296.

[37] V. Dolgachev, B.C. Petersen, A.L. Budelsky, A.A. Berlin, N.W. Lukacs, Pulmonary IL-17E (IL-25) production and IL-17RB+ myeloid cell-derived Th2 cytokine production are dependent upon stem cell factor-induced responses during chronic allergic pulmonary disease, J. Immunol. 183 (9) (2009) 5705–5715.

[38] Pornpimon Angkasekwinai, Heon Park, Yui Hsi Wang, Yi Hong Wang, Seon Hee Chang, David B. Corry, Yong Jun Liu, Zhou Zhu, Chen Dong, Interleukin 25 promotes the initiation of proallergic type 2 responses, J. Exp. Med. 204 (7) (2007) 1509–1517.

[39] Y. Sonobe, H. Takeuchi, K. Kataoka, H. Li, S. Jin, M. Mimuro, Y. Hashizume, Y. Sano, T. Kanda, T. Mizuno, Interleukin-25 expressed by brain capillary endothelial cells maintains blood-brain barrier function in a protein kinase Cepsilon-dependent manner, J. Biol. Chem. 284 (46) (2009) 31834.

[40] Erika A. Rickel, Lori A. Siegel, Rin P. Yoon Bo, James Rottman, David Kugler, David Swart, Penny Anders, Joel E. Tocker, Michael R. Comeau, Alison L. Budelsky, 217 identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25 induced activities, Cytokine 43 (3) (2008) 291.

[41] C.K. Wong, P.F. Cheung, W.K. Ip, C.W. Lam, Interleukin-25-induced chemokines and interleukin-6 release from eosinophils is mediated by p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, and nuclear factor-kappaB, Am. J. Respir. Cell Mol. Biol. 33 (2) (2005) 186–194.

[42] L. Mandik-Nayak, S.J. Seo, C. Sokol, K.M. Potts, A. Bui, J. Erikson, MRL-lpr/lpr mice exhibit a defect in maintaining developmental arrest and follicular exclusion of anti-double-stranded DNA B cells, J. Exp. Med. 189 (11) (1999) 1799–1814.