Elevated IL-37, IL-18 and IL-18BP serum concentrations in patients with primary Sjögren's syndrome

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ABSTRACT

The objectives of this study were to examine the serum levels of interleukin (IL)-37 and its clinical association in patients with primary Sjögren's syndrome (pSS) and to investigate whether or not IL-37 participates in the regulation of the pathogenesis of pSS. ELISA was used to analyse the serum levels of IL-37, total IL-18 and IL-18 binding protein (IL-18BP). The level of free IL-18 was calculated based on the mass action law. The correlations between the IL-37 serum levels with the laboratory values and the total IL-18 and IL-18BP serum levels were analyzed by a Spearman's correlation test. The serum levels of IL-37 in the patients with pSS were significantly increased compared with the healthy controls (HCs). The levels were especially elevated in the patients with pSS with positive anti-Ro/SSA and/or anti-La/SSB antibodies. Furthermore, the patients with pSS showed high serum levels of total IL-18, free IL-18 and IL-18BP compared with the HCs. Strikingly, the IL-37 levels were significantly positively correlated with the antibody levels in the patients with pSS, including rheumatoid factor, anti-Ro/SSA, and anti-La/SSB and the total IL-18 and IL-18BP serum levels. The serum levels of IL-37, which were correlated with antibody production and the serum levels of total IL-18 and IL-18BP, were elevated in the patients with pSS. IL-37, an important anti-inflammatory cytokine, may participate in the regulation of the pathogenesis of pSS.

Primary Sjögren's syndrome (pSS) is a systemic inflammatory autoimmune disorder characterized by extensive lymphocytic infiltration of the salivary and lachrymal glands. However, pSS can also affect systems and organs other than the salivary and lacrimal glands, such as the kidneys, blood system, central nervous system and skin.[1] Interstitial lung disease (ILD) is also a very common symptom of patients with pSS. The prevalence of ILD in patients with pSS is ~5–35%.[2] The EULAR Sjögren's syndrome disease activity index is a clinical index designed to measure disease activity in patients with pSS.[3]

The pathogenesis of pSS is largely unknown and, until now, no universally effective therapy is available. The drug therapy for pSS with severe extraglandular manifestations includes mainly corticosteroids, cyclophosphamide, nucleoside analogs and biological drugs, such as rituximab.[4] Previous reports showed that pSS is a Th1-dominated disease primarily because interferon (IFN)-γ and its related cytokines are consistently found to be highly expressed in patients with pSS.[5] However, the significant hypergammaglobulinemia and high levels of autoantibodies, together with the high expression of interleukin (IL)-10 (another Th2 cytokine), demonstrated a simultaneous activation of the Th2 response.[6]

A cytokine network plays a central role in the regulation of the pathogenesis of pSS. Recent evidence indicated that the imbalance of proinflammatory and anti-inflammatory cytokines resulted in cumulative damage in the glands leading to decreased secretory function.[7 8] IL-18 is a proinflammatory cytokine, which was found to be overexpressed in pSS. IL-18 was detected in acinar cells, ductal cells and macrophages in the salivary glands of the patients with pSS.[7] The IL-18 serum levels in the patients with pSS were increased and correlated with anti-Ro/SSA and anti-La/SSB autoantibodies and C4 hypocomplementemia.[9] The IL-18 binding protein (IL-18BP) is a circulating protein with a high affinity for IL-18, acting as an IL-18 natural antagonist.[10] IFN-γ stimulates the production of IL-18BP, prompting a classical feedback loop whereby IL-18BP neutralizes excessive IL-18 and attenuates the IFN-γ response.[11]

IL-37, a new member of the IL-1 cytokine family, is a natural inhibitor of innate immunity.[12] IL-37 possesses the function of downregulated systemic and local inflammation. IL-37 is mainly induced in an inflammatory context. IL-1β, IL-18, TNF-α, IFN-γ, and TGF-β increase IL-37 synthesis. IL-37 can decrease the production of proinflammatory cytokines and protect mice from inflammatory and autoimmune diseases.[13] Increased IL-37 levels have been associated with many autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in humans.[13–15]

IL-37 can bind to the IL-18 receptor α chain (IL-18Rα), and this binding results in reduced inflammation.[16] Low doses of IL-18BP plus IL-37 effectively reduce the production of inflammatory cytokines induced by IL-18 in vitro.[16] IL-37 acts as an extracellular cytokine

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by binding to IL-18R but using IL-1R8 for its anti-inflammatory properties.[17] Therefore, we inferred that IL-37 acted as an endogenous control mechanism to reduce inflammation in autoimmune diseases by inhibiting IL-18-dependent proinflammatory cytokine production by binding to IL-18R and IL-18BP. In the present study, we measured the serum levels of IL-37, as well as the serum levels of IL-18 and IL-18BP, in patients with pSS to examine the serum levels of IL-37 and its clinical association and to investigate whether or not IL-37 participates in the pathogenesis of pSS.

PATIENTS AND SAMPLES

In this study, 120 patients with pSS and 40 healthy controls (HCs) were enrolled randomly from the First Affiliated Hospital of China Medical University. Patients fulfilled the revised American–European classification, which identifies six criteria.[18] All of the patients were recruited from the baseline and had not yet been treated with steroids and/or disease-modifying antirheumatic drugs before their blood samples were collected. Fully informed written consent was obtained from each patient, and the study was approved by the Ethics Committee of China Medical University. Blood samples were obtained for the determination of erythrocyte sedimentation rate (ESR), C reactive protein (CRP), rheumatoid factor (RF), IgG and anti-Ro/SSA and anti-La/SSB antibody. Serum from all subjects by centrifugation of peripheral blood samples was frozen at −80°C for later analysis. ILD was identified on high-resolution CT.

ELISA FOR IL-37, TOTAL IL-18 AND IL-18BP IN SERUM

The IL-37, total L-18 and IL-18BP levels in serum were determined by ELISA following the manufacturer's instructions (IL-37 Duo-Set ELISA kit, R&D systems; IL-18BP Quantikine ELISA kit, R&D systems). A 96-well microtiter plate had been precoated with a polyclonal-specific antibody. Then 100 μL of different standards or samples (1:2 dilutions) were added to the plate and incubated for 2 hours. After washing four times with phosphate-buffered saline containing 0.05% Tween 20 (T-PBS), 100 μL of a biotin-conjugated polyclonal-specific antibody were added to each microplate well and incubated for 2 hours. The plate was then washed four times with T-PBS and incubated with 100 μL of the working dilution of Streptavidin-horseradish peroxidase for 20 min. Following six washes with T-PBS, 100 μL of 3,3′,5,5′-tetramethylbenzidine was added to each well and incubated for 20 min. The reaction was stopped by the addition of 50 μL of Stop Solution, and the absorbance was examined using a microplate reader at 450 nm. Each sample was measured in triplicate. The level of free IL-18 was calculated based on the mass action law, using a dissociation constant of 400 pM and a stoichiometric ratio of 1:1.[19]

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS V.18.0 software. All of the data were analyzed with a homogeneity test. Results were expressed as mean±SD or median and range. The statistical significance of the differences between the cytokine levels in the patients with pSS and HCs and in the subgroups of the patients with pSS (anti-Ro/SSA+/anti-Ro/SSA− and anti-La/SSB+/anti-La/SSB−) was determined by a Mann-Whitney test. The correlations between the serum levels of IL-37 and laboratory values, as well as other cytokines, were assessed by a Spearman's correlation coefficient. For all experiments, $p<0.05$ was considered significant.

RESULTS

Clinical features of patients with pSS

This study enrolled 120 patients with pSS (112 female and 8 male) and 40 healthy volunteers (38 female and 2 male). Clinical features are indicated in table 1.

Increased serum levels of IL-37, total IL-18, free IL-18 and IL-18BP in patients with pSS

As shown in table 2, the IL-37 serum levels of the patients with pSS (median 438.4 pg/mL (43.71–2338 pg/mL)) were significantly higher than those in the HCs (median 96.31 pg/mL (39.08–328.2 pg/mL); $p<0.001$). Furthermore, the IL-37 serum levels were significantly increased in the anti-Ro/SSA-positive group (median 518.2 pg/mL (43.71–2338 pg/mL)) compared with the anti-Ro/SSA-negative group (median 290.2 pg/mL (59.94–892.6 pg/mL), $p=0.013$). Similarly, the IL-37 serum levels were significantly higher in the anti-La/SSB-positive pSS group (median 557.6 pg/mL (55.92–2338 pg/mL))

Table 1 Clinical data of patients with pSS

Characteristics	pSS	HC
Age (years)	36.2±10.4	33.4±16.1
Sex (F/M)	112/8	38/2
Disease duration (years)	6.3±4.7	–
ESR (mm/hour)	33.8±25.1	–
CRP (mg/L)	19.4±18.2	–
RF (IU/mL)	72.7±693.6	–
IgG (g/L)	18.4±22.7	–
Anti-SSA/Ro	78 (42)	–
Anti-SSB/La	58 (62)	–
ILD	53 (67)	–

CRP, C reactive protein; ESR, erythrocyte sedimentation rate; F, female; HC, healthy control; ILD, interstitial lung disease; M, male; pSS, primary Sjögren's syndrome; RF, rheumatoid factor.

Table 2 Levels of IL-37, IL-18 and IL-18BP in patients with pSS

	pSS n=120	HC n=40	p Value
IL-37 (pg/mL)	438.4 (43.71–2338)	96.31 (39.08–328.2)	p<0.001
Total IL-18 (pg/mL)	389.1 (64.07–1030)	135.9 (40.52–372)	p<0.001
IL-18BP (pg/mL)	1276 (266.4–2732)	727.4 (249.2–1880)	p<0.001
Free IL-18 (pg/mL)	162.13 (45.76–271.05)	73.46 (36.84–190.77)	p<0.01

Values are presented as median and range.
HC, healthy control; IL, interleukin; IL18-BP, interleukin 18 binding protein; pSS, primary Sjögren's syndrome.

compared with the anti-La/SSB-negative group (median 326.9 pg/mL (43.71–1663 pg/mL); p=0.029). There was no difference in the IL-37 levels between the patients with pSS with and without ILD (median 497.1 pg/mL (64.75–2338 pg/mL) vs median 392 pg/mL (43.71–1721 pg/mL); p=0.583; figure 1A). Meanwhile, the total IL-18, free IL-18 and IL-18BP serum levels of the patients with pSS were significantly higher than those in the HCs (table 2). In addition, the total IL-18 and IL-18BP levels were significantly higher in the anti-Ro/SSA-positive and anti-La/SSB-positive pSS groups compared with the anti-Ro/SSA-negative and anti-La/SSB-negative groups. There was no difference in the total IL-18 or IL-18BP levels between the patients with pSS with and without ILD (figure 1B, C).

Correlation between cytokine levels and laboratory values in patients with pSS

Significant positive correlations were found between the IL-37 serum levels and antibody production, including RF and IgG, in the patients with pSS (figure 2A, B). However, there was no correlation between the IL-37 levels and other clinical parameters, including age, ESR, CRP and ILD. We also found that the total IL-18 serum levels were positively correlated with the RF and IgG values (figure 2C, D).

Furthermore, the IL-37 serum levels showed a significant positive correlation with the total IL-18 and IL-18BP serum levels (figure 3A, B).

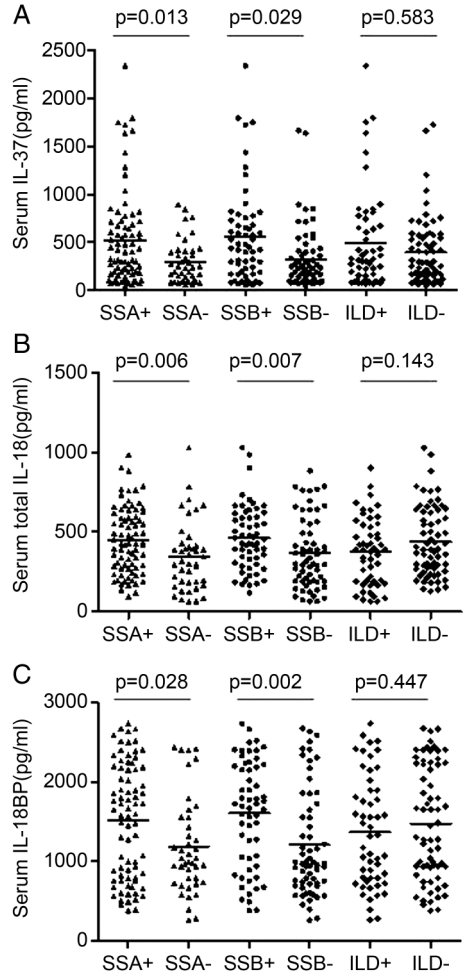

Figure 1 (A) IL-37 serum levels in pSS; (B) total IL-18 serum levels in pSS; (C) IL-18BP serum levels in pSS. IL, interleukin; IL18-BP, interleukin 18 binding protein; pSS, primary Sjögren's syndrome.

DISCUSSION

IL-37 has been detected in various autoimmune diseases. It has been found in synoviocytes from RA, monocytes from patients with SLE,[13] keratinocytes from psoriasis lesions and bowel macrophages from patients with Crohn's disease.[20] However, the association between IL-37 and pSS is still unclear. In this study, we, for the first time, showed that the IL-37 serum levels were elevated in the patients with pSS, particularly in the anti-Ro/SSA-positive and/or anti-La/SSB-positive patients. Anti-Ro/SSA can be found alone in many serums, while anti-La/SSB antibodies are usually accompanied by anti-Ro/SSA.[21] Anti-Ro/SSA antibodies are detectable in 63% of pSS serum samples and in 46% of SLE samples,[22] compared with only 3–15% of patients with RA and 3–11% of patients with SSA-positive systemic sclerosis.[21] Most of the extraglandular complications of pSS are more common among patients with anti-Ro/SSA and anti-La/SSB, including lymphoma, vasculitis, renal tubular acidosis and ILD.[23] Furthermore, we showed that IL-37 is strongly associated with antibody production (immunoglobulin (Ig), RF, SSA, and SSB). Since we know that the significant hypergammaglobulinemia and high levels of autoantibodies are associated with the pathogenesis of pSS and play an important role in organ involvement (such as glands, kidney and blood),[1] we deduced that IL-37 therapy would work due to the strong correlation with antibody production. However, we did not show the correlation between IL-37 and other clinical features, ESR, CRP and ILD. The reason for that may be that ESR and CRP are non-specific inflammation indexes which may be affected by several factors. Although ILD is a common complication in patients with pSS, we did not show the correlation between IL-37 and ILD. Since we know that many other cytokines play roles in the pathogenesis of ILD in autoimmune diseases, such as IL-4, etc, in general we showed a significant correlation between IL-37 and antibody production. It is interesting and IL-37 may be a therapy target especially for those patients with pSS with high Ig.

IL-18, by acting as a proinflammatory cytokine, can regulate autoimmune diseases through its activation of Th1 and Th2 responses.[24 25] IL-18BP, acting as a natural inhibitor of IL-18 activity, is a soluble protein with a high affinity for IL-37.[16] IL-18 was detected in the salivary glands of the patients with pSS and was particularly high in those patients with anti-Ro/SSA and anti-La/SSB autoantibodies.[9] In this study, we also found elevated total IL-18 and IL-18BP serum levels in patients with pSS, which positively correlated with anti-Ro/SSA and anti-La/SSB autoantibodies.

More interestingly, in our study, we first showed that IL-37 associated with total IL-18 and IL-18BP in the patients with pSS.

However, the role of IL-37 in the pathogenesis of pSS has remained elusive. Therefore, we hypothesized that IL-37 possibly plays an important negative regulatory role

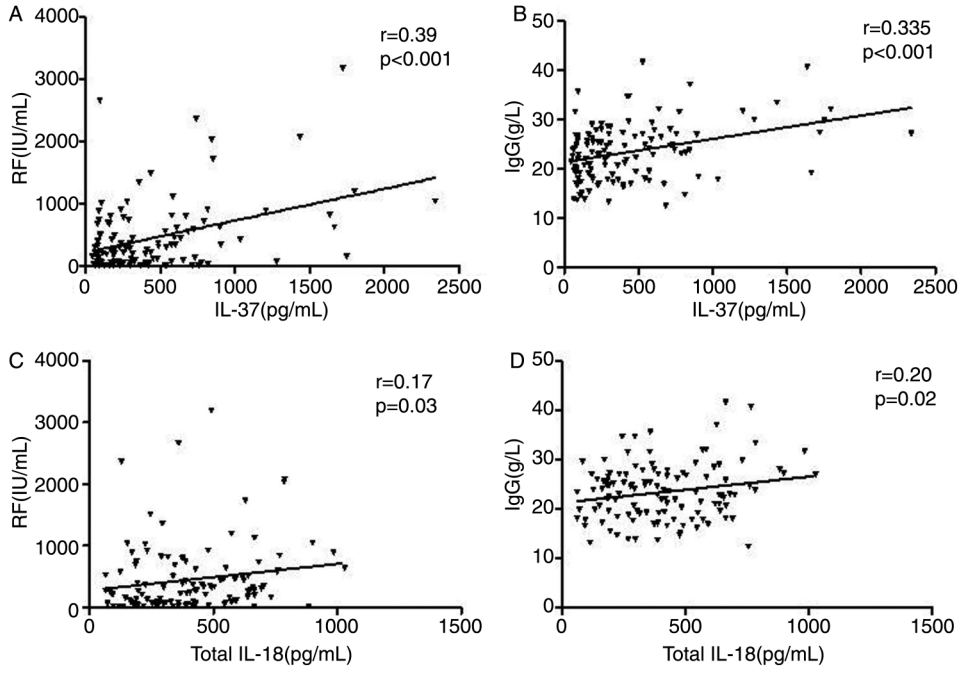

Figure 2 (A) Correlation between IL-37 serum levels and RF in patients with pSS; (B) correlation between IL-37 serum levels and IgG values in patients with pSS; (C) correlation between total IL-18 serum levels and RF in patients with pSS; (D) correlation between total IL-18 serum levels and IgG values in patients with pSS. IL, interleukin; pSS, primary Sjögren's syndrome; RF, rheumatoid factor.

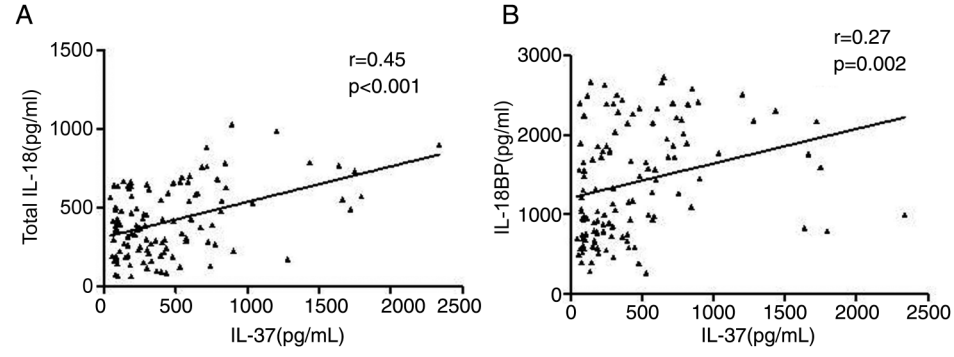

Figure 3 (A) Correlation between IL-37 serum levels and total IL-18 serum levels in patients with pSS; (B) correlation between IL-37 serum levels and IL-18BP serum levels in patients with pSS. IL, interleukin; IL18-BP, interleukin 18 binding protein; pSS, primary Sjögren's syndrome.

as an anti-inflammatory cytokine due to direct and indirect effects.

First, previous reports showed a direct anti-inflammation effect of IL-37 in inflammatory responses. A study in the models of lipopolysaccharide (LPS) shock and chemical colitis suggested that the inflammation is reduced after human IL-37 was expressed in mice.[12] Inhibition of endogenous IL-37 with siRNA in human peripheral blood mononuclear cells (PBMCs), cytokines induced by IL-1β and LPS, increased twofold to threefold, which suggested that endogenous IL-37 serves as a break on inflammation.[12] This study in vitro demonstrates that IL-37 expression in human CD4+CD25+Treg can promote the suppressive effect on T lymphocyte activation.[26]

Second, some previous studies showed that as loss of membrane integrity on cell death, the IL-37 precursor exits from the cell and binds to the IL-18Rα. Accordingly, this binding may result in reduced inflammation. In addition, evidence showed that IL-37 binds to IL-18BP and then binds to the IL-18R-β chain, thus depriving this chain from participating in IL-18 signal transduction.[16] IL-37 bound to IL-18Rα and exploited IL-1R8 to activate a multifaceted intracellular anti-inflammatory programme. The IL-37–IL-1R8–IL-18Rα complex assembled rapidly on the surface of PBMC on stimulation with LPS. IL-37 used IL-1R8 to harness the anti-inflammatory properties and to inhibit the transcription factor nuclear factor-κB, as well as mitogen-activated protein kinases.[17]Accordingly, we hypothesized that IL-37 attenuated the proinflammatory effect of IL-18 in conjunction with IL-18Rα and plays an important negative regulatory role as an anti-inflammatory cytokine.

In conclusion, IL-37 may act as an anti-inflammation cytokine in pSS. Thus, IL-37 may be a novel therapy for pSS.

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