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Increased serum IL-36α and IL-36γ levels in patients with systemic lupus erythematosus: Association with disease activity and arthritis

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ABSTRACT

IL-36 cytokines (IL-36Ra, IL-36α, IL-36β and IL-36γ) belong to the IL-1 family and have been linked to several autoimmune diseases. However, little is known about the relationships between systemic lupus erythematosus (SLE) and IL-36 cytokines. In this study, serum IL-36 cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA), and their associations with SLE-related parameters were analyzed in 72 SLE patients and 63 healthy controls. Additionally, IL-36 cytokine mRNA levels were assessed in 30 of 72 SLE patients and 20 of 63 healthy controls using real-time quantitative reverse transcription polymerase chain reaction (RT-PCR). Compared to healthy controls, SLE patients had significantly decreased serum IL-36Ra levels ($P = 0.001$) and markedly increased serum IL-36α and IL-36γ levels ($P = 0.004$ and $P = 0.001$, respectively). Serum IL-36α and IL-36γ levels were significantly higher in active SLE patients [SLE Disease Activity Index (SLEDAI) score ≥ 5] than in inactive patients (SLEDAI score ≤ 4) ($P = 0.020$ and $P = 0.017$, respectively). Serum IL-36α and IL-36γ levels were strongly correlated with SLEDAI score ($r = 0.308$, $P = 0.008$ and $r = 0.400$, $P = 0.001$, respectively) and complement C3 levels ($r = -0.276$, $P = 0.019$ and $r = -0.314$, $P = 0.007$, respectively). Moreover, SLE patients with arthritis had significantly higher serum IL-36α and IL-36γ levels than those without arthritis ($P = 0.001$ and $P < 0.001$, respectively). Our study indicates that the imbalanced antagonist/agonist profile of IL-36 cytokines may be linked to SLE pathogenesis. Furthermore, IL-36α and IL-36γ may participate in arthritis and may be good biomarkers of SLE disease activity.

1. Introduction

Systemic lupus erythematosus (SLE) is a devastating autoimmune disease that involves multiple organs and systems, including the vascular system, joints, skin, hematopoietic system, kidney and brain. Although the pathogenesis of SLE is not completely understood, significant evidence suggests that the overproduction of autoantibodies, immune complex deposition and the abnormal production of cytokines play vital roles in the onset and development of the disease [1]. Moreover, cytokine dysregulation, such as imbalanced anti-/pro-inflammatory cytokine profiles and imbalanced agonist/antagonist levels, is pervasive in SLE and may be implicated as part of the pathogenic core process of lupus [2].

IL-36 cytokines, which belong to the IL-1 family, were identified through searches of DNA databases for IL-1 homologs approximately one decade ago [3–5]. Members of the IL-36 cytokines include three agonists (IL-36α, IL-36β and IL-36γ) and one receptor antagonist (IL-36Ra). These cytokines are expressed not only in keratinocytes, but also in immune cells, such as monocytes, macrophages, dendritic cells (DCs), T cells and B cells [4,6]. IL-36α, IL-36β and IL-36γ bind to the same receptor composed of the specific subunit IL-36R (IL-1Rrp2) and the common receptor subunit IL-1R accessory protein (IL-1RAcP), leading to the activation of similar intracellular signals, including the NF-κB and MAPK pathways [7]. Through the stimulation of intracellular signals, IL-36α, IL-36β and IL-36γ can promote the production of pro-inflammatory cytokines and act directly on DCs and $CD4^+$ T cells, thus enhancing Th1 responses in an IL-2-dependent manner [8,9]. IL-36Ra is a natural antagonist of the three IL-36 agonists. IL-36Ra can also bind to IL-36R (IL-1Rrp2), but it fails to elicit the subsequent interaction with IL-1RAcP, thus preventing the formation of a functional signaling complex and limiting uncontrolled inflammation and immune responses [10].

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The aberrant expression and function of IL-36 cytokines have been linked to several autoimmune diseases, such as psoriasis [11–13], rheumatoid arthritis [14,15] and primary Sjögren's syndrome [16]. Moreover, in 2015, Chu et al. showed for the first time that serum concentrations of IL-36α and IL-36γ were significantly increased in active SLE patients (SLEDAI score ≥ 6) compared to normal controls and that IL-36α and IL-36γ exerted pro-inflammatory effects by inducing IL-6 and CXCL8 in peripheral blood mononuclear cells (PBMCs) from SLE patients [17].

To the best of our knowledge, there are limited data on the connection between IL-36 cytokines and SLE. We therefore compared the serum and mRNA levels of IL-36Ra, IL-36α, IL-36β and IL-36γ in SLE patients to the levels in healthy controls. In addition, we analyzed the associations between serum levels of IL-36 cytokines and major clinical characteristics of SLE patients.

2. Materials and methods

2.1. Patients and healthy controls

Between September 2016 and August 2017, 72 Chinese patients with SLE were recruited from the Department of Dermatology at Sun Yat-sen Memorial Hospital. All patients fulfilled the American College of Rheumatology (ACR) criteria for the classification of SLE updated in 1997 [18]. In addition, a group of 63 age- and gender-matched healthy individuals without any evidence of infection or autoimmune disease were enrolled as healthy controls. Informed consent was obtained from each participant. This study was approved by the Clinical Research Ethics Committee of Sun Yat-sen Memorial Hospital.

Disease activity was assessed by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Active disease was defined as a SLEDAI score of ≥5, and patients with a SLEDAI score of ≤4 were classified as inactive [19]. Flare was defined as an increase in SLEDAI score of ≥4 from the previous visit [20]. Patients who were recently diagnosed in our hospital without taking glucocorticoids or immunosuppressants were regarded as new-onset patients. Patients were recorded as having arthritis if they fulfilled the ACR criteria for arthritis. Individual patient data concerning demographic characteristics, clinical manifestations and medication were obtained from medical charts. In addition, laboratory test results, including routine blood and urine levels, 24 h urinary protein, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), liver and renal function, serum complement C3 and C4 levels, immunoglobulin levels (IgG, IgM and IgA) and autoantibody detection were also recorded.

2.2. Real-time quantitative reverse transcription polymerase chain reaction (RT-PCR)

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples using density gradient centrifugation. Total RNA was extracted from PBMCs using RNAiso Plus reagent and reverse transcribed to cDNA using PrimeScript™ RT Master Mix. The mRNA expression levels of IL-36 cytokines were determined by real-time fluorescent quantitative PCR using SYBR® Premix Ex Taq™ and the LightCycler® 96 system (Roche, Basel, Switzerland). The reagents listed above were all obtained from Takara Bio Inc., Dalian, China. In addition, the tests were performed in accordance with the manufacturer's instructions. β-Actin was used as an internal reference. The specific primer sequences are listed in Table 1. The mRNA expression levels of target genes were normalized to the levels of β-Actin, and the results were expressed as $2^{-\Delta CT}$ [8,9].

2.3. Enzyme-linked immunosorbent assay (ELISA)

Blood samples were obtained from every participant, and serum was obtained and stored at −80 °C until use. The serum levels of IL-36Ra, IL-36α, IL-36β and IL-36γ were measured using Duoset ELISA kits (all from R&D Systems, Minneapolis, MN, USA). All analyses were performed according to the manufacturer's instructions. Each sample was tested in duplicate.

Table 1
Specific RT-PCR primer sequences.

Genes	Sequences
IL-36Ra	Forward primer: 5′-TCTGACTTAGTGGGCACCTGA-3′ Reverse primer: 3′-CCACAGCAGTAGCACCATCC-5′
IL-36α	Forward primer: 5′-TTCAGGACCAGACGCTCATA-3′ Reverse primer: 3′-TCTTTCTCAAGGGTCTCCACAT-5′
IL-36β	Forward primer: 5′-TGAACCCACAACGGGAGG-3′ Reverse primer: 3′-AATGCTGCGGCTAAGAGGA-5′
IL-36γ	Forward primer: 5′-GACTGGTTCATTGCCTCCTC-3′ Reverse primer: 3′-AGACCAAGCTGCCACCTCTA-5′
β-Actin	Forward primer: 5′-TGGAACGGTGAAGGTGACAG-3′ Reverse primer: 3′-AACAACGCATCTCATATTTGGAA-5′

2.4. Statistical analysis

The data were analyzed using SPSS 20.0 software (SPSS Science, Chicago, USA). The serum levels of the IL-36 cytokines were expressed as the median and interquartile range (IQR), whereas other quantitative data, such as age, were presented as the mean ± standard deviation (SD). The nonparametric Mann-Whitney *U* test was performed to compare the serum levels or mRNA expression levels of IL-36 cytokines between the two different groups, and the Kruskal-Wallis test was used to compare the serum levels of the IL-36 cytokines among the three groups. In addition, correlations between IL-36 cytokines and SLE-related parameters were determined using Spearman's rank correlation test. A P value of < 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 72 SLE patients (64 females and 8 males; mean age, 37.36 ± 15.42 years; range, 17 to 68 years) and 63 healthy controls (57 females and 6 males; mean age, 36.49 ± 15.56 years; range, 16 to 65 years) were recruited for this study. Overall, 27.8% (20/72) of the SLE patients were new-onset, 45.8% (33/72) were in remission and 26.4% (19/72) were in flare. Clinical and laboratory characteristics of the SLE patients are shown in Table 2.

3.2. mRNA expression of IL-36 cytokines in PBMCs from SLE patients and healthy controls

To preliminarily investigate the transcription levels of IL-36 cytokines, mRNA from 30 of 72 SLE patients and 20 of 63 healthy controls was analyzed by RT-PCR. As shown in Fig. 1A, B and C, the mRNA levels of IL-36Ra and IL-36α were decreased, whereas that of IL-36γ was significantly up-regulated in SLE patients compared to healthy controls (all $P < 0.05$). In addition, the mRNA expression of IL-36β was undetectable in both SLE patients and healthy controls.

3.3. Serum levels of IL-36 cytokines in SLE patients and healthy controls

Serum levels of IL-36 cytokines from 72 SLE patients and 63 healthy controls were determined by ELISA. Serum IL-36Ra was detectable (≥93.8 pg/ml) in 6.94% and 23.81% of serum samples in SLE patients and healthy controls, respectively. The frequency of detectable serum IL-36Ra was significantly lower in SLE patients than in healthy controls ($\chi^2 = 7.573$, $P = 0.007$). As shown in Table 3, the median (IQR) of serum IL-36Ra was 0 (0–0) pg/ml in the SLE group and 0 (0–47.50) pg/