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ORIGINAL ARTICLE

Xinfang Huang · Jing Hua · Nan Shen · Shunle Chen

Dysregulated expression of interleukin-23 and interleukin-12 subunits in systemic lupus erythematosus patients

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Abstract The aim of this study was to investigate the regulation of interleukin (IL)-12 and IL-23 expression in the autoimmune disease, systemic lupus erythematosus (SLE). mRNA from healthy subjects and SLE patients were prepared from peripheral blood mononuclear cells (PBMC) and quantitative real-time polymerase chain reaction was performed to quantify IL-23 specific subunit P19, IL-12 specific subunit P35, and their common subunit P40. IL-12 specific subunit P35 mRNA expression in untreated and treated SLE patients was significantly lower than healthy controls (P = 0.015 and 0.000, respectively). Compared with untreated SLE patients, treatment of SLE patients with corticosteroids or corticosteroids plus another immunosuppressor significantly suppressed P40 and P19 expression (P = 0.002 and 0.015, respectively). The mRNA levels of p19, p40, and p35 in active SLE patients (SLEDAT > 10) were significantly higher compared with those in the inactive SLE patients (SLEDAI ≤ 10) (P = 0.000, 0.000, and 0.017, respectively). These results suggest that deficiency of IL-12 and possibly upregulation of IL-23 may contribute to SLE pathogenesis and both cytokines may be therapeutic targets in SLE.

Key words Interleukin-12 · Interleukin-23 · Peripheral blood mononuclear cells (PBMC) · Systemic lupus erythematosus

Introduction

The heterodimeric cytokines, interleukin (IL)-12 and IL-23, share a common p40 subunit, yet they comprise unique p35 and p19 subunits, respectively and have a divergent role in autoimmunity. Many studies have shown that IL-12 was essential for the development of T-cell-dependent immune and inflammatory responses.[1–3] Recently, however, more and more evidence has shown that IL-23 plays a dominant role in Th1-mediated autoimmune disease such as EAE (experimental autoimmune encephalomyelitis) and collagen-induced arthritis.[4,5]

Systemic lupus erythematosus (SLE) is an autoimmune disease in which Th1 and Th2 immunity are imbalanced. The role of IL-12 and IL-23 in human diseases such as SLE remains speculative. The current study aims to investigate the regulation of IL-12 and IL-23 expression in the autoimmune disease, systemic lupus erythematosus (SLE).

X. Huang · J. Hua · N. Shen (✉) · S. Chen
Joint Molecular Rheumatology Laboratory of Shanghai Ren Ji Hospital and Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiao Tong University, 145 Shandong Mid Road, 200001, Shanghai, China
Tel. +86-21-63284623; Fax +86-21-63363475
e-mail: shennand@online.sh.cn

Methods

Peripheral blood mononuclear cells (PBMCs) from SLE patients and healthy controls

This study included 78 Chinese patients (69 women and 9 men, mean age was 30.59 ± 1.32 years). Fifty-one patients were treated with corticosteroids, with or without other immunosuppressive agents. All patients fulfilled the American College of Rheumatology 2000 revised criteria for the classification of SLE.[6] Disease activity was assessed using the SLE Disease Activity index (SLEDAI).[7] Active patients were classified as SLE SLEDAI >10, while the SLEDAI of inactive patients were ≤10. Thirty-six aged-matched healthy controls (33 females, 3 males, mean age 27.86 ± 1.62 years) were also studied. Blood was collected after obtaining informed consent.

Total RNA extraction and preparation

Venous peripheral blood anticoagulated by acid citrate dextrose (ACD) was collected. Erythrocytes were then lysed with a lysis solution containing amine oxalate. RNA was extracted from human peripheral PBMCs with Trizol (Life Technologies, Carlsbad, CA, USA). Conversion of total RNA to cDNA was conducted by reverse transcrip-

tion using SuperScript RNase H Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA).

Quantitative analysis of P19, P35, P40 mRNA

Quantitative analysis of gene expression was performed with the GeneAmp 7900 Sequence Detection System (Applied Biosystems, Fostey City, CA, USA). The primers for IL-12 p40, p19, and p35 were generated using the Primer Express algorithm version 2.0 from published sequences (National Center for Biotechnology Information). All primers were synthesized by the Takara Company (Tokyo, Japan) as follows. The human IL-12 and IL-23 common subunit, p40 (sequence data available from GenBank/EMBL/DDBJ under accession number NM_002187): forward primer CTGGCCAGTACACCTGTCACA; reverse primer GAAGCAGCAGGAGCGAATG. Human IL-12 specific p35 (sequence data available from GenBank/EMBL/DDBJ under accession number NM_000882): forward primer AGGGCCGTCAGCAACATG; reverse primer TCTTCAGAAGTGCAAGGGTAAAATTC. Human IL-23 specific subunit p19 (sequence data available from GenBank/EMBL/DDBJ under accession number NM_016584): forward primer TTCTGC-TTGCAAAGGA TCCA; reverse primer AATATCCGATCCTAGCAGC TTCTC. GAPDH (sequence data available from GenBank/EMBL/DDBJ under accession number BT006893): forward primer 5′-GAAGGTGAAGGTCGGAGTC-3′; reverse primer 5′-GAAGATGGTGATGGGTTTC-3′.

Real-time polymerase chain reactions (PCR) were performed according to the manufacturer's instructions SYBRGreen (Qiagen, Hilden, Germany). We used an Applied Biosystems PRISM 7900 thermal cycler for 2 min at 50°C, 30 min at 60°C, 5 min at 95°C, and 40 cycles of 15 s at 95°C, followed by 60 s at 60°C. Human GAPDH, a housekeeping gene, was used to normalize each sample and gene. The data were analyzed with the Sequence Detection Systems version 2.1.1 software.

Statistical analysis

One-way analysis of variance and independent sample *t*-test were used to compare p40, p35, and p19 gene expression levels with SPSS software version 10.0 (SPSS, Chicago, IL, USA). The data we compared are Avg $-\Delta\Delta$Ct, the higher $-\Delta\Delta$Ct meaning higher mRNA expression.

Results

We analyzed the mRNA levels of p40, p35, and p19 in SLE patients and healthy controls. The values obtained for each gene were normalized to the housekeeping gene, GAPDH. The mean expression of p40 and p19 increased in the untreated SLE patients (1.87 ± 0.56 and 0.18 ± 0.40, respectively) when compared with healthy controls (1.37 ± 0.732 and 0.09 ± 0.58, respectively), although this difference was not statistically significant (both $P > 0.05$) (Fig. 1A,B).

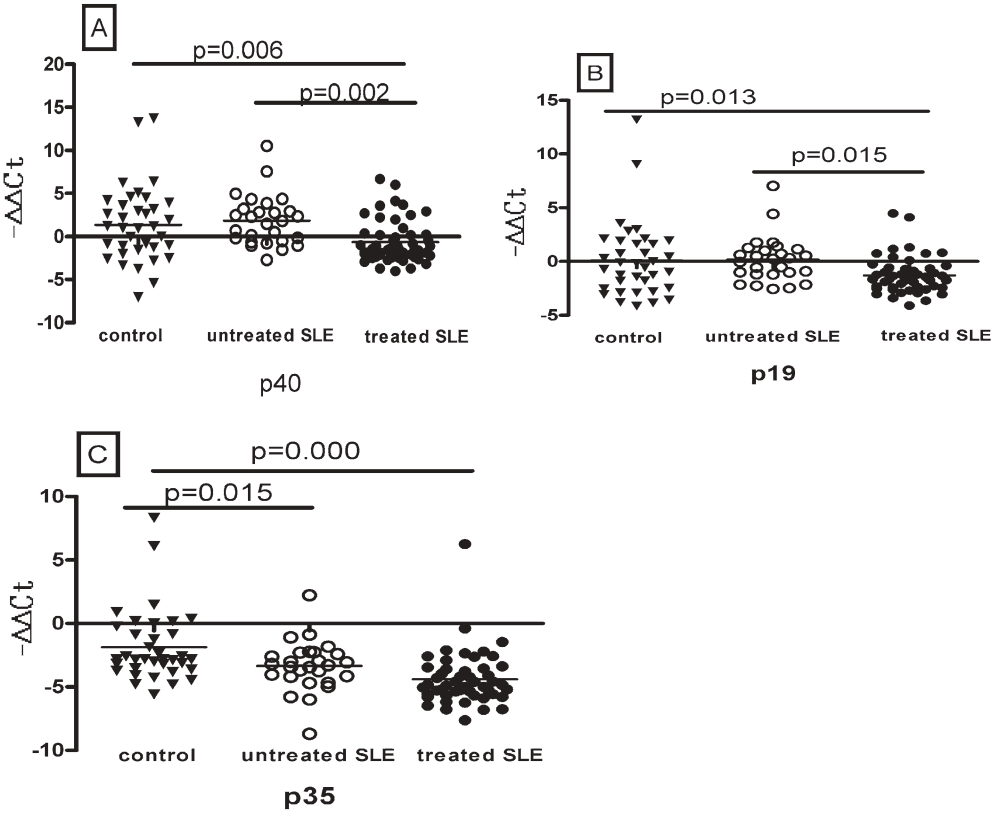

Fig. 1. mRNA level comparison of healthy controls, and untreated and treated SLE patients. *Horizontal bars* represent mean mRNA expression values. The expression of p40 in treated SLE patients was significantly lower than that in healthy controls and untreated SLE patients ($P = 0.006$ and 0.002, respectively; **A**). The expression of p19 in treated SLE patients was significantly lower than those in healthy controls and untreated SLE patients ($P = 0.013$ and 0.015, respectively; **B**). The expression of p35 in untreated SLE patients and treated SLE patients was significantly lower than in healthy controls ($P = 0.015$ and 0.000, respectively; **C**)

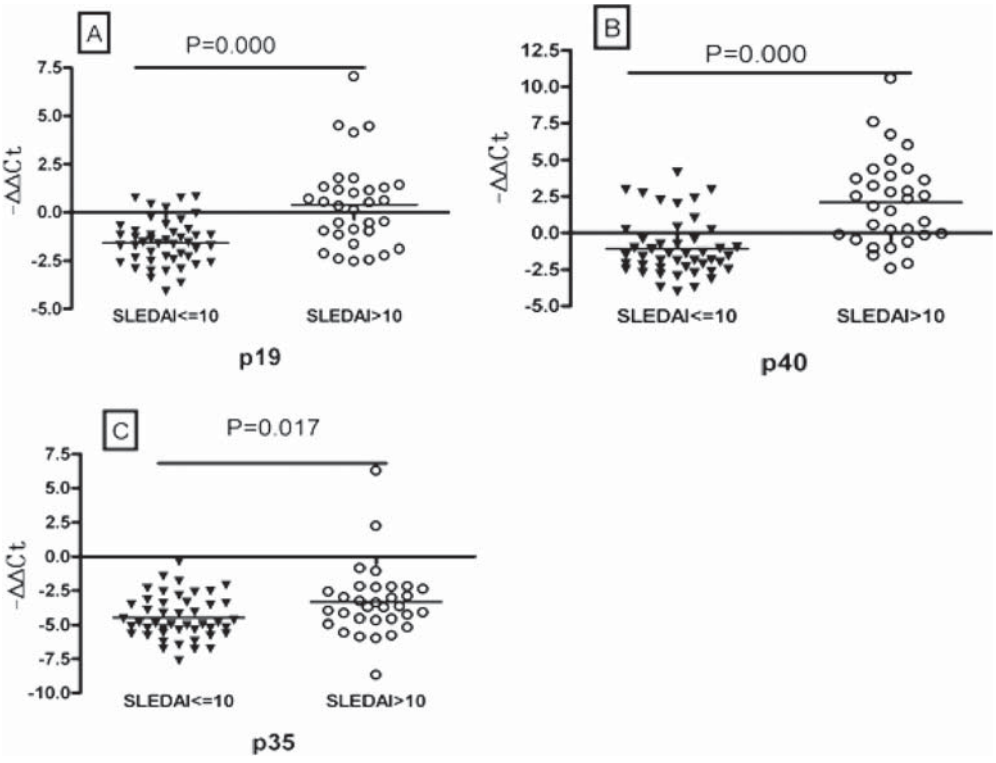

Fig. 2. mRNA level comparisons between active SLE patients (SLEDAI > 10) and inactive SLE patients (SLEDAI≤10). The expression of p19 mRNA is higher in active SLE patients (SLEDAI > 10) relative to inactive SLE patients (SLEDAI ≤ 10) ($P = 0.000$; **A**). The expression of p40 mRNA is higher in active SLE patients (SLEDAI > 10) as compared to inactive SLE patients (SLEDAI ≤ 10) ($P = 0.000$; **B**). The expression of p35 mRNA is higher in active SLE patients (SLEDAI > 10) relative to inactive SLE patients (SLEDAI ≤ 10). Healthy controls are not included in the SLEDAI ≤10 group

However, the mRNA levels of p40 and p19 were obviously lower in the treated SLE (–0.63 ± 0.35 and –1.28 ± 0.23, respectively) compared with healthy controls (1.37 ± 0.732 and 0.09 ± 0.58, respectively) ($P = 0.006$, 0.002) and untreated SLE patients (1.87 ± 0.56 and 0.18 ± 0.40, respectively) ($P = 0.013$ and 0.015, respectively) (Fig. 1A,B). p35 expression was lower both in the untreated (–3.34 ± 0.38) and treated SLE patients (–4.38 ± 0.30) as compared to healthy controls (–1.87 ± 0.47) ($P = 0.015$ and 0.000, respectively) (Fig. 1C).

We also analyzed the mRNA levels in SLE patients with and without lupus nephritis. The mRNA levels of p19, p40, and p35 were unchanged between SLE patients with lupus nephritis and those without lupus nephritis. The mRNA levels of p19, p40, and p35 in active SLE patients (SLEDAT > 10) (0.39 ± 0.40, 2.09 ± 0.53, and –3.35 ± 0.46, respectively) was higher relative to the inactive SLE patients (SLEDAI ≤ 10) (–1.58 ± 0.17, –1.06 ± 0.29, and –4.50 ± 0.23, respectively). The *P* values were 0.000, 0.000, and 0.017, respectively. (Fig. 2A–C).

Discussion

Interleukin-12 and IL-23 share similar functions such as enhancing cellular proliferation, inducing interferon (IFN)-γ production, and promoting Th1 costimulation by dendritic cells (DC). Because IL-12 plays an important role in driving naive T cells into IFN-γ-producing Th1 cells, IL-12 was considered an critical player in the pathogenesis of cell-mediated immunity.[8,9] However, subsequent studies have found that in C57BL/6 mice induced by myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 (MOG35–55), IL-12 p40-deficient (–/–) mice were resistant to EAE and the spinal cords had no abnormal expression, while IL-12p35(–/–) mice were susceptible to EAE and mononuclear cell infiltration and demyelination were found in the spinal cords.[10] Because IL-12 p40 is shared with IL-23, the role of IL-23 was considered in immune disease. Interleukin-23 is expressed by activated dendritic cells derived from peripheral blood monocytes under the stimulation of granulocyte macrophage-colony stimulating factor and IL-4, which was found in both mouse and human. In contrast, the same result was not observed in bone marrow-derived dendritic cells.[11] Similar to IL-12, IL-23 also plays an important role in Th1 cell-mediated immunity and induces much less production of IFN-γ as compared to IL-12. The expression of IL-23 is increased in psoriasis patients, which is an IFN-γ-producing Th1-mediated disease, while IL-12 was not found to be increased, suggesting that IL-23 plays a more important role than IL-12 in psoriasis.[12]

Systemic lupus erythematosus is characterized by an imbalance of Th1/Th2 and Tc1/Tc2 cytokines. It has been reported that there was a relative or absolute deficiency in Th1-type cytokines and a relative or absolute excess in Th2-type cytokines in SLE patients.[13,14]

In our studies, we found that p35 expression was decreased in SLE patients, which suggested that IL-12 expression was decreased in SLE patients. This is also consistent with other reports.[15,16] The significance of lower expression of IL-12 in SLE patient is not clear. It may antagonize IL-23 to regulate autoimmunity.

Other studies also reported that in SLE patients, the level of IL-12 was increased. Interestingly, patients with high levels of IL-12 also had high levels of IFN-gamma,[17] which was inconsistent with our results. Perhaps more replicate research is needed to verify the expression of IL-12 in SLE.

The mean expression of p40 and p19 increased in the untreated SLE patients. Although the decrease was not statistically significant, it was obviously decreased in SLE patients treated with corticosteroids or corticosteroids plus other immunosuppressors compared with untreated patients, which indicated IL-23 was responsive to the therapy. We also found that active SLE patients (SLEDAI > 10) had higher expression of p40 and p19 than the inactive patients, which indicated that IL-23 was associated with SLE activity and might be a good marker for SLE remission. Based on our data, both IL-12 and IL-23 were not related to lupus nephritis. Active SLE patients were characterized with the activation of macrophages and activated dendritic cells, and T and B lymphocytes. Glucocorticoids and immunosuppressants such as methotrexate, hydroxychloroquine/chloroquine, cellcept, and cyclosporine used in SLE patients inhibit activated macrophages, dendritic cells, T lymphocytes, B lymphocytes, and even reduce the number of these activated immune cells. Because activated macrophages and dendritic cells increase the production of IL-12 and IL-23, this might lead to the decreases in IL-12 and IL-23 mRNA expression in treated and inactive SLE patients.

In the future, we should attempt to discern whether IL-23 could serve as a therapeutic target and assess its relationship with lupus activity. These results suggest that an IL-12 deficiency and possibly IL-23 upregulation may contribute to SLE pathogenesis, and both cytokines may serve as SLE therapeutic targets.

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