Xinfang Huang · Jing Hua · Nan Shen · Shunle Chen

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

Received: September 20, 2006 / Accepted: February 6, 2007

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 $\leq 10$) ($P = 0.000$, 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.\(^1\)\(^2\) 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).

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 $\pm$ 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 $\leq 10$. Thirty-six aged-matched healthy controls (33 females, 3 males, mean age 27.86 $\pm$ 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 transcript-
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, Foster 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 TTCTGCTTGAAAGGATCCA; reverse primer AATATCCGATCCTAGCAGCTCTC. 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).