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Short communication

Elevated serum and synovial fluid levels of interleukin-37 in patients with rheumatoid arthritis: Attenuated the production of inflammatory cytokines

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ABSTRACT

Objectives: To measure the serum and synovial fluid (SF) levels of interleukin (IL)-37 in patients with rheumatoid arthritis (RA) and to investigate the effect of recombinant human (rh)IL-37 on inflammatory cytokine production (tumor necrosis factor [TNF]-α, IL-6, IL-17 and IL-10) by peripheral blood mononuclear cells (PBMCs) in RA patients.
Methods: An enzyme-linked immunosorbent assay (ELISA) was used to analyse the serum and SF IL-37 levels. RhIL-37 was used to stimulate RA patient PBMCs. The supernatant TNF-α, IL-17, IL-6 and IL-10 levels were detected with ELISAs.
Results: The serum IL-37 levels in RA patients were significantly increased compared with those of osteoarthritis (OA) and healthy controls (HC), and they were especially elevated in RA patients with positive rheumatoid factor (RF) and anti-citrullinated peptide antibody (CCP) levels. Furthermore, the serum IL-37 levels were positively correlated with RF values. In 20 matched RA SF and serum samples, the SF IL-37 levels were much higher than those in the serum. After anti-TNF-α therapy, the serum IL-37 levels significantly decreased. Additionally, rhIL-37 significantly down-regulated TNF-α, IL-17 and IL-6 production by RA patient PBMCs.
Conclusions: IL-37 is an important anti-inflammatory cytokine in the control of RA pathogenesis by suppressing inflammatory cytokine production. Thus, IL-37 administration may be a novel therapy for RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory disease that is characterized by progressive joint destruction. Multiple pro- and anti-inflammatory cytokine cascades, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-17 and IL-10, have been described in RA, leading to persistent synovitis [1,2]. The anti-inflammatory cytokine levels, however, do not match the increased pro-inflammatory cytokine levels in these patients. Therefore, the anti-inflammatory to pro-inflammatory cytokine imbalance results in synovitis, articular cartilage and bone damage progression, and subsequently, RA onset [3,4].

IL-37, a newly described member of the IL-1 family, has emerged as an anti-inflammatory cytokine that shares the structural pattern of the IL-1 family, particularly that of IL-18 [5]. IL-37 is not constitutively expressed in tissues from healthy subjects; however, it can be induced in peripheral blood mononuclear cells (PBMCs) and dendritic cells (DCs) by a variety of Toll-like receptor (TLR) ligands [6]. IL-1β, TNF-α, interferon (IFN)-γ and IL-18 can induce IL-37 expression in PBMCs [6]. IL-37 expression in synovial tissue and elevated serum IL-37 levels were observed in patients with active RA, suggesting that IL-37 may be involved in RA progression [7,8]. These data begged the questions: What is the exact role of IL-37 in RA? Are there any correlations between IL-37 and other pathogenic cytokines in RA patients? The answers to these questions remain unanswered. In our study, we investigated the serum and synovial fluid (SF) IL-37 levels and correlated with other clinical parameters. Additionally, the TNF-α, IL-6, IL-17 and IL-10 production levels by PBMCs after rhIL-37 stimulation were also investigated.

2. Materials and methods

2.1. Patients

One hundred and fifty RA patients were recruited randomly from the First Affiliated Hospital of China Medical University. All

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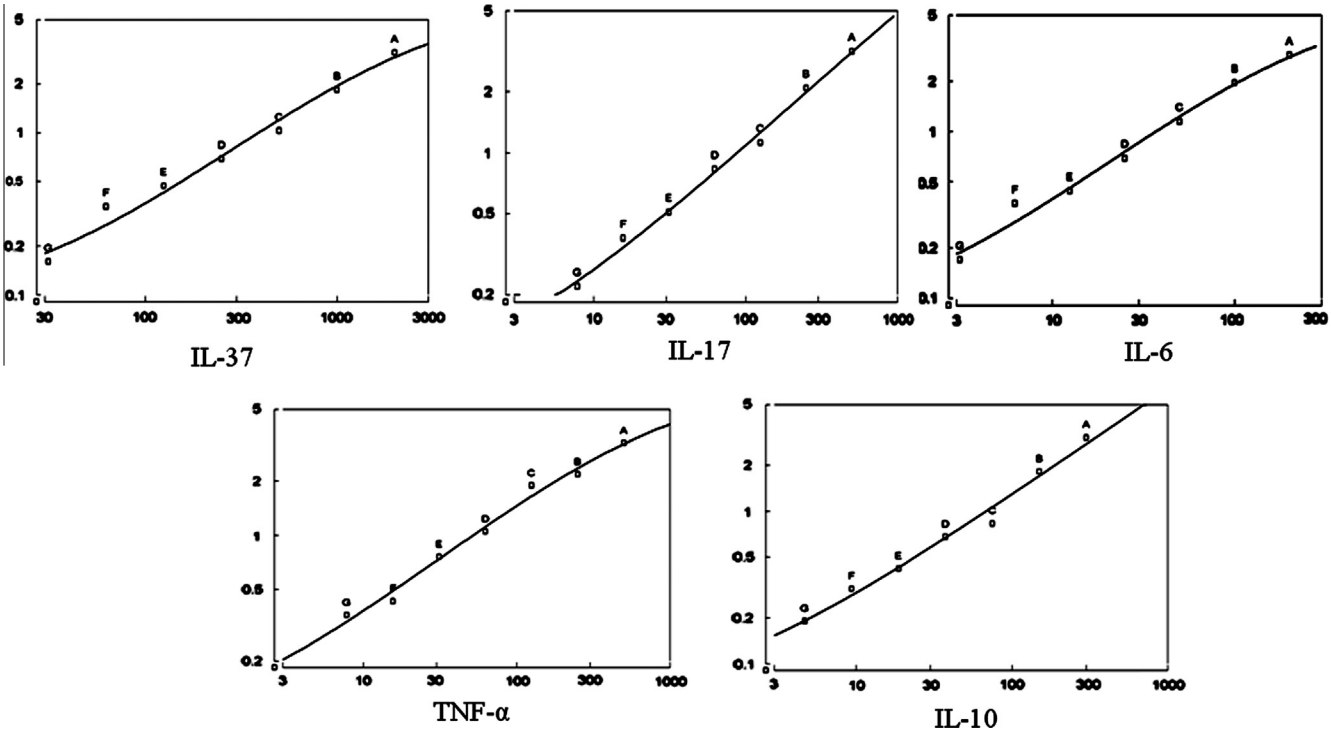

Fig. 1. The IL-37, IL-17, IL-6, TNF-α and IL-10 dilution curves.

of the RA patients fulfilled the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 diagnostic criteria [9]. All of the patients had not received therapy before their blood samples were collected. Fully informed written consent was obtained from each patient, and the ethics committee of the 1st affiliated hospital of China Medical University approved the study. The laboratory assessments included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF and anti-CCP antibody evaluations. SF samples were obtained from 20 RA patients simultaneously. The disease activity was assessed with 28-joint count Disease Activity Score(DAS28) on the sample collection day [10].

2.2. TNF-α blockade therapy

Forty RA patients were treated with infliximab (3 mg/kg, infused a total of 5 times; once at weeks 0, 2, 6, 14 and 22) or etanercept therapies (25 mg twice a week over 22 weeks). The sera samples were collected before and after treatment and stored at −80 °C until use.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Serum and SF sample IL-37 levels were determined with an ELISA following the manufacturer's instructions (R&D systems). A 96-well microtitre plate was pre-coated with a polyclonal antibody that was specific to IL-37. One hundred microliters of different standards or samples were added to the plate and incubated for 2 h. After washing four times with phosphate-buffered saline containing 0.05% tween 20 (T-PBS), 100 μL of a biotin-conjugated polyclonal antibody that was specific for IL-37 was added to each microplate well and incubated for 2 h. The plate was then washed four times with T-PBS and incubated with 100 μl of a streptavidin–horseradish peroxidase (HRP) working dilution for 20 min. Following 6 washes with T-PBS, 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) was added to each well and incubated for 20 min. The reaction was stopped by the addition 50 μL stop solution and the absorbance was examined with a microplate reader at 450 nm. Each sample was measured in triplicate. Because rheumatoid factors may interfere with such assays, we added known quantities of recombinant IL-37 into serum containing rheumatoid factor to determine whether this altered our ability to measure the cytokine. The presence of rheumatoid factor had no significant effect on the measurements.

2.4. Cell isolation and culture

PBMCs were isolated from the peripheral blood of 25 RA patients with a Ficoll–Hypaque gradient following centrifugation (Amersham Pharmacia Biotech). PBMCs (2.0×10^5 cells/ml/well) were stimulated with CD3/CD28 antibody coated beads (2.0×10^5 beads/well; Miltenyi Biotech) for 24 h at 37 °C. The stimulated PBMCs were incubated with rhIL-37 at concentrations of 0, 10, 20, 50, and 100 ng/mL (R & D Systems) for 48 h. Cell-free culture supernatants were collected and stored at −80 °C until use. The supernatants were then assayed for TNF-α, IL-6, IL-17 and IL-10 with ELISA kits, according to the manufacturer's instructions (eBioscience). The IL-37, IL-17, IL-6, TNF-α and IL-10 dilution curves are shown in Fig. 1.

2.5. Statistical analysis

Statistical analysis was performed with the SPSS17.0 and Graph-Pad 6.0 software. The results were expressed as medians and ranges. The statistical significance of the differences between the groups was determined with Mann–Whitney or Wilcoxon matched-pairs signed rank tests. The Spearman's correlation coefficient was used to test for correlations between two variables. For all of the experiments, $P < 0.05$ was considered to be significant.