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Short communication

Elevated frequency of IL-37- and IL-18Rα-positive T cells in the peripheral blood of rheumatoid arthritis patients

Liuqing Wang, Yueying Wang, Liping Xia*, Hui Shen, Jing Lu

Department of Rheumatology, 1st Affiliated Hospital of China Medical University, Shen Yang 110001, China

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ABSTRACT

Objective: To detect the expression of IL-37 and its receptors IL-18Rα and IL-1R8 in $CD4^+$ T cells and total lymphocytes in rheumatoid arthritis (RA) patients and the relationship between autoantibodies and disease activity. To investigate the mechanism of IL-37 and its receptors involved in the pathogenesis of RA. To evaluate the effects of different concentrations of rhIL-37 on peripheral blood mononuclear cells (PBMCs) in RA patients with TNF-α, and IL-6.

Methods: The expression of IL-37 and its receptor IL-18Rα and IL-1R8 in peripheral blood $CD4^+$ T cells and total lymphocytes in RA patients and healthy controls were measured by flow cytometry. The levels of TNF-α and IL-6 in the supernatant were measured by ELISA after rhIL-37 stimulation with PBMCs.

Results: The expression of IL-37 and IL-18Rα in the total lymphocytes, especially in $CD4^+$ T cells in RA patients, was significantly higher than in the healthy control group. There was a positive correlation between the frequency of IL-37- or IL-18Rα-positive $CD4^+$ T cells and ESR, CRP, and DAS28 values. Additionally, rhIL-37 significantly down-regulated TNF-α and IL-6 production in RA patients' PBMCs.

Conclusions: IL-37 plays an important role in the regulation of inflammation in RA. IL-37 and its receptors may play an immunoregulatory role in the activation of lymphocytes, especially $CD4^+$ T cells, in RA patients. IL-37 may represent a therapeutic target for rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial cell proliferation and lymphocyte invasion [1]. The abnormal activation of T lymphocytes, especially CD4-positive ($CD4^+$) T cells, plays an important role in the development of RA [2]. Studies showed that interleukin (IL)-17 producing $CD4^+$ T cells (Th17 cells) contribute to the pathogenesis of rheumatoid arthritis [3].

IL-37 is a newly identified cytokine that belongs to the IL-1 family. IL-37 is also called IL-1F7 [4]. IL-37 has a common constructor model with other members of the IL-1 family, especially IL-18 [5]. It has been demonstrated that IL-37 is a natural suppressor of the innate immune response [6], which has the effect of inhibiting local and systemic inflammatory responses [7]. IL-37 can significantly reduce the production of inflammatory cytokines in LPS-stimulated mouse primary macrophages [8]. Studies showed that IL-37 plays an important role in autoimmune diseases. Elevated serum and plasma levels of IL-37 were observed in RA, systemic lupus erythaematosus (SLE), and ankylosing spondylitis (AS) and were closely associated with disease activity [9]. IL-37 can inhibit the production of inflammatory cytokines by peripheral blood mononuclear cells (PBMCs) in autoimmune diseases [10–13].

IL-37 binds to the receptor-IL-18Rα and recruits the orphan protein IL-1R8 instead of IL-18Rβ to form a three-type domain to silence the TLR joint molecule MyD88, weaken the MyD88 signal, inhibit transcription factors and exert an anti-inflammatory effect [14]. The anti-inflammatory effect of IL-37 is not observed in IL-18Rα knockout mice, and a lack of IL-1R8 primary mouse macrophages and dendritic cells is not associated with anti-inflammatory properties [14,15], which provides the anti-inflammatory activity of a complex consisting of IL-37, IL-18Rα, and IL-1R8. However, how IL-37 exerts immunoregulatory effects in RA has not been thoroughly elucidated to date.

In this study, we measured the frequency of $IL\text{-}37^+$ $CD4^+$ T cells, $IL\text{-}18R\alpha^+$ $CD4^+$ T cells, and $IL\text{-}1R8^+$ $CD4^+$ T cells in RA patients and its association with clinical parameters. In addition, tumour necrosis factor (TNF)-α and IL-6 production levels by PBMCs after stimulation with recombinant human (rh) IL-37 were also investigated.

* Corresponding author.
E-mail address: syxialiping505@sohu.com (L. Xia).

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Table 1
Clinical and laboratory characteristics in patients with RA and HC subjects.

Characteristics	RA(61)	HC(10)
Age (year)	55.36 ± 12.95	53.32 ± 10.32
Sex (F/M)	10/51	3/7
ESR (mm/h)	41.70 ± 26.71	NA
CRP (mg/L)	33.31 ± 45.51	NA
RF (IU/ml)	43(70.49%)	NA
Anti-CCP	41(67.21%)	NA
DAS28-ESR	5.38 ± 1.35	NA

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor. Anti-CCP, anticitrullinated peptide antibody; DAS28, 28-joint count disease activity score.

2. Materials and methods

2.1. Patients

A total of 61 RA patients and 10 healthy controls (HCs) (age and sex matched) were recruited randomly from the Frist Affiliated Hospital of China Medical University. All RA patients fulfilled the American College of Rheumatism (ACR)/European League Against Rheumatism (EULAR) 2010 diagnostic criteria [16]. The laboratory assessments included evaluations of the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF) and anti-cyclic citrullianted-peptide (anti-CCP) antibody. The disease activity was assessed with a 28-joint-count Disease Activity Score (DAS28) on the sample collection day. The study was approved by the local ethics committee of the first affiliated hospital of China Medical University, and written informed consent was given by all patients.

2.2. Flow cytometry

PBMCs were purified from the peripheral blood of 21 RA and 10 HC patients by centrifugation using a Ficoll-Hypaque gradient (GE Healthcare). PBMCs were adjusted to a final concentration of 2×10^6/ml. Three-colour flow cytometry was used to analyse the surface phenotype anti-human CD4-FITC (R&D Systems), receptors anti-human IL-18Rα-PE (R&D Systems), and anti-human SIGIRR-APC (R&D Systems). Appropriately conjugated IgG antibodies were used as isotype controls. After incubation for 30 min at 4 °C, the cells were washed in 2 ml staining buffer (BD) twice. For intracellular staining, PBMCs (2×10^6/ml/well) were stimulated for 5 h with Leukocyte Activation Cocktail (BD) and Goligstop (BD). After stimulation, cells were first stained with anti-human CD4-PE (BD) and later fixed and permeabilized with Perm/Fix solution (BD) at 4 °C for 20 min. Cells were washed with perm/wash buffer (BD) twice and later stained with anti-human IL-37-488 (R&D Systems) for 30 min at 4 °C. Appropriately conjugated IgG antibodies were used as isotype controls. Cell staining was analysed using a FACS Aria II system (BD) and FACS DVIA software (BD).

2.3. Cell isolation and culture

PBMCs were isolated from the peripheral blood of 40 RA patients with a Ficoll–Hypaque gradient following centrifugation (GE Healthcare). The culture medium was RPMI 1640 medium containing 10% foetal bovine serum and 1% penicillin/streptomycin. PBMCs (2×10^5 cells/ml/well) were incubated with rhIL-37 at concentrations of 0, 20, 50, 100 ng/mL (R&D Systems) and LPS1 μg/ml (Sigma) for 24 h. Cell-free culture supernatants were collected and stored at -80 °C until use. The concentration of TNF-α and IL-6 in the culture supernatants of PBMCs from RA patients was measured by human DuoSet ELISA kits (R&D Systems) according to the manufacturer's instructions.

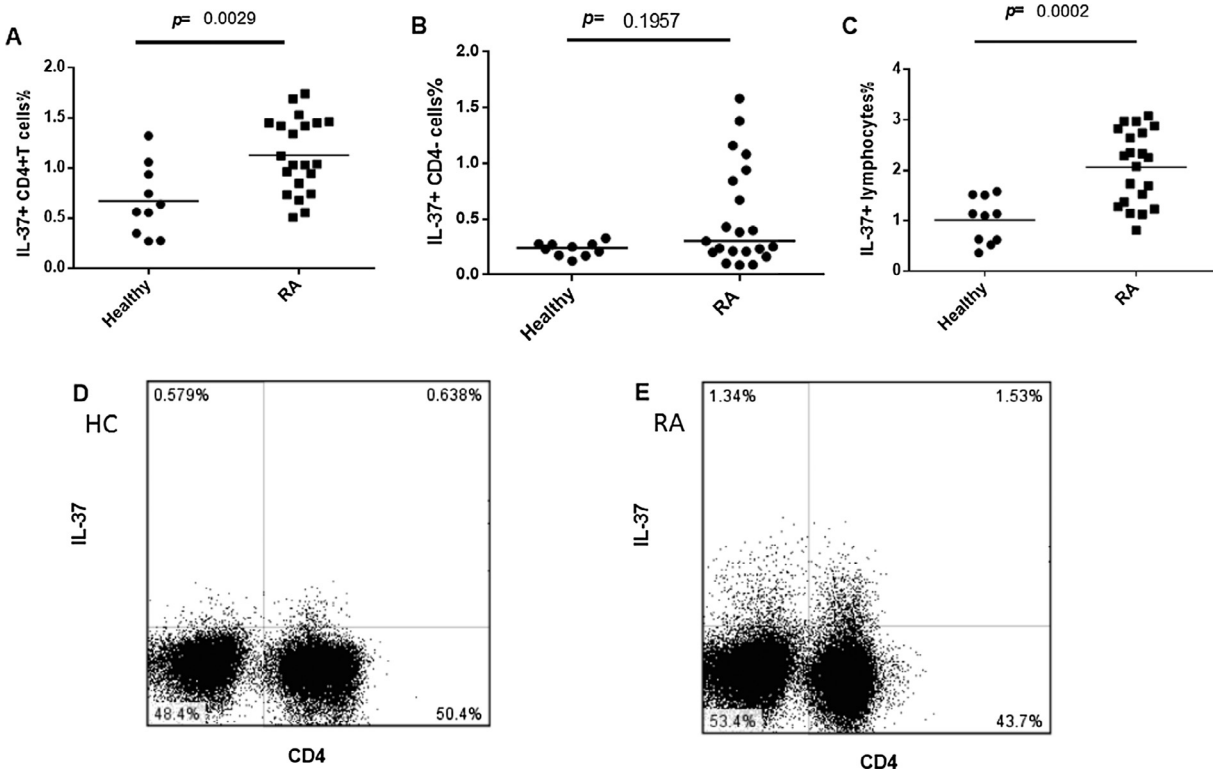

Fig. 1. Increased frequency of IL-37$^+$ CD4$^+$ T cells and IL-37$^+$ total lymphocytes in PBMCs from RA patients. (A) the percentage of IL-37$^+$ CD4$^+$ cells in RA and HCs, (B) the percentage of IL-37$^+$ CD4$^-$ cells in RA and HCs, (C) the percentage of IL-37$^+$ total lymphocytes in RA and HCs, (D) the typical two dimensional scatter diagrams of the frequency of IL-37$^+$ CD4$^+$ Tcells in HCs, (E) the typical two dimensional scatter diagrams of the frequency of IL-37 $^+$ CD4$^+$ T cells in RA.