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Serum levels of IL-17 are elevated in patients with acute gouty arthritis

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ABSTRACT

Acute gouty arthritis (AGA) is one of the most common forms of auto-inflammatory arthritis. IL-17 is a key proinflammatory cytokine which has been implicated in several autoimmune diseases. However, to date little is known about the role of IL-17 in AGA. In the present study, we show that serum IL-17 levels are significantly elevated in AGA patients early in the onset of symptoms of gout, and decrease gradually as symptoms diminish. Correlation analysis indicated that IL-17 expression is not only positively correlated with disease activity, but is also correlated with serum levels of IL-1β which plays a critical role in the differentiation of IL-17$^-$ γδT cells into IL-17$^+$γδT cells. Flow cytometry analysis indicated that γδ T cells are a major source of IL-17 production during the early onset of AGA. We therefore identify IL-17 as a potential novel biomarker for AGA and suggest that targeting the γδ T cell/IL-17 immune axis is a potential strategy for treatment of acute flares of AGA.

1. Introduction

Gout is a systemic disease that results from the deposition of monosodium urate crystals (MSU) in tissue [1]. Acute gouty arthritis (AGA) is one of the most common forms of auto-inflammatory arthritis among men, and is characterized by abrupt, markedly painful arthritis which resolves spontaneously within a week [1,2].

Previous studies have shown that gouty inflammation is mediated by the innate immune system. It is thought that inflammation in AGA is characterized by infiltration of neutrophils and the production of various pro-inflammatory cytokines such as IL-1β, IL-6, IL8, and TNF-α [3–5]. However, the pathogenesis of AGA is incompletely understood.

IL-17 is an important proinflammatory cytokine produced by T helper 17 (Th17) cells, γδT cells and natural killer (NK) cells [6]. In addition to its important role in protective immunity, IL-17 has been shown to exacerbate autoimmune diseases. Several studies have reported that serum or urinary levels of IL-17 are significantly elevated in patients with rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and autoimmune hepatitis [7–10]. Evidence shows that IL-17 is present at sites of inflammatory arthritis and in synergistic interactions amplifies the inflammation induced by other cytokines, including IL-1, IL-6, IL-8, and TNF-α [11,12]. We therefore hypothesized IL-17 is involved in the progress of gouty inflammation. However, to date little is known about the role of IL-17 in AGA.

The aim of this study was to determine whether IL-17 is involved in AGA. Serum IL-17 levels were compared in AGA patients and healthy controls. We then analyzed the correlation of IL-17 expression with serum IL-1β levels, Visual Analogue Scale (VAS) values and serum uric acid (SUA) levels in AGA patients. A follow-up study was performed to observe changes in IL-17 expression from the onset of acute flares of gout to the point where symptoms had completely subsided. We further evaluated the contribution of γδT cells to IL-17 expression during the onset of AGA. Our results suggest that IL-17 has potential as a new biomarker for AGA, and also suggest IL-17$^+$γδT cells are the main source of IL-17 in the early onset of AGA.

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2. Materials and methods

2.1. Study subjects and inclusion criteria

A total of 116 unrelated subjects, including AGA patients (n = 64) and healthy controls (n = 52) were enrolled in this study. All AGA patients were male, and were hospital inpatients or clinic patients in the China-Japan Friendship Hospital. The inclusion criteria used for AGA subjects in this study were in accordance with the 1977 preliminary American Rheumatism Association classification criteria for gout [13]. Healthy controls were defined as gout-free male subjects with no other arthritis-related diseases, and controls were age and sex matched with the AGA patients. The study was performed after obtaining informed consent from all subjects and was approved by the Ethics Committee of the China-Japan Friendship Hospital.

2.2. Clinical and laboratory examination

5 ml fasting venous blood samples were collected from AGA patients at different time points including the interval within 12 h after a gout attack, the 3rd day and 7th day after an attack, and from healthy controls. Blood samples were then centrifuged at 1710 g for 10 min to separate the serum. Metabolic profiling of the analyses including high density lipoprotein (HDL), low density lipoprotein (LDL), SUA, fasting blood glucose (FBG) and serum creatinine (SCR) was carried out using a Hitachi 7600 automatic analyzer (Hitachi, Tokyo, Japan). All reagents were purchased from Beijing Strong Biotechnologies, Inc (Beijing, China). Tests were performed according to Good Clinical Laboratory Practice standards.

2 ml of fasting venous blood were collected into ethylene diamine tetra acetic acid (EDTA) anticoagulant tubes for blood counts and a differential. Blood samples from all subjects were analyzed using XN-9000 systems (Sysmex Co., Kobe, Japan) according to the manufacturer's instructions. The main parameters which were evaluated included total cell count and leukocyte differential count (neutrophils, lymphocytes) in absolute values.

2.3. Measurement of serum levels of IL-17 and IL-1β

IL-17 concentration in serum samples was measured using a commercially available Human IL-17 Immunoassay Quantikine® ELISA kit (lot 317003, Catalog No. D1700, R & D Systems Inc., Minneapolis, MN, USA) following the manufacturer's instructions. Serum IL-1β levels were determined with a chemiluminescence enzyme immunometric kit (Lot, 0182, Immulite IL-1β® DPC, Llanberis, Gwynedd, UK) on an Immulite® 1000 Immunoassay Analyzer. The analytical sensitivities of IL-17 and IL-1β assays were 1.5 pg/mL and 15 pg/mL respectively.

2.4. Flow cytometric analysis

Antibodies used for flow cytometric analysis included TCR PAN α/β-PE (clone IP26A, BECKMAN COULTER, Marseille, France), TCR PAN γ/δ-FITC (clone IMMU510, BECKMAN COULTER, Marseille, France), CD3-FITC/CD (16 + 56)-PE (clone UCHT1/3G8/N901, BECKMAN COULTER, Marseille, France), CD4-FITC/CD8-PE/CD3-PC5 (clone 13B8.2/B9.11/UCHT1, BECKMAN COULTER, Marseille, France), IL-17A-PE (clone N49-653, BD Biosciences). Isotype-matched control antibodies were used to identify background levels of staining.

Fifty microliters of whole blood in EDTA were aliquoted into a micro-centrifuge tube. After fluorescent staining, stained whole blood was mixed with 1 mL of RBC (red blood cell) lysing solution (BECKMAN COULTER, Marseille, France), vortexed, and incubated for 10 min at room temperature in the dark. The mixture was then moved to a 5 mL polystyrene tube, centrifuged for 5 min at 400 × g, and the supernatant was decanted. After one wash, 100 μL of PBS was added to achieve the volume required for data acquisition.

For intracellular staining, 50 μL of samples were first polyclonally stimulated with 50 ng/ml cell stimulation cocktail consisting of a mixture of phorbol 12-myristate 13-acetate at 10 ng/mLandionomycin at 1 μg/mL (catalog 00–4970, eBioscience, San Diego, CA, USA) in the presence of Brefeldin A (at 1 μg/ml, catlog: 00–4506, eBioscience, San Diego, CA, USA) at 37 °C for 5 h. Samples were then prepared for intra-cellular and extra-cellular staining with a PerFix-nc Kit (PN B10825, BECKMAN COULTER, Marseille, France) in accordance with the manufacturer's instructions. Cells were incubated with antibodies for 30 min at room temperature and analyzed on a Cytomics FC 500 Flow Cytometer (BECKMAN COULTER, Inc. Miami, FL, USA) using CellQuest software (BECKMAN COULTERCXP software).

2.5. Statistical analysis

Statistical analysis was performed with Prism 5 (GraphPad Software, San Diego, CA, USA) and IBMSPSS statistics 20 statistical software. Data are shown as mean ± standard deviation (SD) when the number of samples is larger than 30. The data are shown as mean ± standard error of mean (SEM) when the number of samples is less than 30. The two-tailed unpaired *t*-test was used to compare the two groups. Correlation analysis was performed using Pearson's rank correlation coefficients. P values of less than 0.05 were considered to be statistically significant. Significance was defined as p values of <0.05 (*), <0.01 (**), <0.001 (***) or <0.0001 (****).

3. Results

3.1. Clinical characteristics of subjects

Clinical characteristics of the 52 healthy control subjects and 64 AGA patients are summarized in Table 1. The subjects in the two groups are all males of ages 30–70 years (mean age 44). SUA levels, BMI, leukocyte count, neutrophils count were significantly higher in AGA patients than in healthy controls. HDL levels were significantly lower in AGA patients than in healthy controls. There were no statistically significant differences in age, SCR levels, LDL levels, FBG levels or lymphocyte counts in these two groups. VAS values determination was applied only to AGA patients.

Table 1
Clinical Characteristics of AGA patients and healthy controls.

Clinical Characteristics	HC (n = 52)	AGA (n = 64)	Pvalue
Age(years)	42.3 ± 8.4	44.6 ± 8.7	>0.05
SUA(μmol/L)	298.8 ± 46.6	492.8 ± 107.6	<0.01
SCR(μmol/L)	72.8 ± 9.9	75.6 ± 11.6	>0.05
HDL(mmol/L)	1.27 ± 0.39	1.05 ± 0.26	<0.05
LDL(mmol/L)	3.19 ± 1.01	3.43 ± 0.67	>0.05
FBG(mmol/L)	4.6 ± 0.3	4.7 ± 0.40	>0.05
BMI(kg/m^2)	22.9 ± 2.7	25.1 ± 2.9	<0.05
VAS value	ND	6.44 ± 1.25	ND
Leukocyte count (*10^9)	6.07 ± 1.29	7.47 ± 2.50	<0.001
Neutrophils count (*10^9)	3.55 ± 1.01	4.70 ± 2.21	<0.001
Lymphocytes count(*10^9)	1.99 ± 0.52	2.12 ± 0.76	>0.05

All data are presented as mean ± SD and were analyzed with the two-tailed unpaired *t*-test.
AGA = AGA patients; HC=Healthy controls; ND=No determination.