Profile of serum cytokine concentrations in patients with gouty arthritis

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Abstract

Objective: This study aimed to analyze the changes in serum inflammatory cytokines and anti-inflammatory cytokines in patients with gouty arthritis (GA).

Methods: The clinical data and serum samples in patients with gouty arthritis and those in healthy volunteers were collected in China-Japan Friendship Hospital from July 2018 to January 2019. Serum cytokine concentrations in patients with GA and volunteers (controls) were determined by a chemiluminescence method. The differences in cytokine concentrations were compared between the two groups.

Results: Concentrations of serum interleukin-1 beta (IL-1ß), tumor necrosis factor-alpha (TNF-α), IL-6, IL-8, and IL-4 were significantly higher in patients with acute GA than in controls. Serum concentrations of IL-1ß, TNF-α, IL-6, IL-8, and immunoglobulin E in patients with remission of GA were significantly lower, whereas concentrations of IL-10 and interferon-γ were significantly higher, compared with those in patients with acute GA.

Conclusion: This study shows that serum concentrations of IL-1ß, TNF-α, IL-6, IL-8, and IL-4 are significantly elevated in patients with GA, and may be involved in the pathogenesis of GA.

Introduction

Gout is a crystal-associated arthropathy caused by monosodium urate (MSU) deposition, which is directly related to hyperuricemia caused by a purine metabolism disorder and decreased uric acid (UA) excretion.[1] The risk of gout development

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is affected by not only hyperuricemia, but also by sex, weight, age, environmental and genetic factors, and their interactions. In addition to these factors, ABCG2 has stronger effects on the risk of gout than major environmental risk factors, such as obesity and heavy drinking.[2,3] The incidence of gouty arthritis (GA) is highest in male patients with arthritis aged older than 40 years.[4] Clinical manifestations of gout are acute arthritis, chronic arthritis and tophi, gouty nephropathy, joint disability, decreased kidney function, and other diseases associated with metabolic syndrome.[5]

The details of the pathogenesis and self-relief mechanisms of GA are not fully understood. With the progress of research in this field, inflammatory cells, immunoglobulins, and cytokines have been shown to be involved in the pathogenesis of GA during acute gout.[1] As a "danger signal", MSU crystals affect certain immune cells and cytokine production, and stimulate both types of immune responses.[7] MSU crystals activate innate immune cells, such as monocytes/macrophages, mast cells, neutrophils, and natural killer cells, which then secrete pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF).[8] There have been many studies on pro-inflammatory cytokines, but little research on anti-inflammatory cytokines has been performed.[8] Furthermore, little is known about the change in cytokine concentrations in the course of GA. Therefore, this study aimed to examine the concentrations of cytokines and their changes during GA to better understand the roles of those cytokines in the pathogenesis of GA flares and remission.

Materials and methods

Patients

All patients who were treated for an acute gout attack at the Endocrinology Department of the China-Japan Friendship Hospital between July 2018 and January 2019 were included. They were diagnosed with gout in accordance with the diagnostic criteria set forth by the European League Against Rheumatism (2015) and the American College of Rheumatology (2015) conferences.[9,10] We included patients with GA in the GA group. Sex and age-matched healthy adults were recruited as healthy controls (HCs). None of the participants had chronic renal failure, cerebral vascular diseases, heart disease, abnormal liver function, malignant tumors, acute and chronic infectious diseases, or anemia. Patients who were taking any anti-inflammatory medication in the previous 15 days were excluded from the study. The general clinical data of the participants are shown in Table 1. This study was approved by the Ethics Committee of the Institutional Review Board at the China-Japan Friendship Hospital (reference number: 2016-117), and written informed consent was obtained from all participants before the experiment.

Sample collection and measurement

A 5-mL fasting venous blood sample was collected from each patient with GA at different time points, including the interval within 12 hours after a gout attack and the seventh day after an attack. Each participant in the HC group also provided a 5-mL fasting venous blood sample. The serum was separated and high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid (UA), and serum creatinine concentrations were measured. These measurements were carried out using the BeckmanAU5800 automatic analyzer (Beckman Coulter Diagnostics Inc., Chaska, MN, USA). All reagents were purchased from Beijing Strong Biotechnologies, Inc. (Beijing, China).

Table 1. General clinical data in the GA and HC groups.

	HC group (n = 120)	GA group (n = 120)	Reference range	P value
Age (years)	55.4 ± 10.1	54.7 ± 10.7		>0.05
Sex (male/female)	108/12	108/12		>0.05
UA (μmol/L)	301.5 ± 54.1	494.7 ± 71.6	**150–420**	<0.05
sCR (μmol/L)	73.6 ± 13.4	76.9 ± 13.6	**35–106**	>0.05
HDL (mmol/L)	1.47 ± 0.38	1.01 ± 0.36	**1.0–2.2**	<0.05
LDL (mmol/L)	2.86 ± 0.84	3.78 ± 0.87	**≤3.4**	<0.05
Body mass index (kg/m^2)	23.9 ± 2.8	25.1 ± 2.9	**18.5–29.9**	>0.05
Leukocytes (×10^9/L)	5.88 ± 1.62	7.51 ± 1.94	**3.5–9.5**	<0.05
Neutrophils (×10^9/L)	3.82 ± 1.19	5.17 ± 1.78	**1.8–6.3**	<0.05
Lymphocytes (×10^9/L)	1.70 ± 0.71	1.84 ± 0.45	**1.1–3.2**	>0.05
Monocytes (×10^9/L)	0.35 ± 0.11	0.48 ± 0.17	**0.1–0.6**	<0.05

Data are mean ± standard deviation, number, or range.
HC, healthy control, GA, gouty arthritis; UA, uric acid; sCR, serum creatinine; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

The remaining serum was stored at −80°C to measure serum IL-1ß, IL-4, IL-6, IL-8, IL-10, TNF-α, interferon (IFN)-γ, immunoglobulin (Ig) E, and high-sensitivity C-reactive protein (Hs-CRP) concentrations. IgE and hs-CRP concentrations were measured using the latex-enhanced immune transmission turbidimetric method with the IMMAGE800 Immunoturbidimeter (Beckman Coulter Diagnostics Inc.). IL-1ß, IL-6, IL-8, IL-10, and TNF-α concentrations were measured using a chemiluminescence method (Immulite DPC, Gwynedd, UK). A double-antibody sandwich enzyme-linked immunosorbent assay was used to measure serum IL-4 and IFN-γ concentrations (R & D Systems Inc., Minneapolis, MN, USA). A volume of 2 mL of venous blood was collected into ethylenediamine tetra acetic acid anticoagulant tubes for a complete blood count with differential. The total cell count and leukocyte differential count, including neutrophils, lymphocytes, and monocytes in absolute values, were analyzed using the Hematology Analyzer XN-9000 system (Sysmex Co., Kobe, Japan) in accordance with Good Clinical Laboratory Practice standards.

Statistical analysis

Statistical analysis was performed with Prism 5 (GraphPad Software, San Diego, CA, USA) and IBM SPSS statistics 20 statistical software (IBM Corp, Armonk, NY, USA). Data are presented as mean ± standard deviation. A one-way analysis of variance was applied to estimate the mean differences in demographic and biochemical data between the GA and HC groups. An α-value of 0.05 was set as the criteria of significance.

This study's approach was based on the Consolidated Standards of Reporting Trials.[11]

Results

Clinical characteristics of the subjects

There were 120 patients in each group. The mean age of the participants was 55.4 ± 10.1 and 54.7 ± 10.7 years in the HC and GA groups, respectively. There were 12 women and 108 men in each of the HC and GA groups. The GA group had significantly higher mean baseline LDL and UA concentrations, and higher numbers of leukocytes, neutrophils, and monocytes than

those in the HC group (all $P < 0.05$). The mean baseline HDL concentration was significantly lower in the GA group than in the HC group ($P < 0.05$). The mean serum creatinine concentration and body mass index were similar in both groups.

Inflammatory mediator concentrations in the GA and HC groups

The mean IL-1β concentration was significantly higher in the GA group than in the HC group (15.17 ± 6.61 vs 5.24 ± 0.13 pg/mL, $P < 0.001$) (Figure 1). The mean IL-6 concentration was also significantly higher in the GA group than in the HC group (45.81 ± 23.29 vs 4.61 ± 1.74 pg/mL, $P < 0.001$) (Figure 1). Furthermore, mean IL-8, TNF-α, Hs-CRP, IL-4, and IgE concentrations were significantly higher in the GA group than in the HC group ($P = 0.011$, $P = 0.003$, $P = 0.013$, and $P = 0.016$, respectively) (Figure 1). There were no significant

differences in IL-10 or IFN-γ concentrations between the two groups (Figure 1).

Inflammatory mediator concentrations in acute GA, the remission stage of GA, and controls

We investigated variables in different stages of GA. IL-1ß, IL-6, TNF-α, Hs-CRP, and IgE concentrations were significantly higher in patients with acute gout than in those with remission stage gout and HCs (all $P < 0.01$). There were no significant differences in IL-1ß, IL-6, TNF-α, Hs-CRP, or IgE concentrations between patients in the remission stage and HCs. IL-10 and IFN-γ concentrations were significantly higher in patients in the remission stage than in those with acute gout and HCs (all $P < 0.01$). IL-8 concentrations in patients in the remission stage of GA were significantly lower than those in the HC group ($P < 0.05$). There were no significant differences in IL-10 or
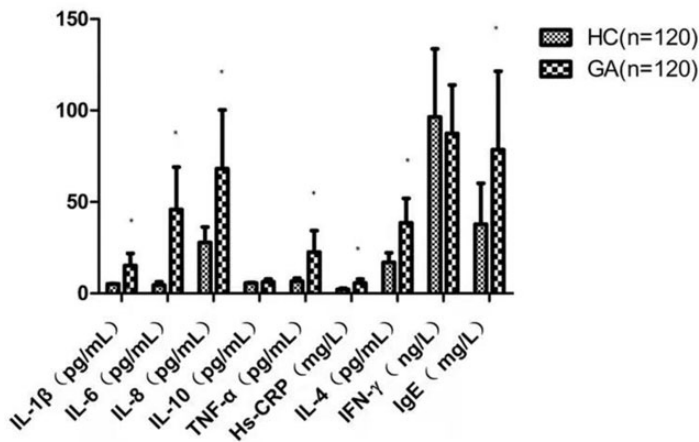

Figure 1. Inflammatory mediator concentrations in the GA and HC groups. *$P < 0.01$ between the two groups.
GA, gouty arthritis; HC, healthy control; IL, interleukin; TNF, tumor necrosis factor; Hs-CRP, high-sensitivity C-reactive protein; IFN, interferon; IgE, immunoglobulin E.

IFN-γ concentrations between patients with acute gout and HCs (Figure 2).

Discussion

Gout is an inflammatory disease. Previous studies have shown that a variety of cytokines are involved in the pathogenesis of GA.[12] However, most of these previous studies focused on the mechanism of acute attack of GA, and little is known regarding the changes in cytokine levels in the course of GA. The mechanism of gouty inflammation is coupled with the formation and activation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome with the subsequent production of pro-inflammatory cytokines. MSU crystals by themselves are not responsible for the induction of expression and assembly of inflammasome components. The first signal in gouty inflammation involves priming monocyte-derived macrophages, which then start binding ligands (S100A8 and S100A9 proteins) to Toll-like receptors. The second signal, triggered by MSU crystals, leads to the activation of multiprotein intracellular NLRP3 inflammasomes, which contain pro-caspase 1. Active caspase 1 then cleaves precursors of IL-1ß and 18 to generate the active forms. The secretion of IL-1ß leads to the recruitment of neutrophils to the site of inflammation, production of additional pro-inflammatory cytokines, and bone/cartilage degradation.[13,14]

IL-1β is a pro-inflammatory cytokine that is mainly produced by monocytes/macrophages. Many studies have shown that IL-1β plays an important role in attacks of GA and tissue damage.[15,16] TNF-α is primarily produced by macrophages and it has multiple physiological functions. TNF-α mediates anti-cancer and regulates the immune function of the body. Additionally, TNF-α is one of the most important mediators of inflammation and participates in many pathological changes in inflammation.[17] IL-8 is an inflammatory chemokine that is mainly secreted by monocytes, macrophages, and endothelial cells. IL-8 has special affinity in activating and recruiting neutrophils to the site of damage. In recent years, many studies have shown that the infiltration of a large number of neutrophils into the articular cavity and their mediation of the
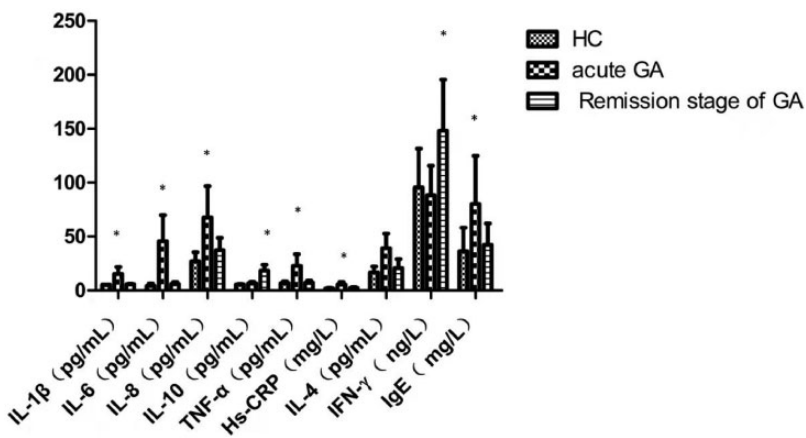

Figure 2. Inflammatory mediator concentrations in acute GA, the remission stage of GA, and HCs. *P < 0.01 compared with HCs.
GA, gouty arthritis; HC, healthy control.

inflammatory response is the core of the pathological mechanism of GA.[18,19] IL-1β, TNF-α, and IL-8 are not only involved in the initiation of GA, but are also inducing factors of persistent inflammation.[20] T helper (Th) cell precursors are stimulated by antigens and differentiate into Th0 cells, and further differentiate into Th1 and Th2 cells under different microenvironments. Th1 cells secret IFN-γ and IL-2, and Th2 cells secrete IL-4.[21,22] Th2 cells can induce B cells to synthesize IgE by secreting IL-4, and type 2 cytokines including IL-4 are essential for promoting Th2 differentiation and for skewing B cell class switching towards IgE.[23] IFN-γ, which is produced by Th1 cells, is an indispensable cytokine for macrophage activation. IFN-γ enhances the ability of phagocytes to phagocytize and kill pathogens, and promotes the production of IgG.[24] IFN-γ also inhibits the synthesis of IgE induced by IL-4, thus inhibiting the production of IgE *in vivo*.[25] IL-10 is mainly secreted by Treg cells, which play a negative regulatory role in the immune response. IL-10 can combine with transforming growth factor-ß to produce a wide range of non-specific anti-inflammatory effects. Additionally, IL-10 inhibits Th1 and Th2 immune responses through contact.[26,27]

In this study, the total numbers of leukocytes, neutrophils, and monocytes in the GA group were significantly higher than those in the HC group. The cytokines IL-1ß, TNF-α, IL-6, and IL-8, which are mainly secreted by monocytes and macrophages, were significantly higher in the GA group than in the HC group Concentrations of IL-4 from TH2 cells and IgE concentrations were also significantly higher in the GA group than in the HC group. These results suggest that innate immune cells and Th2 cells play an important role in promoting inflammation in the early stage of GA.

We also found that serum concentrations of IL-1ß, TNF-α, IL-6, IL-8, and IgE were significantly lower, whereas IL-10 and IFN-γ were significantly higher, in the remission phase than in the acute phase of gout. These finding suggested that proinflammatory factors were lower and anti-inflammatory factors were higher in the remission phase than in the acute phase of gout.

In conclusion, this study shows that cytokines play a role in the pathogenesis of GA and in different courses of this disease. A comprehensive analysis of the differences in the expression of cytokines can help determine the degree of inflammatory activity of GA and provide a theoretical basis for clinical exploration of new effective methods to treat GA. Although the immune mechanism of acute GA is not fully understood, the interaction between inflammatory cells and cytokines in the process of acute gouty inflammation could lead to ideas for the treatment of this disease.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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