Original Article

Research on the Role of Toll-Like Receptor 2 and Toll-Like Receptor 4 and its Signal Pathway in the Pathogenesis of Primary Gout Arthritis

R. J. Cui, M. Li[1], G. Tuerxun[1], Y. Li[2], S. S. Xie[3]

Departments of Rheumatology and Immunology, [1]Endocrinology and [2]Emergency, Sixth Affiliated Hospital of Xinjiang Medical University, [3]Department of Psychological, College of Educational Science, Xinjiang Normal University, Urumqi, China

Abstract

Objectives: The study objective was to research the role of toll-like receptor (TLR) 2 and TLR 4 and its signal pathway in the pathogenesis of primary gout arthritis. **Methods:** Seventy-two patients with primary gout arthritis were selected as the experimental group and 72 healthy controls were selected as the control group. The real-time fluorescence quantitative polymerase chain reaction method was used for testing. Blood uric acid (UA) level, interleukin (IL) IL-1β level, and TLR4 and TLR2 were observed in the two groups. **Results:** Compared with the control group, the levels of UA, IL-1β, and TLR4 in the observation group were significantly higher than those in the control group, and there was a statistically significant difference between the two groups ($P < 0.05$). There was no positive correlation between plasma UA level, IL-1β level, and TLR2 ($P > 0.05$). There was no correlation between TLR2 and serum UA level and IL-1β level in the control group ($P > 0.05$). **Conclusions:** TLR4 and IL-1β signaling acceleration is closely related to gouty arthritis. When the TLR4 signal pathway is activated, the UA salt crystal changes in the patient's body.

Keywords: Blood lipid metabolism, cardiovascular, serum cytokines, winter swimming

Introduction

Gout is a syndrome, which is characterized by elevated urate and monosodium urate (MSU) crystal deposition in tissues, leading to arthritis, soft-tissue masses (i.e., tophi), nephrolithiasis, and urate nephropathy.[1] Toll-like receptors (TLRs) are pattern recognition receptors of innate immunity, which can recognize pathogen-associated molecular patterns and damage-associated molecular patterns.[2] TLR4 is the receptor most thoroughly investigated in the TLR family, and it has been characterized as the receptor for Gram-negative lipopolysaccharide (LPS) and some endogenous ligands such as MSU, heat shock proteins, fragments of hyaluronic acid, and fibronectin.[3]

Overview

Studies have shown that TLRs, especially TLR2 and TLR4, participate in the secretion of inflammatory factors against *Helicobacter pylori* infection.[4] In general, LPS is one of the most significant virulence factors of Gram-negative bacteria, and it is widely accepted that a typical LPS produced by *Escherichia coli* can stimulate the host to initiate a wide range of inflammatory responses via TLR4.[5] However, *H. pylori* LPS exhibits extremely low activity compared to *E. coli* LPS. Furthermore, LPS derived from *H. pylori* can act as an antagonist for TLR4. Hence, it is hypothesized that TLR2 may play a more important role in *H. pylori* infection. In addition, there is still controversy over which of the TLRs is more significant in mediating the host response. Some reports argue that *H. pylori* initiate signals via TLR2, whereas others argue that TLR4 plays a crucial role against *H. pylori* infection. These contradictions may be explained by differences in the cell lines

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Address for correspondence: Dr. R. J. Cui, Department of Rheumatology and Immunology, Sixth Affiliated Hospital of Xinjiang Medical University, Urumqi, China. E-mail: hongguanyu@yahoo.com

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or *H. pylori* strains used.[6] TLR2 and TLR4 cooperatively amplify inducible nitric oxide synthase secretion in GSM06 cell lines infected by *H. pylori*. In contrast, the mutants of TLR2, but not TLR4, inhibited *H. pylori*-induced COX-2 expression in human gastric cancer cell lines AGS. It still remains to be determined which of the TLRs is involved in *H. pylori*-related production of specific cytokines.

Methods

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll–Hypaque density gradient centrifugation from the blood samples. Total RNA was extracted from PBMCs using Trizol reagent (Invitrogen USA – Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States) and reverse transcribed into cDNA using reagents that included random hexamers, superscript II, and dNTP (Invitrogen, USA). The converted cDNA was cryopreserved at −80°C until real-time fluorescence quantitative polymerase chain reaction (RT-qPCR) was performed. RT-qPCR was carried out in a final volume of 20 lL with Power SYBR Green PCR Master (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States). Mixing in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, USA) which containing Power SYBR Green PCR Master Mix (Applied Biosystems, USA) (9 lL), 10 pmol/L each of forward and reverse primers (0.5 lL each), synthesized cDNA sample (1.3 lL) and ddH_2O (8.7 lL). The thermal cycling conditions comprised an initial denaturing step at 95°C for 10 min, 40 cycles of renaturation at 95°C for 15 s, and elongation at 60°C for 1 min. The PCR for each gene was duplicated for each sample, and the mean value was used for further analysis. In addition, the RT-qPCR was run according to a modification of the Cawthon method. The sequences of the primers used for PCR are listed in Table 1.

We used relative quantification to evaluate the mRNA expression of selected genes; housekeeping gene (bactin) was used as an internal control to normalize the mRNA expression of each target gene.

Results

We found that NFjBp65 transcriptional activity and interleukin (IL) IL-1β production both significantly reduced after treated with anti-TLR4 antibody in whole blood from acute gout arthritis (AGA) patients *in vitro* ($P < 0.01$) [Figures 1 and 2], whereas there were no changes in NFjBp65 subtype levels and IL-1β production in whole blood from healthy controls ($P > 0.05$) [Figures 1 and 2]. Interestingly, IL-1β production in AGA patients after treated with anti-TLR4 antibody was upregulated a lot comparing with that in healthy controls ($P < 0.05$).

Figure 2 Effect of TLR4 inhibited on NFjBp65 transcriptional activity and IL-1β production in AGA patients and healthy controls. NFjBp65 transcriptional activity and IL-1β production were significantly reduced in peripheral blood from AGA patients after incubated with 30 lg/mL mouse anti-human TLR4 functional grade purified for 6 h, while not changed in whole blood from healthy controls. HC represents healthy control (blank control group: whole blood incubated with culture media, TLR4 inhibition group: whole blood incubated with anti-TLR4 antibody), GA represents acute gouty arthritis (blank control group: whole blood incubated with culture media, TLR4 inhibition group: whole blood incubated with anti-TLR4 antibody). Data are shown as box plots. Each box represents the upper and lower interquartile range (IQR). Whiskers represent 1.5 times the upper and lower IQRs. Lines inside the boxes represent the median. Statistical significance was set at $P < 0.05$.

In our study, we found that the concentration of uric acid (UA) was much higher in the AGA patients than that in the non-AGA (NAGA) patients and healthy controls and higher in the NAGA patients than that in the healthy controls; body mass index, Glutamic Acid (amino acid), triglyceride, low-density lipoprotein (LDL)-cholesterol, very LDL, and apoB100 were much higher in the AGA and NAGA patients than those in the healthy controls, whereas in the healthy

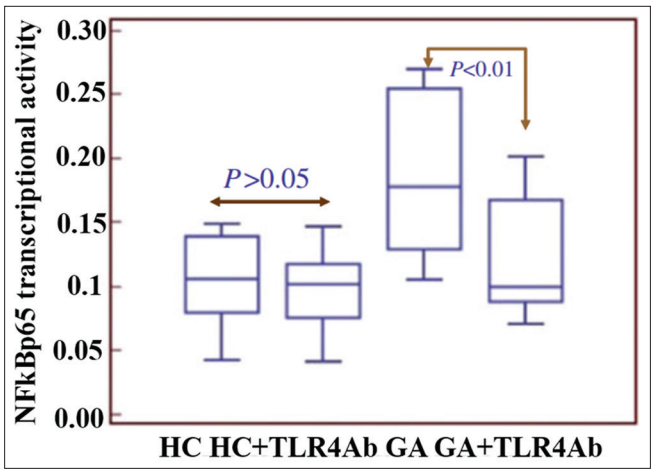

Figure 1: The result of NFjBp65 treated with anti-toll-like receptor 4 antibody in whole blood from acute gout arthritis patients *in vitro*

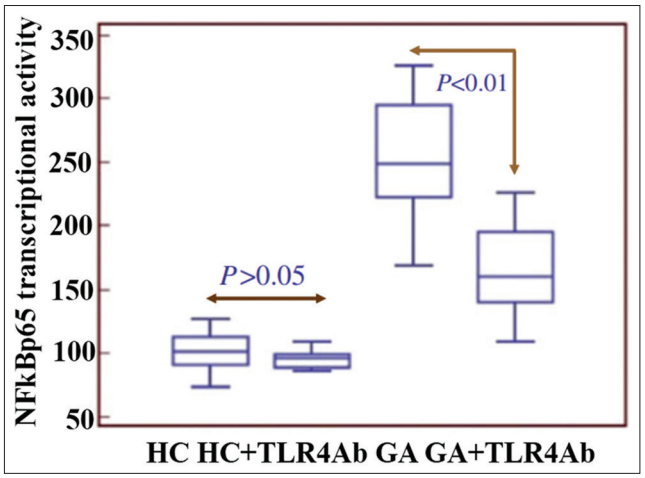

Figure 2: The result of interleukin-1β with anti-toll-like receptor 4 antibody in whole blood from acute gout arthritis patients *in vitro*

Table 1: Sequences of primer used in the reverse transcription-polymerase chain reaction assays

Gene	Forward primer	Reverse primer
TLR4	TCCCTGAACCTATGAAC	CTAAACCAGCCAGACCTT
β-actin	GAGCTACGAGCTGCCTGAGG	GTAGTTTCGTGGATGCCACAG

TLR: Toll-like receptor

controls, high-density lipoprotein-cholesterol and apoA1 levels were elevated. These data suggested that there were metabolic dysfunctions of UA and blood fat in gout patients, and gout is a kind of metabolic disease. Hyperuricemia is the biochemical fundamental of gout development. However, only about 10% of hyperuricemic patients develop into gout eventually, why and how this happens in these patients is unclear. We hypothesize that the inflammation and immunity factors might also contribute to gout development. In our study, the inflammatory parameters of peripheral blood such as neutrophils, erythrocyte sedimentation rate, and C-reactive protein were significantly higher in the AGA patients than those in the NAGA patients; the expression of TLR4, NFjB, and IL-1β production in peripheral blood was much higher in the AGA patients than those in the NAGA patients and the healthy controls and higher in the NAGA patients than those in the healthy controls. Taken together, these results suggested that dysfunctional immunity and inflammation were also involved in gouty inflammation development.

Conclusions

Overall, it is a long way to understand the mechanisms of gout, and also, the mechanisms linked to TLR4–NFjB signaling are activated by endogenous signals (MSU) that have considerable clinical validity. From our study, gout could be classified not only as a type of metabolic disease but also as an auto-inflammatory disease, similar to other autoimmune diseases, mediated by IL-1β specifically.

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Conflicts of interest

There are no conflicts of interest.

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