

Original Article

Relationship between serum inflammatory cytokine levels and NF- κ B expression in rat synovial cells

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Received March 29, 2016; Accepted May 28, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Rheumatoid arthritis (RA) is a type of autoimmune disease caused by cytokines eroding articular synovium induced inflammation. NF- κ B plays a role of switching cell activity in cells. It was suggested that NF- κ B participates in inflammatory response, promotes cell apoptosis, and leads to cell cycle differentiation. It is the main pathological factor of RA. This paper studies the correlation relationship between CIA rats arthritis with NF- κ B and IL-17. A total of 80 SD rats were randomly divided as control and model group. CIA rat model was established in model group. Toe volume and arthritis index (AI) were applied to evaluate joint inflammation. IL-17 level was detected by enzyme-linked immunosorbent method (ELISA). NF- κ B/P65, NF- κ B/P50, and I κ B α expression in synovial cells were tested by western blot. Compared with blank control, the toe volume in model rat was similar in the 1st day and 5th day ($P>0.05$). The toe volume significantly increased at 10th, 15th, and 20th day after modeling compared with control ($P<0.05$). AI showed no statistical difference in the 5th and 10th day after modeling compared with control ($P>0.01$). AI elevated markedly at 15th and 20th day after modeling ($P<0.01$). IL-17 significantly increased in model group compared with control ($P<0.01$). NF- κ B/P65, NF- κ B/P50, and I κ B α expression obviously enhanced in model group compared with control ($P<0.01$). NF- κ B highly expression in CIA rat model synovial cells was related to IL-17 highly expression.

Keywords: Synovial cell, serum inflammatory cytokine, NF- κ B, IL-17

Introduction

As a common immune system disease, rheumatoid arthritis (RA) is characterized as synovial tissue inflammatory hyperplasia, leading to accelerate the joint damage. Nuclear factors - κ B (NF- κ B) can not only transmit signal, but also plays an important role in tumor cells [1]. NF- κ B plays a role of switching cell activity in cells. NF- κ B is in the form of heterologous dimer in static cells, which can prevent I κ B trimer formation in rat joint cells [2]. NF- κ B participates in inflammatory response, promotes cell apoptosis, and regulates cell cycle. Interleukin-17 (IL-17) can accelerate cell damage through suppressing cartilage cell synthesis, thus resulting in joint erosion pathological damage. IL-17 plays a proinflammatory role mainly through binding with IL-17R, leading to produce a variety

of immune responses and accelerate RA disease progress [3]. This study adopts type II collagen induced arthritis (CIA) as model to explore NF- κ B protein expression in synovial cells and serum IL-17 levels, aiming to investigate the correlation among NF- κ B, IL-17, and CIA rat model arthritis.

Materials and methods

Animals

A total of 80 male SD rats with mean weight at 215.14 \pm 10.42 g were provided by Nanjing Lishengfei biotechnology co., LTD (serial number: SCXK 2015-0009). The rats were raised in individual battery with temperature at 21~24°C and relative humidity at 50~60%. All the experiments accorded with animal ethics standards.

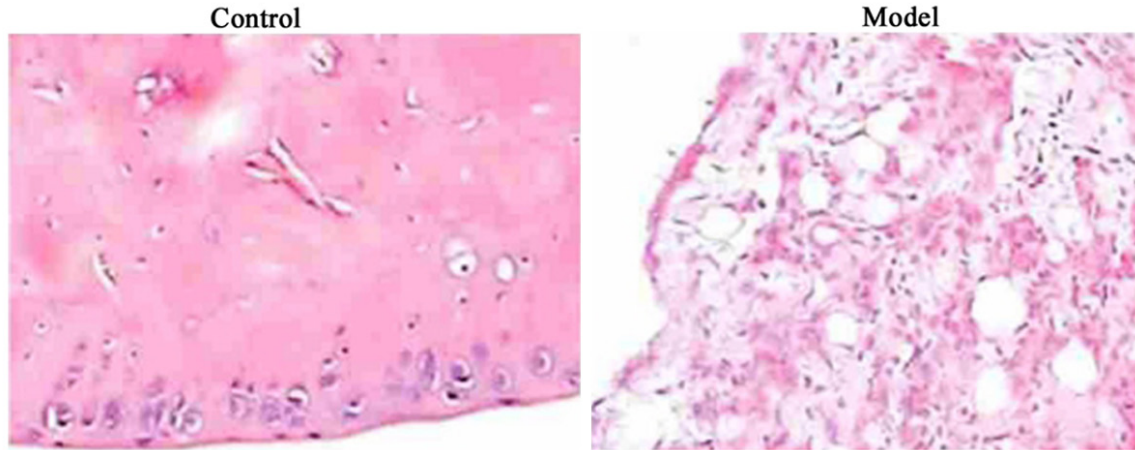


Figure 1. Synovium morphological changes.

Reagents and instruments

Bovine type II collagen was bought from Nanjing Purunli technology co., LTD.; Incomplete Freund's adjuvant was purchased from Nanjing Lishizi biological technology co., LTD. Rabbit anti-rat NF- κ B P65 polyclonal antibody and I κ B α monoclonal antibody were bought from Mego. IL-17 ELISA kit was got from Yangtze river pharmaceutical co., LTD. LD25-2 automatic balance centrifuge was purchased from Shanghai Feili biological technology co., LTD. YLS-7B rat toe volume measurement instrument was got from Tianjin Ruishina biological technology co., LTD. Gel imaging analysis system was purchased from Nanjing Zhongshan biological technology co., LTD. LY70 inverted microscope was purchased from Shanghai KGI biological technology co., LTD.

Grouping and modeling [4]

The SD rats were randomly equally divided into two groups as model group and blank control. CIA rat model was established in model group. A total of 10 mg bovine type II collagen was dissolved in 5 mL of acetic acid solution (mass fraction 0.1 mol/L) and prepared as collagen solution at 2 mg/mL that was stored at 4°C for 24 h. The same volume of Freund's complete adjuvant was applied to generate the collagen emulsion at 1 mg/mL. The solution was subcutaneous injected to the root of rat tail and repeated on the fifth day. In addition, the blank control only received normal saline. No dietary intervention was taken to the two groups. The laboratory temperature was maintained at

20~25°C, while the humidity was controlled at 56%~78%.

Synovial tissue pathological section

Both lower extremities knee synovium and cartilage were extracted and fixed in paraformaldehyde solution. They were then received regular decalcification, dehydration, and embedding. HE staining was performed after section to observe synovial pathological changes.

Toe volume

Toe volume was measured by toe volume measurement instrument at 1st, 5th, 10th, 15th, and 20th day after modeling.

Arthritis index (AI)

Arthritis severity was determined on the 5th day after CIA rat inflammation according to AI scoring [5]. Score 0 means no swelling performance, 1 represents knuckles swelling phenomenon, 2 means toe joints and vola pedis swelling phenomena, 3 stands for paw below ankle swelling phenomenon, 4 means all the paws swelling phenomenon including ankle. AI was calculated before modeling and at 5th, 10th, 15th, and 20th day after modeling. The rats were killed on the 25th day to extract venous blood and joint synovium for detection.

NF- κ B and serum IL-17 detection

The samples or standard substance were added to ELISA plate with three replicates at 37°C. After removing the fluid, the plate was

Table 1. Toe volume comparison (ml)

Group (cases)	1 st day after modeling	5 th day after modeling	10 th day after modeling	15 th day after modeling	20 th day after modeling
Control (40)	1.44±0.12	1.49±0.13	1.52±0.14	1.63±0.15	1.64±0.15
Model group (40)	1.47±0.13	1.50±0.13	1.83±0.16	2.10±0.17	2.21±0.19
t	0.001	0.002	2.126	3.216	3.235
P	P>0.05	P>0.05	P<0.05	P<0.05	P<0.05

Table 2. AI comparison

Group (cases)	5 th day after modeling	10 th day after modeling	15 th day after modeling	20 th day after modeling
Control (40)	0	0	0	0
Model group (40)	3.87±0.67	4.32±0.71	8.21±1.31	8.54±1.87
t	0.006	0.007	5.653	6.681
P	P>0.05	P>0.05	P<0.01	P<0.01

Table 3. Serum IL-17 comparison (ng·ml⁻¹)

Group (cases)	IL-17 (ng·ml ⁻¹)	t	P
Control (40)	21.24±4.21	6.258	P<0.01
Model group (40)	42.21±6.32		

Table 4. NF-κB/P65, NF-κB/P50, and IκBα expression comparison

Group (cases)	NF-κB/P65	NF-κB/P50	IκBα
Control (40)	3.41±1.23	3.51±1.52	5.01±1.61
Model group (40)	6.54±1.71	6.43±1.54	6.89±1.92
t	0.326	5.521	6.641
P	P<0.05	P<0.01	P<0.01

washed by buffer for 5 times and added with enzyme-labelled reagent at 37 °C. After washed by buffer for 5 times, the plate was treated with color developing agent A and B at 37 °C for 10 min. Then the stop buffer was added to each well and the plate was read on microplate reader to test absorbance value (OD value).

Joint synovium was treated by RIPA solution on ice for 40 min. Then the tissue was centrifuged at 4 °C and 12,000×g for 10 min. The protein concentration was determined by Bradford method. A total of 20 μg protein was separated by SDS-PAGE and transferred to NC membrane at 100 V for 1 h. After blocked at 37 °C for 1 h and 4 °C for 24 h, the membrane was incubated in primary antibody and AP-tagged IgG secondary antibody at 37 °C for 1 h after washed by TBST for three times. At last, the membrane was observed to calculate absorbance value.

Statistical analysis

The data was analyzed by SPSS17.0 software. Measurement data was presented as mean ± standard deviation. P<0.05 was considered as statistical significance.

Results

Synovium morphological changes

Pathological results indicated that the synovium from model rat appeared vascular hyperplasia, synovial membrane wall thickening, inflammatory cells increase, cell edema, and synovial hyperblastosis. No angiectasis, hyperplasia, or inflammatory cell production was observed in control rats (**Figure 1**).

Toe volume comparison

Compared with blank control, the toe volume in model rat was similar in the 1st day and 5th day (P>0.05). The toe volume significantly increased at 10th, 15th, and 20th day after modeling compared with control (P<0.05) (**Table 1**).

AI comparison

AI showed no statistical difference in the 5th and 10th day after modeling compared with control (P>0.01). AI elevated markedly at 15th and 20th day after modeling (P<0.01) (**Table 2**).

Serum IL-17 comparison

ELISA revealed that IL-17 significantly increased in model group compared with control (P<0.01) (**Table 3**).

NF-κB expression comparison

NF-κB/P65, NF-κB/P50, and IκBα expression obviously enhanced in model group compared

NF-κB and IL-17 in RA

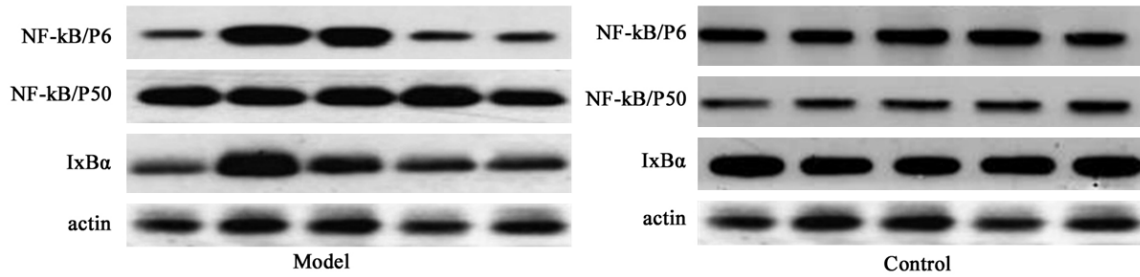


Figure 2. NF-κB/P65, NF-κB/P50, and IκBα expression.

with control ($P < 0.01$). It indicated that NF-κB/P65, NF-κB/P50, and IκBα overexpressed in the synovial cells from model rat (Table 4; Figure 2).

Discussion

RA is a type of autoimmune disease caused by cytokines eroding articular synovium induced inflammation. NF-κB plays a role of switching cell activity in cells. NF-κB is in the inactive form of heterologous dimer in static cells, which can prevent IκB trimer formation in rat joint cells [6]. The process of NF-κB activation is relatively complex, since body can regulate NF-κB activity in the normal physiology [5]. The most common dimer is p65/p50 in the cell, which causes NF-κB binding with p50 to synthesize p65/p50. NF-κB can bind with inhibitory protein IκB in static cells, leading to no regulatory effect on gene [7]. IκB subunit can form NF-κB dimer through binding with IκBα, resulting in inactive NF-κB-IκBα state [8]. In addition, the nuclear-export signal (NES) at the amino terminal of IκBα protein can produce NF-κB-IκBα complex, thereby promoting NF-κB-IκBα complex dynamic balance [9].

As a promoter of NF-κB, IκBα has the sensitivity that can be quickly decomposition and synthesis [10]. IκB can be degraded by TNF-α, IL-1β, IL-6, and IL-17 impact. It then can bind with the κB sequence on DNA after entering cellular nucleus, leading to NF-κB in regulating transcription [11]. Research indicated that NF-κB participates in inflammatory response, promotes cell apoptosis, and leads to cell cycle differentiation. It is the main pathological factor of RA [12-14]. In RA synovial cells, inflammatory factors can promote IKK kinase complex activation. It then accelerates IκB decomposition, leading to numerous NF-κB in cells and various inflammatory mediators (such as TNF-α and

IL-1β) abnormal expression. NF-κB elevation leads to inflammatory factors production. They shows positive correlation, thus contributes to the RA synovial joints involved in the inflammatory response [15-17]. In this study, NF-κB highly expressed in CIA rats synovial tissue, suggesting that NF-κB participates in the pathological damage of RA.

Our results showed that IL-17 can accelerate cell inflammatory reaction. The main reason is that Th17 cells can produce inflammation factors, resulting in promoting neutrophils, T cells, and fibroblasts activation. It causes macrophages and epithelial cells appearance, which further form proinflammatory medium, including (IL-1, IL-6, TNF-α, CO₂ enzyme, IL-8, monocyte chemotactic protein-1, and growth regulatory factors). IL-17 can reduce the formation of cartilage cells and accelerate cells damage, resulting in joint erosion pathological damage. IL-17 plays a proinflammatory role through synthetic IL-17R to produce various immune responses [18]. In RA process, IL-17 can promote IL-1 and TNF-α cytokines production, thus accelerating RA pathology damage [19, 20]. Our experimental results illustrated that compared with control, NF-κB activity obviously enhanced in CIA rats joint inflammation. Serum IL-17 level also elevated, suggesting that IL-17 and NF-κB play a mutual promotion role in RA occurrence and development. However, IκB expression still needs further verification.

To sum up, NF-κB highly expression in CIA rat model synovial cells was related to IL-17 highly expression.

Acknowledgements

This work was supported by Project supported by the National Natural Science Foundation of China (No. 81271948).

Disclosure of conflict of interest

None.

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