IL-1β increases the expression of inflammatory factors in synovial fluid-derived fibroblast-like synoviocytes via activation of the NF-κB-mediated ERK-STAT1 signaling pathway

JIE YANG[1], JUNHU WANG[1], XIAOJUN LIANG[1], HONGMOU ZHAO[1], JUN LU[1], QIANG MA[1], BINGFEI JING[2] and FENG TIAN[1]

[1]Department of Foot and Ankle Surgery, Honghui Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi 710054; [2]Department of Blood Test, Xi'an Blood Center, Xi'an, Shaanxi 710000, P.R. China

Received September 8, 2018; Accepted March 26, 2019

DOI: 10.3892/mmr.2019.10759

Abstract. Interleukin (IL)-1β serves a crucial role in the progression of rheumatoid arthritis. Previous studies have indicated that the ERK/STAT1 signaling pathway may be involved in the inflammatory response in synovial fluid-derived fibroblast-like synoviocytes (sfd-FLSs). However, the molecular mechanisms underlying the pathological effects of the inflammatory factors induced by IL-1β in sfd-FLSs remain unclear. The aim of the present study was to investigate the IL-1β-mediated signaling pathways involved in the expression of inflammatory factors in sfd-FLSs and in a rat model of rheumatoid arthritis. Reverse transcription-quantitative PCR, western blotting, and immunohistochemistry were used to analyze the role of IL-1β in the rat model of rheumatoid arthritis. The results suggested that IL-1β administration exacerbated rheumatoid arthritis, bone injury and increased the expression levels of inflammatory factors, such as IL-17 and tumor necrosis factor α (TNF-α), whereas treatment with anti-IL-1β exhibited opposite effects. *In vitro* experiments in sfd-FLSs further suggested that treatment with IL-1β influenced the expression levels of various inflammatory factors. In specific, IL-1β increased the expression of IL-17 and TNF-α, and decreased the expression of IL-6 and IL-10 in sfd-FLSs. Additionally, treatment with IL-1β increased the mRNA expression and protein phosphorylation of NF-κB, ERK and STAT1 in sfd-FLSs. Treatment with anti-IL-1β exhibited opposite effects on the expression levels of inflammatory factors and suppressed the NF-κB-mediated ERK-STAT1 signaling pathway activation in sfd-FLSs. Finally, treatment with a NF-κB inhibitor suppressed the effects of IL-1β, and NF-κB overexpression reversed the effects of anti-IL-1β on the expression levels of IL-17, TNF-α, NF-κB, ERK and STAT1. In conclusion, the present results demonstrated that treatment with IL-1β increased the expression levels of inflammatory factors in sfd-FLSs via the regulation of the NF-κB-mediated ERK/STAT1 signaling pathway in a rat model of rheumatoid arthritis. Therefore, the NF-κB/ERK/STAT1 signaling pathway may represent a potential target for the development of novel treatments for rheumatoid arthritis.

Correspondence to: Professor Feng Tian, Department of Foot and Ankle Surgery, Honghui Hospital, Xi'an Jiaotong University, 555 Youyi East Road, Xi'an, Shaanxi 710054, P.R. China
E-mail: fengtianxian@yeah.net

Key words: interleukin-1β, inflammation, synovial fluid-derived fibroblast-like synoviocytes, rheumatoid arthritis, NF-κB, ERK, STAT1

Introduction

Rheumatoid arthritis is a chronic autoinflammatory disease characterized by chronic inflammation and bone damage (1-4). Previous studies have demonstrated that rheumatoid arthritis is associated with chronic inflammation of synovial joints, hands and feet (5). Currently, targeted therapy is an available treatment for patients with rheumatoid arthritis (6-9). Numerous studies have demonstrated that targeted therapy for rheumatoid arthritis decreases inflammation, and many anti-inflammatory drugs have been used to improve the prognosis of rheumatoid arthritis, such as non-steroidal anti-inflammatory drugs, methotrexate, glucocorticoid, infliximab, golimumab and adalimumab (10-14). However, identifying the molecular signaling pathways underlying inflammation is required to develop novel treatments for patients with rheumatoid arthritis.

Although the causes underlying rheumatoid arthritis are not fully understood, experimental and clinical evidence suggest that interleukin (IL)-1β may serve an important role in the pathogenesis of rheumatoid arthritis (15-17). A previous study has demonstrated that the human anti-IL-1β monoclonal antibody ACZ885 was effective in blocking inflammatory responses in a mouse model of joint inflammation and in patients with rheumatoid arthritis (18). Theoretically, blocking the IL-1β pathway using specific anti-IL-1β antibodies would suppress the inflammatory process, limiting joint damage (19-21). In addition, patients with rheumatoid arthritis present high circulating levels of pro-inflammatory IL-1, and clinical trials have revealed that an IL-1 antagonist presented beneficial effects in patients with rheumatoid arthritis (22).

Furthermore, a previous study revealed that treatment with an IL-1 receptor antagonist was safe and well-tolerated, and was able to regulate immune responses, thus providing clinical benefits (23). ERK and STAT pathways have been identified as potential molecular targets in the treatment of rheumatoid arthritis (24-26). Additionally, NF-κB activity is associated with the severity of rheumatoid arthritis and a decreased response to infliximab (27). A previous study has reported that synovial fluid-derived fibroblast-like synoviocytes (sfd-FLSs) can be used as an *in vitro* model to evaluate the inflammatory processes in rheumatoid arthritis (28). Therefore, understanding the role of IL-1β signaling in sfd-FLSs may be crucial for an improved understanding of rheumatoid arthritis. Previous studies demonstrated that blocking NF-κB, ERK and STAT1 expression may be beneficial for the treatment of human rheumatoid arthritis (24,29,30). Therefore, the present study investigated the expression levels of NF-κB, ERK and STAT1 in sfd-FLSs to explore the role of IL-1β in rheumatoid arthritis.

In the present study, the expression, the role and the molecular mechanism underlying IL-1β in sfd-FLSs and in a rat model of rheumatoid arthritis were investigated. The findings identified that IL-1β was a pro-inflammatory factor upstream of NF-κB, which regulated the ERK/STAT1 pathway in sfd-FLSs and in a rat model of rheumatoid arthritis.

Materials and methods

Establishment of a rat model of rheumatoid arthritis. A total of 30 8 week-old female Sprague Dawley rats (200-250 g body weight) were purchased from The Experimental Animal Center of Jinzhou Medical University (Jinzhou, China). All rats were housed at 23±1˚C, 50±5% humidity with a 12 h light/dark cycle and free access to food and water. The induction of type II collagen-induced arthritis was achieved as previously described (31), by the subcutaneous injection of 2 mg collagen (ModiQuest Research) per rat (n=10 in each group). Rats were treated with IL-1β (10 mg/kg, Sigma-Aldrich; Merck KGaA), PBS (control; equal volume) or anti-IL-1β (10 mg/kg, ACZ885, Sigma-Aldrich; Merck KGaA) by subcutaneous injection every 4 days for a total of seven times.

Evaluation of arthritis. Rats were examined 28 days after collagen injection, and an arthritis score was assigned to each rat. The arthritis scores of experimental rats were evaluated using a scale of 0-2 for each paw, with a maximum total score of 8, as previously described (32). A score for each paw was assigned as follows: 0, normal paw; 0.25, 1-2 swollen toes; 0.5, 3-4 swollen toes; 0.75, slightly swollen footpad or ankle; 1, swollen footpad or ankle; 1.25, 1-2 swollen toes and swollen footpad or ankle; and 2.0, swollen toes and swollen footpad and ankle.

H&E staining. The tibias in experimental rats (n=5 per group) were fixed in 4% paraformaldehyde for 24 h, decalcified in 10% EDTA (pH = 7.4) for 5 days and embedded in paraffin. The tibias were cut into 4 μm tissue sections and then stained with 1% haematoxylin and eosin (H&E) for 15 min at room temperature. The tissue sections were imaged using a light microscope (TE2000S; Nikon Corporation).

ELISA. Blood samples were collected from all rats 28 days after collagen injection. Samples were centrifuged at 4,000 x g for 15 min at 4˚C. The circulating levels of TNF-α (cat. no. RTA00, R&D Systems, Inc.) and IL-17 (cat. no. HS170, R&D Systems, Inc.) were analyzed using ELISA kits according to the manufacturer's protocol.

Immunohistochemical staining. Synovial membranes were collected from rats 28 days after collagen injection. Tissues were fixed with 4% paraformaldehyde at room temperature for 12 h. Paraffin-embedded tissue samples of synovial membranes were obtained and cut into 4 μm sections, deparaffinized and rehydrated using a descending alcohol series. Sections were prepared and epitope retrieval was performed using Tris-HCl buffer (cat. no. AP-9005-050; Thermo Fisher Scientific, Inc.) for 30 min at 37˚C. Tissue sections were stained H&E (Sigma-Aldrich) for 15 min at room temperature. Sections were treated with 3% hydrogen peroxide for 15 min at 37˚C and subsequently blocked with 5% BSA (Sigma-Aldrich; Merck KGaA) for 2 h at 37˚C. Sections were washed with PBS and incubated with rabbit anti-rat IL-17 (1:1,000; ab193955; Abcam), TNF-α (1:1,000; ab109332; Abcam), ERK (1:1,000; ab32537; Abcam), phosphorylated ERK (pERK; 1:1,000; ab201015; Abcam) and STAT1 (1:1,000; ab2071; Abcam) at 4˚C overnight. Sections were washed three times and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG; 1:2,000; cat. no. 1706515; Bio-Rad Laboratories, Inc.) for 1 h at 37˚C. Diaminobenzidine was used as substrate for the immunohistochemical reaction. Tissue sections were visualized at x200 magnification using a confocal microscope (LSM780; Carl Zeiss AG).

sfd-FLSs culture. The sfd-FLS line HIG-82 (American Type Culture Collection cat. no. 1832) was purchased from BeNa Culture Collection. sfd-FLSs were grown in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. Cells were treated with IL-1β (1 mg/ml; cat no. SRP6551; Sigma-Aldrich; Merck KGaA), anti-IL-1β (1 mg/ml; cat no. PRS4877; Sigma-Aldrich; Merck KGaA) and/or NF-κB inhibitor (1 mg/ml; cat no. 481412; Sigma-Aldrich; Merck KGaA) for 12 h at 37˚C for further analysis.

Cells transfection. sfd-FLSs were seeded in 6-well plates at a density of $1x10^4$ cells/well in 2 ml RPMI-1640 supplemented with 10% FBS. Cells were cultured for 12 h and washed with PBS three times. NF-κB cDNA was cloned into a pcDNA3.1 plasmid (pcDNA3.1-NF-κB; Thermo Fisher Scientific, Inc.), and the empty plasmid pcDNA3.1 (Thermo Fisher Scientific, Inc.) served as control. sfd-FLSs were transfected with pcDNA3.1-NF-κB (5 μg) or empty pcDNA3.1 (5 μg) using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Cells were harvested after 72 h for further analysis.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from sfd-FLSs using the RNAeasy mini kit (Qiagen GmbH) according to manufacturer's protocol. RNA was reverse transcribed into cDNA using the QuantiTect

Reverse Transcription Kit (Qiagen GmbH) at 42˚C for 2 h according to on the manufacturer's instrument. All forward and reverse primers were purchased from Invitrogen (Thermo Fisher Scientific, Inc.) and are listed in Table I. qPCR was performed as follows: Initial denaturation at 95˚C for 2 min, followed by 45 cycles of 95˚C for 30 sec, 59˚C for 30 sec and 72˚C for 30 sec. The total volume of each reaction was 25 μl and contained 50 ng of cDNA, 200 μM dNTP, 2.5 units of Taq DNA polymerase (Takara Biotechnology, Co., Ltd.) and 200 μM primers using the SYBR® Premix Ex Taq™ kit (Takara Biotechnology, Co., Ltd.). Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (33). The results are presented as fold-change relative to the expression level of β-actin, used as internal control.

Western blotting. sfd-FLSs were homogenized using RIPA lysis buffer (Thermo Fisher Scientific, Inc.). Protein concentration was measured using a bicinchoninic acid assay kit (Thermo Fisher Scientific, Inc.). Subsequently, protein samples (20 μg in each lane) were separated by 12.5% SDS-PAGE. Protein were blotted on a nitrocellulose membrane and the membranes were incubated with primary antibodies anti-IL-17 (1:1,000; ab193955; Abcam), TNF-α (1:1,000; ab109332; Abcam), ERK (1:1,000; ab32537; Abcam), pERK (1:1,000; ab201015; Abcam), STAT1 (1:1,000; ab2071; Abcam), pSTAT1 (1:1,000; ab30645; Abcam) and β-actin (1:1,000; ab8226; Abcam) for 12 h at 4˚C, after blocking with 5% BSA (Sigma-Aldrich; Merck KGaA) for 1 h at 37˚C. Subsequently, the membranes were incubated with HRP-conjugated goat anti-rabbit IgG (1:5,000; cat. no. PV-6001; OriGene Technologies, Inc.) for 24 h at 4˚C. The blots were visualized using an enhanced chemiluminescence detection system (cat. no. 32209; Pierce; Thermo Fisher Scientific, Inc.). Densitometric quantification was performed using Quantity-One software (version 1.2; Bio-Rad Laboratories, Inc.).

Statistical analysis. Data are presented as the mean ± SD. Differences were evaluated for significance using one-way ANOVA followed by Tukey's post hoc test. Data were analyzed using GraphPad Prism (version 6.0; GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Table I. Primers used in the present study.

Gene symbol	Primer sequence (5'-3')
TNF-α	F: CCCTCACACTCAGATCATCTTCT
	R: GCTACGACGTGGGCTACAG
IL-17	F: GGGCCTGGCTTCTGTCTGA
	R: AAGTTCGTTCTGCCCCATCA
NF-κB	F: CACCCTCACCCTCCAACAAA
	R: TTCTCTTTCGTTCCCGGTGG
ERK	F: TGGTCCAGGGGTCTTACTCC
	R: TAAAGCCATGCCAATCTC
STAT1	F: GCAGGTTCACCAGCTTTATGA
	R: TGAAGATTACGCTTGCTTTTCCT
β-actin	F: CGGAGTCAACGGATTTGGTC
	R: AGCCTTCTCCATGGTCGTGA

TNF-α, tumor necrosis factor α; IL-17, interleukin 17; F, forward; R, reverse.

Results

Effects of anti-IL-1β on inflammation and NF-κB-mediated ERK-STAT1 signaling =in a rat model of rheumatoid arthritis. The effects of IL-1β and of an IL-1β inhibitory antibody (anti-IL-1β) on inflammation were investigated in a rat model of rheumatoid arthritis. The results suggested that treatment with anti-IL-1β decreased the rheumatoid arthritis score, whereas treatment with IL-1β exacerbated rheumatoid arthritis *in vivo* (Fig. 1A). Histopathological analysis demonstrated decreased synovial hyperplasia and bone erosion in the anti-IL-1β group compared with the control and IL-1β-treated groups. Treatment with anti-IL-1β decreased the bone injury score, whereas IL-1β increased the bone injury score compared with the control group (Fig. 1B). Treatment with anti-IL-1β increased the total body weight compared with the control group (Fig. 1C). Anti-IL-1β treatment decreased the serum levels of IL-17 and TNF-α in the rheumatoid arthritis rats, whereas IL-1β treatment increased the serum levels of IL-17 and TNF-α (Fig. 1D). Furthermore, the present results indicated that treatment with anti-IL-1β downregulated the gene and protein expression levels of NF-κB, ERK and STAT1, whereas treatment with IL-1β exhibited the opposite effects (Fig. 1E and F).

Anti-IL-1β downregulates the expression levels of the inflammatory factors IL-17 and TNF-α in sfd-FLSs. The effects of anti-IL-1β on the expression levels of various inflammatory factors were analyzed in sfd-FLSs *in vitro*. The results suggested that treatment with anti-IL-1β decreased the mRNA and protein expression levels of the pro-inflammatory factors IL-17 and TNF-α in sfd-FLSs (Fig. 2A and B). By contrast, treatment with anti-IL-1β increased the expression levels of the anti-inflammatory factors IL-6 and IL-10 in sfd-FLSs (Fig. 2C and D). Treatment with IL-1β exhibited the opposite effects (Fig. 2).

Anti-IL-1β downregulates the NF-κB-mediated ERK/STAT1 pathway in sfd-FLSs. The effects of anti-IL-1β on the NF-κB-mediated ERK/STAT1 signaling pathway were analyzed in sfd-FLSs *in vitro*. The results indicated that treatment of sfd-FLSs with anti-IL-1β decreased the mRNA expression levels and the protein phosphorylation of NF-κB, ERK and STAT1 (Fig. 3A and B). Conversely, treatment with IL-1β exhibited the opposite effects (Fig. 3A and B). Treatment with an NF-κB inhibitor (NF-κBIR) suppressed the IL-1β-mediated increase in the mRNA expression levels of NF-κB, ERK and STAT1 in sfd-FLSs (Fig. 3). Additionally, NF-κBIR inhibited the IL-1β-mediated increase in pNF-κB/NF-κB, p-ERK/ERK and pSTAT1/STAT1 protein expression ratios in sfd-FLSs (Fig. 3D). Conversely, NF-κB overexpression (NF-κBOR) suppressed the anti-IL-1β-mediated decrease in the mRNA expression and protein phosphorylation levels of NF-κB, ERK and STAT1 (Fig. 3E and F).

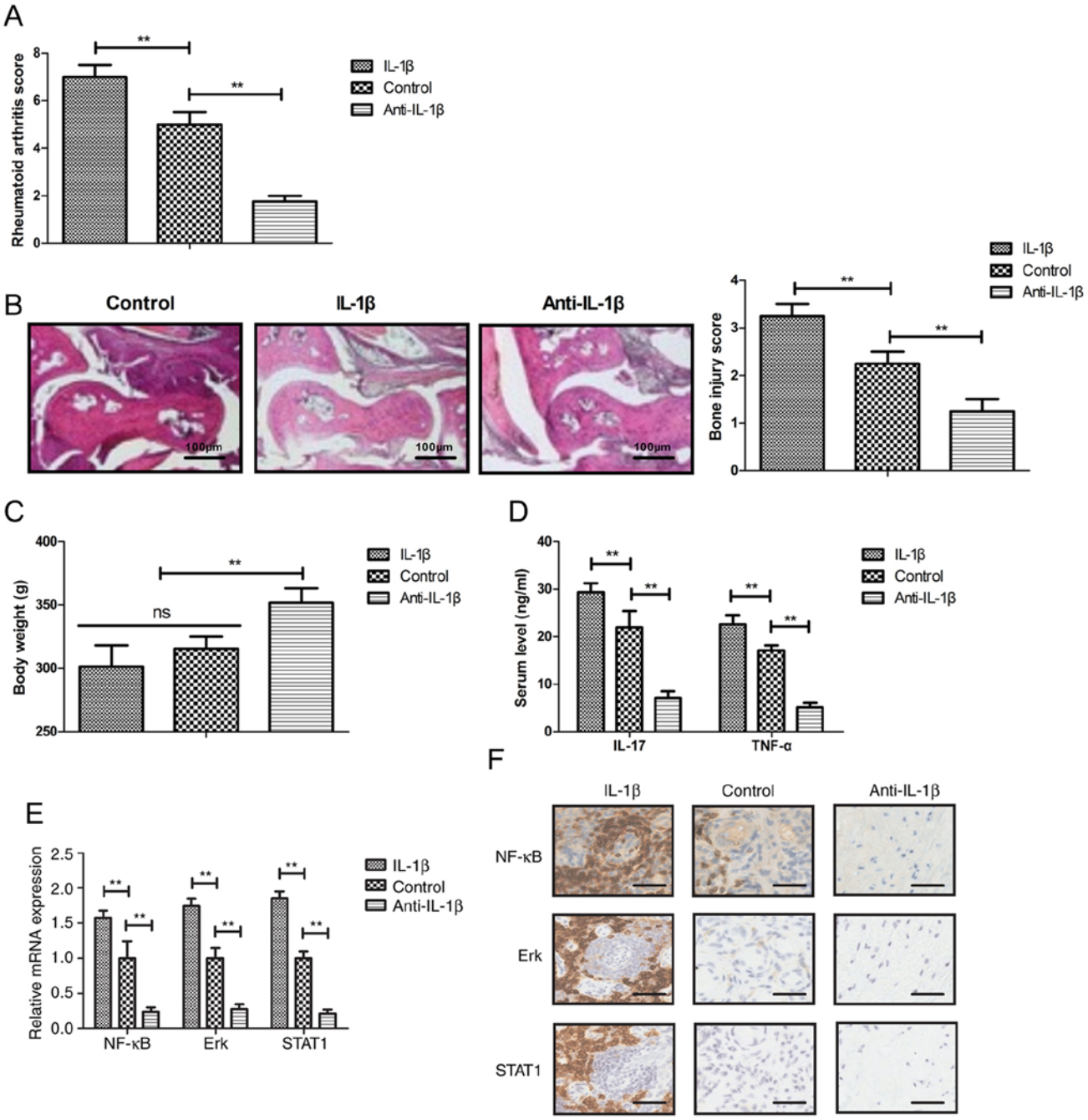

Figure 1. Effects of IL-1β and anti-IL-1β treatment on inflammation and NF-κB, ERK and STAT1 expression in a rat model of rheumatoid arthritis. (A) Therapeutic effects of anti-IL-1β on rheumatoid arthritis determined by analysis of the rheumatoid arthritis score. (B) Bone injury in rat models of rheumatoid arthritis. Magnification x40. (C) Body weight in the rheumatoid arthritis experimental rats. (D) Effects of treatments on the serum levels of IL-17 and TNF-α in the rheumatoid arthritis rats. Scale bar, 100 μm. (E) Effects of treatments on the mRNA expression levels of NF-κB, ERK and STAT1 in joint tissue from the rheumatoid arthritis rats. (F) Representative immunohistochemistry results showing the protein expression levels of NF-κB, ERK and STAT1. Scale bar=100 μm. **P<0.01, with comparisons indicated by brackets. IL, interleukin; NS, not significant; TNF-α, tumor necrosis factor α.

IL-1β increases the expression levels of inflammatory factors in sfd-FLSs via the NF-κB-mediated ERK/STAT1 signaling pathway. The mechanism underlying IL-1β-mediated inflammation was further investigated in sfd-FLSs. The results suggested that NF-κB inhibition suppressed the IL-1β-mediated increase in the mRNA and protein expression levels of IL-17 and TNF-α in sfd-FLSs (Fig. 4A and B). Similarly, NF-κB overexpression inhibited the anti-IL-1β-mediated decrease in the mRNA and protein expression levels of NF-κB, IL-17 and TNF-α in sfd-FLSs (Fig. 4C and D).

Discussion

Rheumatoid arthritis affects the function of joints and tissues, which may lead to various pathological symptoms, including fatigue, general discomfort and body weight loss (34). A previous study has demonstrated that NF-κB and various pro-inflammatory cytokines are involved in the inflammation of the joints through multiple signaling pathways both *in vivo* and *in vitro* (35). In the present study, the role of the pro-inflammatory cytokine IL-1β was investigated *in vitro,*

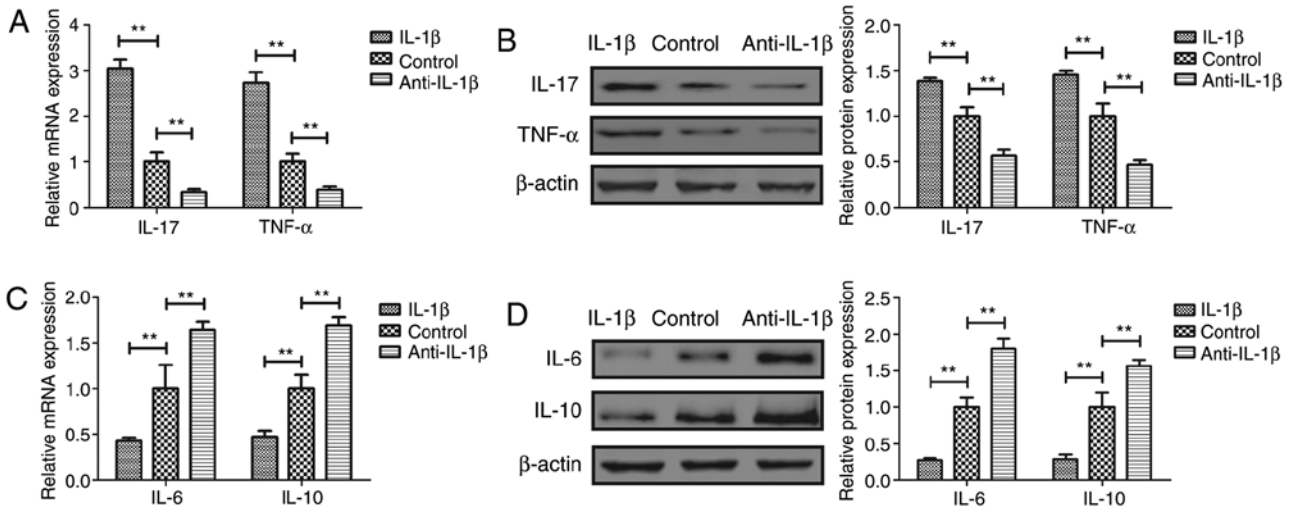

Figure 2. Effects of IL-1β and anti-IL-1β on the expression levels of various inflammatory factors in sfd-FLSs *in vitro*. (A) mRNA and (B) protein expression levels of IL-17 and TNF-α. (C) mRNA and (D) protein expression levels of IL-6 and IL-10. **P<0.01, with comparisons indicated by brackets. sfd-FLSs, synovial fluid-derived fibroblast-like synoviocytes; IL, interleukin; TNF-α, tumor necrosis factor α.

using sfd-FLSs, and *in vivo*, using a rat model of rheumatoid arthritis. The present study suggested the importance of the NF-κB-mediated ERK/STAT signaling pathway in rheumatoid arthritis and revealed a novel mechanism by which IL-1β inhibition ameliorated inflammatory factor expression through inhibition of NF-κB in sfd-FLSs. The decrease in the activity of the ERK/STAT pathway induced by anti-IL-1β was identified to protect rheumatoid arthritis rat against arthritic inflammation, possibly by inhibiting the IL-1β-mediated activation of the NF-κB signaling pathway.

Elevated serum levels of IL-1β have been reported in patients with rheumatoid arthritis (36). Decreasing the expression levels of IL-1β could decrease inflammation and facilitate the treatment of rheumatoid arthritis (37). The present results suggested that inhibition of IL-1β using a IL-1β blocking antibody decreased the mRNA and protein expression levels of IL-17 and TNF-α in sfd-FLSs and in rat models of rheumatoid arthritis. *In vivo* experiments suggested that blocking IL-1β decreased the rheumatoid arthritis score, bone injury and increased the body weight in rheumatoid arthritis rat. Although treatment with IL-1β affected the serum levels of various cytokines and the pathology of rheumatoid arthritis, it did not affect the body weight of the animals. Notably, further experiments are required to determine the cellular specificity of the protective effects of anti-IL1β treatment by generating transgenic rodents presenting cell-specific IL-1β inhibition.

In the present study it was hypothesized that the inflammatory response induced by IL-1β was able to promote a positive feedback loop leading to the upregulation of IL-17 and TNF-α, which may be potential targets in the treatment of rheumatoid arthritis. Previous studies have reported that the expression levels of IL-6 and IL-10 are downregulated in patients with rheumatoid arthritis (38-40). The present data suggested that IL-1β decreased IL-6 and IL-10 expression, whereas anti-IL-1β increased IL-6 and IL-10 expression in sfd-FLSs, which further indicated the therapeutic potential of anti-IL-1β in treating rheumatoid arthritis. Inhibition of IL-6 modulated type III collagen and C-reactive protein degradation in patients with rheumatoid arthritis exhibiting an inadequate response to anti-TNF therapy (41). IL-6 is an independent predictive factor of drug survival after dose escalation of infliximab in patients with rheumatoid arthritis (38). Additionally, STAT3 increases the expression level of IL-10 in a subset of regulatory B cells in patients with rheumatoid arthritis (42), suggesting that IL-10 may promote the occurrence and progression of rheumatoid arthritis (43). The present results suggested that anti-IL-1β markedly upregulated IL-6 and IL-10 in sfd-FLSs, suggesting that blocking IL-1β may have anti-inflammatory effects that may be beneficial for the treatment of rheumatoid arthritis. Notably, our results demonstrated that anti-IL-1β treatment increased the total body weight compared with the control group, which may suggest contributed to body weight loss of patients with rheumatoid arthritis. The increased total body weight of experimental animals in anti-IL-1B treatment may due to the reduction of inflammation.

NF-κB signaling is essential for the development and progression of rheumatoid arthritis (44). A previous study found that the ERK signaling pathway served a central role in the initiation and progression of rheumatoid arthritis and ERK inhibitors were described as novel potential treatments for rheumatoid arthritis (24). STAT1 expression is increased in inflammatory arthritis, suggesting that its pro-apoptotic and anti-inflammatory effects are not able to effectively counteract inflammation (45-47). In the present study, the mRNA and protein expression levels of NF-κB, ERK and STAT1 were analyzed and the results suggested that anti-IL-1β treatment downregulated NF-κB, ERK and STAT1 expression in sfd-FLSs and in a rat model of rheumatoid arthritis. NF-κB inhibitor suppressed IL-1β-mediated upregulation of IL-17 and TNF-α in sfd-FLSs, whereas NF-κB overexpression suppressed anti-IL-1β-mediated downregulation of IL-17 and TNF-α in sfd-FLSs. In addition, NF-κB overexpression suppressed the anti-IL-1β-mediated decrease in the mRNA expression and protein phosphorylation levels of NF-κB, ERK and STAT1, indicating that anti-IL-1β may regulate the ERK/STAT1 pathway by targeting NF-κB. Therefore, the present results suggested that NF-κB may be involved in

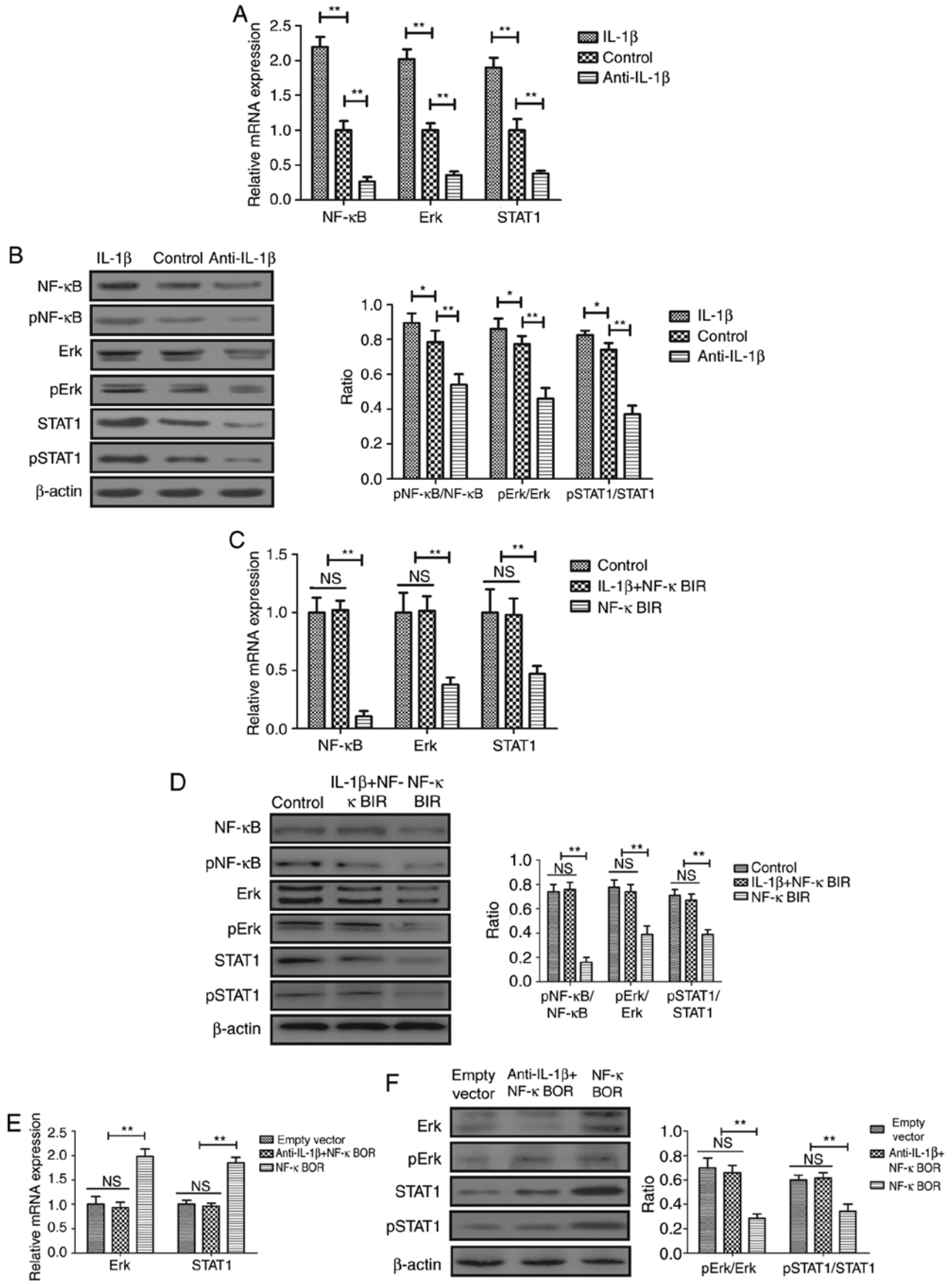

Figure 3. Effects of IL-1β and anti-IL-1β on the activity of the NF-κB-mediated ERK/STAT1 signaling pathway in sfd-FLSs *in vitro*. (A) mRNA expression levels of NF-κB, ERK and STAT1 in the different cell groups. (B) Protein expression levels of phosphorylated and total NF-κB, ERK and STAT1 in the different cell groups. (C) Effects of NF-κBIR on IL-1β-mediated increase in the mRNA expression and the (D) protein phosphorylation levels of NF-κB, ERK and STAT1 in sfd-FLSs. (E) Effects of NF-κBOR on anti-IL-1β-mediated decrease in the mRNA expression and (F) protein phosphorylation levels of ERK and STAT1 in sfd-FLSs. $^{*}P<0.05$ and $^{**}P<0.01$, with comparisons indicated by brackets. sfd-FLSs, synovial fluid-derived fibroblast-like synoviocytes; NF-κBIR, NF-κB inhibitor; NF-κBOR, NF-κB overexpression; IL, interleukin; p, phosphorylated; NS, not significant.

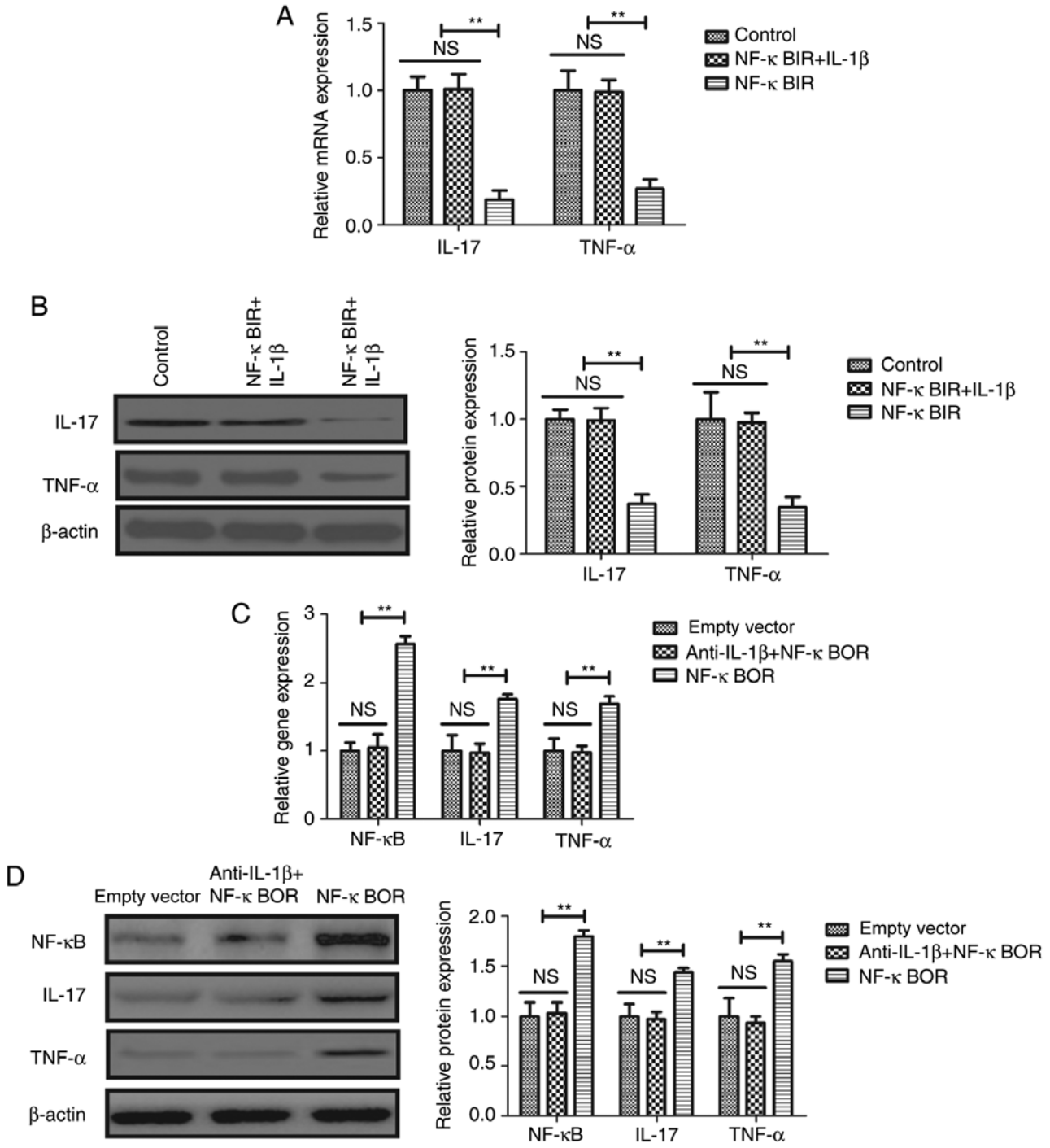

Figure 4. IL-1β enhances inflammatory factor expression via the NF-κB-mediated ERK/STAT1 signaling pathway in sfd-FLSs. (A) Effects of NF-κBIR on IL-1β-mediated increase in the mRNA and (B) protein expression levels of IL-17 and TNF-α in sfd-FLSs. (C) Effects of NF-κBOR on anti-IL-1β-mediated decrease in the mRNA and (D) protein expression levels of NF-κB, IL-17 and TNF-α in sfd-FLSs. **P<0.01, with comparisons indicated by brackets. sfd-FLSs, synovial fluid-derived fibroblast-like synoviocytes; IL, interleukin; TNF-α, tumor necrosis factor α; NF-κBIR, NF-κB inhibitor; NF-κBOR, NF-κB overexpression; NS, not significant.

the pathogenesis of IL-1β-induced rheumatoid arthritis mediated by the ERK/STAT1 signal pathway, and that anti-IL-1β improved the symptoms associated with rheumatoid arthritis by inhibiting the NF-κB signaling pathway.

Collectively, systemic administration of anti-IL-1β decreased arthritis severity and tissue inflammation in a rat model of rheumatoid arthritis. In addition, IL-1β increased the expression levels of inflammatory factors via the upregulation of the NF-κB-mediated ERK/STAT1 signaling pathway. The present results suggested that IL-1β may be a crucial inflammatory factor involved in rheumatoid arthritis and that the NF-κB-mediated ERK/STAT1 signaling pathway may represent a potential therapeutic target for the treatment of rheumatoid arthritis.

Acknowledgements

Not applicable.

Funding

The present study was supported by The Xi'an Health and Family Planning Commission (grant no. J20161008) and The Study of Structural Changes of Subchondral Bone in Post-Traumatic Arthritis In Rabbits (grant no. XA20170502).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JY performed all experiments in the present study. JW, XL, HZ, QM and BJ analyzed the experimental data. FT designed the present study. JL performed the experiments and wrote the manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethic Committee of Honghui Hospital, Xi'an Jiaotong University (approval no. JS20160215X).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sharma J, Bhar S and Devi CS: A review on interleukins: The Key manipulators in rheumatoid arthritis. Mod Rheumatol 27: 723-746, 2017.
2. Lage-Hansen PR, Lindegaard H, Chrysidis S and Terslev L: The role of ultrasound in diagnosing rheumatoid arthritis, what do we know? An updated review. Rheumatol Int 37: 179-187, 2017.
3. Tarp S, Furst DE, Dossing A, Østergaard M, Lorenzen T, Hansen MS, Singh JA, Choy EH, Boers M, Suarez-Almazor ME, *et al*: Defining the optimal biological monotherapy in rheumatoid arthritis: A systematic review and meta-analysis of randomised trials. Semin Arthritis Rheum 46: 699-708, 2017.
4. Jiang M, Ren F, Zheng Y, Yan R, Huang W, Xia N, Luo L, Zhou J and Tang L: Efficacy and safety of down-titration versus continuation strategies of biological disease-modifying anti-rheumatic drugs in patients with rheumatoid arthritis with low disease activity or in remission: A systematic review and meta-analysis. Clin Exp Rheumatol 35: 152-160, 2017.
5. Haus E, Sackett-Lundeen L and Smolensky MH: Rheumatoid arthritis: Circadian rhythms in disease activity, signs and symptoms, and rationale for chronotherapy with corticosteroids and other medications. Bull NYU Hosp Jt Dis 70 (Suppl 1): S3-S10, 2012.
6. Steunebrink LM, Versteeg GA, Vonkeman HE, Ten Klooster PM, Kuper HH, Zijlstra TR, van Riel PL and van de Laar MA: Initial combination therapy versus step-up therapy in treatment to the target of remission in daily clinical practice in early rheumatoid arthritis patients: Results from the dream registry. Arthritis Res Ther 18: 60, 2016.
7. Bernard NJ: Rheumatoid arthritis: Choline kinase-more than a cancer therapy target? Nat Rev Rheumatol 10: 699, 2014.
8. Iwata S, Nakayamada S, Fukuyo S, Kubo S, Yunoue N, Wang SP, Yoshikawa M, Saito K and Tanaka Y: Activation of syk in peripheral blood B cells in patients with rheumatoid arthritis: A potential target for abatacept therapy. Arthritis Rheumatol 67: 63-73, 2015.
9. Bernard NJ: Rheumatoid arthritis: Are FcRL4+ B cells the next target for RA biologic therapy? Nat Rev Rheumatol 10: 127, 2014.
10. Pincus T and Castrejon I: Evidence that the strategy is more important than the agent to treat rheumatoid arthritis. Data from clinical trials of combinations of non-biologic DMARDs, with protocol-driven intensification of therapy for tight control or treat-to-target. Bull Hosp Jt Dis 71 (Suppl 1): S33-S40, 2013.
11. Rabquer BJ and Koch AE: NK4 therapy: A new approach to target angiogenesis and inflammation in rheumatoid arthritis. Arthritis Res Ther 15: 119, 2013.
12. Sakai R, Tanaka M, Nanki T, Watanabe K, Yamazaki H, Koike R, Nagasawa H, Amano K, Saito K, Tanaka Y, *et al*: Drug retention rates and relevant risk factors for drug discontinuation due to adverse events in rheumatoid arthritis patients receiving anti-cytokine therapy with different target molecules. Ann Rheum Dis 71: 1820-1826, 2012.
13. Svanstrom H, Lund M, Melbye M and Pasternak B: Concomitant use of low-dose methotrexate and NSAIDs and the risk of serious adverse events among patients with rheumatoid arthritis. Pharmacoepidemiol Drug Saf 27: 885-893, 2018.
14. Best JH, Kong AM, Lenhart GM, Sarsour K, Stott-Miller M and Hwang Y: Association between glucocorticoid exposure and healthcare expenditures for potential glucocorticoid-related adverse events in patients with rheumatoid arthritis. J Rheumatol 45: 320-328, 2018.
15. Pasi S, Kant R, Gupta S and Surolia A: Novel multimeric IL-1 receptor antagonist for the treatment of rheumatoid arthritis. Biomaterials 42: 121-133, 2015.
16. Yang HQ, Liu XG, Yang X, Chen T and Yu SG: Effect of different types of moxibustion intervention on expression of inflammatory cytokines IL-1 and TNF-alpha in rabbits with rheumatoid arthritis. Zhen Ci Yan Jiu 38: 134-139, 2013 (In Chinese).
17. Adachi M, Okamoto S, Chujyo S, Arakawa T, Yokoyama M, Yamada K, Hayashi A, Akita K, Takeno M, Itoh S, *et al*: Cigarette smoke condensate extracts induce IL-1-beta production from rheumatoid arthritis patient-derived synoviocytes, but not osteoarthritis patient-derived synoviocytes, through aryl hydrocarbon receptor-dependent NF-kappa-B activation and novel NF-kappa-B sites. J Interferon Cytokine Res 33: 297-307, 2013.
18. Alten R, Gram H, Joosten LA, van den Berg WB, Sieper J, Wassenberg S, Burmester G, van Riel P, Diaz-Lorente M, Bruin GJ, *et al*: The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. Arthritis Res Ther 10: R67, 2008.
19. Kalliolias GD and Liossis SN: The future of the IL-1 receptor antagonist anakinra: From rheumatoid arthritis to adult-onset Still's disease and systemic-onset juvenile idiopathic arthritis. Expert Opin Investig Drugs 17: 349-359, 2008.
20. Settas LD, Tsimirikas G, Vosvotekas G, Triantafyllidou E and Nicolaides P: Reactivation of pulmonary tuberculosis in a patient with rheumatoid arthritis during treatment with IL-1 receptor antagonists (anakinra). J Clin Rheumatol 13: 219-220, 2007.
21. Botsios C, Sfriso P, Ostuni PA, Todesco S and Punzi L: Efficacy of the IL-1 receptor antagonist, anakinra, for the treatment of diffuse anterior scleritis in rheumatoid arthritis. Report of two cases. Rheumatology (Oxford) 46: 1042-1043, 2007.
22. Nikfar S, Saiyarsarai P, Tigabu BM and Abdollahi M: Efficacy and safety of interleukin-1 antagonists in rheumatoid arthritis: A systematic review and meta-analysis. Rheumatol Int 38: 1363-133, 2018.
23. Niu X, He D, Deng S, Li W, Xi Y, Xie C, Jiang T, Zhang JZ, Dong C and Chen G: Regulatory immune responses induced by IL-1 receptor antagonist in rheumatoid arthritis. Mol Immunol 49: 290-296, 2011.
24. Ohori M: ERK inhibitors as a potential new therapy for rheumatoid arthritis. Drug News Perspect 21: 245-250, 2008.
25. Isomaki P, Junttila I, Vidqvist KL, Korpela M and Silvennoinen O: The activity of JAK-STAT pathways in rheumatoid arthritis: Constitutive activation of STAT3 correlates with interleukin 6 levels. Rheumatology (Oxford) 54: 1103-1113, 2015.
26. Boyle DL, Soma K, Hodge J, Kavanaugh A, Mandel D, Mease P, Shurmur R, Singhal AK, Wei N and Rosengren S: The JAK inhibitor tofacitinib suppresses synovial JAK1-STAT signalling in rheumatoid arthritis. Ann Rheum Dis 74: 1311-1316, 2015.
27. Torices S, Julia A, Munoz P, Varela I, Balsa A, Marsal S, Fernández-Nebro A, Blanco F, López-Hoyos M, Martinez-Taboada V and Fernández-Luna JL: A functional variant of TLR10 modifies the activity of NFkB and may help predict a worse prognosis in patients with rheumatoid arthritis. Arthritis Res Ther 18: 221, 2016.

28. Trzybulska D, Olewicz-Gawlik A, Sikora J, Frydrychowicz M, Kolecka-Bednarczyk A, Kaczmarek M and Hrycaj P: The effect of caveolin-1 knockdown on interleukin-1β-induced chemokine (C-C motif) ligand 2 expression in synovial fluid-derived fibroblast-like synoviocytes from patients with rheumatoid arthritis. Adv Clin Exp Med 27: 1491-1497, 2018.
29. Yin G, Wang Y, Cen XM, Yang M, Liang Y and Xie QB: Lipid peroxidation-mediated inflammation promotes cell apoptosis through activation of NF-κB pathway in rheumatoid arthritis synovial cells. Mediators Inflamm 2015: 460310, 2015.
30. Gordon RA, Grigoriev G, Lee A, Kalliolias GD and Ivashkiv LB: The interferon signature and STAT1 expression in rheumatoid arthritis synovial fluid macrophages are induced by tumor necrosis factor alpha and counter-regulated by the synovial fluid microenvironment. Arthritis Rheum 64: 3119-3128, 2012.
31. Nakajima H, Takamori H, Hiyama Y and Tsukada W: The effect of treatment with interferon-gamma on type II collagen-induced arthritis. Clin Exp Immunol 81: 441-445, 1990.
32. Saha S, Qi J, Wang S, Wang M, Li X, Kim YG, Núñez G, Gupta D and Dziarski R: PGLYRP-2 and Nod2 are both required for peptidoglycan-induced arthritis and local inflammation. Cell Host Microbe 5: 137-150, 2009.
33. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
34. Murray E, Ellis A, Butylkova Y, Skup M, Kalabic J and Garg V: Systematic review and network meta-analysis: Effect of biologics on radiographic progression in rheumatoid arthritis. J Comp Eff Res 7: 959-974, 2018.
35. Wang QH, Lv SW, Guo YY, Duan JX, Dong SY, Wang QS, Yu FM, Su H and Kuang HX: Pharmacological effect of caulophyllum robustum on collagen-induced arthritis and regulation of nitric oxide, NF-κB, and proinflammatory cytokines in vivo and in vitro. Evid Based Complement Alternat Med 2017: 8134321, 2017.
36. Ruscitti P, Cipriani P, Cantarini L, Liakouli V, Vitale A, Carubbi F, Berardicurti O, Galeazzi M, Valenti M and Giacomelli R: Efficacy of inhibition of IL-1 in patients with rheumatoid arthritis and type 2 diabetes mellitus: Two case reports and review of the literature. J Med Case Rep 9: 123, 2015.
37. Chabaud M, Page G and Miossec P: Enhancing effect of IL-1, IL-17, and TNF-alpha on macrophage inflammatory protein-3alpha production in rheumatoid arthritis: Regulation by soluble receptors and Th2 cytokines. J Immunol 167: 6015-6020, 2001.
38. Takasugi K, Nishida K, Natsumeda M, Yamashita M, Yamamoto W and Ezawa K: IL-6 is an independent predictive factor of drug survival after dose escalation of infliximab in patients with rheumatoid arthritis. Mod Rheumatol 28: 452-460, 2018.
39. Yamana J, Yamamura M, Okamoto A, Aita T, Iwahashi M, Sunahori K and Makino H: Resistance to IL-10 inhibition of interferon gamma production and expression of suppressor of cytokine signaling 1 in CD4+ T cells from patients with rheumatoid arthritis. Arthritis Res Ther 6: R567-R577, 2004.
40. Sabry D, Elamir A, Mahmoud RH, Abdelaziz AA and Fathy W: Role of LncRNA-AF085935, IL-10 and IL-17 in rheumatoid arthritis patients with chronic Hepatitis C. J Clin Med Res 9: 416-425, 2017.
41. Juhl P, Thudium CS, Gudmann NS, Karsdal MA, Bay-Jensen AC and Siebuhr AS: IL-6 receptor inhibition modulates type III collagen and C-reactive protein degradation in rheumatoid arthritis patients with an inadequate response to anti-tumour necrosis factor therapy: Analysis of connective tissue turnover in the tocilizumab RADIATE study. Clin Exp Rheumatol 36: 568-574, 2018.
42. Banko Z, Pozsgay J, Szili D, Tóth M, Gáti T, Nagy G, Rojkovich B and Sármay G: Induction and differentiation of IL-10-Producing regulatory B cells from healthy blood donors and rheumatoid arthritis patients. J Immunol 198: 1512-1520, 2017.
43. Ouyang BS, Che JL, Gao J, Zhang Y, Li J, Yang HZ, Hu TY, Wu YJ and Yang M: Effects of electroacupuncture and simple acupuncture on changes of IL-1, IL-4, IL-6 and IL-10 in peripheral blood and joint fluid in patients with rheumatoid arthritis. Zhongguo Zhen Jiu 30: 840-844, 2010 (In Chinese).
44. Li G, Xia Z, Liu Y, Meng F, Wu X, Fang Y, Zhang C and Liu D: SIRT1 inhibits rheumatoid arthritis fibroblast-like synoviocyte aggressiveness and inflammatory response via suppressing NF-κB pathway. Biosci Rep 38: BSR20180541, 2018.
45. Scheinman RI, Trivedi R, Vermillion S and Kompella UB: Functionalized STAT1 siRNA nanoparticles regress rheumatoid arthritis in a mouse model. Nanomedicine (Lond) 6: 1669-1682, 2011.
46. Walker JG, Ahern MJ, Coleman M, Weedon H, Papangelis V, Beroukas D, Roberts-Thomson PJ and Smith MD: Expression of Jak3, STAT1, STAT4, and STAT6 in inflammatory arthritis: Unique Jak3 and STAT4 expression in dendritic cells in seropositive rheumatoid arthritis. Ann Rheum Dis 65: 149-156, 2006.
47. Kasperkovitz PV, Verbeet NL, Smeets TJ, van Rietschoten JG, Kraan MC, van der Pouw Kraan TC, Tak PP and Verweij CL: Activation of the STAT1 pathway in rheumatoid arthritis. Ann Rheum Dis 63: 233-239, 2004.