NF-κB and I-κB overexpression in articular chondrocytes with progression of type II collagen-induced arthritis in DBA/1 mouse knees

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Abstract

Objective

To investigate the roles of NF-κB and I-κB in chondrocytes in the cartilage destruction of mouse collagen-induced arthritis.

Methods

DBA/1 mice were immunized with bovine type II collagen (C II) emulsified with Freund's incomplete adjuvant. Histological abnormalities in the mouse knees were evaluated in sections stained with hematoxylin and eosin, or toluidine blue. Immunostaining using anti-NF-κB and anti-I-κB antibodies was performed to observe the expression and nuclear shift of NF-κB and I-κB in articular chondrocytes.

Results

Loss of metachromasia of perichondrial cartilage was found in 8 out of 10 knees of the mice at 5 weeks, and in all 10 knees at 7 weeks after C II-immunization. The mean percentage of NF-κB-positive chondrocytes at 3, 5 and 7 weeks and in the control was 33.6 ± 7.6, 54.9 ± 2.7, 75.9 ± 1.0 and 27.9 ± 3.8%, respectively. The mean percentage of NF-κB-positive nuclei was 9.1 ± 0.7, 19.5 ± 3.7, 47.5 ± 3.0 and 8.6 ± 0.1%, respectively. NF-κB-positive chondrocytes significantly increased from 3 to 7 weeks in a time-dependent manner ($p < 0.0001$). The percentage of I-κB-positive chondrocytes at 3, 5 and 7 weeks and in the control was 15.5 ± 1.7, 41.1 ± 2.5, 51.2 ± 5.7 and 12.1 ± 2.1%, respectively. I- κB-positive chondrocytes significantly increased from 3 to 5 weeks ($p < 0.005$).

Conclusion

These findings suggest that nuclear shift of NF-κB localization causes cartilage destruction in the early stage of arthritis in DBA/1 mice immunized with C II.

Key words

NF-κB, I-κB, collagen-induced arthritis, DBA/1 mouse, cartilage destruction.

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Introduction

NF-κB is reported to be a critical transcription factor involved in the production of major proinflammatory cytokines and adhesion molecules (1-4), which is thought to be a key regulatory element in rheumatoid arthritis (RA), and to consist of hetero- or homodimers containing p50 and/or p65 subunits (5). The complex normally resides in the cytoplasm of cells and is bound by as constitutively produced inhibitor known as I-κB (6). Following activation by various stimuli, I-κB is phosphorylated and degraded by the ubiquitin-proteosome system (7). The released NF-κB translocates to the nucleus and binds to specific NF-κB DNA binding sites and initiates gene expression.

The articular chondrocytes in inflamed joints are reported to produce proinflammatory cytokines, adhesion molecules (8, 9), and various enzymes (10, 11). In the early stage of RA, it is reported that cartilage destruction is caused by activation of cellular elements including chondrocytes as well as synoviocytes (10, 11).

Collagen-induced arthritis (CIA) has been established as an experimental autoimmune diseases induced by immunization of a susceptible mouse or rat strain with type II collagen, with cartilage destruction and synovitis resembling RA (12). In this study, we investigated the expression and nuclear shift of NF-κB and I-κB in chondrocytes of mice with collagen-induced arthritis, to elucidate their role in cartilage destruction.

Materials and methods

Animals and induction of collagen-induced arthritis (CIA)

DBA/1 male mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Thirty-five 9-week-old mice (22-25 g) were immunized with bovine type II collagen (Elastin Products Co., Owensville, MO, USA). Type II collagen was dissolved at 2 mg/ml in 10 mM acetic acid and emulsified in an equal volume of Freund's incomplete adjuvant (Difco, Detroit, MI, USA). The emulsion, 0.1 ml per mouse, was injected subcutaneously at the base of the tail, followed by a booster injection 3 weeks after the initial immunization. Control mice were given an injection of Freund's incomplete adjuvant emulsified with 10 mM acetic acid in the same manner.

Evaluation of arthritis

The 140 limbs of 35 mice were observed once a week after the initial immunization up to 7 weeks. Arthritis was defined as visible joint swelling and/or erythema, for which clinical scoring (arthritis index) was performed according to Verdrengh *et al.* (13). Macroscopic inspection yielded a score of 0-3 points for each paw (0 = normal appearance; 1 = mild swelling and erythema; 2 = moderate swelling and erythema; 3 = marked swelling and erythema). The arthritis index was calculated by adding the points from all four paws in each mouse. Maximum score per mouse was 12 points.

Histological analysis

Five mice were sacrificed at 3, 5 and 7 weeks after the initial immunization, and control mice were sacrificed at 6 and 7 weeks for histological analysis. Histological analysis of the joints was performed on the bilateral knees. All specimens were fixed in 10% neutralized formalin, decalcified with 0.5 M EDTA, and embedded in paraffin prior to cutting then into 5 μm sagittal sections. Sections were stained with hematoxylin and eosin (HE), or toluidine blue. Immunostaining with rabbit polyclonal anti-NF-κB p65 antibodies and rabbit polyclonal anti-I-κBα antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed on 10 knees in each group using the avidine-biotin-glucose complex method (Vectastain ABC-GO kit; Vector Laboratories Inc., Burlingame, CA, USA).

Statistical analysis

The significance of differences between two groups was assessed by Student's t-test, and the time-dependent significance of differences was assessed by ANOVA and Scheffe's F test. P values less than 0.05 were considered to be statistically significant.

Results

Macroscopic assessment of arthritis

Swelling and erythema in the paws of collagen-immunized mice were observed from 3 weeks after the initial immunization. The onset and progression of arthritis were evaluated once a week using the arthritis index. The mean (± SE) index at 0-3, 4, 5, 6 and 7 weeks after C II-immunization was 0 ± 0, 0.2 ± 0.4,2.7 ± 0.5, 4.4 ± 1.0 and 7.2 ± 2.9 points, respectively. The index of control mice was 0 ± 0, 1.1 ± 1.5, 0.7 ± 0.7, 0.7 ± 0.7 and 1.8 ± 1.3 points, respectively. The index was significantly higher at 5 weeks after the initial immunization compared to that at 4 weeks ($p < 0.05$).

Pathological findings of arthritis

At three weeks after C II-immunization, hyperplasia of the synovial lining layer was observed in one knee, and loss of metachromasia of superficial cartilage was observed in 2 out of 10 knees. These histological abnormalities were found in all 10 knees at 5 and 7 weeks. Both loss of metachromasia of the perichondrial cartilage in toluidine blue stained sections and an irregular cartilage surface in HE stained sections were found in 8 out of 10 knees of the mice at 5 weeks and in all 10 knees at 7 weeks, while they were not found in the mice at 3 weeks (Fig. 1, Table I).

Table I. Pathological findings in knees of mice immunized with type II collagen.

	Weeks after immunization		
	3	5	7
Hyperplasia of lining layer of synovium	1	10	10
Loss of metachromasia in superficial cartilage	2	10	10
Loss of metachromasia in perichondrial cartilage	0	8	10
Disruption of smooth cartilage surface	0	8	10
Pannus formation	0	3	7

Values are numbers of knees in 5 mice immunized with type II collagen.
Metachromasia of cartilage was evaluated in sagittal sections stained with toluidine blue, and other pathological findings were evaluated in sections stained with hematoxylin and eosin.

Immunohistological analysis

NF-κB appeared to be located in chondrocytes in the superficial layer of articular cartilage from 3 weeks at the earliest, while it was observed in chondocytes in the deep layer at 5 weeks (Fig. 2). The mean (± SE) percentage

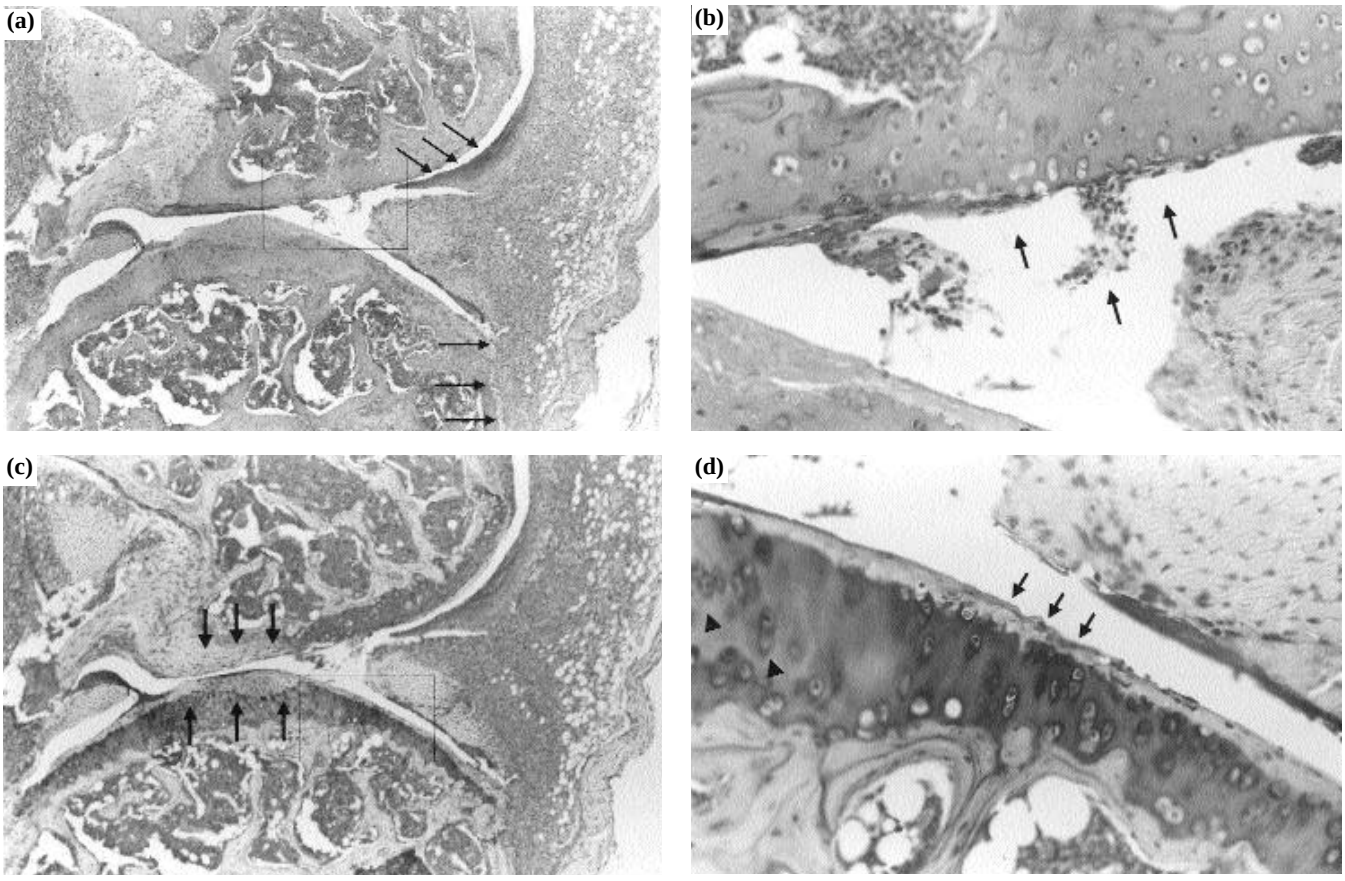

Fig. 1. Histological findings in a DBA/1 mouse knee at 7 weeks after type II collagen-immunization. Sequential sections were stained with hematoxylin and eosin (**a, b**), or with toluidine blue (**c, d**). Note the synovial hyperplasia (**a**), the cartilage destruction facing the pannus with cell infiltration (**b**), the loss of metachromasia of cartilage (**c**) indicated by arrows. Proteoglycan loss indicated by a reduction in metachromasia of superficial cartilage (**arrows**), and of perichondrial cartilage (**arrowheads**) was seen (**d**). Original magnification x40 (**a, c**); x200 (**b, d**).

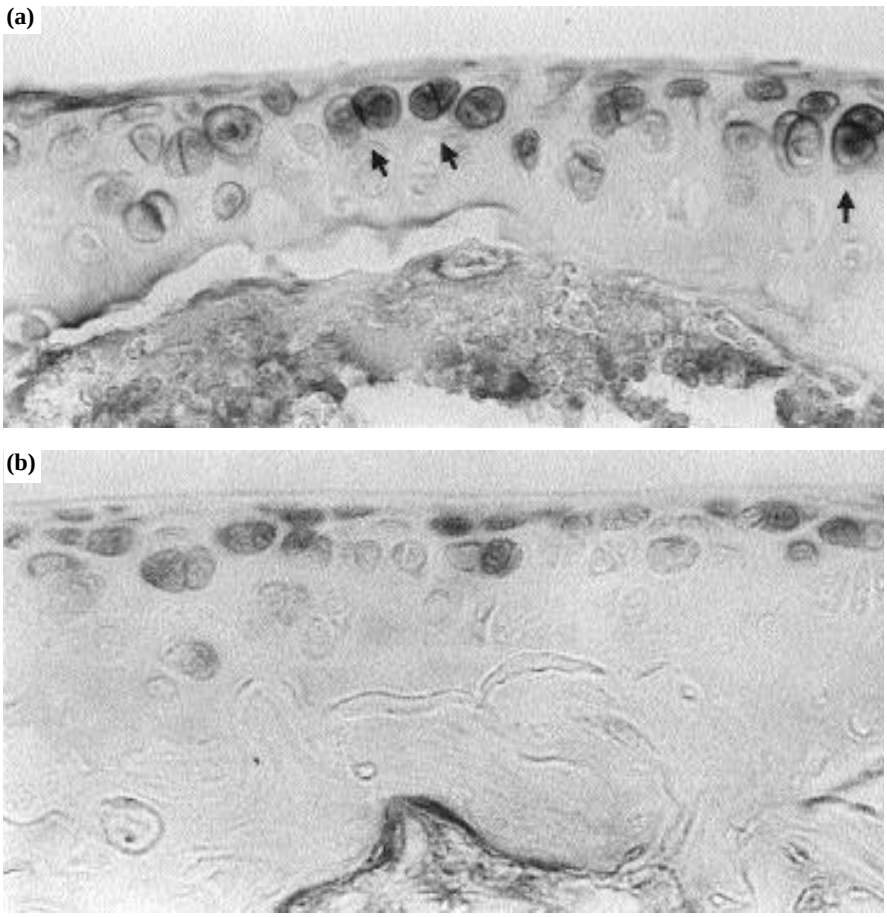

Fig. 2. NF-κB positive chondrocytes of the knee from a mouse sacrificed at 7 weeks after type II collagen-immunization (**a**),and a control mouse injected with adjuvant without collagen (**b**). Note NF-κB expression in chondrocytes of the superficial cartilage layer (**a, b**), and in chondrocytes of the deep layer (**a**). Arrows indicate NF-κB-positive chondrocytes. Sagittal section, original magnification x200.

of NF-κB-positive chondrocytes (cytoplasm and nuclei) out of total articular chondrocytes at 3, 5 and 7 weeks and in the control was 33.6 ± 7.6, 54.9 ± 2.7, 75.9 ± 1.0 and 27.9 ± 3.8%, respectively. The percentage of NF-κB-positive nuclei was 9.1 ± 0.7, 19.5 ± 3.7, 47.5 ± 3.0 and 8.6 ± 0.1%, respectively (Fig. 3). The percentage of NF-κB-positive chondrocytes at 7 weeks increased to 136% of that at 5 weeks, while NF-κB-positive nuclei at 7 weeks increased to 243% of that at 5 weeks. NF-κB-positive chondrocytes and NF-κB-positive nuclei significantly increased from 3 to 7 weeks in a time-dependent manner ($p < 0.001$). NF-κB-positive chondrocytes and arthritis index increased significantly after 5 weeks, and loss of metachromasia in perichondrial cartilage and disruption of the smooth cartilage surface were found after 5 weeks.

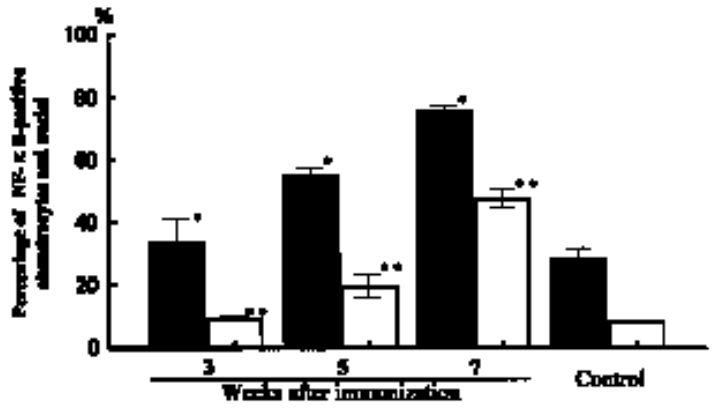

Fig. 3. Percentage of NF-κB-positive chondrocytes and nuclei out of total articular chondrocytes. Note the time-dependent significant increase in percentage of NF-κB-positive chondrocytes and nuclei (*, **: $p < 0.001$). Percentage of NF-κB-positive chondrocytes ( ) and NF-κB-positive nuclei ( ).

I-κB was located in chondrocytes in the superficial layer of articular cartilage of all mouse knees (Fig. 4). The mean (± SE) percentage of I-κB-positive chondrocytes out of total articular chondrocytes at 3, 5 and 7 weeks and in the control was 15.5 ± 1.7, 41.1 ± 2.5, 51.2 ± 5.7 and 12.1 ± 2.1%, respectively (Fig. 5). I-κB-positive chondrocytes increased significantly from 3 to 7 weeks in a time-dependent manner ($p < 0.001$). The percentage of NF-κB-positive chondrocytes was significantly higher than that of I-κB-positive chondrocytes at 5 ($p < 0.05$) and 7 weeks ($p < 0.0005$).

Discussion

In RA, cartilage destruction is not only a result of synovitis, but is also caused by activation of its cellular element, chondrocytes (10, 11). Chondrocytes are reported to produce various molecules (8, 9, 11, 14). These molecules may contribute to the destruction of pericellular matrix and make it easy for chondrocytes to interact with proinflammatory agents in synovial fluid (10, 15), resulting in further destruction of cartilage.

NF-κB is a transcription factor that likely plays a key role in the pathogenesis of inflammatory disease (16). Many studies have been performed and most of them were focused on synoviocytes. However, the localization of NF-κB in articular chondrocytes and its critical role in cartilage degeneration of arthritis are not clear. In the present study, arthritis index of CIA increased significantly from 5 weeks, while NF-κB expression in articular chondrocytes increased from 3 to 7 weeks in a time-dependent manner.

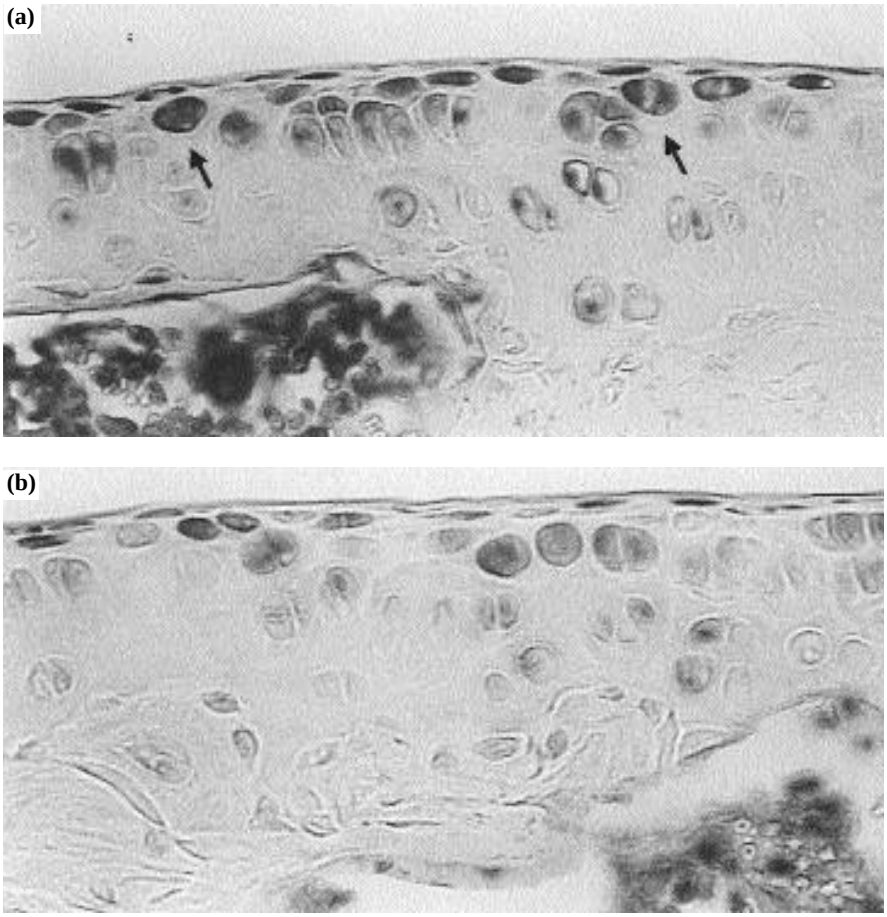

Fig. 4. I-κB-positive chondrocytes in the knee from a mouse sacrificed at 7 weeks after type II collagen-immunization (**a**), and from a control mouse injected with adjuvant without collagen (**b**). Note I-κB expression in chondrocytes of the superficial layer (**arrows**). Sagittal section, original magnification x200.

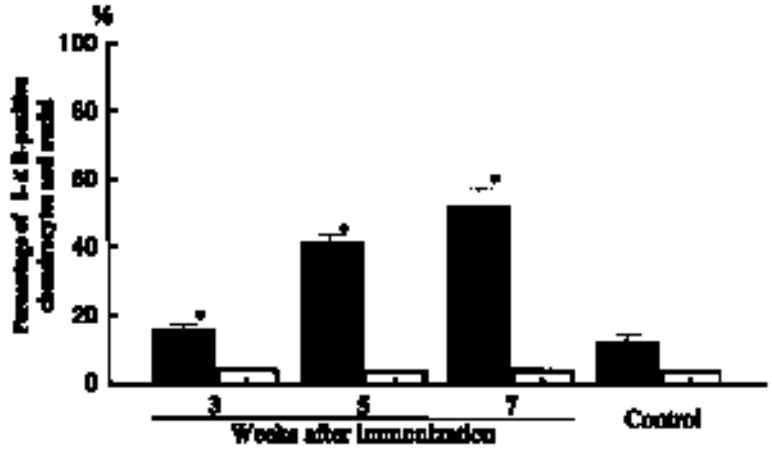

Fig. 5. Percentage of I-κB-positive chondrocytes and nuclei out of total articular chondrocytes. Immunohistological analysis was performed on 10 knees in each group. Note the time-dependent significant increase in percentage of I-κB-positive chondrocytes (*: $p < 0.001$). Percentage of I-κB-positive chondrocytes ( ) and I-κB-positive nuclei ( ).

Moreover, the expression of NF-κB started in superficial chondrocytes in the articular cartilage. Reduction of metachromasia, evidence of loss of proteoglycan, started in the superficial cartilage, at 3 weeks. These findings suggest that NF-κB expression in chondrocytes increased with the progression of cartilage degeneration in the superficial layer, which is likely to be an initial target of chondrocyte-activators in synovial fluid.

I-κB might have an NF-κB binding site in the promoter area of its DNA, and up-regulation of NF-κB may initiate the transcription of I-κB to suppress NF-κB activation as a negative-feedback system. In the current study, the expression of I-κB in chondrocytes, as well as NF-κB, increased in a time-dependent manner, but the percentage of NF-κB-positive chondrocytes was significantly higher than that of I-κB - positive chondrocytes at 5 and 7 weeks. NF-κB overexpression relative to I-κB may induce the progression of collagen-induced arthritis. The TNF-α, IL-1 and IL-6 expression were observed in cartilage in the early stage of CIA (17). NF-κB was reported to inhibit apoptosis (18, 19) and to induce proinflammatory cytekines in synovium (20). It was also reported that NF-κB induces mRNA and protein expression of TNF-α, IL-1 and matrix metalloproteinases in chondrocytes of RA (21). The interaction between the inflammatory agents of chondrocytes and synoviocytes may induce severe inflammation to joint destruction. The expression of NF-κB and I-κB were detected in the control cartilage. NF-κB also expressed in the control of the previous studies (18, 19), I-κB binding with NF-κB may normally reside in chondrocyte as the inactivated form. Further analysis of the time-course of transcription factors in CIA is of great interest in understanding the mechanism of the early stage of cartilage degeneration in arthritis.

Recently, modulation of NF-κB has been focused on its effectiveness in the treatment of arthritis. Inhibition of NF-κB nuclear translocation led to increased apoptosis of human RA synovial fibroblasts (19). Inhibition of NF-κB by transfection of its decoy oligonucleotide suppressed the production of IL-1 and TNF-α in the synovium of CIA rat joints (20). Intraarticular gene transfer of I-κB kinase β ameliorated synovial inflammation of adjuvant

arthritis (22). According to these studies, the modification of NF-κB expression in articular chondrocytes could be a therapeutic intervention for arthritis, including RA. Further investigation on the roles of NF-κB and I-κB in the early stage of CIA would help to solve the mechanism of joint destruction in RA.

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