# Blockade of IL-17 alleviated inflammation in rat arthritis and MMP-13 expression

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**Abstract. – OBJECTIVE: Rheumatoid arthritis (RA) is one systemic auto-immune disorder featured as chronic synovitis and can destruct joint cartilage. Fibroblast-like synoviocyte (FLS) secretes various factors affecting chondrocyte matrix and degradation. This study thus investigated the effect of interleukin-17A (IL-17A) on FLS and osteoclast.**

**MATERIALS AND METHODS: Type II collagen-induced arthritis (CIA) rats were assigned to CIA model, CIA + IgG1 isotype, and CIA + Anti-Rat IL-17A groups. Tissue volume and arthritis index (AI) evaluated arthritis condition. ELISA and flow cytometry measured IL-17A content and Th17 cell percentage in joint cavity fluid. Matrix metallopeptidase 13 (MMP-13) and collagen type II alpha 1 (COL2A1) expression in synovial tissues were compared. FLS-osteoclast co-culture system was treated with IL-17A + IgG1 Isotype or CIA + Anti-Rat IL-17A. MMP-13 and COL2A1 expression were compared.**

**RESULTS: CIA model rats had significantly higher IL-17A and Th17 cell ratio in joint cavity fluid. Injection of Anti-Rat IL-17A decreased AI and tissue volume in model rats, decreased MMP-13 while increased COL2A1 expression in synovial or cartilage tissues. IL-17A treatment remarkably up-regulated MMP-13 mRNA or protein expression in chondrocytes. Anti-IL-17A weakened effects of IL-17A on FLS or chondrocytes.**

**CONCLUSIONS: IL-17A inhibits COL2A1 mRNA and protein expression of chondrocyte in the co-culture system via inducing MMP-13 expression in FLS, thus enhancing collagen degradation and playing a role in RA-related cartilage injury.**

Key Words:
Rheumatoid arthritis, Chondrocytes, FLS, IL-17A, MMP-13, COL2A1.

## Introduction

Rheumatoid arthritis (RA) is one systemic autoimmune disorder featured with chronic synovitis. It has refractory recurrence and causes threatens for patient's life quality and working ability[1]. As one common systemic autoimmune disease, RA is caused by over-proliferation of fibroblast-like synoviocyte (FLS) in synovial tissues, plus abnormal hyperplasia of microvascular endothelial cells and subsequent pannus. The infiltration of large amounts of inflammatory cells into joint cartilage and peripheral tissues further cause cartilage destruction and bone invasion, eventually leading to deficits of bone-joint function and structural deformation, causing high morbidity[2]. RA is described as one chronic inflammation that can destruct joint cartilage injury and bone loss. Although major inflammatory site of RA locates in the synovial tissues, destruction of joint cartilage and bone tissues are the major reasons leading to joint dysfunction of RA patients[3]. Interleukin-17 (IL-17) is one recently identified pro-inflammatory factor, and is mainly secreted by Th17 cells[4]. IL-17 plays critical roles in systemic lupus erythrocyte[5], auto-immune thyroiditis[6] and multiple sclerosis[7]. During RA pathogenesis, IL-17 can facilitate proliferation of synovial cells, induce the release of inflammatory factor/chemotactic factor by synoviocyte, facilitate aggregation and infiltration of inflammatory cells, inhibit cartilage formation, and facilitate bone destruction, thus facilitating RA pathogenesis[4,8,9]. Matrix metallopeptidase 13 (MMP-13) belongs to collagenase family of MMPs, and it is one strong enzyme specifically targeting type II collagen to degrade extracellular matrix of

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chondrocytes. It can directly degrade type II collage, which is one featured and most abundantly distributed component in cartilage matrix, thus playing the predominant role in cartilage matrix degradation[10]. Synoviocyte is the major secretory cell of MMP-13, and may play a role in joint injury of RA patients. This study injected type II collagen into rats to establish a collagen-induced arthritis (CIA) model, which mimicked human RA pathogenesis. We further investigated the role of IL-17 in RA pathogenesis. Moreover, this study also established a co-culture system of FLS-chondrocyte, to explore if IL-17 plays a role in affecting MMP-13 expression in synovial cells and degradation of chondrocytes.

## Materials and Methods

### Experimental Animals

Healthy male SPF-grade Wistar rats were purchased from Wenzhou Medical University. All rats were 6-8 weeks age, with body weight between 220 and 250 g. Housing and procedures of animals followed Animal Experimental Ethics Guideline.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of The Second Affiliated Hospital of Wenzhou Medical University.

### Major Reagent and Materials

Dulbecco's Modified Eagle Medium (DMEM), DMEM/F12, fetal bovine serum (FBS) and collagenase type II were purchased from Gibco (Rockville, MD, USA). Bovine type II collagen was purchased from Chondrex (San Diego, CA, USA). In complete Freund's Adjuvant (IFA) was purchased from MP BioMedicals (Santa Ana, CA, USA). Recombinant Rat IL-17A Protein was purchased Bio-Rad Laboratories (Hercules, CA, USA). Anti-Rat IL-17A (Catalogue No. 64DEC17) and IgG1 Isotype antibody (Catalogue No. MOPC-21) were purchased from eBioscience (Santiago, CA, USA). IL-17A (Catalogue No. ELM-IL17-1) and MMP-13 ELISA kits (Catalogue No. OKBB00228) were purchased from RayBiotech (Norcross, GA, USA). PCR primer was synthesized by Sangon (Shanghai, China). Fluorescent quantitative PCR test kit and SYBR Green dye were purchased from Toyobo Co. Ltd. (Osaka, Japan). Vimentin antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). MMP-13, alpha-1 chain of type II collagen (COL2A1) was purchased from Abcam Biotechnology (Cambridge, MA, USA). Secondary antibody was purchased from Boster (Wuhan, China).

### CIA Model Preparation and Grouping

Under sterile conditions, bovine type II collagen was solved in 0.1 mol/L acetic acid solution at 4 mg/L concentration. After kept in 4°C fridge overnight, the solution was mixed with equal volume of IFA for complete emulsification to prepare 2 mg/L collagen emulsion, which was stored at 4°C for further use. Collagen protein emulsion (0.5 mL) was injected subcutaneously at multiple sites at rat-tails. 0.3 mL emulsion was injected 7 days later to potentiate immunity. Control rats received equal volume of saline. CIA model rats were divided into three groups: CIA model group, CIA + IgG1 Isotype antibody group, which received 20 μg IgG1 isotype antibody into bilateral knee joint cavity 7 days after CIA preparation; CIA + Anti-Rat IL-17A antibody group, which received 20 μg anti-rat IL-17A antibody into bilateral knee joint cavity 7 days after CIA preparation.

### Calculation of Arthritis Index (AI) and Tissue Volume

Rat AI was calculated at 7, 14, and 21 days after preparing CIA model. AI was scored based on following criteria: 0 score, no swelling/reddish of joint; 1 score, swelling of toe joint; 2 scores, swelling of toe joint and footpad; 3 scores, swelling of paw below ankle; 4 scores, swelling of all paws including ankle. The highest score is 4 for each joint. The summation score of all four-ankle joints was AI for this rat. Tissue volume from fore- and hind-paws was measured at 7, 14 and 21 days after model preparation.

### FLS Separation and Culture

At 21 days after modeling, rats were sacrificed and collected for bilateral knee joint synovial tissues. Surface fat tissues were removed. Tissues pieces were digested in 0.1% collagenase type II at 37°C for 2.5 h. Tissues were then digested in 0.05% trypsin for 10 min, followed by quenching in 10% FBS in DMEM medium. The mixture was filtered and centrifuged at 1000 r/min for 10 min. Cells were then re-suspended in DMEM medium containing 10% FBS and 1% streptomycin-penicillin, and were cultured in 37°C chamber with 5% $CO_2$. Culture medium was changed at 3-4 days later. Unattached cells were removed

and medium was changed every 2 days later. When cells reached confluence, they were passed at 1:3 ratio. The 5th generation of cells was used for identification and further experiments.

### Immunofluorescence for Vimentin Expression

The 5th generation of synovial cells was rinsed twice with phosphate-buffered saline (PBS), and was fixed in 4% formaldehyde for 15 min. After washing with PBS for three times, cells were treated with 0.1% Triton X-100 at room temperature for 30 min. Cells were then rinsed with PBS for three times (5 min each), followed by 1% bovine serum albumin (BSA) blocking at room temperature for 60 min. Vimentin antibody (1:300) was added for 4°C overnight incubation after PBS washing. Alexa Flour 488 labeled secondary antibody (1:200) was then added for 60 min incubation at room temperature. 0.1% DAPI was added for 1 min staining following PBS washing. Coverslips were mounted for observation under a fluorescent microscope.

### Separation and Culture of Chondrocytes

At 21 days after model preparation, rats were sacrificed to collect bilateral knee joint synovial tissues along with joint cartilage, which were cut into 1-3 $mm^3$ pieces under sterile conditions. Tissues were digested in 0.25% trypsin at 37°C for 2.5 h with 0.2% type II collagenase. Tissue mixture was filtered and cells were re-suspended in DMEM/F12 medium containing 10% FBS and 1% streptomycin-penicillin. Cells were cultured at 37°C with 5% $CO_2$ in the chamber. Culture medium was changed every 2-3 days. Experiments were performed when cells reaching 70%-80% confluence.

### ELISA for Cytokine Contents

At 21 days after model preparation, rat joint cavity fluid was collected and centrifuged at 2000 r/min for 10 min. The supernatant was saved and kept at -80°C fridge for IL-17A assay. In ELISA assay, 100 μL gradient diluted IL-17A standards or joint cavity fluid was added into 96-well plate, which was cultured at room temperature for 3 h. The plate was then washed in 100 μL 1 × wash solution for 4 times, with the addition of 100 μL biotin-labeled secondary antibody for 60 min and incubated at room temperature. After removing secondary antibody, 100 μL 1 × wash solution was used for 4 times of washing. Each well was then filled with 100 μL streptavidin solution for 45 min room temperature incubation. Each well was washed 4 times again using 100 μL 1 × wash solution. 100 μL TMB one-step substrate reagent was then added to each well for 30 min room temperature incubation, followed by the addition of 50 μL stop solution. Absorbance value at 450 nm was measured.

### Co-culture of FLS-chondrocytes

Chondrocytes were inoculated at upper chamber of transwell, whose lower chamber was filled with FLS cells. 4 different groups, including control, IL-17A (5 ng/mL), isotype antibody treatment (5 ng/mL IL-17A + 15 ng/mL IgG1 Isotype antibody), IL-17A antibody blocking (5 ng/mL IL-17A + 15 ng/mL Anti-Rat IL-17A antibody) were adopted based on treatment in lower FLS cells. After 72 h of co-culture, cells from upper and lower chambers were collected, along with the supernatant. ELISA kit was used to measure MMP-13 content in supernatant from the culture system, using the method abovementioned.

### qRT-PCR Assay

Total RNA was extracted from cells. cDNA was synthesized using random primers and oligdT primers. Using cDNA as the template, PCR amplification was performed under the direction of TaqDNA polymerase, using primer sequences: COL2A1$P_F$: 5'-TGGAC GCCAT GAAGG TTTTC T-3'; COL2A1$P_R$: 5'-TGGGA GCCAG ATTGT CATCT C-3'; MMP-13$P_F$: 5'-CCAGA CTTCA CGATG GCATT G-3'; MMP-13$P_R$: 5'-GGCAT CTCCT CCATA ATTTG GC-3'; β-actin$P_F$: 5'-GAACC CTAAG GCCAA C-3'; β-actin$P_R$: 5'-TGTCA CGCAC GATTT CC-3'. In a total 10 μL PCR reaction system, we added 5.0 μL 2× SYBR Green Mixture, 0.5 μL forward and 0.5 μL reverse primer, 1 μL cDNA, and 3 μL dd$H_2O$. The reaction conditions were: 95°C denature for 5 min, followed by 95°C 15 s, and 60°C 1 min for 40 cycles. PCR was performed on ABI ViiA7 Real-time fluorescent quantitative PCR cycler.

### Western Blot Assay

RIPA solution was used to extract tissue or cell proteins, which were quantified by BCA approach. 40 μg protein was mixed with loading buffer for boiled denature (5 min). Proteins were loaded onto 12% separation gel and 5% condensing gel, and were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for 3 h. Proteins were then

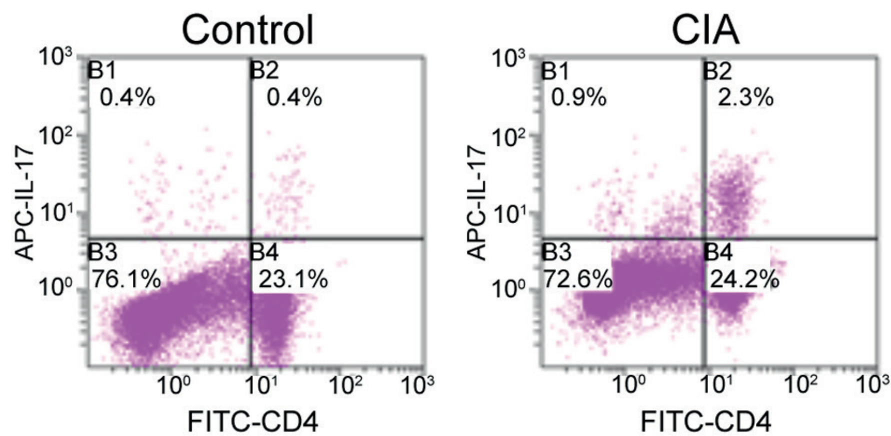


**Figure 1.** Flow cytometry for Th17 cell ratio in joint cavity fluid.

transferred to polyvinylidene fluoride (PVDF) membrane, which was blocked in phosphate buffered saline and Tween 20 (PBST) containing 5% defatted milk powder for 60 min under room temperature. Primary antibody (COL2A1 at 1:200, MMP-13 at 1:200 and β-actin at 1:500) was added for overnight incubation at 4°C overnight. The goat anti-mouse (Catalogue No. sc-2005; 1:5000; Santa Cruz Biothechnology (Santa Cruz, CA, USA) or goat anti-rabbit (Catalogue No. ab97200; 1:5000; Abcam Biotechnology, Cambridge, MA, USA), was then added for 60 min room temperature incubation after 3 times of PBST washing. The member was developed by ECL reagent, and was exposed and scanned for imaging.

### Statistical Analysis

SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used for data input and statistical analysis. Measurement data were presented as mean±standard deviation (SD). Student *t*-test or Tukey's post hoc test was used for comparing measurement data between groups. Statistical significance was defined when $p<0.05$.

## Results

### Significant Elevation of IL-17A Contents in Joint Cavity Fluid in CIA Model Rats

CIA model rats had swelling of bilateral hindlimb joints 7 days after immunization. With elongated treatment time, joint swelling was further aggravated, whilst no swelling was observed in control group. AI score showed significantly higher AI in CIA model group at 7, 14 and 21 days after immunization compared to control group (Table I). Results showed that this study successfully generated a rat CIA model for further experiments. ELISA results showed remarkably elevated IL-17A content in joint cavity fluid of CIA model rats compared to control group (Table II). Flow cytometry results showed remarkably elevated Th17 cell percentage in joint cavity fluid of CIA model rats compared to control rats (Figure 1).

### Blockade of IL-17A Decreased AI Score and Tissue Volume of CIA Model Rats

Compared to CIA model group, IgG1 Isotype antibody injection into joint cavity did not sig-

**Table I.** AI score and tissue volume of rats.

| | Control (n=10) | | CIA model (n=10) | |
|---|---|---|---|---|
| | AI score | Tissue volume | AI score | Tissue volume |
| Day 7 | 0 | 1.56±0.13 | 3.91±0.68* | 1.67±0.14* |
| Day 14 | 0 | 1.60±0.14 | 5.11±0.72* | 1.92±0.16* |
| Day 21 | 0 | 1.64±0.14 | 7.62±0.85* | 2.17±0.18* |

Note: *, $p<0.05$ comparing between CIA group and control group.

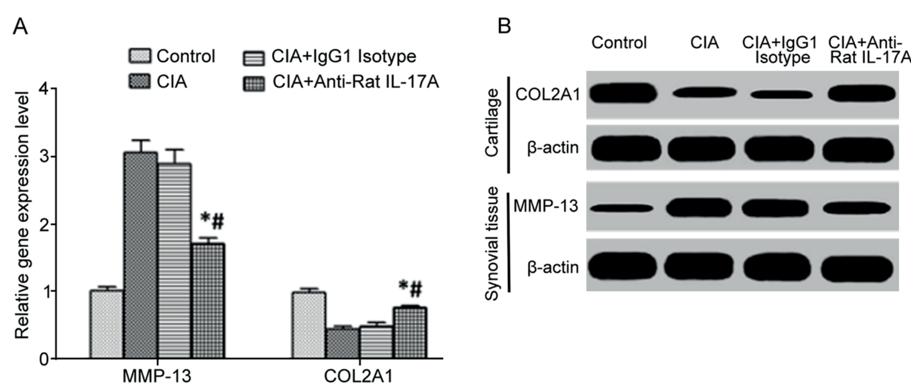


**Figure 2.** Blockade of IL-17A decreased rat synovial tissue MMP-13 and cartilage COL2A1 expression. ***(A)*** qRT-PCR for synovial tissue MMP-13 and cartilage COL2A1 gene expression; ***(B)*** Western blot for synovial tissue MMP-13 and cartilage COL2A1 protein expression. *, $p<0.05$ compared to CIA group; #, $p<0.05$ compared to CIA + IgG1 Isotype group.

**Table II.** IL-17A contents in joint cavity fluids.

| | Control (n=10) | CIA model (n=10) |
|---|---|---|
| IL-17A (pg/mL) | 19.51±2.77 | 157.41±30.23* |

Note: *, $p<0.05$ comparing between CIA group and control group.

nificantly change AI score or tissue volume at 7, 14 and 21 days after modeling ($p>0.05$ compared to model group, Table III and Table IV). The injection of Anti-Rat IL-17A neutralizing antibody into joint cavity effectively decreased joint swelling of CIA model rats, and remarkably decreased AI score and tissue volume ($p<0.05$, Table III and Table IV).

### Blockade of IL-17A Decreased MMP-13 Expression in Rat Synovial Tissues and COL2A1 Expression in Cartilage Tissues

Compared to control group, CIA model rats had significantly elevated MMP-13 expression in synovial tissues (Figure 2A and 2B). COL2A1 expression in cartilage tissues was significantly depressed (Figure 2A and 2B). The injection of IL-17A neutralizing antibody into joint cavity of model rats remarkably decreased MMP-13 expression in synovial tissues, and elevated COL2A1 expression in chondrocytes (Figure 2A and 2B). Results indicated that blockade of IL-17A effectively inhibited MMP-13 expression in synovial tissues, and suppressed the degradation of cartilage collagen.

### Blockade of IL-17A Elevated COL2A1 Expression of Chondrocytes in Co-culture System

Immunofluorescence assay showed that almost all cells culture until 5$^{th}$ generation had Vimentin expression, indicating successful obtaining of FLS (Figure 3A). In a co-culture system, application of IL-17A significantly up-regulated MMP-13 mRNA (Figure 3B) and protein expression (Figure 3D) in FLS, and markedly enhanced MMP-13 content in supernatant (Figure 3C), whilst decreased COL2A1 mRNA or protein expression in chondrocytes. After neutralizing IL-17A effects by antibody, MMP-13 expression in FLS was significantly decreased (Figure 3B and 3D), accompanied with lower MMP-13 contents in supernatant (Figure 3C) and elevated COL2A1 expression in chondrocytes (Figure 3B and 3D).

**Table III.** Comparison of AI score.

| | CIA | CIA+IgG1 Isotype | CIA+Anti-Rat IL-17A |
|---|---|---|---|
| Day 7 | 3.87±0.59 | 3.76±0.62 | 1.89±0.32*# |
| Day 14 | 5.22±0.81 | 5.31±0.79 | 2.55±0.29*# |
| Day 21 | 7.73±0.89 | 7.82±0.90 | 3.11±0.35*# |

Note: *, $p<0.05$ comparing to CIA group; #, p<0.05 compared to CIA + IgG1 Isotype group.

**Table IV.** Comparison of rat tissue volume.

| | CIA | CIA+IgG1 Isotype | CIA+Anti-Rat IL-17A |
|---|---|---|---|
| Day 7 | 1.71 ± 0.15 | 1.72 ± 0.14 | 1.59 ± 0.14*# |
| Day 14 | 1.99 ± 0.14 | 2.05 ± 0.18 | 1.71 ± 0.15*# |
| Day 21 | 2.34 ± 0.17 | 2.36 ± 0.18 | 1.79 ± 0.16*# |

Note: *, $p<0.05$ comparing to CIA group; #, $p<0.05$ compared to CIA + IgG1 Isotype group.

## Discussion

RA is a chronic disease featured with proliferative synovitis. Over-proliferation of synoviocyte and pannus formed by angiogenesis are important patho-physiological grounds for destruction of cartilage and bones of RA patients[11]. Synoviocyte consists of macrophage-like synoviocyte and fibroblast-like synoviocyte (FLS), the latter of which is major components of synovial tissues. Under RA pathological conditions, over-proliferation and activation of FLS can secrete multiple cytokines such as vascular endothelial growth factor (VEGF) to facilitate vascular endothelial proliferation, angiogenesis and formation of pannus, to release multiple inflammatory mediators, chemotactic factors, to induce chemotaxis of inflammatory cells or aggregation and infiltration, leading to cartilage injury and bone destruction. Moreover, the direct expression and secretion of multiple proteinase or matrix degrading enzymes directly impair joint cartilage and bone tissues[12]. Therefore, FLS plays a critical role in mediating cartilage injury, bone invasion and loss of RA patients. Matrix metalloproteinase (MMP) is one type of proteinase sharing similar structures with wide distribution in mesenchymal tissues, and can be secreted by fibroblast, synovial cell, macrophage, and neutrophil, thus playing a critical role in degradation of extracellular matrix (ECM) and is closely correlated with bone formation and cartilage development[13]. Over-

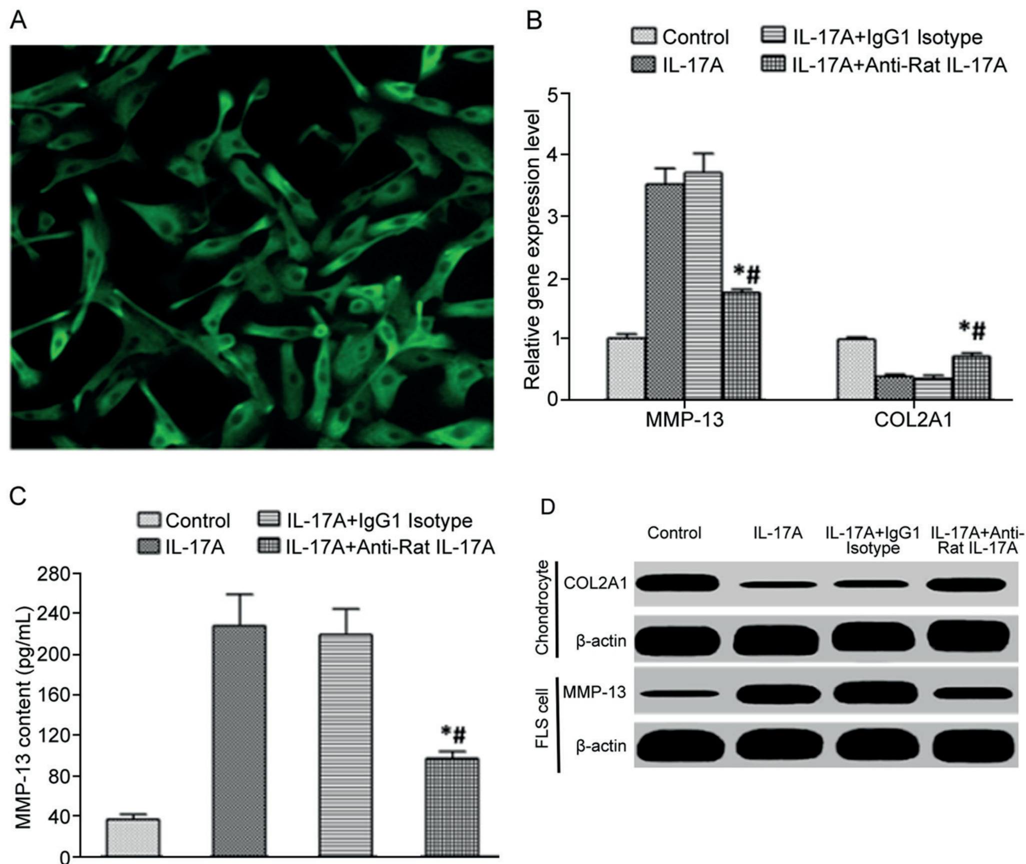


**Figure 3.** Blockade of IL-17A decreased MMP-13 expression in FLS and increased COL2A1 expression in chondrocytes. ***(A)*** Immunofluorescence for Vimentin expression assay; ***(B)*** qRT-PCR for MMP-13 expression in FLS and COL2A1 gene expression in chondrocytes; ***(C)*** ELISA for MMP-13 content in culture supernatant; ***(D)*** Western blot for MMP-13 in FLS and COL2A1 protein expression in chondrocytes. *, $p<0.05$ compared to IL-17A group; #, $p<0.05$ compared to IL-17A + IgG1 Isotype group.

expression or abnormally elevated expression of MMPs can increase matrix degradation in joint cartilage and bone tissues, causing erosion and destruction of cartilage or bone tissues, thus playing a crucial role in occurrence of multiple joint disorder such as RA or knee arthritis. MMP-13 belongs to collagenase family in MMPs, and is one potent enzyme that can degrade cartilage ECM specifically targeting type II collagen, thus can degrade such protein with most featured and sufficient distribution in cartilage matrix[10]. MMP-13 has about 5-10 folds higher potency in degrading type II collagen, thus playing a predominant role in cartilage matrix degradation. With the discover of Th17, a novel T cell sub-group, secreted IL-17 has drawn research interests and has become new focus. In addition to Th17 cells, IL-17 is also secreted by immune cells including CD8+ cell, NKT cell, mast cell and neutrophile[4]. IL-17 protein family consists of six subtypes (from A to F), among which IL-17A is the widest studied with definitive pro-inflammatory cytokine. IL-17A can facilitate synovial cell proliferation, induce its release of inflammatory factor/chemotactic factor, potentiate inflammatory cell aggregation and infiltration, inhibit cartilage formation, and facilitate bone destruction, thus enhancing RA pathogenesis[14]. This study established a co-culture system of CIA rat and FLS-OC, and investigated the role of IL-17A in RA pathogenesis from the perspective of synovial cells and chondrocytes. AI and tissue volume all indicated successful generation of CIA rat model in this study. We further tested IL-17A contents and Th17 expression in joint cavity fluid form CIA and control rats. Results showed significantly higher IL-17A content and Th17 cell ratio in joint cavity fluid from CIA model rats compared to control ones, indicating the involvement of abnormally elevated IL-17A expression in RA pathogenesis. Sandoghchian et al[15] showed that CIA model significantly elevated IL-17 expression in mouse serum and spleen tissues. Ju et al[16] found higher IL-17 expression and secretion in T cells of CIA model animals compared to control group. Lubberts et al[17] found remarkably higher IL-17 content in synovial fluid in CIA model mice compared to control group. In this study, abnormal elevation of IL-17A content in CIA model rat's joint cavity fluid is consistent with Lubberts et al[17]. Comparing to CIA model group, injection of IgG1 Isotype into joint cavity did not effectively alleviate joint inflammation or swelling condition of model rats, whilst injection of Anti-Rat IL-17A neutralizing antibody significantly decreased joint swelling condition of CIA model rats, significantly decreasing AI and tissue volume. Lubberts et al[17] also showed that injection of IL-17 expressing adenovirus into joint cavity significantly enhanced synovial inflammation and joint injury in CIA model mice. After blocking IL-17 effects, CIA model mice had significantly decreased synovial inflammation and joint injury[17]. Nakae et al[18] found that knockout of IL-17 gene significantly alleviated joint injury condition of CIA model mice. Moreover, Koenders et al[19] found that injection of soluble IL-17 receptor (sIL-17R) significantly decreased joint inflammatory response or cartilage injury condition of CIA rats. This study found that antagonizing IL-17A effectively relieved collagen-induced rat joint inflammation or joint swelling, as consistent with Lubberts et al[17] and Koenders et al[19]. Further tests showed that CIA model rats had significantly higher MMP-13 expression in synovial tissues compared to control animals, whilst COL2A1 protein expression in joint cartilage tissues was lower, indicating that CIA induced degradation of cartilage matrix, and decreasing collagen content in cartilage tissues. Injection of IL-17A neutralizing antibody into rat joint cavity significantly decreased MMP-13 expression in CIA model rat's synovial tissues, and elevated type II collagen content in cartilage tissues. Results showed that IL-17A might induce expression and secretion of MMP-13 in synovial tissues, thus inducing degradation of collagen in cartilage tissues and cartilage injury. For further investigation, we established a co-culture system including FLS and chondrocytes, on which the role of IL-17A was discussed. Results showed that IL-17A significantly up-regulated MMP-13 expression in co-culture system and release of MMP-13 into supernatants, and down-regulated COL2A1 gene/protein expression in chondrocytes. The blockade of IL-17A by antibody weakened the effect of IL-17A on FLS and chondrocytes. Koenders et al[19] found that injection of IL-17A expressing adenovirus into joint cavity significantly up-regulated S100A8, IL-1β and MMPs expression levels in mouse synovial and cartilage tissues, and induced synovitis or cartilage injury. In this research, IL-17A significantly up-regulated MMP-13 expression in synoviocyte in co-culture system, and inhibited COL2A1 expression in chondrocytes, as similar

with Koenders et al[19]. Our *in vitro* study also replenished the conclusion drawn by Koenders et al[19]. However, other investigations showed that IL-17A directly affected chondrocyte differentiation, cartilage formation and matrix degradation. Sylvester et al[20] showed that IL-17 could induce MMP-3 and MMP-13 expression in chondrocytes via activating MAPK, AP-1 and NF-kappaB signal molecules, thus playing an important role in mediating matrix degradation and cartilage injury. Tanigawa et al[21] also observed that IL-17 could reduce TIMP-2 or TIMP-4 expression via directly up-regulating MMP-1 and MMP-13 expression in chondrocytes, and decrease type II collagen content and induce cartilage degradation. Schminke et al[22] found that IL-17A can inhibit the differentiation of chondrocyte precursor cells toward mature cells via direct up-regulation of RUNX2, IL-6 and MMP-3 expression in precursor cells, thus inhibiting cartilage formation and playing a role in RA pathogenesis. Therefore, this study does not exclude the potentially direct regulation of IL-17A on chondrocytes. The effect of IL-17A on regulating MMP-13 expression in synoviocyte to indirectly affect chondrocyte matrix synthesis and degradation, however, cannot be neglected.

## Conclusions

IL-17A can inhibit COL2A1 expression and increase collagen degradation in the co-culture system via inducing MMP-13 expression in FLS, thus playing a role in RA-related cartilage injury.

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**Conflict of Interest**
The Authors declare that they have no conflict of interests.

## References

1) Guo G, Fu T, Yin R, Zhang L, Zhang Q, Xia Y, Li L, Gu Z. Sleep quality in Chinese patients with rheumatoid arthritis: contributing factors and effects on health-related quality of life. Health Qual Life Outcomes 2016; 14: 151.
2) Goldring SR. Differential mechanisms of de-regulated bone formation in rheumatoid arthritis and spondyloarthritis. Rheumatology (Oxford) 2016; 55: ii56-ii60.
3) Kotrych D, Dziedziejko V, Safranow K, Pawlik A. Lack of association between CXCL9 and CXCL10 gene polymorphisms and the outcome of rheumatoid arthritis treatment with methotrexate. Eur Rev Med Pharmacol Sci 2015; 19: 3037-3040.
4) Cho ML, Jung YO, Kim KW, Park MK, Oh HJ, Ju JH, Cho YG, Min JK, Kim SI, Park SH, Kim HY. IL-17 induces the production of IL-16 in rheumatoid arthritis. Exp Mol Med 2008; 40: 237-245.
5) Crispin JC, Tsokos GC. IL-17 in systemic lupus erythematosus. J Biomed Biotechnol 2010; 2010: 943254.
6) Horie I, Abiru N, Sakamoto H, Iwakura Y, Nagayama Y. Induction of autoimmune thyroiditis by depletion of CD4+CD25+ regulatory T cells in thyroiditis-resistant IL-17, but not interferon-gamma receptor, knockout nonobese diabetic-H2h4 mice. Endocrinology 2011; 152: 4448-4454.
7) Pollinger B. IL-17 producing T cells in mouse models of multiple sclerosis and rheumatoid arthritis. J Mol Med (Berl) 2012; 90: 613-624.
8) Ryu S, Lee JH, Kim SI. IL-17 increased the production of vascular endothelial growth factor in rheumatoid arthritis synoviocytes. Clin Rheumatol 2006; 25: 16-20.
9) Miossec P. IL-17 in rheumatoid arthritis: a new target for treatment or just another cytokine? Joint Bone Spine 2004; 71: 87-90.
10) Zhang P, Feng K, Xue Y, Zhang CX, Wang Y, Li XL. Clinical applications of haploidentical hematopoietic stem cell transplantatoin in severe aplastic anemia. Eur Rev Med Pharmacol Sci 2017; 21: 155-161.
11) Zhu SL, Huang JL, Peng WX, Wu DC, Luo MQ, Li QX, Li ZX, Feng XX, Liu F, Wang MX, Chen WQ, Olsen N, Zheng SG. Inhibition of smoothened decreases proliferation of synoviocytes in rheumatoid arthritis. Cell Mol Immunol 2015; 14: 214-222.
12) Li XJ, Xu M, Zhao XQ, Zhao JN, Chen FF, Yu W, Gao DY, Luo B. Proteomic analysis of synovial fibroblast-like synoviocytes from rheumatoid arthritis. Clin Exp Rheumatol 2013; 31: 552-558.
13) Wang X, Zhao X, Tang S. Inhibitory effects of EGb761 on the expression of matrix metalloproteinases (MMPs) and cartilage matrix destruction. Cell Stress Chaperones 2015; 20: 781-786.
14) Baek SH, Lee SG, Park YE, Kim GT, Kim CD, Park SY. Increased synovial expression of IL-27 by IL-17 in rheumatoid arthritis. Inflamm Res 2012; 61: 1339-1345.
15) Sandoghchian Shotorbani S, Zhang Y, Baidoo SE, Xu H, Ahmadi M. IL-4 can inhibit IL-17 production in collagen induced arthritis. Iran J Immunol 2011; 8: 209-217.
16) Ju JH, Cho ML, Jhun JY, Park MJ, Oh HJ, Min SY, Cho YG, Hwang SY, Kwok SK, Seo SH, Yoon CH, Park SH, Kim HY. Oral administration of type-II collagen suppresses IL-17-associated RANKL expression of CD4+ T cells in collagen-induced arthritis. Immunol Lett 2008; 117: 16-25.
17) Lubberts E, Joosten LA, Oppers B, van den Bersselaar L, Coenen-de Roo CJ, Kolls JK, Schwarzenberger P,

van de Loo FA, van den Berg WB. IL-1-independent role of IL-17 in synovial inflammation and joint destruction during collagen-induced arthritis. J Immunol 2001; 167: 1004-1013.

18) Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J Immunol 2003; 171: 6173-6177.

19) Koenders MI, Marijnissen RJ, Devesa I, Lubberts E, Joosten LA, Roth J, van Lent PL, van de Loo FA, van den Berg WB. Tumor necrosis factor-interleukin-17 interplay induces S100A8, interleukin-1beta, and matrix metalloproteinases, and drives irreversible cartilage destruction in murine arthritis: rationale for combination treatment during arthritis. Arthritis Rheum 2011; 63: 2329-2339.

20) Sylvester J, Liacini A, Li WQ, Zafarullah M. Interleukin-17 signal transduction pathways implicated in inducing matrix metalloproteinase-3, -13 and aggrecanase-1 genes in articular chondrocytes. Cell Signal 2004; 16: 469-476.

21) Tanigawa S, Aida Y, Kawato T, Honda K, Nakayama G, Motohashi M, Suzuki N, Ochiai K, Matsumura H, Maeno M. Interleukin-17F affects cartilage matrix turnover by increasing the expression of collagenases and stromelysin-1 and by decreasing the expression of their inhibitors and extracellular matrix components in chondrocytes. Cytokine 2011; 56: 376-386.

22) Schminke B, Trautmann S, Mai B, Miosge N, Blaschke S. Interleukin 17 inhibits progenitor cells in rheumatoid arthritis cartilage. Eur J Immunol 2016; 46: 440-445.