Interleukin 17 (IL-17) Increases the Expression of Toll-like Receptor-2, 4, and 9 by Increasing IL-1β and IL-6 Production in Autoimmune Arthritis

JUN-HEE LEE, MI-LA CHO, JU-IN KIM, YOUNG-MEE MOON, HYE-JWA OH, GEUN-TAE KIM, SUN-RYU, SEUNG-HOON BAEK, SUN-HEE LEE, HO-YOUN KIM, and SUNG-IL KIM

ABSTRACT. ***Objective.*** To examine the effect of interleukin 17 (IL-17) on the expression of Toll-like receptor (TLR)-2, 4, and 9 in collagen-induced arthritis (CIA) in mice.

Methods. On Days 28 and 32 after induction of CIA in mice, phosphate-buffered saline (PBS group) or IL-17 (IL-17 group) was injected into both knee joints. On Day 35, mice were sacrificed. The severity of knee joint arthritis, synovial inflammation, and bone destruction was measured by a scoring system using macrography and histological analysis. Synovial expression of TLR-2, 4, 9, IL-17, IL-1ß, tumor necrosis factor-α (TNF-α), and IL-6 was determined by real-time PCR and immunohistochemistry. Synoviocytes of CIA mice were cultured with IL-17 and with neutralizing antibodies to cytokine, and the expression of TLR-2, 4, 9, IL-1ß, TNF-α, and IL-6 was determined by real-time RT-PCR.

Results. In CIA mice, knee arthritis scores, synovial inflammation, bone destruction scores, and expression of synovial TLR-2, 4, and 9, IL-17, IL-1ß, TNF-α and IL-6 were higher in the IL-17 and PBS groups than in normal DBA1 mice. These variables were also significantly higher in the IL-17 group than in the PBS group. In CIA synoviocytes, IL-17 increased the expression of TLR-2, 4, and 9, and this effect was significantly alleviated by neutralizing antibodies to IL-17, IL-1ß, and IL-6.

Conclusion. IL-17 aggravates joint inflammation and destruction, and increases the synovial expression of TLR-2, 4, and 9 by increasing IL-1ß and IL-6. These results imply that the IL-17-induced increase in expression of TLR-2, 4, and 9, and IL-1ß and IL-6 production are involved in the IL-17-induced aggravation of arthritis. (First Release Feb 15 2009; J Rheumatol 2009;36:684–92; doi:10.3899/ jrheum.080169)

Key Indexing Terms:
TOLL-LIKE RECEPTORS COLLAGEN-INDUCED ARTHRITIS
INTERLEUKIN 1ß INTERLEUKIN 6 INTERLEUKIN 17

Rheumatoid arthritis (RA) is chronic systemic autoimmune inflammatory disease that affects predominantly synovial joints. Although the exact pathophysiological mechanisms of RA remain largely unknown, numerous inflammatory cells, such as T and B cells, fibroblast-like synoviocytes, and antigen-presenting cells and their extensive production of proinflammatory mediators are implicated[1].

Toll-like receptors (TLR) are pattern-recognition receptors that are involved in the uptake and processing of various exogenous and endogenous antigens. TLR mediate the maturation of dendritic cells and promote naive T cells toward a Th0, Th1, or Th2 phenotype[2–4], and they play a crucial role in the regulation of innate and adaptive immune responses. TLR are highly expressed in synovial tissue, and their activation contributes to autoimmune and chronic inflammatory diseases such as RA[5–7]. A recent study reported that treatment with an inhibitor of the TLR signaling pathway is effective and safe in patients with RA[8].

Interleukin 17 (IL-17) was recently shown to be the central cytokine produced by a newly identified subset of T helper cells, called "Th17," that are generated following signals from transforming growth factor ß, IL-6, and IL-23. IL-17 activates many of the same signaling events as innate cytokines such as tumor necrosis factor-α (TNF-α) and IL-1ß, and is thus considered an important bridging mole-

From the Department of Internal Medicine, College of Medicine, Pusan National University, Busan; Pusan National University Hospital Research Institutes; and The Rheumatism Research Center, Catholic University of Korea, Seoul, South Korea.

Supported by a grant (R11-2002-098-05001-0) from the Korea Science and Engineering Foundation through the Rheumatism Research Center at The Catholic University of Korea and by a project grant from Pusan National University Hospital Institute.

S-I. Kim, MD, PhD; S-H. Lee, MD, PhD; J-H. Lee, MD, PhD; G-T. Kim, MD; S-H. Baek, MD, Department of Internal Medicine, College of Medicine, Pusan National University; S-Ryu; J-I. Kim, Pusan National University Hospital Research Institutes; M-L. Cho, PhD; Y-M. Moon; H-J. Oh; H-Y. Kim, MD, PhD, The Rheumatism Research Center, Catholic University of Korea.

M-L. Cho and J-H. Lee were co-first authors.

Address reprint requests to Dr. S-I. Kim, Division of Rheumatology, Department of Internal Medicine, College of Medicine, Pusan National University, 1-10, Ami-Dong, Seo-Gu, Busan, South Korea.
E-mail: ksimd@pusan.ac.kr

Accepted for publication November 23, 2008.

cule between the adaptive and innate immune systems[9]. IL-17 is involved in both initiation and progression of collagen-induced arthritis (CIA). IL-17 affects bone turnover, particularly in inflammatory arthritis[10–12]. Blocking IL-17 in rodent models of arthritis reduces inflammation and bone damage, whereas excess IL-17 exacerbates disease. IL-17 knock-out mice are resistant to CIA, and other mouse models also resistant to CIA show defects in IL-17 production. Therefore, IL-17 and its receptor are considered attractive therapeutic targets for inflammatory diseases. IL-17 is produced spontaneously by RA synovial membrane cultures, and elevated levels of IL-17 have been detected in the synovial fluid of patients with RA[13–17].

We examined the effects of IL-17 on the expression of TLR-2, 4, and 9 in CIA in mice.

MATERIALS AND METHODS

Animals. Male DBA1 mice were purchased from SLC Inc., Shizuoka, Japan. Mice were housed in filter-top cages and were used in experiments at age 7–9 weeks.

Cytokines and antibodies. Recombinant mouse IL-17 and mouse monoclonal antibodies to IL-17, IL-1ß, TNF-α, and IL-6 were purchased from R&D Systems, Minneapolis, MN, USA.

Induction of CIA. Bovine type II collagen (CII; Chondrex, Seattle, WA, USA) was prepared and diluted in 0.05 M acetic acid to a concentration of 2 mg/ml and then emulsified in equal volumes of Freund's complete adjuvant (Chondrex). DBA1 mice were immunized at the base of the tail with 100 µg bovine CII. On Day 21, mice received a booster injection in the tail of 100 µg CII dissolved in incomplete Freund's adjuvant.

Assessment of knee arthritis. Mice were considered to have arthritis when significant changes in redness or swelling were noted in the digits or in other parts of the paws. Knee joint arthritis was scored visually after knee joint skin dissection under intraperitoneal anesthesia using a scale of 0–2 (0 = no inflammation, 1 = mild inflammation, 1.5 = severe inflammation, and 2 = very severe inflammation). Scoring was done by 2 independent observers with no knowledge of the experimental group, on Days 28, 32, and 35.

Frequency of knee arthritis. To investigate the frequency of knee arthritis, CIA was induced in 40 mice. The mice were anesthetized by intraperitoneal injection, and the frequency of knee arthritis was assessed on Days 28, 32, and 35.

Study protocol. The test subjects were 10 normal mice and 120 mice with CIA. Normal mice were observed up to Day 35, and CIA was induced as described. Twenty-eight days after the first immunization, mice were anesthetized and the knee joint arthritis was assessed. Forty-five mice with a knee arthritis score > 1 were selected and assigned to 3 groups. The first 15 mice were not given an injection into the knee joint (control group). The second group of 15 mice were injected with 10 µl phosphate-buffered saline (PBS) into both knee joints. The third group of 15 mice were injected with 200 ng IL-17 in 10 µl PBS (R&D Systems) on Days 28 and 32. On Day 35, all mice were sacrificed and the knee joints were isolated.

Histological analysis. Mice were anesthetized and the whole knee joint was removed and fixed for 4 days in 10% formalin. After decalcification in 5% formic acid, the specimens were processed for paraffin embedding. Tissue sections (6 µm) were stained with hematoxylin and eosin. Histopathological changes were scored using the following measures. Inflammation of synovium was scored on a scale of 0–5 depending of infiltration of inflammatory cells into the synovium (0 = no cells; 1 = very mild; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe) from both sides of the pannus adjacent to the femur and tibia (Figure 1A). Severity of bone erosion was scored on a scale of 0–5 (0 = no erosion; 1 = decrease of bone width less than one-quarter of the total thickness; 2 = decrease of bone width between one-quarter and half the total thickness; 3 = decrease of bone width between half and three-quarters of the total thickness; 4 = decrease of bone width more than three-quarters of the total thickness; 5 = complete loss of bone) from pannus adjacent to the femur and tibia (Figure 1B). Scoring was performed on both knee joints of 5 mice from each experimental group by 2 observers with no knowledge of the experimental group.

Isolation of knee joint synovium and real-time reverse transcription-polymerase chain reaction (RT-PCR). After isolation of the knee joint, the synovium was isolated as reported[18] and immersed immediately in liquid nitrogen. Total RNA was extracted from the frozen synovium using TRIzol solution (Invitrogen Life Technologies, Carlsbad, CA, USA). Each solution containing 1 µg RNA was heated at 65˚C for 15 min, and a mixture containing reverse transcriptase was added to each solution. cDNA was obtained by running cycles at 25˚C for 10 min, 42˚C for 60 min, 99˚C for 5 min, and 4˚C for 5 min. A First Strand cDNA Synthesis Kit for RT-PCR (AMV) (Roche Applied Science, Indianapolis, IN, USA) was used for the reactions described above, and real-time PCR was done using the LightCycler instrument (Roche). Into the microcapillary tubes were added LightCycler-DNA Master SYBR-Green I (Roche), cDNA template, each primer, and 25 mM $MgCl_2$ to make a final volume 20 µl. PCR conditions comprised 50 cycles, with 10 s predenaturation at 95˚C, 5 s annealing at 60˚C, and 20 s primer extension at 72˚C. Primers for IL-17, TLR-2, 4 and 9, IL-1ß, TNF-α, and IL-6 were purchased from Bioneer, Daejun, Korea; base sequences are shown in Table 1. The threshold cycle (CT), an indication of the amount of mRNA, was determined by monitoring the fluorescent signal for each cycle. Knee joint synovia from 9 mice were used from each experimental group; each group was divided into 3 subgroups, each comprising knee joint synovia from 3 mice. Real-time RT-PCR was performed on the combined synovia for each subgroup.

Immunohistochemistry for TLR-2, 4, and 9. The whole knee joints from mice in the IL-17 group were removed and fixed for 1 day in 4% paraformaldehyde. After decalcification in 10% EDTA, specimens were processed for paraffin embedding, and blocks 6-µm thick were cut. Each section was dewaxed and placed in 3% hydrogen peroxide in methanol for 10 min at room temperature. The Vector Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) was used for immunohistochemistry. Tissue sections were incubated with 1.5% normal goat serum for 30 min to block proteins and then reacted with primary antibody to TLR-2 (sc-10739), TLR-4 (sc-10741), or TLR-9 (sc-25468) at 4˚C for 14–16 h. All primary antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA. Slides were incubated with biotinylated secondary antibody solution followed by an avidin–biotinylated enzyme complex. PBS containing 0.05% Tween 20 was used for washing after each process. Chromogenic reactions were visualized with 3,3'-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA), and the nuclei were counterstained with hematoxylin. Slides were mounted in permanent mounting media (Dako, Glostrup, Denmark).

Isolation and culture of CIA mouse synoviocytes. Synoviocytes were isolated by enzymatic digestion of joint synovial tissues obtained from knee joints of arthritic mice. Tissues were minced into 2–3 mm pieces and treated for 4 h with 4 mg/ml type I collagenase (Worthington Biochemical, Freehold, NJ, USA) in Dulbecco's modified Eagle's medium (DMEM) at 37˚C in 5% CO_2. Dissociated cells were centrifuged at 500 g, resuspended in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin, and plated in 75 cm^2 flasks. After overnight culture, nonadherent cells were removed, and adherent cells were cultivated in DMEM supplemented with 20% FBS. Cultures were kept at 37˚C in 5% CO_2, and the medium was replaced every 3 days. When cells approached confluence, they were passed after diluting 1:3 with fresh medium; synoviocytes from passages 2–3 were used in the experiments. The cells were morphologically homogeneous and exhibited the appearance of synovial fibroblasts, with typical bipolar configuration

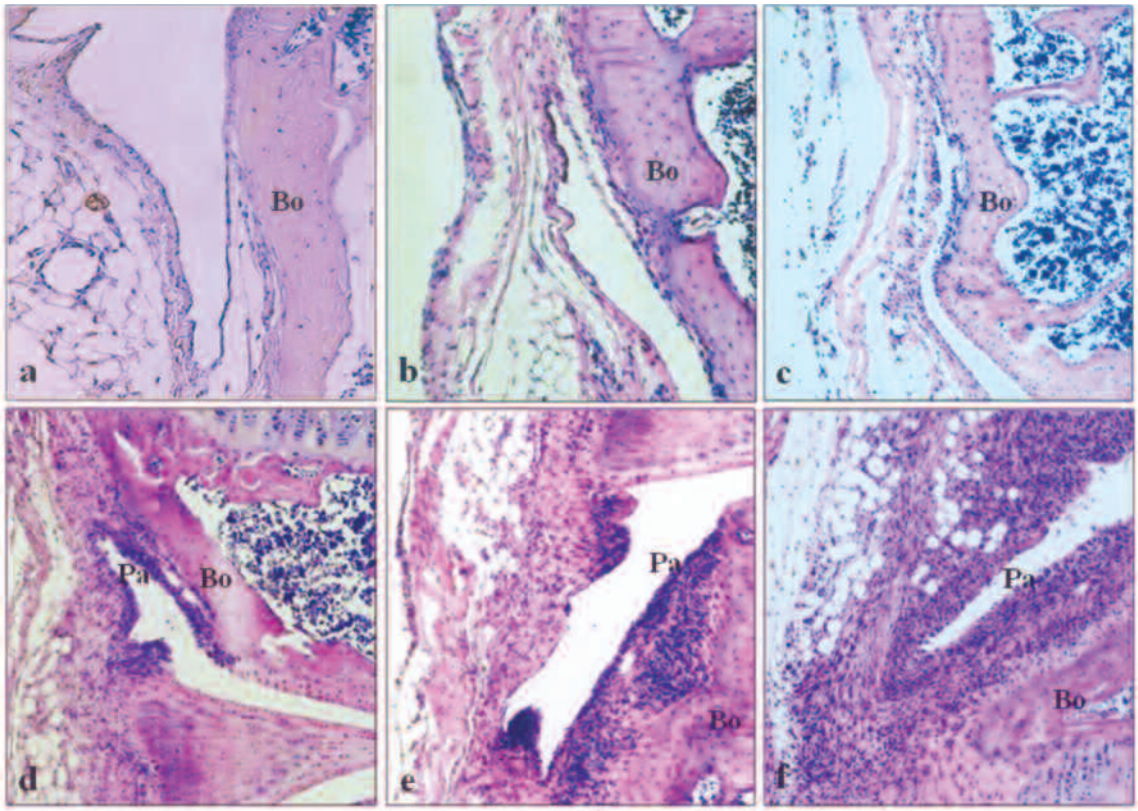

Figure 1A. The scoring method to quantify synovial inflammation in CIA. a: normal (score 0); b: very mild (score 1); c: mild (score 2); d: moderate (score 3); e: severe (score 4); f: very severe (score 5). Bo: bone adjacent to pannus; Pa: pannus.

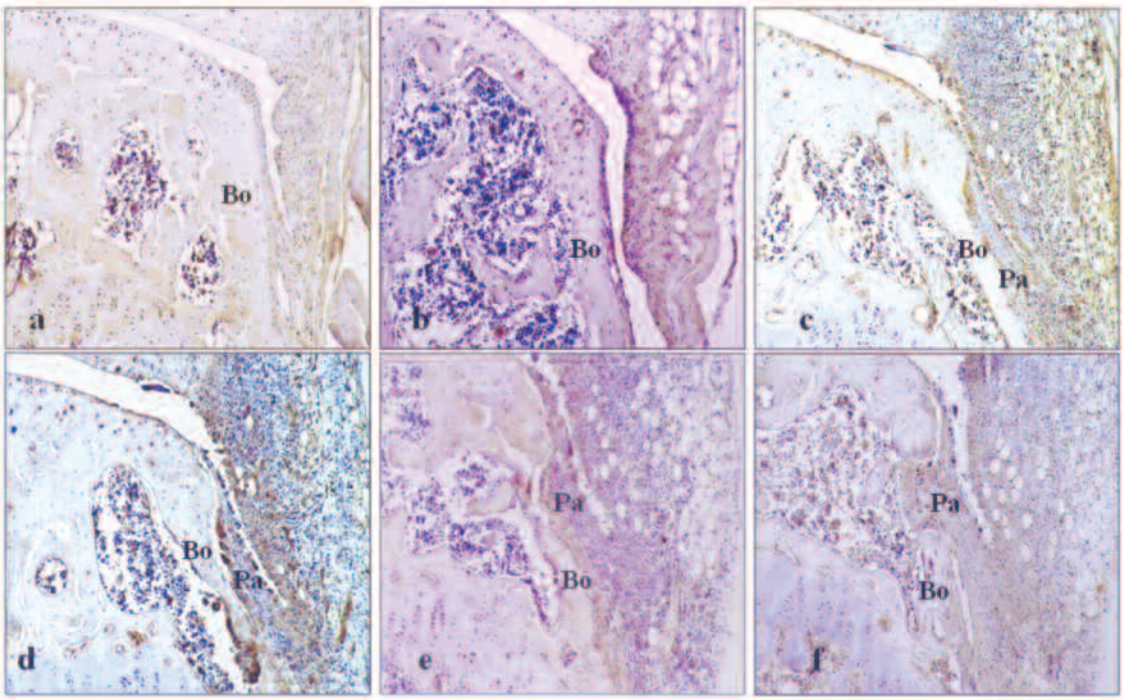

Figure 1B. The scoring method to quantify bone destruction in CIA. a: no erosion of bone (score 0); b: decrease of bone width less than one-quarter of the total thickness (score 1); c: decrease of bone width between one-quarter and half the total thickness (score 2); d: decrease of bone width between half and three-quarters of the total thickness (score 3); e: decrease of bone width more than three-quarters of the thickness (score 4); f: complete loss of bone (score 5). Bo: bone adjacent to pannus; Pa: pannus.

Table 1. Primers used for real-time RT-PCR.

Primer	Stream	Sequence
ß-actin	Sense	5'–ATC TGG CAC CAC ACC TTC TAC AAT GAG C-3'
	Antisense	5'–GTC ATA CTC CTG CTT GCT TGA TCC ACA TC-3'
IL-17	Sense	5'–TCT CAT CCA GCA AGA GAT CC-3'
	Antisense	5'–AGT TTG GGA CCC CTT TAC AC-3'
TLR-2	Sense	5'–GCC ACC ATT TCC ACG GAC T-3'
	Antisense	5'–GGC TTC CTC TTG GCC TGG-3'
TLR-4	Sense	5'–CCT CTG CCT TCA CTA CAG AGA CTT T-3'
	Antisense	5'–TGT GGA AGC CTT CCT GGA TG-3'
TLR-9	Sense	5'–ACT GAG CAC CCC TGC TTC TA-3'
	Antisense	5'–AGA TTA GTC AGC GGC AGG AA-3'
IL-1ß	Sense	5'–ATG GCA ACT GTT CCT GAA CTC AAC T-3'
	Antisense	5'–CAG GAC AGG TAT AGA TTC TTT CCT TT-3'
TNF-α	Sense	5'–CAA ACC ACC AAG TGG AGG AG-3'
	Antisense	5'–AGA TAG CAA ATC GGC TGA CG-3'
IL-6	Sense	5'–CCA TCC AGT TGC CTT CTT G-3'
	Antisense	5'–AAG TGC ATC ATC GTT GTT CAT AC-3'

under inverse microscopy. Synoviocytes were cultured with IL-17 (1–10 ng/ml) and neutralizing antibody to IL-17, IL-1ß, TNF-α, or IL-6 (10 µg/ml each) for 12 h. The cytotoxicity of the doses of neutralizing antibody was evaluated by the 3-C4,5-dimethylthiazol-2,5,-diphenyltetrazolium bromide assay. The mRNA expression of TLR-2, 4, and 9 was evaluated using SYBR-Green-based real-time PCR (Synergy Brands, Chicago, IL, USA).

Detection of cytokine production by ELISA. To determine the amounts of IL-1ß, TNF-α, and IL-6 in supernatants, antibodies against mouse IL-1ß, TNF-α, and IL-6 and biotinylated anti-mouse IL-1ß, TNF-α, and IL-6 antibodies (R&D Systems) were used as capture and detection antibodies, respectively. Horseradish peroxidase–avidin (R&D Systems) was used for color development. The amounts of cytokines present in test samples were determined from standard curves established with serial dilutions of recombinant murine IL-1ß, TNF-α, and IL-6 (R&D Systems).

Statistical analysis. Differences between experimental groups were tested using the Mann–Whitney U test and Student's t test according to the parameter. The data are expressed as the mean ± SEM.

RESULTS

Frequency of knee joint arthritis in the CIA model. We performed a preliminary experiment to determine the frequency of knee arthritis in 40 mice with CIA. The frequency of knee joint arthritis was 40% (16 mice) by Day 28, 65% (26 mice) by Day 32, and 68% (27 mice) by Day 35. The knee arthritis scores were 1.2 ± 0.2 on Day 28, 1.4 ± 0.4 on Day 32, and 1.5 ± 0.3 on Day 35.

IL-17 aggravated knee arthritis, synovial inflammation, and bone destruction. On Day 28, the knee arthritis scores were the same (1.2 ± 0.2) in the IL-17, PBS, and control groups. On Day 32, the scores were 1.9 ± 0.2 for IL-17 mice, 1.4 ± 0.4 for PBS mice, and 1.4 ± 0.2 for the control mice. On Day 35, the scores were 2.0 ± 0.1 for IL-17 mice, 1.5 ± 0.3 for PBS mice, and 1.5 ± 0.3 for control mice. The scores were significantly higher on Days 32 and 35 in the IL-17 group than in the other 2 groups, but did not differ significantly between the PBS and control groups (Figure 2A).

The scores for synovial inflammation were significantly higher in the IL-17 mice than in PBS and control mice (Figure 2B). Inflammation scores were 4.8 ± 0.4, 2.5 ± 0.8, and 2.4 ± 0.7 in the IL-17, PBS, and control groups, respectively ($p < 0.05$). Bone destruction was also significantly greater in the IL-17 mice than in the PBS and control mice (Figure 2C). Bone destruction scores were 4.8 ± 0.6, 2.3 ± 0.8, and 2.4 ± 0.6 in the IL-17, PBS, and control groups, respectively ($p < 0.05$). The numbers of inflammatory cells and the extent of cartilage erosion and bone destruction in the joints of IL-17 group mice were more severe than for CIA (PBS or control) mice (Figure 2D). These results indicate that IL-17 induces aggravation of joint inflammation and destruction.

IL-17 increased synovial expression of TLR-2, 4 and 9, IL-17, IL-1ß, TNF-α, and IL-6 in CIA mice. TLR-2, 4, and 9 expression was significantly higher in the IL-17, PBS, and control groups than in the normal group, and higher in the IL-17 group than in the PBS and control groups (Figure 3). The relative synovial expression of TLR-2 was 41.7 ± 3.8 in IL-17 mice, 25.2 ± 6.7 in PBS mice, 23.6 ± 3.9 in control mice, and 1.0 in normal mice. The corresponding values were 15.5 ± 1.5, 4.7 ± 2.1, 4.5 ± 0.1, and 1.0 for TLR-4 expression, and 12.6 ± 2.9, 4.6 ± 0.2, 4.7 ± 0.6, and 1.0 for TLR-9 expression.

The relative synovial expression of IL-17 was significantly higher in the IL-17, PBS, and control groups than in the normal group, and higher in the IL-17 group than in the control and PBS groups (Figure 3). The relative synovial expression of IL-17 was 15.1 ± 2.3, 2.3 ± 0.2, 2.1 ± 0.2, and 1.0 in the IL-17, PBS, control, and normal groups, respectively. IL-1ß expression was 357.4 ± 62.4, 33.4 ± 3.4, 33.7 ± 1.1, and 1.0 in the IL-17, PBS, control, and normal groups, respectively. The corresponding values for TNF-α expression were 3.7 ± 0.2, 2.0 ± 0.1, 2.1 ± 0.3, and 1.0. The corresponding values for IL-6 expression were 159.8 ± 37.2, 88.7 ± 11.9, 90 ± 4.3, and 1.0. These results show that IL-17

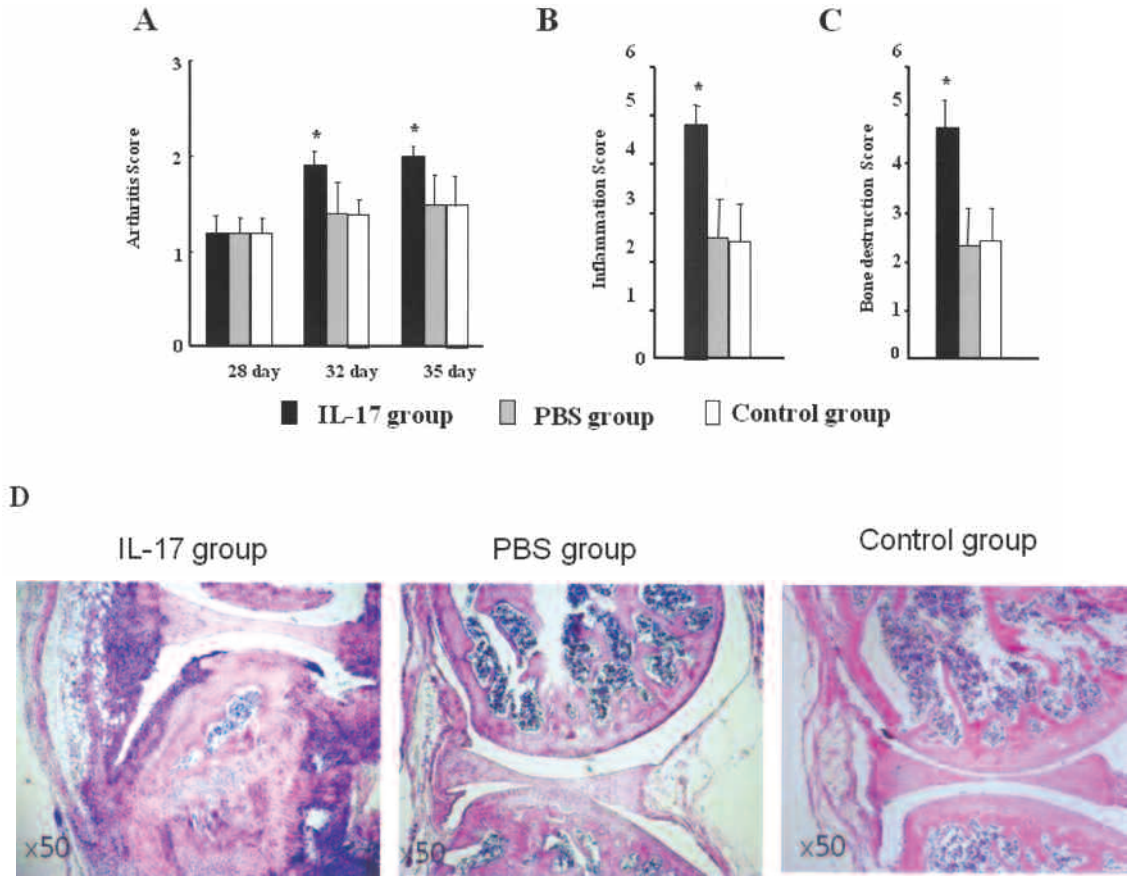

Figure 2. Scores for arthritis (A), synovial inflammation (B), and bone destruction (C) of the knee joint in CIA mice. A: The knee joint arthritis score was significantly higher in the IL-17 group than in the PBS and control groups. B: The synovial inflammation score was significantly higher in the IL-17 group than in the PBS and control groups. C: The bone destruction score was significantly higher in the IL-17 group than in the PBS and control groups. *$p < 0.05$. D: Histological sections of knee joints from CIA (PBS or control group) and IL-17 group mice. Hematoxylin–eosin staining; original magnification ×100.

increases synovial expression of TLR-2, 4, and 9 and inflammatory cytokines in CIA.

TLR-2, 4, and 9-positive cells were distributed in synovial lining layers and pannus adjacent to bone erosion. Immunohistochemistry showed that TLR-2, 4, and 9-positive cells were distributed in the synovial lining layers and pannus adjacent to bone erosion (Figure 4).

IL-17 increases expression of TLR-2, 4 and 9, IL-1β, TNF-α, and IL-6 in CIA mouse synoviocytes. In CIA mouse synoviocytes cultured with IL-17 (1, 5, or 10 ng/ml), expression of TLR-2, 4 and 9, IL-1ß, IL-6, and TNF-α was significantly higher than in the control cultures. Addition of anti-IL-17-neutralizing antibody (10 ng/ml) significantly decreased the expression of TLR-2, 4 and 9, IL-1ß, IL-6, and TNF-α compared with culture with IL-17 (5 ng/ml) alone (Figure 5A). Production of IL-1ß, IL-6, and TNF-α was also significantly decreased via addition of anti-IL-17-neutralizing antibody (Figure 5B).

IL-17 increased expression of TLR-2, 4, and 9 by increasing production of IL-1ß and IL-6 in CIA mouse synoviocytes. The IL-17-induced increase in TLR-2 expression was blocked by neutralizing antibodies to IL-1ß, IL-6, and TNF-α, and TLR-9 expression was blocked by neutralizing antibodies to IL-1ß and IL-6, and this effect was enhanced significantly by combining neutralizing antibodies to IL-1ß and IL-6. The IL-17-induced increase in TLR-4 expression was blocked significantly by neutralizing antibodies to IL-1ß and IL-6 (Figure 6). These results indicate that IL-17 increases the expression of TLR-2, 4, and 9 by increasing production of IL-1ß and IL-6 in CIA mouse synoviocytes.

DISCUSSION

We demonstrated that IL-17 aggravates joint inflammation and destruction, and increases the synovial expression of TLR-2, 4, and 9 in CIA mice. We also showed that IL-17 increases the expression of TLR-2, 4, and 9 by increasing the production of IL-1ß and IL-6 in CIA mice synoviocytes. These results imply that the IL-17-induced increase in the expression of TLR-2, 4, and 9 occurs through a process involving IL-1ß and IL-6 production, and that this may mediate the aggravating effect of IL-17 on joint inflammation and destruction in CIA.

In vitro studies have shown that IL-17 stimulates the production of proinflammatory cytokines, chemokines, and

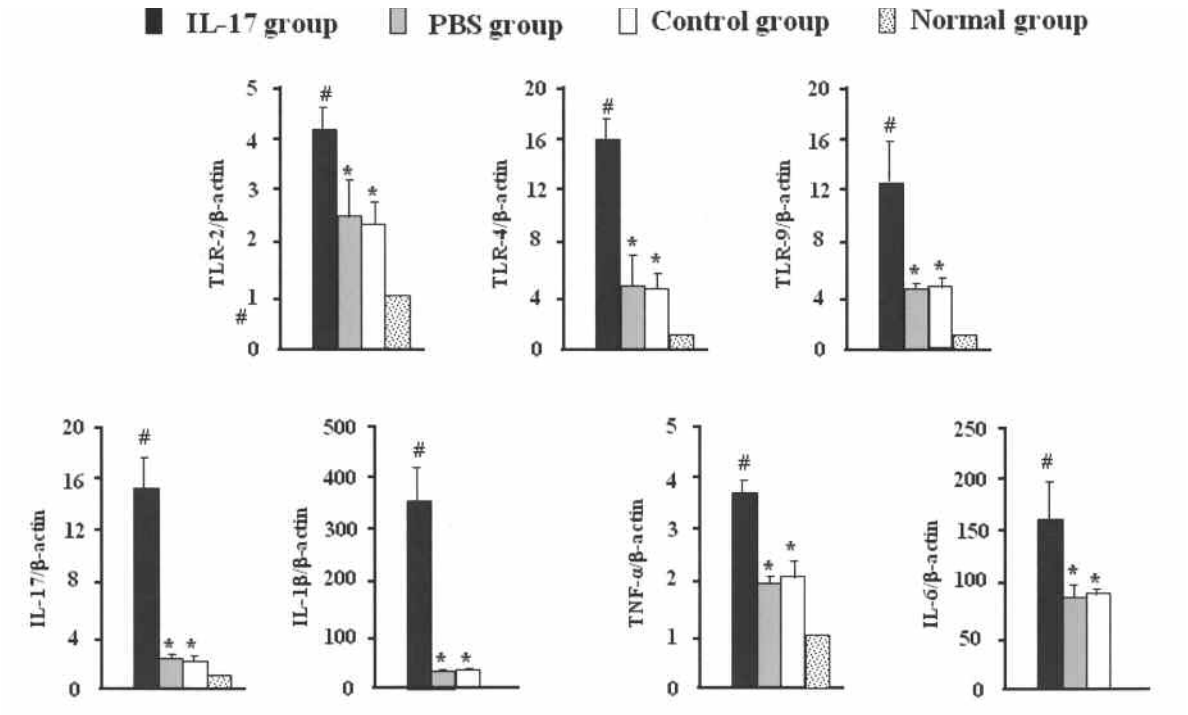

Figure 3. Relative synovial expression of TLR-2, 4 and 9, IL-17, IL-1ß, TNF-α, and IL-6 in CIA mice. Levels were significantly higher in the IL-17, PBS, and control groups than in the normal group. Levels were significantly higher in the IL-17 group than in the PBS and control groups. #p < 0.05 compared with the PBS and control groups; *p < 0.05 compared with the normal group.

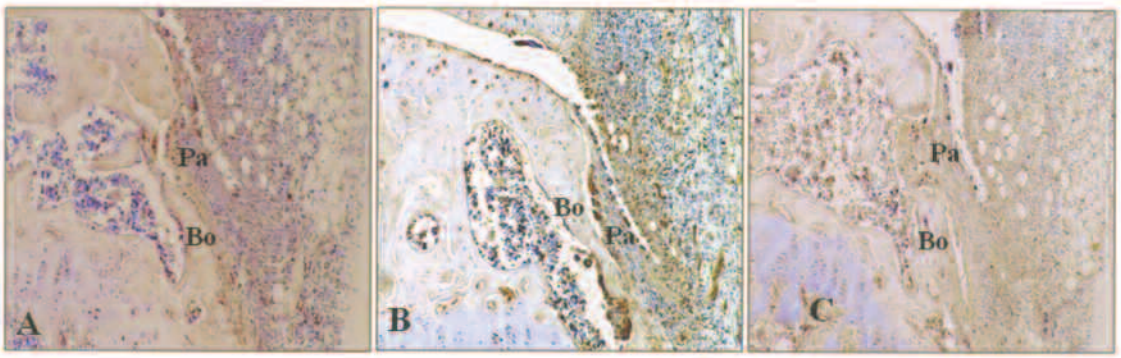

Figure 4. Immunohistochemistry for TLR-2 (A), TLR-4 (B), and TLR-9 (C) from knee joints of the IL-17 group with CIA. TLR-2, 4, and 9-positive cells are present in synovium. Original magnification x100. Bo: bone; Pa: pannus.

angiogenic factors such as IL-1, TNF, IL-6, IL-8, macrophage inflammatory protein-1α, and vascular endothelial growth factor[19–21]. IL-17 has synergistic effects with IL-1 and TNF on the production of cytokines, chemokines, and angiogenic factors by cultured synovial fibroblasts, and with TNF to induce cartilage destruction[21–24]. *In vivo* studies have shown that IL-17 is involved in the initiation and progression of experimental murine arthritis[9–12]. We found that IL-17 increased the production and expression of IL-1ß, TNF-α, and IL-6 in CIA mouse synoviocytes and aggravated joint inflammation and destruction.

TLR are highly expressed in synovial tissues of patients with RA, and TLR signaling by specific exogenous or endogenous ligands has been postulated as essential for the development of autoimmunity[5-7,25,26]. We found that IL-17 significantly increased joint inflammation and destruction and the synovial expression of TLR-2, 4, and 9 in CIA. These results suggest that the IL-17-induced increase in TLR-2, 4, and 9 expression is involved in the IL-17-induced aggravation of joint inflammation and destruction. We hypothesize that the mechanisms responsible for the IL-17-induced increase in TLR-2, 4, and 9 expression in CIA are as follows. First, increased endogenous TLR ligands produced by aggravated synovial inflammation in response to IL-17 increase

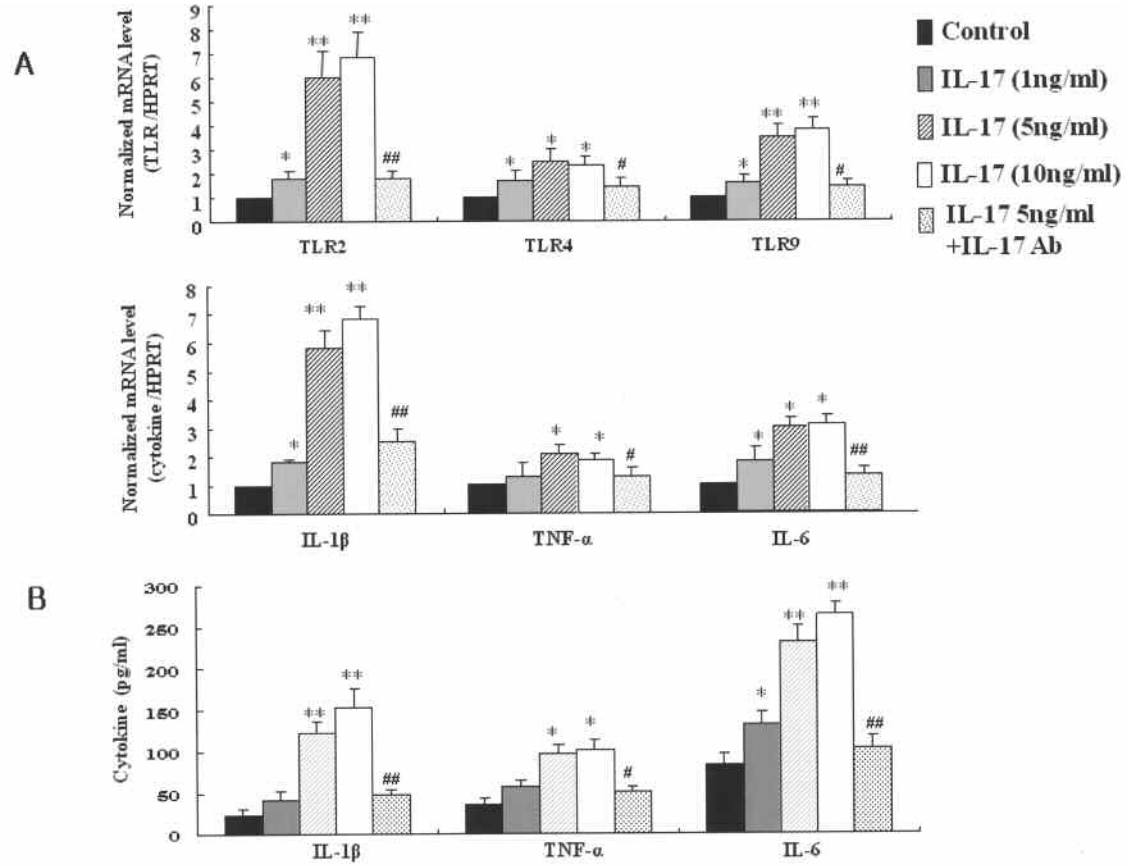

Figure 5. IL-17 increased the expression of TLR-2, 4 and 9, IL-1ß, TNF-α, and IL-6 in CIA mouse synoviocytes. A. Synoviocytes were cultured with IL-17 (1, 5, or 10 ng/ml) and anti-IL-17-neutralizing antibody (10 µg/ml) for 24 h, and mRNA expression was determined by real-time RT-PCR. B. Synoviocytes were cultured with IL-17 (1, 5, or 10 ng/ml) and anti-IL-17-neutralizing antibody (10 µg/ml) for 48 h. IL-1ß, TNF-α, and IL-6 in the culture supernatants were measured by ELISA. *$p < 0.05$ compared with control; **$p < 0.01$ compared with control; $^{\#}p < 0.05$ compared with 5 ng/ml of IL-17; $^{\#\#}p < 0.01$ compared with 5 ng/ml of IL-17.

the expression of TLR. This opinion is supported by the significantly higher expression of inflammatory cytokines such as IL-17, IL-1ß, TNF-α, and IL-6 in our IL-17 group compared with the PBS and control groups. Second, IL-17 may directly increase TLR-2, 4, and 9 expression by TLR-2, 4, and 9-expressing cells. Third, the first and second mechanisms may work in combination. We showed that IL-17 increases expression of TLR-2, 4, and 9, IL-17, IL-1ß, TNF-α, and IL-6, and that these effects were blocked by neutralizing antibodies to IL-17, IL-1ß, and IL-6 in CIA mouse synoviocytes. These results indicate that IL-17 increases the expression of TLR-2, 4, and 9 by stimulating the production of IL-1ß and IL-6. Chabaud and Miossec found that combination therapy acting on more than one cytokine may increase the percentage of responding patients as well as the degree of individual patient response[27]. They evaluated the efficacy of soluble TNF-α receptor (sTNFR) treatment alone (as in current therapy) and determined whether combining it with type II sIL-1R (sIL-1RII) and sIL-17R would lead to an enhanced effect[27]. We also speculate that administration of these antibody combinations (TNF-α, IL-1ß, IL-6, or IL-17 antibodies) is able to slow down CIA progression *in vivo* after CIA induction and lead to an enhanced effect.

Interestingly, expression of IL-17 gene was enhanced after IL-17 injection into joints. Coury, *et al* found an IL-17-dependent pathway of dendritic cell fusion and unexpected IL-17 synthesis by dendritic cells[28]. Therefore, IL-17 induced dendritic cell activation, fusion, and cytokine expression (such as TNF-α, IL-1ß, IL-6, and also IL-17). They also respond either to their autocrine IL-17 or to exogeneously added IL-17.

We have demonstrated that IL-17 increases the expression of TLR-2, 4, and 9 by increasing IL-1ß and IL-6 production, and that this aggravates joint inflammation and destruction. These results suggest that IL-17 aggravates joint inflammation and destruction by increasing the expression of TLR-2, 4, and 9 through its stimulation of IL-1ß and IL-6 production.

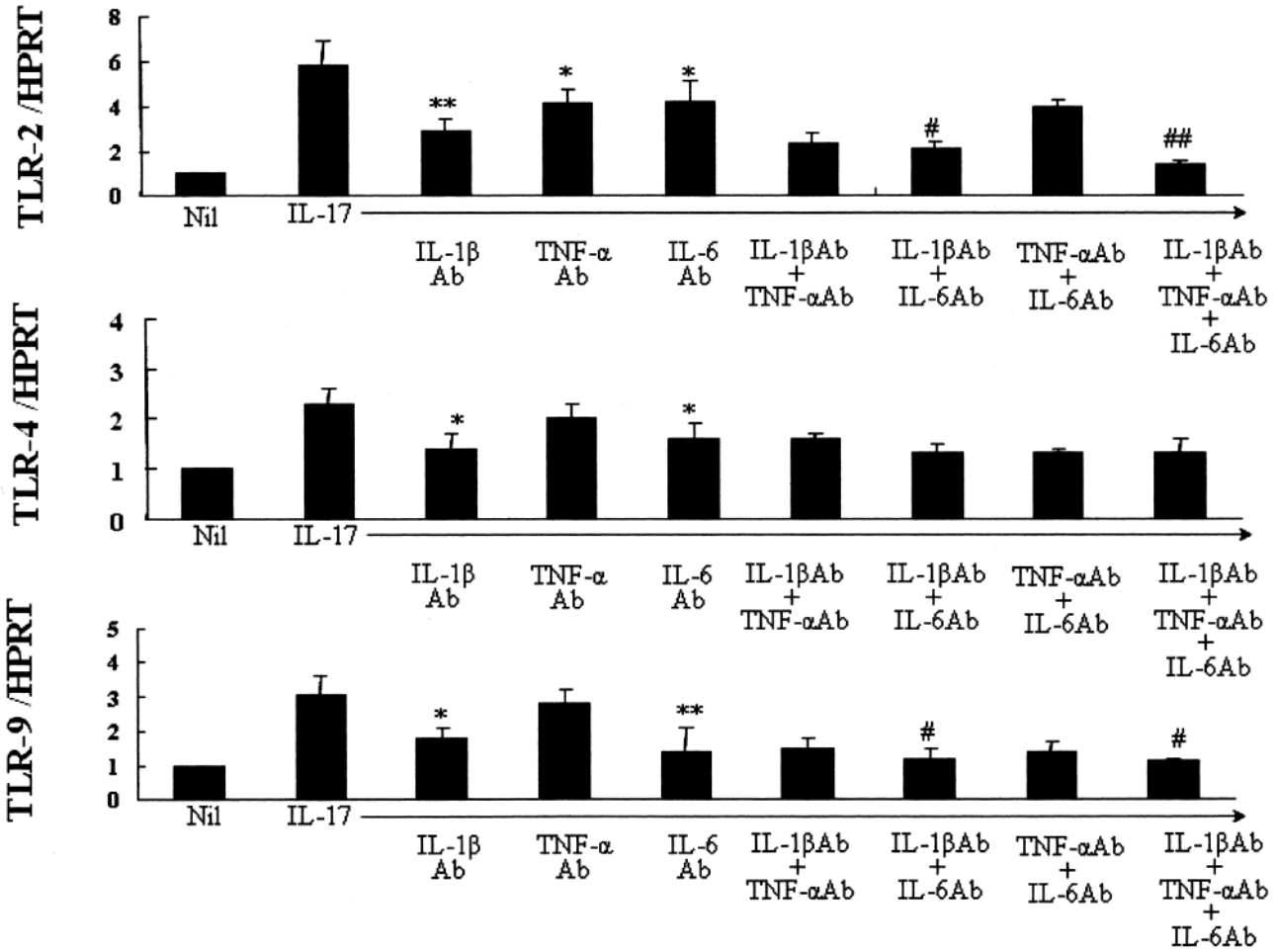

Figure 6. IL-17 increased TLR-2, 4, and 9 expression by increasing IL-1ß and IL-6 production in CIA mouse synoviocytes. Synoviocytes were cultured with IL-17 (5 ng/ml) and neutralizing antibodies to IL-1ß, anti-TNF-α, or IL-6 (10 μg/ml) for 24 h, and mRNA expression was determined by real-time RT-PCR. *$p < 0.05$ compared with 5 ng/ml IL-17; **$p < 0.01$ compared with 5 ng/ml IL-17; $^{\#}p < 0.05$ compared with 5 ng/ml IL-17 with anti-IL-1ß antibody; $^{\#\#}p < 0.01$ compared with 5 ng/ml IL-17 with anti-IL-1ß antibody.

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