Morphological Changes of Bones and Joints with Rheumatoid Arthritis and Osteoarthritis

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ABSTRACT

Arthritis is a common disease in aged people, and is clinically divided into rheumatoid arthritis (RA) and osteoarthritis (OA). Although common symptoms such as pain are present, the underlying pathological mechanisms are slightly different. Therefore, the objectives of the present study were to compare joint damage induced by RA and OA by analyzing the major morphological and molecular differences, and to propose a suitable therapeutic intervention based on the pathophysiological conditions of bones and joints. For the RA animal model, 8-week-old DBA1/J mice were immunized with bovine type II collagen emulsified in complete Freund's adjuvant (CFA). Normal C57BL/6 mice (over 2 years of age) were used for OA. The clinical arthritis score was calculated using a subjective scoring system, and paw thicknesses were measured using calipers. The serum TNF α level was analyzed using an ELISA kit. Micro-CT was used to identify pathological characteristics and morphological changes. In collagen-induced RA mice, there were increased ankle joint volumes and clinical scores ($p<0.01$). The concentration of TNF α was significantly increased from 3 to 7 weeks after immunization. Micro-CT images showed trabecular bone destruction, pannus formation, and subchondral region destruction in RA mice. OA among aged mice showed narrowed joint spaces and breakdown of articular cartilage. This study suggests that a careful therapeutic intervention between RA and OA is required, and it should be based on morphological alteration of bone and joint.

(Key words : Rheumatoid arthritis, Collagen, TNF α, Therapeutic intervention)

INTRODUCTION

Arthritis is a frequent disease in aged populations of men and women. It is divided into two types: osteoarthritis (OA) is an age-related disease, and rheumatoid arthritis (RA) is a well-known autoimmune disease. RA is an inflammatory disease, mainly destroying the synovium of peripheral joints. The major symptoms associated with arthritis are joint pain, stiffness, and reduced strength and endurance. Arthritis induces declined activity, gait alterations, and articular destruction. It is estimated that RA affects approximately 1% of the adult population worldwide, and is most prevalent among men and women of approximately 40 years of age. In spite of its low prevalence, patients with RA are physically, emotionally, and socially affected (Feldmann *et al.*, 1996; Yelin *et al.*, 1987).

On the other hand, OA is the most frequent arthritis in elderly people over 60 years. The breakdown of articular cartilage is known as a primary cause of OA. This leads to rubbing among bones, joint stiffness, limitation of movement (LOM), and pain. At the cellular level, infiltration of macrophages and perivascular T and B lymphocytes in core areas are observed (Benito *et al.*, 2005; Oehler *et al.*, 2002; Goldenberg *et al.*, 1982). Obesity and excessive catabolism contribute to the accelerated pathogenesis of RA. Recently, it was reported that knee pain in OA patients is strongly associated with loss of joint cartilage from cohort studies (Neogi *et al.*, 2009) and that early knee OA is related to the series of inflammatory cytokines frequently seen in RA (Scanzello *et al.*, 2009).

Both RA and OA exhibit similar symptoms, including induction of upregulation of extracellular matrix (ECM) proteinase, matrix metalloproteinase (MMP) iso-

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forms, and aggrecanases (ADAMTS), thereby leading to a breakdown of joint structures. Previous studies reported that an increase of tumor necrosis factor α (TNF-α) could be associated with secretion of these proteinases (Murphy and Nagase, 2008). Moreover, upregulation of both cytokines and chemokines are accompanied by fibrillation of the cartilage and localized production of fibrocartilage (Goldring and Goldring, 2010). Furthermore, abnormal joint structures lead to pain during walking, performing activities of daily living, and working in patients suffering from arthritis. This significantly reduces quality of life (Jakobsson *et al.*, 2007).

However, some characteristics, such as etiology, pathogenesis, and intervention, are completely different. A primary response is initiated in the synovium in the pathogenesis of RA, and several immune cells, including regulatory T-cells (Treg), are hyperactivated as a cascade. This induces the excess production of pro-inflammatory cytokines. An imbalance between pro- and anti-inflammatory cytokines causes an immune response within the synovium, resulting in so-called "pannus". Pannus is derived from the Latin word meaning "clotting", and is defined as hyperplasia of cells within a synovial lining, the interface between articular cartilage and synovium, and the subchondral region (Otero and Goldring, 2007). In contrast to RA, articular cartilage has been suggested to be a target tissue in OA, thereby inducing bone destruction. The most frequent region affected differs between the two types. RA often occurs in distal joints such as wrist joints, while OA appears in weight-bearing joints such as knee joints (McQueen, 2007).

TNF α could be involved in a common pathogenesis. The TNF gene is closely linked to the major histocompatibility complex (MHC) locus on murine chromosome 17 (human 6) (Dentener and Wouters, 2006). Recently, novel interventions using either anti-TNF α antibody or drugs against TNF α have been suggested as an effective treatment. From meta analysis results, Alonso-Ruiz *et al.* reported that administration of anti-TNF α drugs could decrease symptoms at lower dosages than recommended (Alonso-Ruiz *et al.*, 2008).

The purposes of the present study were to compare the morphological changes in bones and joints with RA or OA, distinguish the characteristics of RA from OA, and summarize the pathophysiology involved in each.

MATERIALS AND METHODS

Animals

The Ethical Committee for Animal Care and Use at Inje University approved all of the animal procedures performed. For the RA model, male DBA1/J mice weighing 18 to 20 g (6 weeks of age) were purchased from Orient (Korea), and all animals were serologically negative for common pathogens. For the OA animal model, normal C57BL/6 mice (over 2 years of age) were used. Mice were housed at 23.1±1℃ during a 12-hour light/dark cycle (light on: 06:00) and allowed free access to food and water.

Induction of RA and Measurements

For the RA model, mice immunized with collagen were used as previously described (Ohno *et al.*, 2008). Eight-week-old male DBA/1J mice were initially injected with 100 μg bovine type II collagen dissolved in acetic acid (Chondrex) containing complete Freund's adjuvant (CFA, Sigma) containing 1 mg/mL of heat-killed *Mycobacterium tuberculosis*. On day 21 from the first immunization, a boosting injection was administered with a collagen mixture containing incomplete Freund's adjuvant (Sigma, Fig. 1). The ankle joint volume was measured by calipers once every week. To indicate the degree of inflammation, the clinical scores for the paws were measured (Table 1). The sum of the scores for all paws from each mouse was used as the total clinical score.

Tissue Collection

Collagen-induced RA animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) and sacrificed at the indicated time. Serum was obtained to analyze the concentration of TNF α, and both limbs were removed from the animals in each group.

Analysis of Serum TNFα Concentration

Table 1. Scoring system for subjective evaluation of arthritis severity (Malfait *et al.*, 2001)

Severity score	Degree of inflammation
0	No evidence of erythema and swelling
0.5	Erythema and mild swelling confined to the tarsals or ankle joint
1	Erythema and mild swelling extending from the ankle to the tarsals
1.5	Erythema and moderate swelling extending from the ankle to metatarsal joints
2	Erythema and severe swelling encompass the ankle, foot & digits, or ankylosis of the limb

Mice were bled on weeks 1, 3, 5, 7, and 10 after the initial immunization. The collected blood samples were centrifuged at 2,000 *g* for 10 minutes at 4℃. The serum layer was separated and kept at −80℃, which was performed prior to week 2. Freezing/thawing cycles were avoided, and the serum TNF α level was quantitated by ELISA according to the manufacturer's protocol (BD Biosciences).

Micro-Computed Tomography

Micro-computed tomography (micro-CT) was performed in the hind limbs using a Skyscan 1076 scanner (Skyscan). For maximal image quality, scanning was performed at 100 kV and 100 μA. Each limb was scanned through a 360° scan rotation with an exposure time of 590 ms. In total, 1800 projected images were obtained at a resolution of 9 μm/pixel.

Statistical Analysis

Data were collected from repeated experiments and presented as mean±SEM. One-way ANOVA and Student's *t*-test were used for statistical analysis. Differences were considered significant at $p<0.05$. All analyses were performed using SPSS ver. 18.0.

RESULTS

Characterization of Collagen-Induced RA Animals

Clinical Signs of Collagen-Induced RA

Body weight

Body weight of mice with and without RA were measured once every week (Fig. 2A). The body weight was definitely augmented in all groups in a time-dependent manner. Compared with the positive and negative controls, there was a significant increase in body weight induced by bovine type II collagen at week 1 from the initial immunization ($p<0.05$). No significant changes were shown among the positive and negative controls and the collagen-induced RA group at week 10.

Clinical Score

We measured the clinical arthritis score, including swelling and reddening, based on previous studies (Fig. 2B). No significant differences were shown in clinical scores before initiation of immunization (week 0). Compared with the negative controls, the arthritis score as an index of disease development was dramatically increased from the first immunization to the termination of the experiment ($p<0.01$). There was a significant difference between the positive controls and the collagen-treated group from disease onset time to week 10 ($p<0.01$). CFA induced an increased clinical score in either the collagen-induced RA group or the positive control group at week 4 ($p<0.01$). The arthritis score was signi-

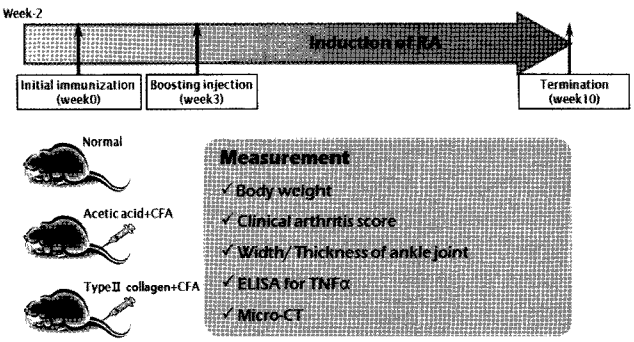
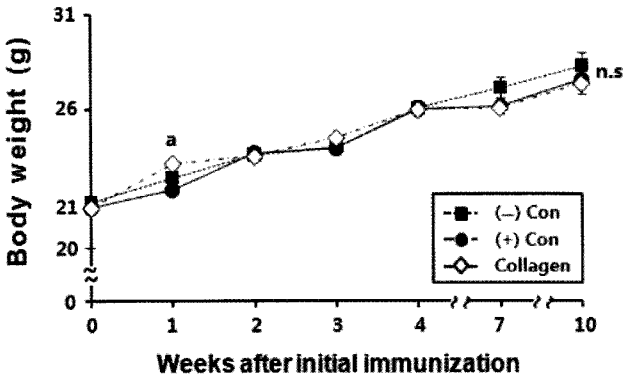
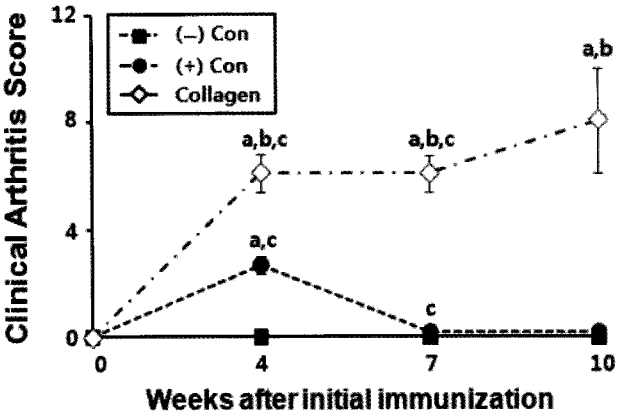


Fig. 1. Schematic diagram of the experimental design.



(A)



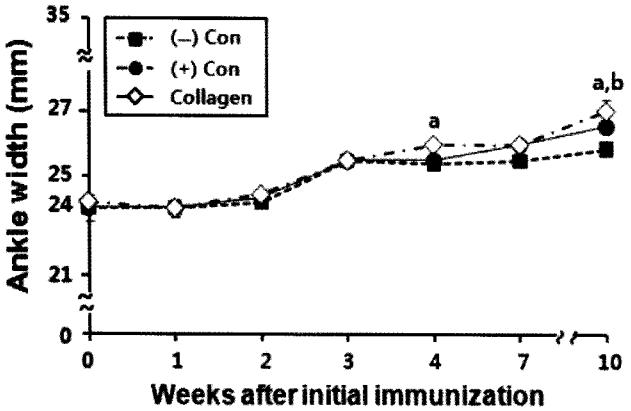
(B)

Fig. 2. Changes in physiological parameters in collagen-induced RA animals. Mice as a positive control (n=15) were treated with 0.1 N acetic acid and CFA. Mice (n=60) were intradermally immunized with bovine type II collagen and CFA at week 0. (A) Changes in body weight. (B) Alterations in clinical arthritis scores as an indicator of disease development. [a] $p<0.01$, negative control vs. collagen at the same time; [b] $p<0.01$, positive control vs. collagen at the same time; [c] $p<0.01$, immediately previous time vs. indicated time.

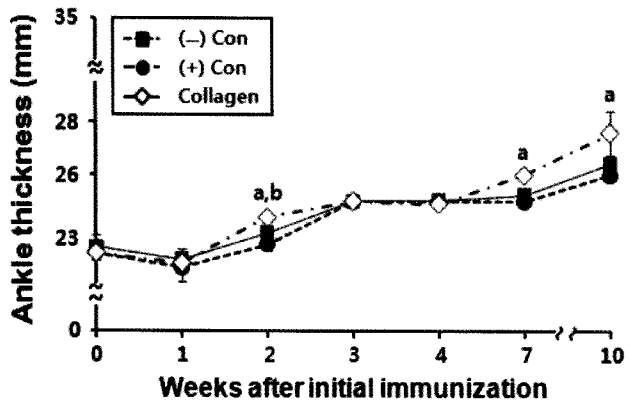
ficantly decreased to baseline at week 7 and consistently maintained ($p<0.01$), and no statistically significant difference between weeks 0 and 10 was shown in the positive control group. However, there was a continuous increase in the score induced by collagen ($p<0.01$).

Ankle Joint Volume

Joint volume, a special indicator for swelling, was determined by both ankle width and thickness. There was no significant variation in width or thickness among all groups before immunization (Fig. 3A). Compared with the positive and negative control groups, ankle width was definitely increased in collagen-immunized mice at week 4 ($p<0.01$). At week 10, a significant increase in ankle width was induced by collagen, compared with the negative control group ($p<0.01$). Finally, there was an increase in width in the collagen-treated group at week 10 *vs.* week 7 ($p<0.01$). Ankle thickness in collagen-induced RA mice was definitely increased at weeks 2, 7, and 10, compared with that in the control mice ($p<0.01$) (Fig. 3B). In the collagen-induced RA group, a critical increase in thickness was shown from weeks 1 to 2 ($p<0.01$).



(A)



(B)

Fig. 3. Alteration in ankle joint thickness and width. [a] $p<0.01$, negative control vs. collagen at the same time; [b] $p<0.01$, immediately previous time vs. indicated time within collagen-induced RA mice.

Alteration of the Serum TNFα Level

We analyzed the TNF α serum level immediately after sacrifice at the indicated time (Fig. 4). The normal TNF α level in serum was approximately 50 pg/mL before immunization; there was no significance among the groups. However, the serum TNF α concentration was significantly increased in either the positive control or the collagen-treated group at week 3 after immunization ($p<0.01$). At week 7 after the initial immunization, collagen dramatically augmented TNF α within serum, compared with control groups ($p<0.01$). No significant changes between control and collagen-immunized mice were shown at any time point, excluding weeks 3 and 7. Compared with week 0, immunization with bovine type II collagen definitely increased the serum TNF α concentration at weeks 3 and 7 ($p<0.01$). In addition, a significant increase in TNF α was induced by acetic acid injection with CFA at week 3 ($p<0.01$).

Changes in Morphology of Bones and Joints of Mice with RA or OA

We used micro-CT to determine the abnormal architecture of the subchondral bones and joints and to discriminate between the morphological characteristics of RA and OA (Fig. 5). In collagen-induced RA animals, pannus, which is a medical term for a hanging flap of tissue, was observed between both articular cartilages at week 4 after the first immunization. However, significant destruction of cartilage and great loss of trabecular bone was induced by aging (Fig. 5A). There was a dramatic decrease in knee joint space in OA-induced mice (>2 years of age) and a loss of cortical bone, compared with RA mice (12 weeks of age) (Fig. 5B). Images in the sagittal plane imply that the break of the subchondral region, including the growth plate, was initiated by RA and OA. Aging, a common cause of OA, resulted in a severe depletion of trabecular bone (Fig. 5C).

DISCUSSION

The aims of this study were to identify arthritis type-dependent changes in function and morphology, and to suggest target pharmacological and physical interventions between RA and OA. We used collagen-induced RA animals and compared them with age-induced OA mice. For RA induction, the DBA1/J (MHC-H2q) strain was selected because it is more susceptible to collagen-induced arthritis than is the DBA-2/J (MHC-

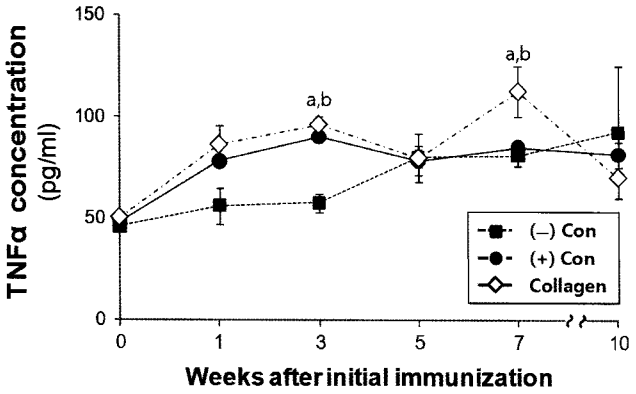


Fig. 4. Alteration in serum TNF α concentration in DBA/1J mice with and without collagen injection. [a] $p<0.01$, negative vs. positive control, collagen-treated group at the same time; [b] $p<0.01$, before injection (week 0) vs. indicated time within collagen-injected DBA1/J mice.

H2d) strain (Donate *et al.*, 2010). Collagen dissolved in acetic acid was emulsified in CFA and administered to DBA1/J mice. Because CFA contains 1 mg/mL heat-killed mycobacterium, the positive control group showed mild inflammatory symptoms, including increases in the clinical arthritis score and concentration of TNF-α until week 4. However, this response was transient and was minimized after the acute phase. Both the inflammatory score and ankle volume continuously increased in a time-dependent manner, and this was considered to be an indicator of disease development.

TNF α is mainly secreted by macrophages and mast cells, and plays important roles in the physiology of several organs: an increase in C-reactive protein in the liver, stimulation of phagocytosis of macrophages, and action as a potent chemoattractant for neutrophils (Locksley *et al.*, 2001). In this study, the TNF α level was significantly increased in the positive control and collagen-treated group until week 3. TNF α in the positive control group was recovered to the basal level after week 3, while a continuous increase was shown in the collagen-induced RA group until week 7.

Micro-CT images showed at least three differences between RA and OA. First, a definite destruction of articular cartilage was shown in OA; RA mice showed a relatively intact cartilage. Second, a loss of compact bone was shown in both types of arthritis, but it was more severe in OA than in RA. Third, the joint space between the femur and tibia was significantly reduced in OA animals. In both types, the growth plate co-

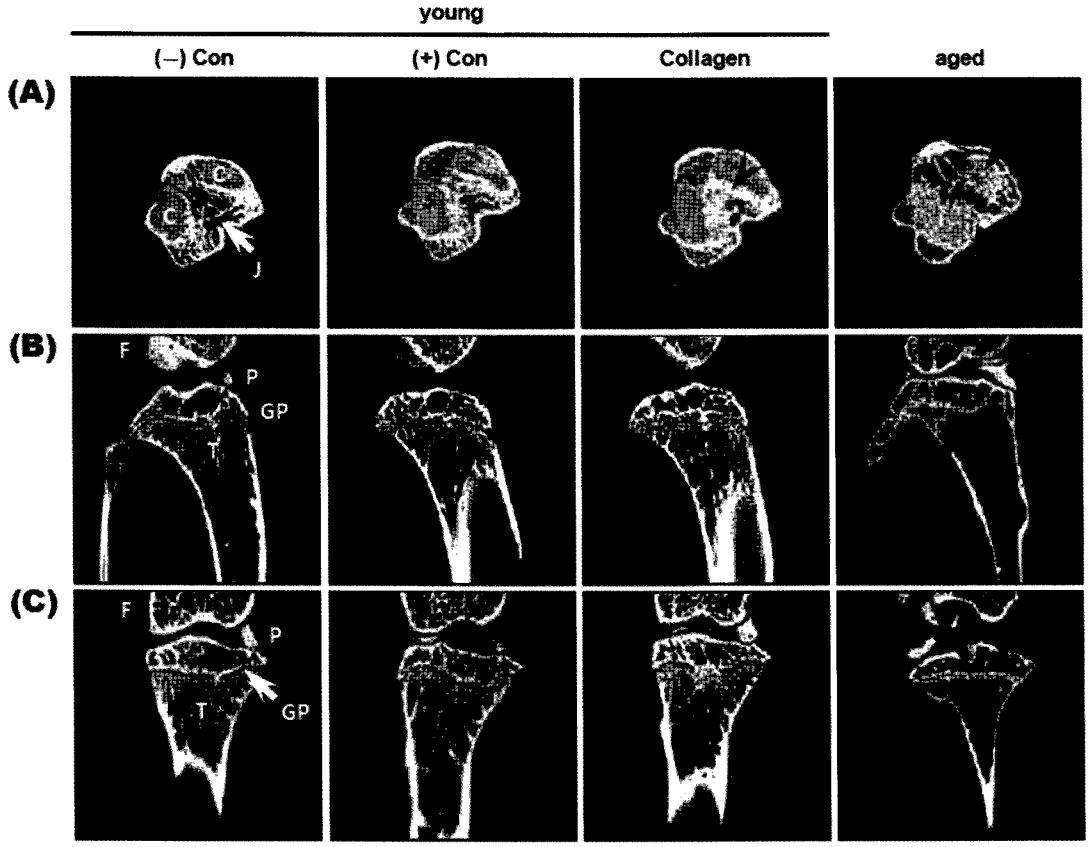


Fig. 5. Representative CT images of knee joints from mice with either collagen-induced RA or age-induced OA. (A) Horizontal, (B) Coronal, and (C) Sagittal sections are shown. Abbreviations: C, articular cartilage; J, joint; F, femur; P, patella; GP, growth plate; T, trabecular bone.

Table 2. Features of different forms of chronic arthritis (Lories and Lyuten, 2009)

	Rheumatoid arthritis	Osteoarthritis
Prevalence	0.5~1%	Radiographic Hand OA 27.2% Knee OA 13.8% Hip OA 27.0% Symptomatic Hand OA 6.8% Knee OA 4.9% Hip OA 9.2%
Onset	Peak onset between 3rd and 6th decade	Peak onset after 6th decade
Male/Female ratio	0.04	0.87 (radiographic OA) 0.79 (symptomatic OA)
Peripheral arthritis	Many small joint of hand and feet, symmetrical	Single joint
Autoantibodies	Yes, ACPA and RF	No
Associated genes	TRAF1, CD40, STAT4 HLA-DR genes	GDF5, ASPN, PTGS2
Hypothetical primary target tissue	Synovium	Cartilage/subchondral bone

nnecting the epiphysis and shaft was greatly reduced in both RA and OA animals, compared with young and normal animals. These differences resulted from the different characteristics (Table 2).

RA is an inflammatory autoimmune disease that destroys the synovium of peripheral joints. It comprises a syndrome of joint pain, stiffness, and reduced strength and endurance. Helper T cells (Th), including Th17 and Th1, are automatically hyperactivated, thereby leading to a chronic inflammatory process. Tregs, which normally maintain activities of Th1 and Th17, were reportedly deficient in patients who suffered from RA, inclining the immune system toward a pro-inflammatory condition. When the Th1 activity was excessive, cellular pathologies, including multiple sclerosis, diabetes, and RA, developed (Chen, 2010).

Joint destruction was observed to be a consequence of RA. Clinically, it was induced by at least two factors: pannus and osteoclasts. First, synoviocytes were transformed into a pannus. Normal synovium is a cluster of synoviocytes with fibroblast- and macrophage-like shapes that produces a component of ECM called as cadherins. Cadherins within synovial fluid mediate cell-to-cell adhesions, preventing hyperproliferation of synoviocytes. The overexpression of cadherins may be present in the synovium of RA patients, and Lee reported that monoclonal cadherin-11 antibody prevented the progression of RA (Lee *et al.*, 2007). The proliferating cells within pannus tissue can penetrate the ECM of the subchondral region as well as the cartilage, destroying them. Second, differentiation of osteoclasts results in local resorption of bone. In the normal condition, there is a balance between bone formation by osteoblasts and destruction by osteoclasts. Differentiated multinuclear osteoclasts are involved in the monocyte/macrophage lineage, decaying collagen within the bone matrix by secreting proteases (Goronzy and Weyand, 2009). These two components can be potent inducers of the breakdown of joints in RA patients.

Although joint demolition may be present in OA patients, it is mainly focused on the articular cartilage. Articular cartilage is organized into two distinct regions: the superficial and deep zones. Chondrocytes in the superficial zone express proteoglycan-4, which plays a role in boundary lubrication. Chondrocytes exist under low oxygen conditions, especially within the deep zones, and they upregulate hypoxia-inducible factor-1 α (Murphy *et al.*, 2009). The pathogenesis of OA is characterized by chondrocyte clustering. It results from increased cell proliferation and production of specific ECM proteins. As the process continues, upregulated production of destructive proteinase genes occurs. Analyses of OA cartilage in humans have shown increased catabolic proteins such as MMP and ADAMTS (Goldring and Goldring, 2010).

The present study mainly focused on morphological and pathological aspects between RA and OA. Further experiments are required to determine the effect of arthritis on functional aspects, including pain, balance, and gait. Moreover, analysis of molecular markers that represent the metabolic state of joint architecture is required.

This study compared both the molecular and morphological characteristics between RA and OA, and there were similarities and differences as following: 1) The onset of RA is earlier than that of OA, and the most frequent site is slightly different. 2) A primary target of RA is the synovium, while OA mainly focuses on the

articular cartilage. A severe loss of cartilage is mainly present in OA, based on micro-CT images, while the formation of pannus is present in RA. 3) Joint destruction is induced by cytokines, degradative proteinase, and osteoclasts in both types of arthritis, and arthritis type-specific molecules exist. Based on these results, we suggest that considerable interventions are needed to treat arthritis because the pathogenesis differs between the two types.

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