# Development and characteristics of pannus-like soft tissue in osteoarthritic articular surface in rat osteoarthritis model

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## Abstract

### Objective

*Pannus is invasive granulation tissue found on the articular cartilage having rheumatoid arthritis (RA). However, pannus-like tissue has also been found in osteoarthritis (OA). Our previous study showed that pannus-like tissue in OA (OA pannus) was frequently found in human OA samples. The purpose of the study is to investigate the development and the characteristics of OA pannus in a rat OA model.*

### Design

*Ligaments of the knee joint were transected in Wister rats to induce OA. The knee joints were removed at weeks 1, 2, 4 and 6, and subjected to histological study. Samples were stained with hematoxylin and eosin (HE), Safranin-O and immuno-stained for vimentin, CD34, type II collagen and MMP-3. The whole knee joint of OA rats was implanted in SCID mice and kept for a further 3 weeks. Then the histological findings were evaluated in HE sections.*

### Result

*OA pannus appeared at week 2 and extend over the articular surface. OA pannus cells were positive for vimentin and/or CD34. At week 6, a part of articular surface was restored with matrix. OA pannus cells expressed MMP-3 as well as type II collagen. Histological study of rat OA knees implanted in SCID mice showed that OA pannus cells filled the joint space and invaded articular cartilage.*

### Conclusions

*The presence of OA pannus was found in a rat OA model and its features were similar to those in human OA. OA pannus had both catabolic and reparative features, and the latter feature were speculated to be dominant in the later phase of the disease under a certain environmental condition.*

**Key words**

Pannus, osteoarthritis, rat, SCID mouse, histology.

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*Competing interests: none declared.*

## Introduction

Osteoarthritis (OA) is the most common form of chronic arthritis characterized by pain, deformity and increasing disability. OA involves almost all the components of synovial joints encompassing cartilage, synovial tissue and subchondral bone. Pathological signs of OA include degraded articular cartilage associated with subchondral sclerosis, osteophyte formation and synovitis. The articular cartilage degradation is initiated by loss of proteoglycan and cleavage of type II collagen resulting in fibrillation and cleft formation in the articular surface (1-4).

Pannus has been known as invasive granulation tissue observed on articular cartilage of joints having rheumatoid arthritis (RA), however, pannus-like tissue has also been found in OA samples and even been listed as one of the items in the histological score of OA (5-8). Moreover, our previous study showed that pannus-like tissue in OA (OA pannus) was frequently found in OA cartilage examined (9). Unlike typical invasive pannus in RA, OA pannus did not include inflammatory cells and macrophages expressing CD68.

It has been speculated that pannus-like tissue in OA might be derived from bone marrow, as it gained access to joint surface through gaps in the subchondral bone plate and OA pannus cells expressed vimentin. Although OA pannus might develop for repairing articular defect, it had a catabolic feature expressing IL-1β and MMP-3 (9. 10). As characteristics of OA pannus were deduced from investigation using advanced human OA, these data provide a little information regarding the origin and the fate of OA pannus.

Ligament transection animal models have been developed to investigate OA *in vivo* using rabbits (11), dogs (12) and mice (13). Rats have been also used as an OA model (14, 15) and recent study showed that an anterior cruciate ligament transaction (ACLT) rat had pannus-like tissue 28 days after the surgery in some cases (16).

The aim of the present study is to investigate the development and the characteristics of OA pannus using an animal model. To this end, whole rat joints with OA were implanted in SCID mice to elucidate a property of OA pannus.

## Materials and methods

*Animals and ligament transaction*

Fifteen male Wister rats (9 weeks old) were obtained from Charles River, Japan. A ligament transaction model was prepared according to Stoop *et al.* (14) with some modifications. Briefly, the left knee joint was shaved and the skin was disinfected under anesthesia. The medial collateral ligament was transected, a part of medial meniscus was removed and then the anterior cruciate ligament was transected under the direct vision. This procedure permitted a secure operation and complete instability in the knee joints. After the instability was confirmed, the joint capsule and the skin were closed with nylon suture. Sham operation was performed in the contralateral knee joint. Three rats each were sacrificed at weeks 1, 2, 4 and 6 after the surgery and the knee joints were subjected to the histological study. The remaining 3 rats were used for the following experiments.

*Implantation of the rat knee joint to SCID mice*

Three SCID mice (FOX CHASE SCID CD-17/Icr-scid/Jcl) were purchased from CLEA Japan, Inc. The mice were kept permanently under sterile conditions. The knee joints of the rats were operated on, as mentioned above, to induce OA. After 3 weeks, the rats were sacrificed and both the OA and intact knee joints were resected from the lower extremities by cutting the distal femur and the proximal tibia. Then the specimens were carefully skinned without damaging the joint capsule. The SCID mice were anesthetized and the isolated joints of the rats were implanted subcutaneously in the back of SCID mice. Three weeks later, the implanted knees were removed from the SCID mice and subjected to histological study. These animal studies were approved by Institutional Animal Care and Use Committee.

*Sample preparation*

To investigate the development and the characteristics of OA pannus, the tibia was separated, fixed with 4% buffered

formalin for 5 days and decalcified with 10% EDTA for 3 days. Then the tibia was cut coronary in the center of articular surface for coronal section and embedded in paraffin. The retrieved knee joints from the SCID mice were prepared similarly as a whole joint without separating the tibia from the femur.

*Histochemical and immunohistochemical staining*

All samples were cut at 5μm and stained with hematoxylin and eosin (HE). For immunohistochemical staining, the sections were deparaffinized, treated with 0.4mg/ml of proteinase K (Wako Pure Chemical Industry, Japan) for 10 minutes, incubated with 3% hydrogen peroxide for 30 minutes followed by washing in PBS and blocked by Block-ace (Snow Brand, Japan) to prevent non-specific reaction for 10 minutes. Then sections were incubated with mouse anti-CD34 antibody (Santa Cruz Biotechnology, CA, USA) at a dilution of 1:500, mouse anti-vimentin antibody (SIGMA, USA) at a dilution of 1:500, rabbit anti MMP-3 antibody (SIGMA, USA) at a dilution of 1:500, and mouse anti-collagen type II antibody (Chemicon, CA, USA) at a dilution of 1:500 overnight at 4°C. As negative controls, sections were incubated with non-immune serum of respective animals. The reaction of first antibody was followed incubation with biotinylated link antibody (DAKO JAPAN, Japan) for 30 minutes and peroxidase-labelled streptavidin (DAKO JAPAN, Japan) for 30min. After each step, sections were washed by PBS extensively. Staining was completed after incubation with substrate-chromogen solution (DAKO JAPAN, Japan). Lastly, sections were counterstained with hematoxylin and mounted for stable specimen.

*Histomorphologic assessment*

Histomorphologic changes of articular cartilage both in the medial and lateral tibial condyles were assessed using the Mankin scoring system (8). The development of OA pannus was evaluated as percentage of the length of OA pannus divided by the length of whole articular surface in each HE section. The results were expressed as the mean and SD.

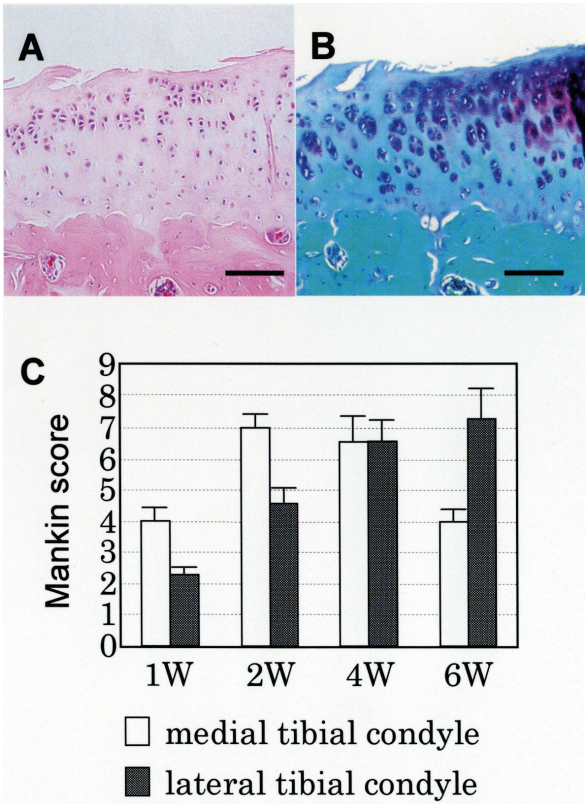


**Fig. 1.** Histological findings of the medial tibial condyle of the rat 4 weeks after the surgical intervention. Sections were stained with HE (**A**) and Safranin-O (**B**). The time course of articular degeneration of the medial and lateral tibial condyles were expressed as Mankin scores (**C**). Scale bar = 100μm. Data are the mean ± SD (n=3).

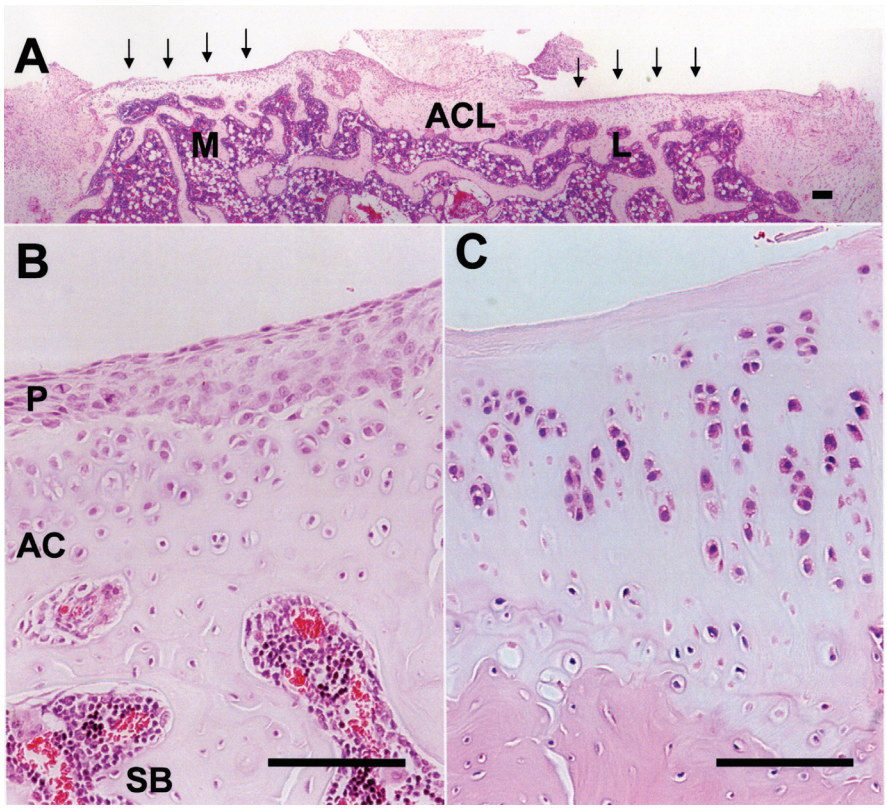


**Fig. 2.** OA pannus on the surface of the tibial condyle of rat OA models. **A**, Representative coronal section of the tibial condyle 2 weeks after the surgical intervention). In this specimen, both condyles wee covered by OA pannus. **B**, Higher magnification view of OA pannus at week 4. **C**, Higher magnification view of articular surface of the medial tibial condyle at week 6. M: the medial tibial condyle, L: the lateral tibial condyle, ACL: attachment of anterior cruciate ligament, P: OA pannus, AC: articular cartilage, SB: subchondral bone. Arrows: OA pannus, Scale bar = 100μm

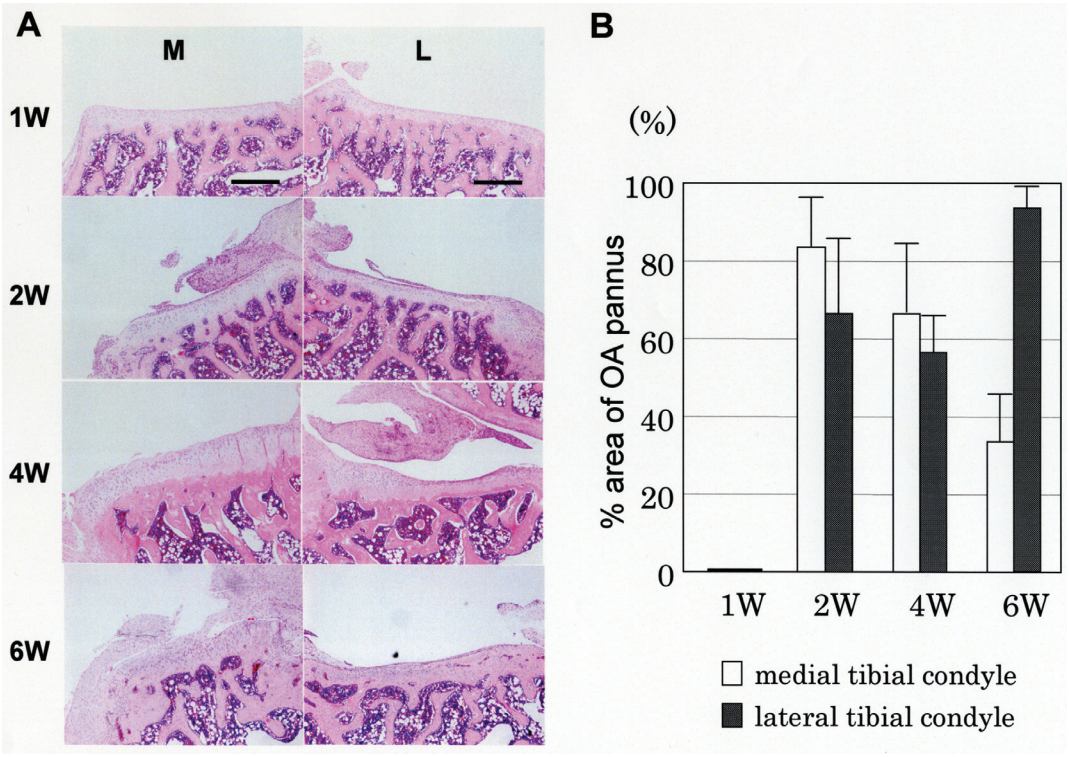


**Fig. 3.** Development of OA pannus. **A**, Representative histological views of the medial and lateral condyle of rat OA models at 1, 2, 4 and 6 weeks after the surgery. **B**, The time course of pannus development is shown. The values indicate the average percentages of articular surface covered by OA pannus. M: the medial tibial condyle, L: the lateral tibial condyle. Scale bar = 500μm. Data are the mean ± SD (n=3).

## Results

*Development of OA and OA pannus in the rat OA model*

In the rat OA model, fibrillation of articular surface, clustering of chondrocytes and proteoglycan depletion expressed as loss of Safranin-O staining were found in the medial tibial condyle similar to human OA in some cases (Figs. 1A, B). Articular degradation indicated by Mankin scores proceeded as a peak at week 2 in the medial tibial condyle and progressively in the lateral tibial condyle (Fig. 1C). Distinct OA pannus appeared at week 2 and then, extended allover the articular surface (Fig. 2A). Histologically, OA pannus was about 100μm in depth and clearly discriminated from hyaline cartilage. OA pannus cells were flat in the surface and round in the deeper area with large nucleus (Fig. 2B). However, OA pannus disappeared at week 6 leaving amorphous and acellular matrix in the medial tibial condyle (Fig. 2C). Figure 3 shows a representative time course of OA pannus development in both condyles. OA pannus was absent at week 1. The findings were rather variable after 2 weeks. A section of the medial condyle in one of the 3 rats at week 4 just showed typical fibrillation without soft tissue, whereas in other rats at week 4, articular cartilage of the medial condyle was covered by OA pannus. A section at week 6 showed an amorphous layer covering hyaline cartilage and in other sections, articular cartilage was covered in part by OA pannus. Collectively, the average percentage of articular surface covered by OA pannus was about 80% at week 2 and decreased to 30% at week 6 in the medial tibial condyle, whereas in the lateral condyle, 60% at week 2 and increased to about 90% at week 6 (Fig. 3B).

*Origin of OA pannus*

In the medial tibial condyle, OA pannus appeared at week 2 around the joint margin and extended over the articular surface (Fig. 4a, b). As a marker of mesenchymal phenotype and of bone marrow derived cells, sections were stained with vimentin and CD 34, respectively. Although, vimentin positive cells and CD34 positive cells were confined to the bone marrow at week 1 (data not shown), they appeared in the marginal area of articular surface and extended to the center of joint (Fig. 4e, f, I, j). Bone marrow preferentially in the subchondral area and OA pannus tissue were positive for vimentin (Fig. 4f, g). In addition to superficial cells, matrix was immune-positive for vimentin in OA pannus tissue. Cytoplasm of OA pannus cells as well as bone marrow cells were positive for CD34 (Fig. 4j, k). Chondrocytes in hyaline cartilage were consistently negative for vimentin nor CD34. Moreover, vimentin and CD34 positive cells in the bone marrow seemed to travel onto the articular surface through a gap at the juxtaarticular area (Fig. 4a, d, e, h, i, l). Negative controls showed no staining (Fig. 4m, n).

*Characteristics of OA pannus cells*

To identify the characteristics of OA pannus cells, samples were stained by anti MMP-3 antibody and anti type II collagen antibody. OA pannus cells around the border with hyaline cartilage expressed MMP-3 (Fig. 5a, c). Matrix of OA pannus was positive for type II collagen and OA pannus cells also strongly expressed type II collagen (Fig. 5b, d). These findings were similar to those of human OA pannus (9) and suggested that OA pannus had both catabolic and anabolic properties.

*OA pannus in the SCID mice*

As properties of OA pannus cells might be influenced by *in vivo* factors such as

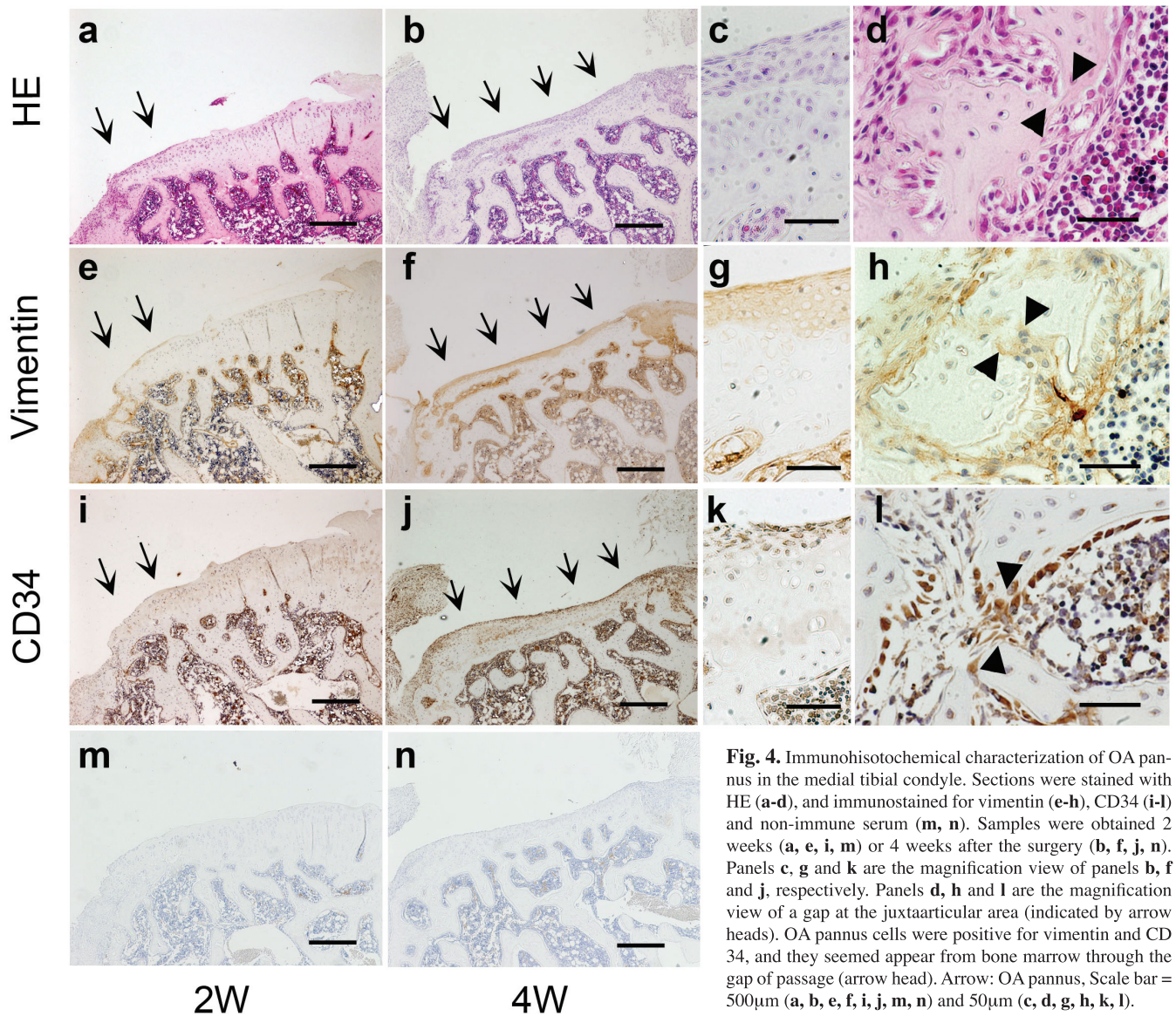


**Fig. 4.** Immunohisotochemical characterization of OA pannus in the medial tibial condyle. Sections were stained with HE (**a-d**), and immunostained for vimentin (**e-h**), CD34 (**i-l**) and non-immune serum (**m, n**). Samples were obtained 2 weeks (**a, e, i, m**) or 4 weeks after the surgery (**b, f, j, n**). Panels **c**, **g** and **k** are the magnification view of panels **b, f** and **j**, respectively. Panels **d, h** and **l** are the magnification view of a gap at the juxtaarticular area (indicated by arrow heads). OA pannus cells were positive for vimentin and CD 34, and they seemed appear from bone marrow through the gap of passage (arrow head). Arrow: OA pannus, Scale bar = 500μm (**a, b, e, f, i, j, m, n**) and 50μm (**c, d, g, h, k, l**).

joint movement and mechanical stress over time, rat knees were implanted in the SCID mice to further identify the properties of OA pannus at week 3. Joint space of 3 OA rat knees implanted in SCID mice were filled with fibrous tissue (Fig. 6a, b, c) whereas control knees had little such fibrous tissue in the join space (Fig. 6c). The fibrous tissue was shown to be derived from bone marrow and invaded into articular cartilage with multinuclear cells (Fig. 6e).

## Discussion

The presence of soft tissue on the surface of OA cartilage has been described as fibrous connective tissue, chondroid or reparative cartilage and they were considered to be reparative tissue for injured cartilage (5, 17). In our previous study, pannus-like tissue was found in more than 80% of OA samples and it had a catabolic property rather than a reparative one (9, 10). We speculated that the tissue was reparative in the earlier stage of OA but that it became deleterious after the long exposure to catabolic factors such as cytokines or growth factors that involved in OA. We also considered that the origin of OA pannus in human seemed bone marrow mesenchymal cells, as it had continuity with bone marrow and it expressed vimentin. A limitation of our previous studies was that the samples were obtained from patients with advanced OA and there was no information regarding OA pannus in earlier stages of the disease.

An animal model makes it possible to investigate early events and natural course of arthritis. In the present OA model, OA pannus developed at week 2 as if it emerged from bone marrow through a gap in the juxtaarticular area. Expression of vimentin or CD34 in OA pannus cells as well as bone marrow cells reinforced the hypothesis that OA pannus was derived from bone marrow cells. Pluripotential mesenchymal stem cells (MSC) are present in the bone marrow at a density of $1/10^4$-$10^5$ bone marrow cells (18) and CD34 is a marker of hematopoietic progenitor cells. As OA pannus cells exclusively expressed CD34 in articular cartilage, they were suggested to originate from bone marrow.

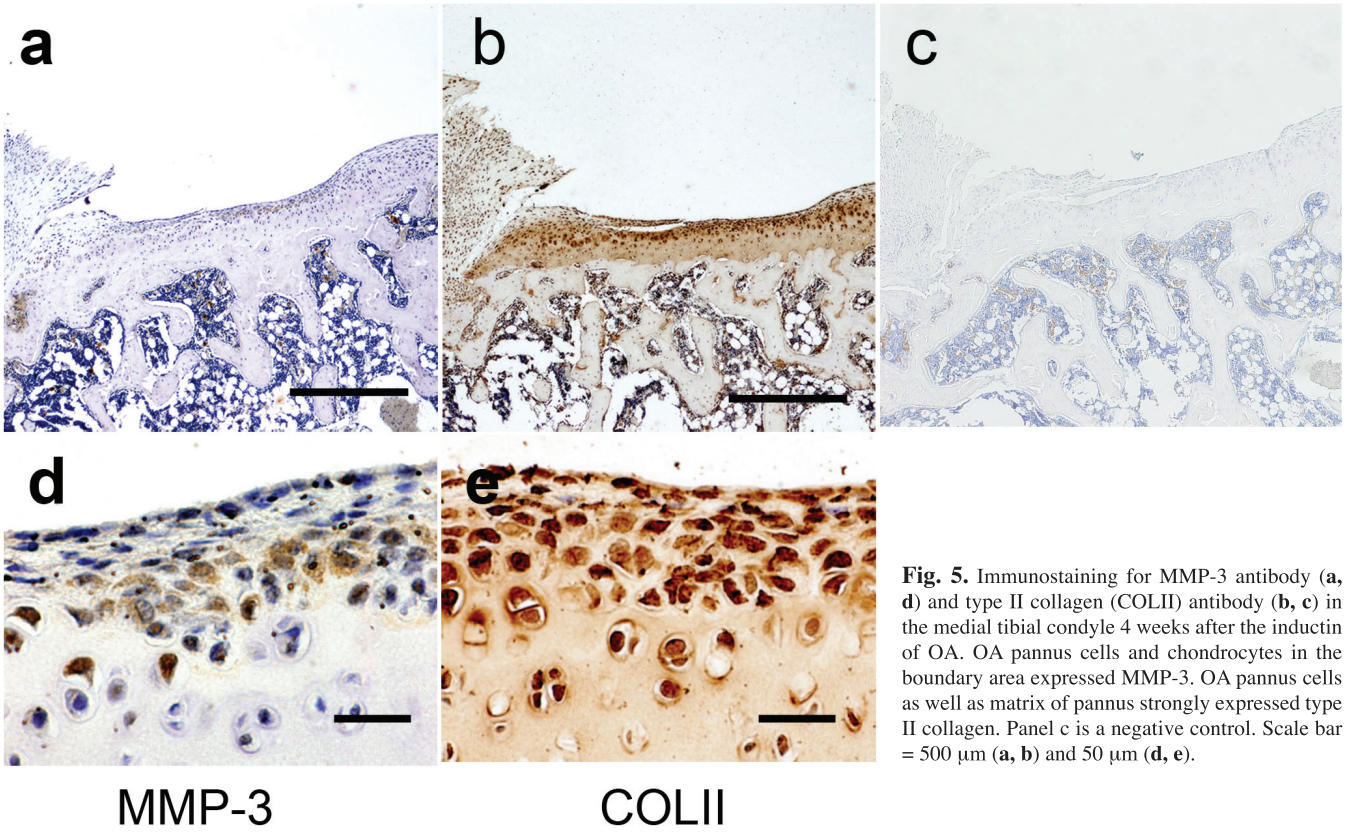


**Fig. 5.** Immunostaining for MMP-3 antibody (**a, d**) and type II collagen (COLII) antibody (**b, c**) in the medial tibial condyle 4 weeks after the inductin of OA. OA pannus cells and chondrocytes in the boundary area expressed MMP-3. OA pannus cells as well as matrix of pannus strongly expressed type II collagen. Panel c is a negative control. Scale bar = 500 μm (**a, b**) and 50 μm (**d, e**).

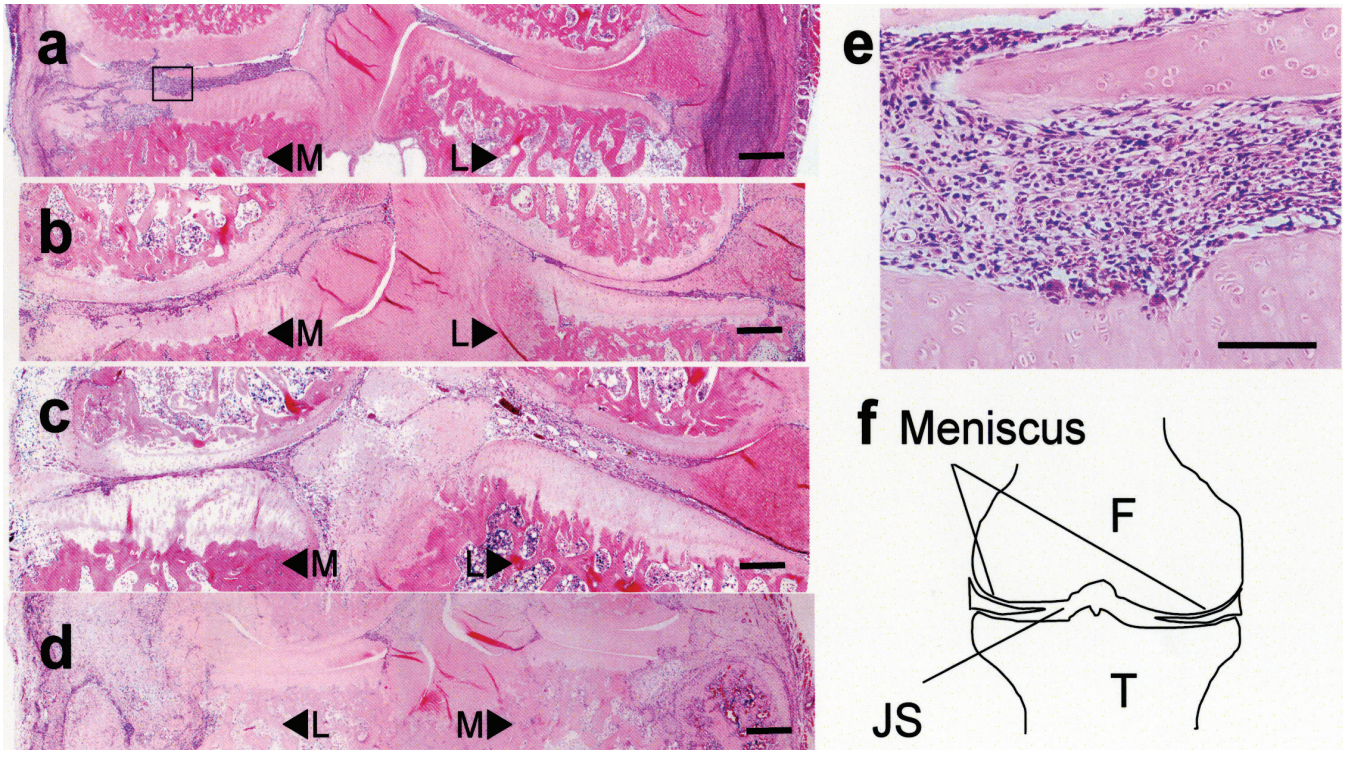


**Fig. 6.** Coronal section of the knee joints that were implanted in SCID mice for 3 weeks (**a, b, c, d**). Three rats underwent excision of ligaments to induce OA on the left knee and the right knee was served as a control. The rats were sacrificed at week 3 and the excised whole knee joints were implanted in SCID mice for 3 weeks. Joint space of OA knees were filled with fibrous tissue (**a, b, c**), whereas that of the control knee have little such fibrous tissue (**d**). Panel **d** is a representative section of a control knee. The fibrous tissue invaded into articular cartilage with multinuclear cells (e). Panel **e** is a magnification view of the indicated area in panel **a**. An anatomical drawing of the knee joint is shown (**f**). M: medial, L: lateral, F: femur, T: tibia, JS: joint space. Scale bar = 500 μm (**a, b, c, d**) and 50 μm (e).

Similar to human OA, OA pannus cells around the border with hyaline cartilage were positive for MMP-3, suggesting that OA pannus in a rat had a catabolic feature. On the contrary, OA pannus cells had an anabolic feature expressing type II collagen. OA pannus disappear and the articular surface seemed repaired with amorphous matrix at week 6 in the medial tibial condyle and devastating articular erosion characteristic of human OA hardly occur in the rat OA model. These findings suggested that cartilage of the rat was resistant to development of OA and OA pannus tissue might play a reparative role in a part.

Implantation of the whole rat knees into SCID mice was conducted to disclose the fate and the feature of OA pannus. The knee joints from OA rats were implanted in SCID mice at week 3, as previous our experiments showed that OA pannus was well organized around that time. In addition, the whole joint was prepared for implantation to prevent the rat cartilage from direct invasion by mouse tissue. Implanted knees were retrieved from SCID mice 3 weeks after the implantation, when 6 weeks had passed from induction of OA. At that time, bone marrow derived tissue occupied the joint space exclusively in OA knees and the tissue was considered to be derived from OA pannus. The tissue invaded into the cartilage with multinuclear cells suggesting that it had a catabolic feature. A question was raised why only OA pannus that was implanted SCID mice remained catabolic, while OA pannus in the living joint showed some reparative feature at week 6. One of the reasons was that the living joints kept moving and the articular cartilage was subjected to mechanical loading. Adequate exercise and mechanical loading had beneficial effects on articular cartilage *in vivo* and *in vitro*. Moderate exercise on ACLT rats increase the expression of Hsp70 that protects chondrocytes from apoptosis (16), and intermittent hydrostatic pressure decreased the production of MMPs from chondrocytes (19). Adequate movement might provide beneficial influence on OA pannus. Another mechanism would be a mechanical exclusion of invading tissue by joint motion. Besides these local mechanical factors, the systemic factors of rats such as growth factors, hormonal mediators and immunological mediators might affect the OA process, which were absent in the SCID mice.

In the juxtaarticular area, a gap was found, through which bone marrow cells traveled onto the articular surface, suggesting that the origin of OA pannus cells were bone marrow cells. Chondrogenic mesenchymal stem cells support the growth of hematopoietic progenitors cells by secreting a number of hematopoietic cytokines and do not express CD34 (20). Thus, early OA pannus cells were highly immature and included various progenitor cells, which might permit OA pannus to develop reparative cartilage.

The limitation of the present study was a deviation of histological findings. To solve this problem, lager number of rats, an identical surgical procedure and a uniform condition of feeding would be necessary. To investigate the factors that affect OA pannus would remain as a future subject.

In conclusion, we confirmed the presence of pannus-like tissue in a rat OA model and its features were similar to those in human OA in a part. OA pannus had both catabolic and reparative features, and the latter feature was speculated to be dominant in the later phase of the disease by a certain environmental factors. Mechanical factors and systemic factors might have beneficial roles in transformation of OA pannus to cartilaginous tissue resulting in restoration of articular cartilage.

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