Presence of pannus-like tissue on osteoarthritic cartilage and its histological character


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Summary

Objective: To investigate and characterize pannus-like tissue which is often present on osteoarthritic articular cartilage.

Design: Cartilage specimens from 15 knee and five hip joints of patients with osteoarthritis (OA) undergoing arthroplasty were stained for HE and Safranin-O. They were also immunostained by antitype I collagen, type II collagen, CD68, IL-1β and MMP3 antibodies.

Results: Ninety percent of joints have pannus-like tissue on the articular surface, preferentially in a marginal area. The articular cartilage was divided into three regions according to the location: the marginal zone, the intermediate zone and the para-eroded zone. Pannus-like tissue in OA knee joint occurred 45.9%, 27.5% and 11.1% of the surface of each region respectively. Histologically, pannus-like tissue was divided into three regions according to the location: the marginal zone, the intermediate zone and the para-eroded zone. Pannus-like soft tissue covering articular cartilage in rheumatoid arthritis (RA) and after the microscopic observation, pannus was revealed to be invasive granulation tissue. It is composed of aggressive macrophage- and fibroblast-like mesenchymal cells6 brought about by immature cells which include fibroblast-like cells6–8, macrophage-like cells and other inflammatory cells8 arising from the junction between synovial tissue and cartilage (bare area). These contribute to cartilage degradation by the release of collagenolytic enzymes8.

In fact, soft tissue covering articular surface of OA is often found during joint surgery and in articular specimens. As in previous papers, the soft tissue covering articular cartilage was termed as ‘granulation tissue’2, ‘reparative fibrocartilage’3, ‘reparative tissue’4 and ‘pannus’2,5, we named the tissue as pannus-like tissue in this paper. Regarding the function and the origin of pannus-like tissue of OA joint, some previous studies suggest that it arises directly from the bone marrow2,3,11 or from synovial membrane5,4 and that it may contribute to cartilage erosion. However, frequency, origin, function and fate of pannus-like tissue in OA have not yet been analysed well. In this paper, we focused on the tissue to clarify its frequency and its implication in OA.

Introduction

Osteoarthritis (OA) is a complex of interactive, degenerative and repair processes in cartilage, bone, and synovium, with secondary components of inflammation1. Pathologic changes of osteoarthritic joints are characterized by osteophyte formation, softening, fibrillation and abrasion of the cartilage following denudation of underlying bone. Previous investigations for OA have focused mainly on cartilage degradation by loss of matrix and reduction of chondrocytes; however, soft tissue is frequently observed on the articular surface of OA articular cartilage. Fassbender described a pannus and synovial tissue and cartilage (bare area). These contribute to cartilage destruction by the release of collagenolytic enzymes5.

Key words: Osteoarthritis, Pannus-like tissue, MMP3, IL-1β.© 2003 Published by Elsevier Science Ltd on behalf of OsteoArthritis Research Society International.
Materials and methods

CARTILAGE SAMPLES

Articular cartilage specimens were obtained from 15 knee joints and five hip joints from 17 patients with primary OA undergoing arthroplasty. The average age at operation was 71.0 years old (from 54 to 79 years). The diagnosis was established according to the American College of Rheumatology criteria. They had no episode of injury and no radiological and serological evidence suggesting other arthritis such as RA. All the samples were obtained with informed consent from patients and the institutional ethical committee approved the study protocol.

PREPARATION OF CARTILAGE SPECIMENS

From two to 22 cartilage specimens were resected from articular samples (Fig. 1). As many specimens as possible were prepared from each individual sample and were managed to include articular cartilage ranging from a marginal region to an eburnated region if possible. Specimens were finished at about 3 mm width and then fixed in 4% paraformaldehyde/0.01 M phosphate buffered saline (4% PFA/PBS) for 2 h. After defatting in 99.5% ethanol for 5 days, and decalcification in 10% ethylenediamine-N,N,N′,N′-tetraacetic acid, disodium salt, dihydrate (EDTA2Na) for 3 days, the sections were embedded in paraffin and 5 μm sections were prepared. For immunostaining with antitype I collagen and type II collagen antibodies, samples were fixed for 2 h, carefully separated from subchondral bone, and snap frozen in liquid nitrogen. Then, 6 μm frozen sections were prepared.

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STAINING

All specimens were examined with HE staining and Safranin-O staining with standard procedure. Representative 18 to 21 specimens from 10 knee joints were examined by immunohistochemistry as follows. After deparaffinization, the sections were incubated with 3% hydrogen peroxide/methanol for 5 min, followed by digestion with proteinase K (DAKO, CA, U.S.A.) without dilution for 5 min. Then sections were incubated with antihuman IL-1β polyclonal antibody (ENDOGEN, MA, U.S.A.), at a dilution of 1:500, antihuman MMP-3 monoclonal antibody (Fuji Chemical, Japan), at a dilution of 1:200, antihuman CD68 monoclonal antibody (DAKO), at a dilution of 1:200, antihuman type I collagen monoclonal antibody (SIGMA, MO, U.S.A.), at a dilution of 1:200, and antihuman type II collagen polyclonal antibody (SANBIO, Uden, The Netherlands), at a dilution of 1:500. As negative controls, sections were incubated with non-immune serum of respective animals. After incubation with the first antibody, each step was followed by extensive washing in PBS. Reaction with primary antibody was then followed by incubation for 30 min with horseradish peroxidase (HRP)-conjugated antimouse IgG (Nichirei, Japan) at a concentration of 4 μg/ml at 3 points for MMP3, CD68 and type I collagen, HRP-conjugated antirabbit IgG (Nichirei, Japan) at a concentration of 4 μg/ml for IL-1β and HRP-conjugated antigoat IgG (Nichirei, Japan) at a concentration of 4 μg/ml for type I collagen. Then, the sections were visualized with 3,3′-Diaminobenzidine tetrahydrochloride (DAKO) and counterstained with Mayer’s Hematoxylin (Wako Chemicals, Japan).

The number of IL-1β and MMP3 positive cells was evaluated by counting up to 200 cells in each area of vascular or fibrous type of pannus-like tissue and underlying cartilage.

STATISTICAL ANALYSIS

The frequency was expressed as the mean±standard error. To compare the values between different zones, Wilcoxon signed-ranks test was used for statistical analysis.

Result

FREQUENCY OF PANNUS-LIKE TISSUE

Histological observation showed that pannus-like tissue was found in 14 out of 15 (93.3%) knee joints and four out of five (80%) hip joints. Accordingly, 90% of OA joints had pannus-like tissue (Table I). Pannus-like tissue was macroscopically evident in one hip and 4 knee specimens. Picture of pannus-like tissue is shown in (Fig. 1).
HISTOLOGICAL FINDINGS OF PANNUS-LIKE TISSUE

Histologically, pannus-like tissue could be classified into two types: the vascular type and the fibrous type (Fig. 2). The vascular type was characterized by hypervascularity, hypercellularity and distinct border between cartilage and pannus-like tissue. Some cells in vascular type tissue accumulated in the junction between cartilage and pannus-like tissue [Fig. 2(b)]. Contrary to the vascular type, the fibrous type was characterized by avascularity, hypocellularity and indistinct junction. In the fibrous type, fibroblastic cells were arranged scattered and no vessels were seen [Fig. 2(e)].

LOCALIZATION, FREQUENCY AND EXTENTION OF PANNUS-LIKE TISSUE

To clarify the localization of pannus-like tissue, we divided the surface of OA cartilage into three zones [Fig. 3(a)]. The region within 5 mm from joint margin was defined as zone I (marginal zone), the region within 5 mm from eburnated bone was defined as zone III (paraeburnated zone) and the intermediate region between them was defined as zone II (intermediate zone) where cartilage keeps its thickness and morphology well. Frequency of pannus-like tissue was calculated in individual specimens. The overall frequency was the average of the frequency of each specimen. Regarding to OA knee joint, pannus-like tissue occurred in 45.9±6.7% (mean±S.E.) in zone I, 27.5±5.3% in zone II and 11.1±4.5% in zone III. Vascular type tissue occurred in 20.0±5.6%, 8.3±3.0% and 3.6±2.5% of zones I, II and III respectively. Fibrous type tissue occurred in 34.0±6.5%, 23.5±5.4% and 11.1±4.5% of zones I, II and III respectively. Regarding to OA hip joint, pannus-like tissue occurred in 61.3±21.4 % (mean±S.E.) in zone I, 28.9±11.8% in zone II and 10.0% in zone III. Vascular type tissue occurred in 55.0±21.0%, 2.2±2.2% and 10.0% of zones I, II and III respectively. Fibrous type tissue occurred in 15.0±11.9%, 27.8±11.5% and 10.0% of zones I, II and III respectively. The frequency of zone I was highest compared to the other zones, especially in the vascular type tissue. In zone II, fibrous type tissue was predominant (Table II).

To clarify the extension of pannus-like tissue on cartilage, we measured the length of pannus-like tissue and cartilage of knee samples with microscope in each knee.
was dominant in zone II*. Fibrous type tissue occupied predominantly [Fig. 3(b)].

In other zones, especially in the vascular type tissue. In zone I, the % area of zone I was also highest compared to the other zones, especially in the vascular type tissue. In zone III, % area 10.1±3.0% and 3.1±1.3% of zones I, II and III respectively. Fibrous type tissue occupied in 16.6±3.1%, 4.2±1.9% in zone III. Vascular type tissue occupied the % area of each specimen. Percent area of pannus-like tissue was calculated in individual specimens. The overall % area was the average of the % area of each specimen. Pannus-like tissue occupied 27.6±4.7% (mean±s.e.) in zone I, 12.5±4.0% in zone II and 4.2±1.9% in zone III. Vascular type tissue occupied 11.0±3.2%, 2.4±1.2% and 0.9±0.7% of zones I, II and III respectively. Fibrous type tissue occupied in 16.6±3.1%, 10.1±3.0% and 3.1±1.3% of zones I, II and III respectively.

The % area of zone I was also highest compared to the other zones, especially in the vascular type tissue. In zone II, fibrous type tissue occupied predominantly [Fig. 3(b)].

CHARACTERISTICS OF PANNUS-LIKE TISSUE

To clarify the extracellular matrix composition of pannus-like tissue, tissues were stained by Safranin-O and immunostained by antitype I and type II collagen antibodies. Extracellular matrix of both vascular and fibrous types were negative for Safranin-O staining and type II collagen, and positive for type I collagen (Fig. 4). This result indicates that pannus-like tissue does not include proteoglycan and type II collagen, suggesting it is not mature hyaline cartilage, but has fibrous property.

IMPLICATION OF PANNUS-LIKE TISSUE

To know the destructive nature of pannus-like tissue, specimens were immunostained by anti-IL-1β and MMP3 antibodies. Many pannus-like tissue cells as well as chondrocytes expressed IL-1β and MMP3, in the vascular type tissue, the positive rate of IL-1β and MMP3 were 40% and 34% respectively. The rates rose up to 77% and 55% in the area of junction. In the fibrous type tissue, the positive rates were 63% and 42%, and positive cells were diffusely arranged (Fig. 5, Table III).

ORIGIN OF PANNUS-LIKE TISSUE

Some samples showed that bone marrow cells gave birth to vascular type tissue in zone III and proliferative connective tissue on bare area had continuity with pannus-like tissue in zone I (Fig. 6). CD68 positive cells in pannus-like tissue were very few in all specimens examined (data not shown), whereas a lot of CD68 positive cells infiltrated into RA pannus tissue. Figure 7 shows representative microphotographs of expressing CD68 in pannus-like tissue of OA and RA pannus tissue. These findings suggest that the origin of pannus-like tissue of OA is different from RA pannus and that vascular type tissue cell was presumably originated from mesenchymal cells of bone marrow or around diaphyseal tissue. Fibrous type tissue exists predominantly in zone II; intermediate zone [Fig. 3(b), Table II].

Discussion

In previous articles written in the 1970s and 1980s, pannus-like tissue of OA was described in histological studies as just one of the histological findings of OA cartilage. These examinations were done with HE, Safranin-O and toluidine blue staining and the authors did not show any distinct evidence that suggested the function of pannus-like tissue. In the present study, we further analysed pannus-like tissue in terms of characteristics, implication and localization by histochemistry and immunohistochemistry. The following novel results were found: (1) Pannus-like tissue preferentially located in the marginal area; (2) Pannus-like tissue was classified into two different tissue types, the vascular type and the fibrous type; (3) Most of cells in pannus-like tissue expressed catabolic factors, IL-1β and MMP3; (4) There were only a few CD68 positive cells in pannus-like tissue.

Matrix metalloproteinase (MMPs) are reported to play important roles in cartilage destruction in RA and OA. They are produced both by chondrocytes and synovocytes especially stimulated by IL-1 and TNF-α. They are also produced by RA pannus, which is implicated in cartilage destruction in RA.

Some previous articles suggested that pannus-like tissue in OA might contribute to cartilage erosion without evidence about how it affected cartilage destruction. Others described pannus-like tissue as regenerative tissue. At first, we also considered that pannus-like tissue was able to repair injured cartilage. On the contrary, it expressed no cartilaginous matrices, moreover pannus-like tissue cells expressed catabolic factors such as IL-1β and MMP3. These findings strongly suggest that pannus-like tissue contributes to cartilage erosion. In the vascular type tissue, there was high accumulation of IL-1β and MMP3 positive cells in the area of junction, which strongly suggested its direct effect on cartilage erosion. In the fibrous type tissue, IL-1β and MMP3 expressing cells were arranged diffusely.

Pannus-like tissue cells seem one of resources of pro-inflammatory cytokines and proteases like chondrocytes and synovocytes in OA joint. Synovocytes are one of the possible candidates for pannus in RA. In RA, continuity between proliferated...
Table II
Frequency of pannus-like tissue in each zone

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Fig. 4. Characteristics of extracellular matrix of pannus-like tissue on OA cartilage. (a)–(c) Vascular type tissue. (d)–(f) Fibrous type tissue. Characteristics were estimated by Safranin-O staining (a),(d), by type I collagen immunostaining (b),(e), and by type II collagen immunostaining (c),(f). Pannus-like tissue was negative in both Safranin-O staining and type II collagen and positive by type I collagen.

Fig. 5. Immunohistochemistry with IL-1β and MMP3 in OA cartilage. (a),(b) Vascular type tissue. (c),(d) Fibrous type tissue. (e),(f) Negative control. (a),(c),(e) IL-1β immunostaining, (b),(d),(f) MMP3 immunostaining. In vascular type, many pannus-like tissue cells expressed IL-1β and MMP3. The expression was intense in the area of junction between cartilage and pannus-like tissue (arrows). IL-1β and MMP3 positive cells spreaded diffusely in fibrous type. P: pannus-like tissue. C: cartilage. Bar=100 μm.
synovial tissue and RA pannus is often found; however, such continuity was lacking in our series. Moreover, there were far fewer CD68 positive cells in pannus-like tissue in OA, compared to RA pannus (Fig. 6), suggesting that their origin was different. Considering the findings that showed the continuity between pannus-like tissue and bone marrow, mesenchymal cells are the most probable candidate for vascular type tissue.

Two candidates of origin are expected in fibrous type tissue. The first candidate is mesenchymal cells, because fibrous type tissue was usually continuous with vascular type tissue. Vascular type tissue gradually loses its vascularity and hypercellularity, and is transformed into fibrous type tissue. The second candidate is hyaline cartilage, because the junction is indistinct and fibrous type tissue seemed to be altered from true cartilage [Fig. 2(d)]. Chondrocytes might be transformed in the pathogenic circumstance in OA, causing fibrous metaplasia.

Although these cells may infiltrate to repair injured cartilage, they may change to destructive phenotype under the specific circumstance, as the joints we examined were at highly advanced stage. It is possible that chondrocytes change their phenotype and original cartilage transforms its property. The vascular type tissue may transformed to the fibrous type tissue. In any case these two types of tissue have catabolic feature.

We need to focus on the fact that pannus-like tissue has direct and indirect catabolic functions to OA cartilage and pannus-like tissue becomes the novel therapeutic target.

Acknowledgments

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