Osteoarthritis and Cartilage (2002) **10,** 156–162
© 2002 OsteoArthritis Research Society International
doi:10.1053/joca.2001.0494, available online at http://www.idealibrary.com on IDEAL®
1063–4584/02/020156+07 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Increased cellular infiltrate in inflammatory synovia of osteoarthritic knees

I. Saito, T. Koshino, K. Nakashima, M. Uesugi and T. Saito
Department of Orthopaedic Surgery, Yokohama City University School of Medicine, Yokohama, Japan

Summary

Objective: To determine the nature and origin of synovial inflammation in knees with osteoarthritis (OA).

Method: Synovial samples were obtained from 21 medial compartmental knee OAs from 19 patients. First, using 11 medial knee synovial samples from 9 patients, a quantitative estimation of synovitis was made with both ordinary and immunohistochemical staining. Second, from the other 10 knees, tissue samples were taken from both the medial and the lateral compartments to quantify cells that infiltrated into the synovium. Medial synovium was immunostained using antibodies to anti-type II collagen, CD68, CD2, CD4, CD8, CD15, CD19, CD25, HLA-DR, CD1a and LN5. The lateral synovium was immunostained with anti-type II collagen, CD68, HLA-DR and CD4 antibody as a control.

Result: Denatured cartilaginous detritus was found captured by synovial lining cells with a strong immunoreactivity to CD68 antibody, and whose phagocytic potential was activated. The number of anti-type II collagen-positive fragments in the medial compartment of the knee was larger than that found in the lateral compartment. Moreover, the population of CD68-positive cells in synovial tissue and HLA-DR-positive cells in the lining layer was larger in the medial compartment than in the lateral compartment. The number of CD4-positive cells (defined as helper/inducer T lymphocytes) was greater in medial synovium than in lateral synovium.

Conclusion: Overall, this study strongly supports the concept that the synovitis observed in patients with knee OA might be induced by an immunological mechanism involving, to some extent, a macrophage/helper T cell interaction. © 2002 OsteoArthritis Research Society International

Key words: Osteoarthritis of the knee, Synovitis, Cartilage fragment, Cellular infiltration.

Introduction

Primary osteoarthritis (OA) of the knee is one of the most common knee disorders among the elderly. It is usually bilateral and induces pain, restriction of motion and progressive varus or valgus deformity. It has been deemed a less inflammatory disease than rheumatoid arthritis (RA)[1]. Pathological findings of RA synovium are synovial proliferation with inflammatory cell infiltrates. A detailed subset of infiltrated lymphocytes in synovitis was studied[2,3,4], and the relationship between the cell infiltrates and the chemical mediator, such as IL-1, IL-8, IL-10, IL-15, IFN-γ and TNF-α were reported[5,6,7,8,9,10]. Synovium of OA has been used as a control for RA. However, several investigators pointed out the presence of prominent inflammatory pathology in the synovial membrane of knees with OA. According to our observation during joint surgery, the surface of the synovial membrane often shows signs of inflammation, including synovial proliferation with villous formation and congestion, and the severity of the synovitis varies depending on whether the medial, the lateral or the suprapatellar portion of the knee joint is affected. The characteristic features of synovial changes in knee OA are thought to be mild hyperplasty of synovial lining cells, interstitial edema, increased vascularization and moderate cellular infiltration of subsynovial tissue[11,12,13,14].

Patients with medial compartmental OA of the knee usually complain of pain, the main location of which is the anterior part of the medial joint space. Inflammation may be closely related to pain provocation[15]. Synovitis can be induced by multiple factors, for instance, cartilage fragments and debris, joint instability and neurogenic factors[15,16]. The causes of synovitis are still unclear. Thus, during surgery we obtained synovial samples from the medial side of 21 knees which were almost equally affected by medial compartmental OA, and investigated the nature and the origin of synovitis using an immunohistochemical staining technique with monoclonal antibodies.

Received 27 July 2000; revision requested 16 October 2000; revision received 8 March 2001; accepted 2 September 2001.

Correspondence to Tomoyuki Saito, MD, PhD, Associate Professor, Department of Orthopaedic Surgery, Yokohama City University School of Medicine, 3–9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. Tel: +81-45-787-2655; Fax: +81-45-781-7922; E mail: t_saito@med.yokohama-cu.ac.jp

Materials and methods

PATIENTS

Synovial samples were obtained from the medial compartment of 21 knees in 19 patients (14 women, 5 men) during joint surgery. From 10 of these knees synovial samples were also taken from lateral compartments. The patients fulfilled the criteria defined by the American College of Rheumatology[17] and the knees were classified as medial compartmental knee OA according to Albäck's classification[18]. The patients gave informed consent for enrollment into the study and had surgical interventions (19 high tibial osteotomies, 2 unicompartmental knee arthroplasties). The patient's mean age at surgery was 64 years,

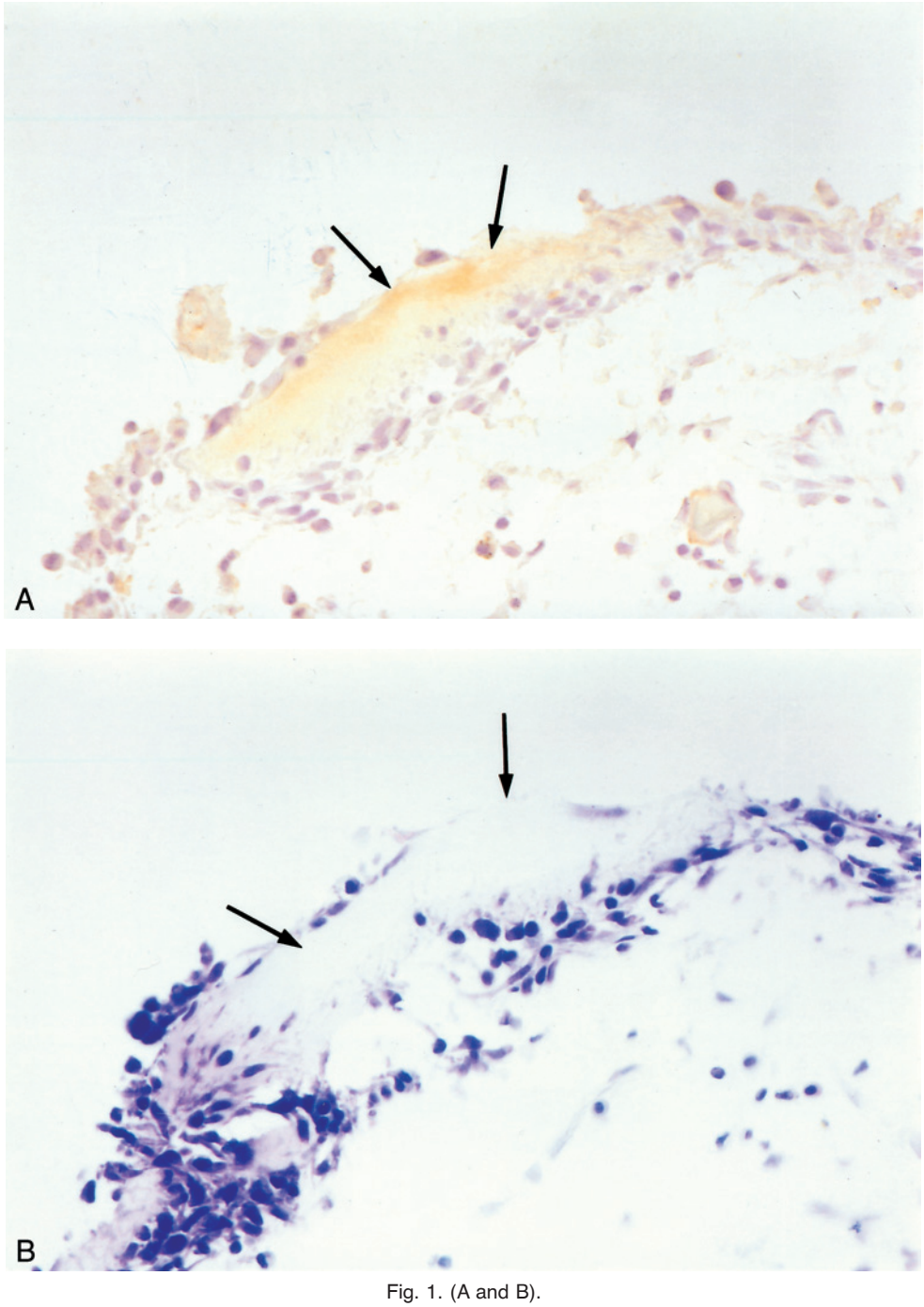

Fig. 1. (A and B).

ranging from 47 to 78 years. All patients complained of pain at rest around the medial joint space and disability caused by severe pain when walking and descending or ascending stairs. Walking ability was restricted to less than 200 m in most patients.

Synovial tissue samples measuring 7 mm square were obtained with a sharp scalpel under a direct vision at the time of surgery from a site 1 cm proximal to the medial or lateral meniscus. The medial synovium was constantly exposed to mechanical stimuli exerted by osteophytes that had developed around the medial femoral condyle. Ten samples taken from the lateral compartments of the knees were used as a control. Tissue samples were immediately frozen in optimal cutting temperature (OCT) compound (Tissue-Tek; Miles Laboratories, Naperville, IL) and stored at −80°C in a deep freezer. The frozen tissue samples were sliced at a thickness of 5 to 6 μm at −30°C using a cryostat (Sakura Coldtome CM-502, Sakura Seiki, Tokyo) and mounted onto slides coated with poly-L-lysin (Sigma Diagnostics, St Louis, MO) as adhesive.

MONOCLONAL ANTIBODIES

The specimens were immunostained using the following mouse monoclonal antibodies: anti-type II collagen antibody (Fuji Yakuhin, Toyama, Japan); anti-CD68 antibody,

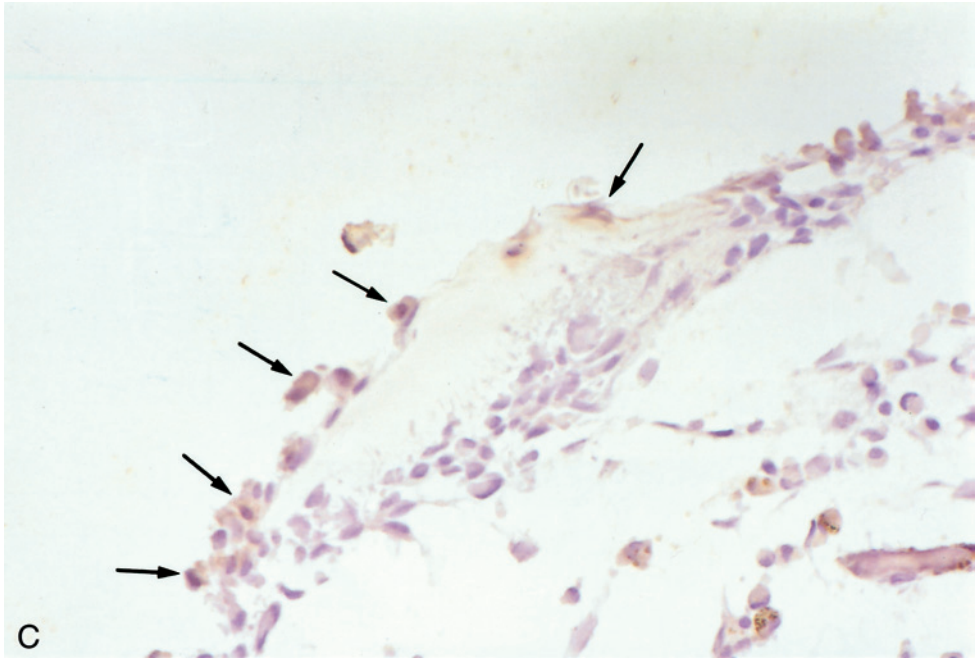

Fig. 1. (C).

Fig. 1. Medial synovium from the medial compartmental osteoarthritic knee of a 68-year-old woman (original magnification, 400×). (A) Photomicrograph showing a large type II collagen-positive cartilage fragment entrapped by synoviocytes in the synovial lining layer (arrows) (counter stained with hematoxylin). (B) Photomicrograph showing that the fragment did not show any metachromasia even after toluidine blue staining (arrows), indicating loss of glycosaminoglycans (toluidine blue stain). (C) Photomicrograph revealing that the cells enriching the denatured fragment reacted positively with anti CD68-antibody (arrows), and that their phagocytic activity was up-regulated.

specific for macrophage lineage cells[19,20] (EBM11, DAKO, Glostrup, Denmark); anti-CD2, for T cell identification (IMMUNOTECH, Cedex, France); anti-CD8, a cytotoxic/suppressor T cell one (IMMUNOTECH, Cedex, France); anti-CD19 for all B cells (IMMUNOTECH, Cedex, France); anti-CD4 for a helper/inducer T cell subset (IMMUNOTECH, Cedex, France); anti-CD15 for granurocytes (IMMUNOTECH, Cedex, France); anti-CD1a for dendritic cells (IMMUNOTECH, Cedex, France); anti-CD25 for T and B cells with IL-2 receptor[21] (IMMUNOTECH, Cedex, France); anti-HLA-DR for enumeration of cell subsets of the immune system expressing the Class II antigen[21] (IMMUNOTECH, Cedex, France); and LN-5 for macrophages and histiocytes in hematopoietic organs[22] (mouse monoclonal antibody Macrophages, IMMUNOTECH, Cedex, France).

IMMUNOHISTOCHEMICAL STAINING

First, using 11 tissues samples from 9 patients, thin slices of tissue mounted on glass slides were fixed in 10% neutral buffered formalin and stained with hematoxylin and eosin, and toluidine blue for quantitative estimation of synovitis. Other frozen sections from these 11 samples mounted on slides were air-dried for 1 to 2 h at room temperature and then fixed in cold acetate at 4°C for 10 min. Immunohistochemical staining was carried out using anti-CD68 antibody and mouse anti-type II collagen antibody.

Second, the remaining 10 samples from the medial compartments were used to quantify the population of cells infiltrated into the subsynovial tissue using immunohistochemical staining with mouse monoclonal antibodies against the epitopes of CD68, CD2, CD4, CD8, CD15, CD19, CD25, HLA-DR, CD la and LN5. Cartilagenous fragments entrapped by lining cells were counted in the sections immunostained with anti-type II collagen.

Third, synovial samples obtained from the lateral compartments of these same 10 knees were immunostained with type II collagen antibody, CD68, HLA-DR and CD4 in the same manner. Toluidine blue-staining was also performed on 10 pairs of medial and lateral samples.

In the process of immunohistochemical staining endogenous peroxidase activity at first was quenched with H_2O_2. The sections were incubated with properey diluted primary antibody at room temperature for 30 min in a humidity chamber. The tissue samples were immunostained according to the avidin-biotin-peroxidase complex technique using Vector Elite ABC Kit (Vector Laboratories, Burlingame, CA) and the color was developed with diaminobenzidine (Dojindo, Kumamoto, Japan) and H_2O_2. The sections were counterstained with hematoxylin or methyl green. As a negative control, nonspecific purified mouse immunoglobulin G (Charles River, South Bridge, MA) was used instead of the monoclonal antibodies.

HISTOLOGICAL ANALYSIS

In the first 11 samples, CD68-positive cells and anti-type II collagen immunoreactive cartilage fragments were counted in five visual fields randomly selected in each specimen under a microscope (BH-2, Olympus, Tokyo). Among 5 obtained values, the highest one was recorded as the representative for each sample. In the other 10 samples in order to compare the difference in the populations of

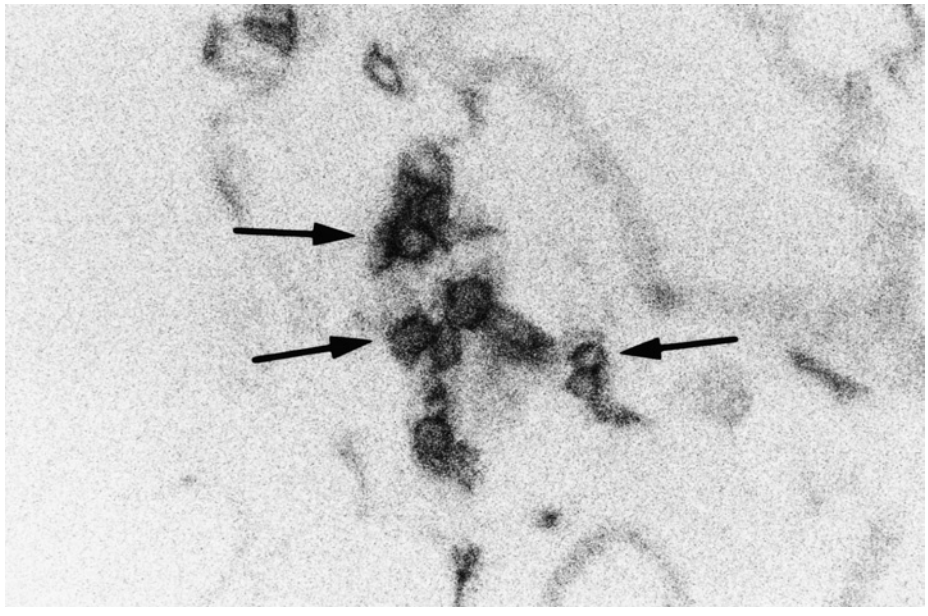

Fig. 2. Medial synovium taken from the medial compartmental osteoarthritic knee of a 66-year-old woman. Photomicrograph showing moderate infiltration of CD4-positive cells in the subsynovial tissue (arrows), as revealed by immunohistochemical staining with anti-CD4 antibody (original magnification, 200×).

Table I
Number of cells positive to primary antibody in medial synovium in medial compartment of osteoarthritic knees

Primary antibody	Synovial layer	Number of positive cells† (mean±S.D.)	Ratio* (mean±S.D.)
CD4§	Lining	3.2±2.4	0.09±0.05
	Sublining	4.97±3.4	0.10±0.41
CD8	Lining	0.54±0.38	0.03±0.04
	Sublining	1.8±1.4	0.02±0.03
CD2	Lining	4.7±3.7	0.12±0.16
	Sublining	5.4±4.3	0.18±0.15
CD25	Lining	1.9±3.2	0.08±0.18
	Sublining	2.9±5.2	0.06±0.08
CD1a	Lining	3.9±5.3	0.13±0.08
	Sublining	5.4±8.8	0.10±0.14
CD19	Lining	2.0±2.7	0.06±0.18
	Sublining	2.6±3.8	0.04±0.06
HLA-DR	Lining	13.6±6.6	0.38±0.16
	Sublining	13.6±8.2	0.23±0.08
CD15	Lining	1.8±1.6	0.06±0.06
	Sublining	2.1±2.0	0.05±0.05
LN5	Lining	6.3±9.5	0.14±0.14
	Sublining	9.1±16.1	0.11±0.10
CD68	Lining	18.1±4.4	0.50±0.12
	Sublining	9.9±3.2	0.23±0.10

†The average number of positive cells per field under 400× magnification.
*Ratio of the number of positive cells to total cells.
§CD4-positive round cells defined as helper/inducer T-lymphocytes.

Table II
Number of cells positive to primary antibody in lateral synovium in medial compartment of osteoarthritic knees

Primary antibody	Synovial layer	Number of positive cells† (mean±S.D.)	Ratio* (mean±S.D.)
CD4§	Lining	1.8±1.1	0.07±0.04
	Sublining	1.9±1.0	0.07±0.03
HLA-DR	Lining	6.7±4.6	0.23±0.12
	Sublining	5.7±2.8	0.24±0.07
CD68	Lining	5.8±3.7	0.25±0.13
	Sublining	3.3±1.5	0.12±0.04

†The average number of positive cells per field under 400× magnification.
*Ratio of the number of positive cells to total cells.
§CD4-positive round cells defined as helper/inducer T-lymphocytes.

antibody-positive cells between the medial and lateral compartments, five visual fields with a large number of positive cells were chosen in each specimen. Under a 400× magnification, the number of positive cells and the number of total cells appearing in each field were counted. The ratio of average number of positive cells to average number of total cells was calculated for each sample. Among CD4-positive cells, only cells with a round shape and small size, defined as helper/inducer T-lymphocytes, were counted since CD4 antigens also emerged on some macrophage lineage cells as well as helper/inducer T-lymphocytes[23]. Type II collagen-positive cartilage fragments were counted in five randomly selected visual fields in each specimen under 400× magnification and the average number was recorded.

STATISTICAL ANALYSIS

All measured variables were entered in a computer database (StatView for Windows, Version 4.58; Abacus Concepts Inc, Berkeley, CA). Fisher's PLSD (non-parametric) was employed for multiple comparisons. The Student's paired T test was used to compare the difference between two variables. *P* values less than 0.05 were considered significant.

Result

HISTOLOGICAL FINDINGS OF SYNOVITIS IN THE MEDIAL COMPARTMENT

In the first 11 synovial specimens stained with hematoxylin and eosin, the most predominant histological finding in the medial synovium of knee OAs was vascular proliferation in the edematous subsynovial tissue. Scattered or moderate cellular infiltrates were observable in all specimens. All specimens presented focal or diffuse villous hyperplasia on the superficial layer of the synovium. However, 5 of the 11 specimens showed multiple layers synovial lining cells. In 5 specimens stained with toluidine blue, there were round-shaped mast cells containing a large amount of toluidine blue-staining material. Mast cells were mainly present in the immediate vicinity of blood vessels, which averaged 9 per field under 400× magnification.

Immunohistochemical staining using anti-type II collagen showed the positive-staining cartilaginous fragments entrapped by the synovial lining cells in the proliferating synovial villi in all specimens. The size of the fragments varied greatly and some of the fragments did not show metachromasia even after staining with toluidine blue [Fig. 1(A–C)]. The number of these fragments per field under 400× magnification ranged from 3 to 26 with an average of 9.1±6.2.

Immunoreactivity against the CD68 antibody was detectable on the surface of cells present in the synovial lining layer and the subsynovial tissue in all 11 specimens. The proliferating lining cells in the synovial villi which surrounded cartilage fragments showed strong immunoreactivity to the CD68 antibody. The mean number per field of these CD68-immunoreactive cells was 30.8±11.0 in the synovial lining layer, ranging from 17 to 55 cells. Whereas, it was 14.5±9.6 (range 5 to 32) in the subsynovial tissue. Therefore, the immunoreactivity and the number of CD68-positive cells were more predominant in the synovial lining layer than in the subsynovial tissue.

COMPARISON OF CELL POPULATION BETWEEN THE MEDIAL AND LATERAL COMPARTMENTS

There were scattered or moderate cellular infiltrates present in all of the 10 synovial tissue samples taken from the medial compartments. Immunohistochemical staining revealed that the majority of the cells in the lining layer of the medial compartment were CD68-positive cells that had lisosomal activity, followed by HLA-DR-positive cells that were antigen-presenting cells. There was a significantly higher emergence rate of CD68- and HLA-DR-immunoreactive cells than other types of antibody-positive cells in the medial synovium ($P<0.005$). The average ratio of CD68- and HLA-DR-positive cells to the total cells in a visual field was 0.5±0.12 and 0.16±0.18 in the lining layer, and 0.23±0.10 and 0.23±0.08 in the sublining layer, respectively. In the sublining layer of the medial synovium, distribution of HLA-DR-positive cells showed the same pattern as CD68-positive cells.

The CD4-positive round cells were present as a scattered infiltrate in the synovial sublining layer from the medial compartments (Fig. 2). This represented approximately 30% of the total CD4-positive cells. The other subset of T-lymphocytes, namely CD8, CD2 and CD19-positive cells, that defined all B cells, were in low numbers

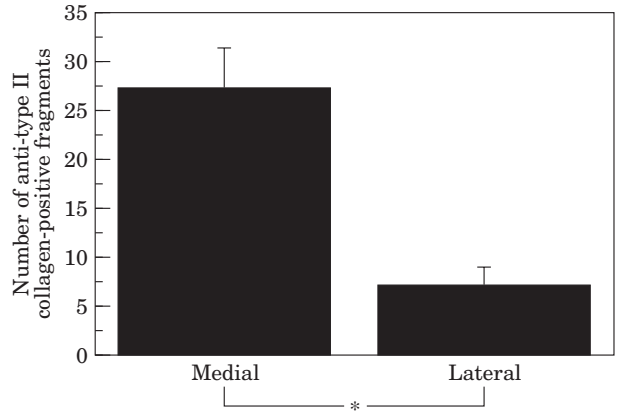

Fig. 3. Comparison between the medial and lateral compartments of the number of anti-type II collagen-positive cartilage fragments in the synovium. The longitudinal axis indicates the average number per field of anti-type II collagen fragments at 400× magnification. There is a significant difference in the number of fragments between the medial and the lateral synovium. *$P<0.005$.

in the lining and sublining layers. The average ratio of CD4-positive round cells to the total cells was significantly higher than that of CD8 positive cells (cytotoxic/suppressor T lymphocytes) (Table I).

In the lateral synovium CD68- and HLA-DR-positive cells were also found. However, the average ratio of CD68-positive cells to total cells was 0.25±0.13 for the lining layer and 0.12±0.04 for the sublining layer. This was almost one half the ratio in the medial synovium (0.5±0.12 for the lining layer and 0.23±0.10 for the sublining layer). There was a significant difference in the average ratio of CD68-positive cells between the medial and the lateral synovium ($P<0.05$). The average ratio of HLA-DR-positive cells to total cells was 0.38±0.16 for the lining layer and 0.23±0.08 for the sublining layer in the medial synovium. It was 0.23±0.12 for the lining layer and 0.24±0.07 for the sublining layer in the lateral synovioum, indicating a significant difference only in the lining layer ($P<0.05$).

The average ratio of CD4-positive cells to total cells in the medial synovium was 0.09±0.05 for the lining layer and 0.10±0.41 for the sublining layer, and it was 0.07±0.04 for the lining layer and 0.07±0.03 for the sublining layer in the lateral synovium (Tables I and II). This constituted no significant difference in the ratios. However, the number of the cells in both layers of the medial synovium was significantly larger than that of the lateral synovium ($P<0.05$).

Regarding cartilage fragments entrapped by synovial lining cells, the average number was 27.4±11.6 (range 16.6 to 56.6) in the medial synovium, and 7.2±5.8 (range 0 to 18.6) in the lateral synovium. This was a significant difference ($P<0.005$) (Fig. 3).

Discussion

The materials used in this study were synovial tissue obtained from patients with advanced medial compartmental osteoarthritis of the knee at the time of surgery. Their medial femoral condyle showed massive cartilaginous loss on the weight bearing area and eburnation of subchondral bone. Although the cartilage was yellowish, the articular surface of the lateral compartment was well maintained. Synovitis in osteoarthritic knees has been reported as being confined to the synovial membrane located close to

the degenerative cartilage[13,24]. In the present study, macroscopic observation also revealed that the synovium in the medial compartment showed villous formation of a reddish color due to increased circulation, and was thicker than the synovium in the lateral compartment. These findings indicate that the degree of synovitis may vary depending on the site. In patients with medial compartmental osteoarthritis of the knee, medial soft tissues, including the medial collateral ligament and capsule, are more contracted than the lateral soft tissues[25]. Therefore, the medial synovium is impinged between horizontally protruded osteophytes around the medial condyle of the tibia and the contracted medial collateral ligament which tightens on knee extension. Moreover, varus-deformed knees show adduction movement (lateral thrust)[26] and the tibia is externally rotated when standing (screw-home movement). Thus, during movement, the medial synovium and the articular cartilage are biomechanically stimulated, contributing to the occurrence of synovitis.

Under these circumstances, our histopathological study of the medial synovium affected by osteoarthritis of the knee clearly demonstrated the presence of synovitis, which agreed with the findings described in previous reports[11,12,13,14]. Furthermore, our immunohistochemical study using several antibodies sensitive to cells present in synovial tissue clarified the nature and origin of the synovitis in osteoarthritic knees. The synovial membrane had incorporated fragments of cartilage that originated from the worn-out articular surface. Although these fragments immunoreacted with anti-type II collagen antibody, the fragments lacked metachromasia when stained with toluidine blue, indicating loss of glycosaminoglycsans[27]. The denatured cartilaginous detritus was recognized as a foreign body and entrapped by the synovial lining cells that had a strong positive reaction with anti-CD68 antibody. The cellular immunoreactivity against anti-CD68 antibody has been reported as indicating a high specificity for cells of the macrophage lineage and to react primarily with lysosomes in these cells. The expression of CD68 indicated up-regulation of the phagocytic activity of these cells[19]. Moreover, from the results of the current study, HLA-DR-expressing cells were found to be abundant in the synovium and to locate mainly in the superficial layer of the synovium. The MHC class II molecules, that is HLA-D, especially react to B-cells, antigen-presenting cells and macrophages[21]. Therefore, it is assumed that the HLA-DR-positive cells found in the synovium may have originated from the macrophage-like cells immunostained with anti-CD68 antibody in the synovial lining.

Although patients recruited for this study complained of pain on the medial side of the knee and suffered from medial compartmental knee OA, this study did not demonstrate significant differences in the ratio of CD4-positive cells between the medial and lateral compartments, though the actual number of CD4-positive cells in the medial synovium was found to be significantly larger than in the lateral synovium. Furthermore, CD4-positive cells were detected more frequently than CD8-positive cells in the medial synovium. While it is said that the ratio of CD4-positive cells to CD8-positive ones is about 2:1 in peripheral blood, this study showed that the ratio of CD4-positive cells to CD8-positive ones in the sublining layer of the medial synovium was much higher (5:1). Tak *et al.* pointed out that there was a specific subset of CD4-positive cells which played a very important role in the pathogenesis of synovitis in RA[23]. Therefore, this group of CD4-positive cells may increase, which is likely to be closely related to the development of synovitis in osteoarthritic knees. The number of B-lymphocytes that secreted immunoglobulins was quite small and they were found scattered in the subsynovial tissue of knees with osteoarthritis. These findings were similar to one of the synovial abnormalities of RA[28].

Generally speaking, it is recognized that exogenous antigens are taken up by macrophages and broken down in lysosomes. The antigens reappear on the surface of macrophages as a processed peptide associated with the MHC class II[21]. In this study as the macrophage-like cells found among synovial lining cells had taken up fragments of type II collagen, it was thought that the fractions of the collagen were broken down in lysosomes of lining cells, and the peptide-originated fraction of the collagen emerged on the surface of the cells with the HLA-D locus, HLA-DR, and stimulating helper/inducer T lymphocytes[21,29]. Furthermore, it was suggested that highly activated lining cells may modulate synovial inflammation and destruction of cartilage by releasing IL-1, TNF-α, and other soluble cytokines[10,30].

There have been several reports on the antigenicity of collagen and cellular reactivity in human disease[31,32,33]. Lymphocytes from patients with rheumatoid arthritis were thought to exhibit cellular sensitivity to native human type II and type III collagens, whereas those obtained from other kinds of arthritis, including osteoarthritis, did not show any response to collagen[32]. Therefore, to provoke and propagate inflammation of the synovium in a knee with OA, the existence of antigen-presenting macrophages derived from the synovial lining cells may be mandatory to transmit the message of activated synovial cells that engulf denatured cartilaginous fragments into T lymphocytes.

From the results of the authors' comprehensive investigation of cell characterization in synovium of osteoarthritic knees, it was concluded that severity of synovitis depended on the involved site in a knee joint, and synovitis may be induced by an immunological mechanism involving macrophage/helper T-cell interaction to some extent, as well as local mechanical factors.

Acknowledgments

This work was supported by Grant-in-Aid for Scientific Research (C) of The Ministry of Education, Science, Sports and Culture.

References

1. Goldenberg DL, Cohen AS. Synovial membrane histopathology in the different diagnosis of rheumatoid arthritis, gout, pseudogout, systemic lupus erythematosis, infectious arthritis and degenerative joint disease. Med 1978;57:239–52.
2. Shimaoka Y, Attrep JF, Hirano T, Ishihara K, Suzuki R, Toyosaki T, *et al*. Nurse-like cells from bone marrow and synovium of patients with rheumatoid arthritis promote survival and enhance function of human B cells. J Clin Invest 1998;102:606–18.
3. Kiener HP, Baghestanian M, Dominkus M, Walchshofer S, Ghannadan M, Willheim M, *et al*. Expression of the C5a receptor (CD88) on synovial mast cells in patients with rheumatoid arthritis. Arthritis Rheum 1998;41:233–45.

4. Cush JJ, Pietschmann P, Oppenheimer-Marks N, Lipsky PE. The intrinsic migratory capacity of memory T cells contributes to their accumulation in rheumatoid synovium. Arthritis Rheum 1992;35: 1434–44.
5. Cauli A, Yanni G, Panayi GS. Interleukin-1, interleukin-1 receptor antagonist and macrophage populations in rheumatoid arthritis synovial membrane. Br J Rheumatol 1997;36:935–40.
6. Konig A, Krenn V, Gillitzer R, Glockner J, Janssen E, Gohlke F, *et al*. Inflammatory infiltrate and interleukin-8 expression in the synovium of psoriatic arthritis—an immunohistochemical and mRNA analysis. Rheumatol Int 1997;17:159–68.
7. Cohen SB, Katsikis PD, Chu CQ, Thomssen H, Webb LM, Maini RN, *et al*. High level of interleukin-10 production by the activated T cell population with the rheumatoid synovial membrane. Arthritis Rheum 1995;38:936–52.
8. Morita Y, Yamamura M, Kawashima M, Harada S, Tsuji K, Shibuya K, *et al*. Flow cytometric single-cell analysis of cytokine production by CD4+ T cells in synovial tissue and peripheral blood from patients with rheumatoid arthritis. Arthritis Rheum 1998;41:1669–76.
9. McInnes IB, al-Mughales J, Field M, Leung BP, Huang FP, Dixon R, *et al*. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. Nat Med 1996;2:175–82.
10. Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor α in synovial tissues and at the catilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991;34:1125–32.
11. Winkinson LS, Pistsillides AA, Edwards JC. Giant cells in arthritic synovium. Ann Rheum Dis 1993;52:182–4.
12. Goldenberg DL, Egan MS, Cohen AS. Inflammatory synovitis in degenerative joint disease. J Rheumatol 1982;9:204–9.
13. Lindblad S, Hedfors E. Arthroscopic and immunohistlogic characterization of knee joint synovitis in osteoarthritis. Arthritis Rheum 1987;30:1081–8.
14. Revell PA, Mayston V, Lalor P, Mapp P. The synovial membrane in osteoarthritis: a histological study including the characterization of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. Ann Rheum Dis 1988;47:300–7.
15. Brandt KD. Pain synovitis, and articular cartilages in osteoarthritis. Semin Arthritis Rheum 1989;18:77–80.
16. Saito T, Koshino T. Distribution of neuropeptides in synovium of the knee with osteoarthritis. Clin Orthop 2000;376:172–82.
17. Cooper C. Osteoarthritis and related disorders. Epidemiology. In: Klippel JH, Dieppe PA, Eds. Rheumatology, II. London: Mosby International 1998:8.2.1–7.
18. Ahlbäck S. Osteoarthritis of the knee. A radiographic investigation. Acta Radiol Diagn(Stockh) Suppl 1968;277:7–72.
19. Greywoode GIN, McCarthy SP, McGee JOD. Labelling of cells of the mononuclear phagocyte system in routinely processed archival biopsy specimens with monoclonal antibody EBM/11. J Clin Pathol 1990;43:992–6.
20. Kelly PM, Bliss E, Morton JA, Burns J, McGee JO. Monoclonal antibody EBM/11: High cellular specificity for human macrophages. J Clin Pathol 1988;41:510–5.
21. Roitt IM. Essential immunology. 7th edn. London: Blackwell Scientific Publications 1991:115–7.
22. Bhoopat L, Turner RR, Stathopoulos E, Meyer PR, Taylor CR, Marder RJ, Epstein AL. Immunohistochemical characterization of two new monoclonal antibodies (LN-4, LN-5) reactive with human macrophage subsets and derived malignancies in B5-fixed, paraffin-embedded tissues. Blood 1988;71:1079–85.
23. Tak PP, Smeets TJ, Daha MR, Kluin PM, Meijers KA, Brand R, *et al*. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis Rheum 1997;40:217–25.
24. Klareskog L, Johnell O, Hulth A, Holmdahl R, Rubin K. Reactivity of monoclonal anti-type II collagen antibodies with cartilage and synovial tissue in rheumatoid arthritis and osteoarthritis. Arthritis Rheum 1986;29:730–8.
25. Freeman MAR. The surgical anatomy and pathology of the arthritic knee. In: Freeman MAR, Eds. Arthritis of the knee. Clinical features and surgical management. Berlin Heidelberg New York: Springer-Verlag 1980: 41–52.
26. Koshino T, Tsuchiya K. The effect of high tibial osteotomy on osteoarthritis of the knee. Clinical and histological observations. Int Orthop 1979;3:37–45.
27. Reinmann I, Christein SB, Dimer NH. Observaions of reversibility of glicosaminoglycan depletion in articular cartilage. Clin Rel Res 1982;168:258–64.
28. Young CL, Adamson TC, Vaughan JH, Fox RI. Immunohistologic charactarization of synovial membrane lymphocyte in rheumatoid arthritis. Arthritis Rheum 1984;27:32–9.
29. Klareskog L, Forsum U, Kabelitz D, Ploen L, Sundstrom C, Nilsson K. Immune functions of human synovial cells. Phenotypic and T cell regulatory properties of macrophage-like cells that express HLA-DR. Arthritis Rheum 1982;25:488–501.
30. Deleuran BW, Chu CQ, Field M, Brennan FM, Katsikis P, Feldmann M, *et al*. Localization of interleukin-1, type 1 interleukin-1 receptor and interleukin-1 receptor antagonist in the synovial membrane and cartilage/pannus junction in rheumatoid arthritis. Br J Rheumatol 1992;31:801–9.
31. Klareskog L, Johnell O, Hulth A, Holmdahl R, Rubin K. Reactivity of monoclonal anti-type II collagen antibodies with cartilage and synovial tissue in rheumatoid arthritis and osteoarthritis. Arthritis Rheum 1986;29:730–8.
32. Solinger AM, Bhatnagar R, Stobo JD. Cellular, molecular, and genetic characteristics of T cell reactivity to collagen in man. Proc Natl Acad Sci U S A 1981 Jun;78:3877–81.
33. Trentham DE, Dynesius RA, Rocklin RE, David JR. Cellular sensitivity to collagen in rheumatoid arthritis. N Engl J Med 1978;299:327–32.