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Characterization of infiltrating T cells and Th1/Th2-type cytokines in the synovium of patients with osteoarthritis

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Summary

Objective: It has been suggested that osteoarthritis (OA) is induced by mechanical stress followed by cartilage destruction, and it is generally accepted that there is little involvement of an immune response in OA compared with that in rheumatoid arthritis (RA). We have previously found clonally expanded transcripts of Vβ chain of the T cell receptor (TCR) in the synovium of patients with OA. To test the hypothesis that an immune response is involved in OA, we determined: (a) whether $CD3^+$, $CD4^+$, and $CD8^+$ T-cells were infiltrating the synovial membrane of patients with OA; (b) the Th1/Th2-type cytokines produced at the protein level in the synovium of patients with OA.

Methods: Immunohistochemical analysis was performed to identify T-cells that infiltrated the synovium of patients with OA using specific antibodies against $CD3^+$, $CD4^+$, and $CD8^+$ T-cell differential antigens, interferon-gamma (IFN-γ as a marker for Th1 cells, and interleukin-4 (IL-4) as a marker for Th2 cells.

Results: $CD4^+$ T-cells were strongly detected in the sublining layer of the synovium of patients with OA compared with the number detected in the same synovial layer of normal subjects. The number of IFN-γ^+ cells was significantly higher than that of IL-4^+ cells in the synovium of patients with OA ($P<0.05$).

Conclusions: These observations suggest that Th1 cells predominate in the synovium of patients with OA, which clearly indicates that immune regulation occurs and may play critical roles in inflammation and cartilage destruction in patients with OA. © 2002 OsteoArthritis Research Society International

Key words: Osteoarthritis, $CD4^+$ T cells, Synovium, Th1/Th2-type cytokines.

Introduction

Osteoarthritis (OA) is a disease causing locomotor dysfunction in aged people. Surgery is the method mainly used to treat OA, and to date there are few drugs that can arrest the progression of cartilage destruction. It is therefore necessary to determine the detailed mechanism underlying cartilage destruction in order to develop a drug therapy for OA. According to recent reports, various cytokines produced by T-cells have been implicated in the process of cartilage damage[1,2]. Furthermore, our group previously found clonally expanded transcripts of Vβ chain of the T cell receptor (TCR) in the synovium of patients with OA[3].

T helper cells were classified into Th1 and Th2 cells based on their patterns of cytokine production. Th1-type cytokines, interleukin-2 (IL-2) and interferon gamma (IFN-γ) are associated with macrophage activation, enhancement of cell-mediated cytotoxicity, delayed-type hypersensitivity responses, and effective responses to intracellular pathogens[4,5]. Th2-type cytokines, IL-4 and IL-10 are associated with allergic diseases, helminthic infections, and progressive infections by intracellular bacteria[5]. A biased cytokine pattern is observed in animal models of autoimmune disease. For example, in experimental allergic encephalomyelitis, the levels of IL-2 and IFN-γ that decrease with the concomitant IL-4 production are restored to original levels following the administration of IL-4 to prediabetic mice, which prevents the development of diabetes[6].

A Th1/Th2-type cytokine imbalance with Th1-type cytokines predominating has been suggested to be of pathogenetic significance in rheumatoid arthritis (RA)[7,8]. Infiltrating T-cells producing Th1-type cytokines play crucial roles in local inflammation in RA[7]. In this study, to determine whether OA is an inflammatory disease we determined the numbers of $CD3^+$, $CD4^+$ and $CD8^+$ T cells, as well as those of cells expressing IFN-γ and IL-4 in the synovium of patients with OA.

Materials and methods

SYNOVIAL TISSUE PREPARATION

During total knee arthroplasty, synovial tissue samples were obtained from the knee joints of 10 patients with RA (mean age: 69±8 years old, seven female and three male patients) who were diagnosed according to established RA classification[9] and ten OA patients (mean age: 72±6 years old, six female and four male patients) who were diagnosed according to X-ray diagnostics. Ten samples from patients with shelf disorder of the knee were obtained during arthroscopic surgery; these patients were considered normal subjects in this study. Informed consent was obtained from

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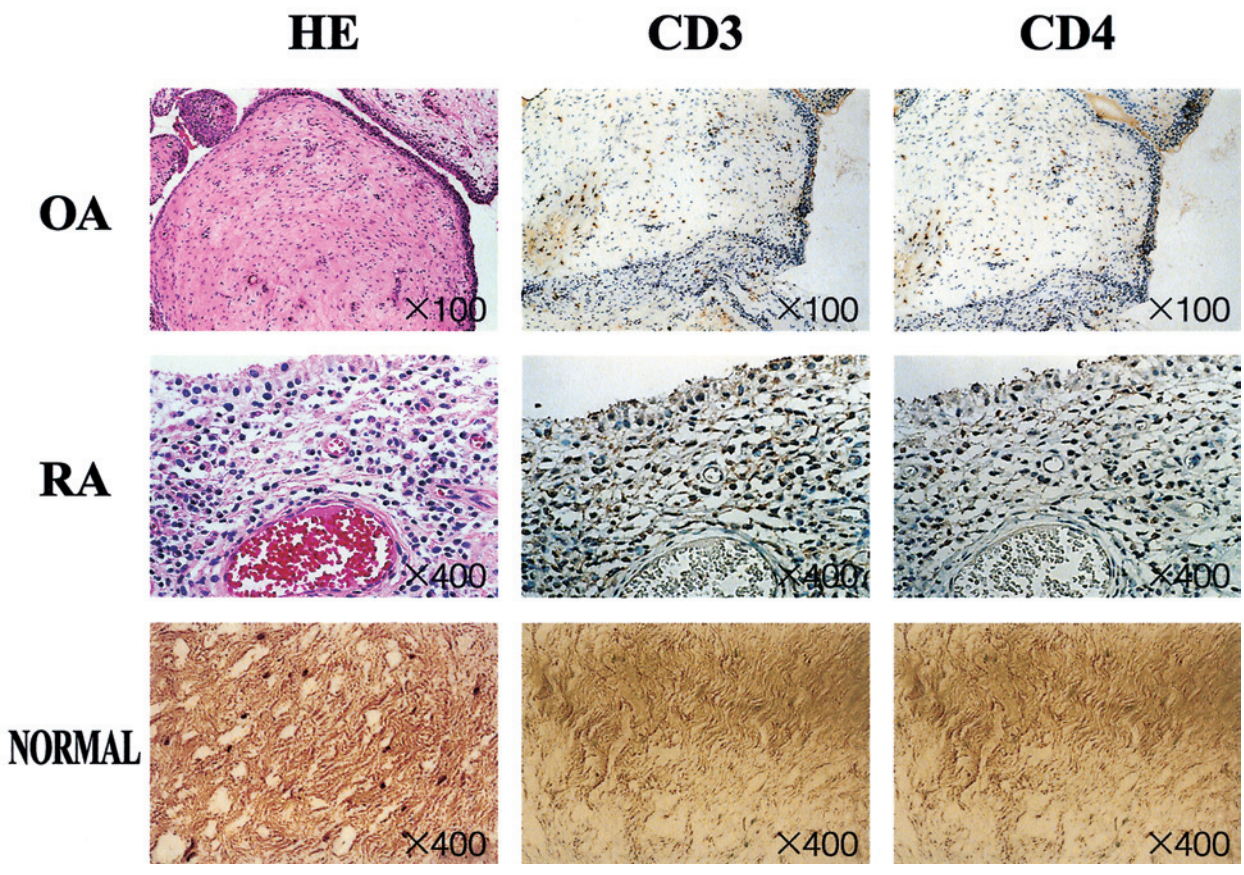

Fig. 1. Detection of $CD3^+$ and $CD4^+$ T cells in synovium of patients with OA and RA. Specimens from OA and RA patients, and normal subjects were stained with anti-CD3 antibody (center panel) and anti-CD4 antibody (right panel), and counterstained with hematoxylin solution. Results of counterstaining of sequential specimens with Meyer's hematoxylin and eosin (HE) are shown in the left panel. Shown in the middle and left panels are images representing serial sections of the synovium from one OA patient, one RA patient and one normal subject, and these pairs are representative of all samples. Original magnifications were ×100 for OA; ×400 for RA and NORMAL.

all the patients. The study was approved by our college ethics committee.

IMMUNOHISTOCHEMICAL STAINING

The synovial tissue samples were frozen with OCT compound (Sakura, Tokyo, Japan) immediately after surgery, and 5-μm-thick sections were prepared on glass slides (DAKO, Denmark). Specimens were fixed in acetone-ethanol (1:1) for 10 min. For immunohistochemistry, mouse monoclonal anti-human CD3, CD4 and CD8 antibodies (DAKO, Denmark), a mouse monoclonal anti-human IL-4 antibody (DAKO, Denmark) and a mouse monoclonal anti-human IFN-γ antibody (DAKO, Denmark) at 1:100 dilutions were applied as primary antibodies to the sections for 15 min. An isotype-matched non-specific mouse immunoglobulin G was used as a negative control. After washing in phosphate-buffered saline containing 0.1% Tween-20 (PBS-T), the glass slides were incubated for 15 min with a biotinylated goat antimouse immunoglobulin antibody (DAKO) diluted 1:1000 with PBS-T, washed again, and finally incubated with streptavidin peroxidase (DAKO) for 3 min. Staining was enhanced with 3,3′-diaminobenzidine tetrahydrochloride (DAB; DAKO), and the glass slides were counterstained with Meyer's hematoxylin and eosin (HE) (DAKO).

MICROSCOPIC EXAMINATION

Immunohistochemically stained sections were examined under a microscope by a single qualified investigator who was blind to the clinical data and histologic diagnoses. Infiltrating T-cells ($CD3^+$, $CD4^+$, and $CD8^+$ T cells), IFN-γ^+ and IL-4^+ cells in the synovium of patients with OA, and RA, and of normal subjects were counted under a high-power field (hpf) of 400× and data were expressed as means±S.E.M. cells/hpf of 10 hpfs that were randomly selected for each group.

STATISTICAL ANALYSIS

Differences in the number of infiltrating cells among the three study groups were tested by one-way analysis of variance. *P* values less than 0.05 were considered significant.

Results

EXPRESSION OF CD3+ AND CD4+ T-CELLS THAT INFILTRATED THE SYNOVIUM OF PATIENTS WITH OA AND RA

At first, we determined that T-cells infiltrated the synovium and found that they expressed the cell surface

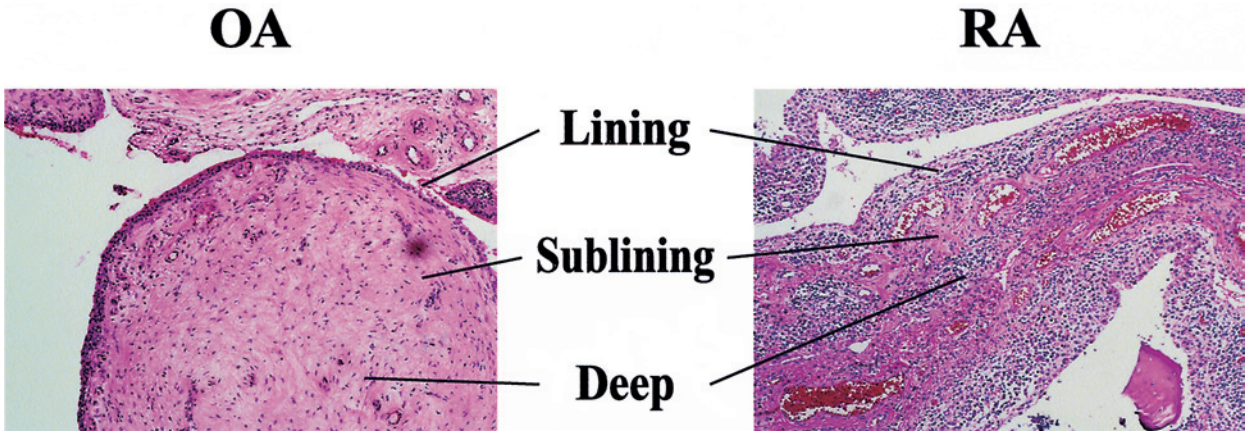

Fig. 2. Three layers of the synovium of patients with OA and RA. Frozen sections of specimens from OA and RA patients were stained with HE solution, and the representative images of the lining layer, sublining layer and the deep layer are shown. Original magnification was ×100.

Table I
*Number of CD3$^+$, CD4$^+$, CD8$^+$ T cells, and IFN-γ^+ and IL-4$^+$ cells infiltrating the three layers of synovium of patients with OA, and RA, and of normal subjects**

Group	Layer	CD3$^+$	CD4$^+$	CD8$^+$	IFN-γ^+	IL-4$^+$
OA (*N*=10)	Lining	22±7	14±5	10±3	18±7‡	3±1
	Sublining	231±71†	168±61†	101±33†	33±15†	7±2
	Deep	18±10	9±3	5±2	4±2‡	2±1
RA (*N*=10)	Lining	239±89	156±55	102±29	55±23§	12±5¶
	Sublining	344±111	298±132	178±93	67±19§	13±4¶
	Deep	241±88	135±55	83±35	58±23§	12±3¶
Normal (*N*=10)	Lining	0	0	0	0	0
	Sublining	0	0	0	0	0
	Deep	0	0	0	0	0

*Data of each group are presented as means±S.E.M. CELLS/HIGH-POWER FIELD (HPF) OF 10 HPFS. IFN-γ=interferon-gamma. IL-4=interleukin-4.
†P<0.01 vs CD3$^+$, CD4$^+$, and CD8$^+$ cells in the lining layer and deep layer of OA.
‡P<0.01 vs IL-4$^+$ cells in OA.
§P<0.01 vs IFN-γ^+ cells in OA.
¶P<0.01 vs IL-4$^+$ cells in OA.

markers, CD3 and CD4. As shown in Fig. 1, several CD3$^+$ and CD4$^+$ T-cells were detected in the synovium of patients with OA and there were many CD3$^+$ and CD4$^+$ T-cells in the synovium of patients with RA. These observations suggest that CD3$^+$ and CD4$^+$ T-cells infiltrated the synovium of patients with OA and RA but not that of normal subjects.

LOCALIZATION OF T CELLS THAT INFILTRATED THE SYNOVIUM OF PATIENTS WITH OA AND RA

We then determined the localization of T-cells that infiltrated the synovium of patients with OA and RA. As shown in Fig. 2, the synovium is composed of three layers: the lining layer, sublining layer and deep layer. CD3$^+$, CD4$^+$ and CD8$^+$ T-cells were detected in the three layers immunohistochemically using specific antibodies. CD3$^+$, CD4$^+$ and CD8$^+$ T-cells were predominantly detected in the sublining layer and to some extent in the deep layer of the synovium of patients with OA, while all layers were positively stained for these cells in the synovium of patients with RA (Table I).

EXPRESSION OF TH1/TH2-TYPE CYTOKINES IN THE SYNOVIUM OF PATIENTS WITH OA AND RA

We next performed immunohistochemical staining with an anti-IFN-γ antibody to detect INF-γ, a TH1-type cytokine, and with an anti-IL-4 antibody to detect IL-4, a Th2-type cytokine. INF-γ^+ and IL-4$^+$ cells were detected in the synovium of patients with OA although they were fewer in number than those in the synovium of patients with RA. Results of a representative staining are shown in Fig. 3.

NUMBERS OF IFN-γ^+ AND IL-4$^+$ CELLS IN THE SYNOVIUM OF PATIENTS WITH OA AND RA

As shown in Table I, the number of IFN-γ^+ cells was significantly higher than that of IL-4$^+$ cells in the synovium of patients with OA as well as RA. We found that the number of IFN-γ^+ cells was approximately five-fold greater than that of IL-4$^+$ cells in the synovium of patients with OA. It is also interesting to note that the numbers of IFN-γ^+ and IL-4$^+$ cells in the synovium of patients with OA were about three-fold less than those in the synovium of patients with

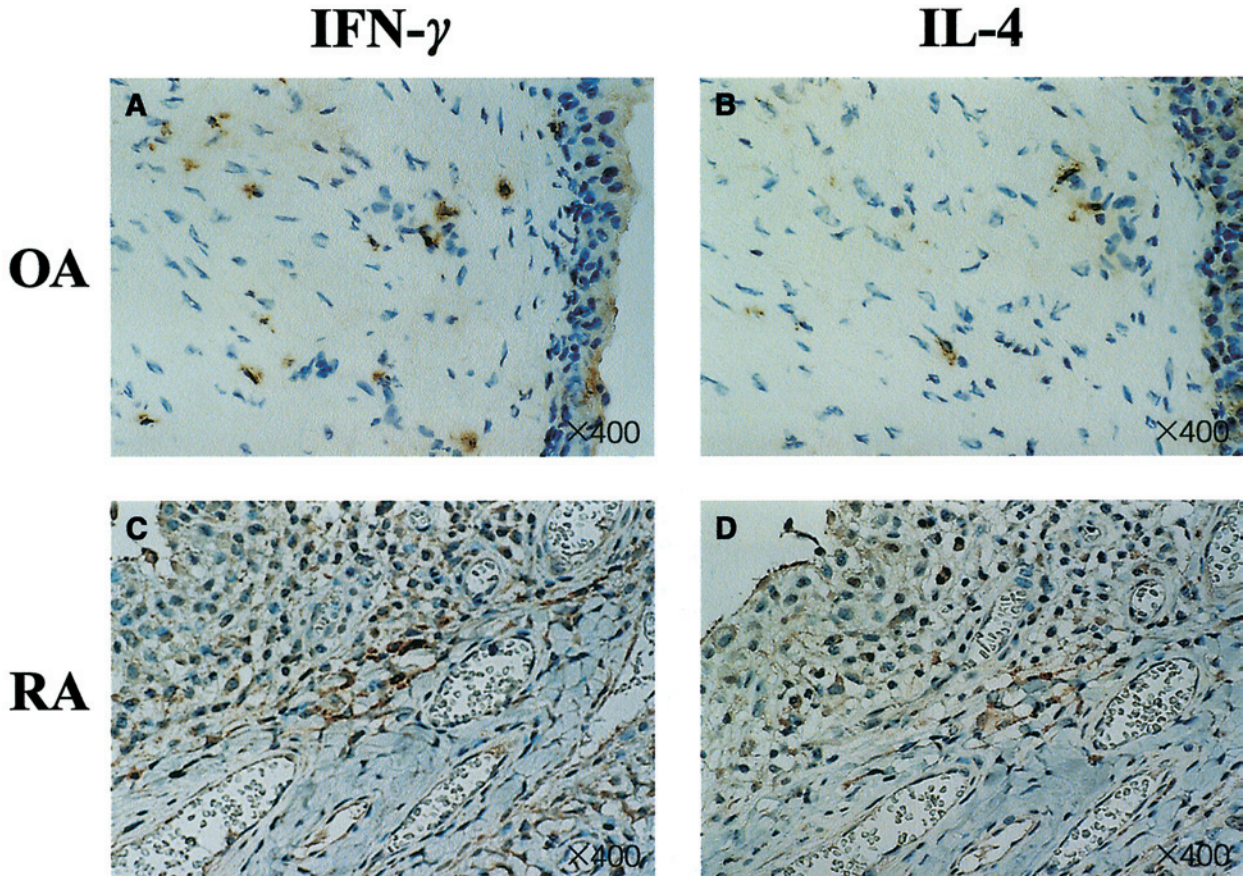

Fig. 3. Detection of IFN-γ^+ and IL-4^+ cells in the synovium of patients with OA and RA. Frozen sections of specimens from OA (A, B) and RA (C, D) patients were stained with an anti-IFN-γ (A, C) or IL-4 (B, D) monoclonal antibody. Nuclear counterstaining was performed using hematoxylin solution. Representative data from the OA and RA patients are shown. Original magnification was ×400.

RA. No positive staining was observed in the synovium of normal subjects.

Discussion

In autoimmune diseases, such as RA and systemic lupus erythematosus (SLE), autoantigens are considered to induce T-cell activation and they play critical roles in autoreactive immune response. In contrast, the pathogenesis of OA is considered mainly to be mechanical stress. OA, as well as RA, is characterized by severe inflammation that induces cartilage destruction. To date, several groups have implicated T-cells in the pathogenesis of OA[1,2,10]. In collagen-induced arthritis in which type II collagen acts as an autoantigen, cartilage destruction resembles that in OA. Furthermore, some disease-modifying antirheumatic drugs (DMARDs) used for RA therapy are also effective against OA[3]. Thus, we have previously suggested that an immune response involving T-cells driven by autoantigens is a feature of the pathogenesis of OA. We previously analysed the repertoire of $CD4^+$ T-cells that infiltrated the synovium of patients with OA and found clonally expanded transcripts of Vβ chain of the TCR[3]. The transcripts are different from those in the synovium of patients with RA, and these results suggest the existence of a specific antigen derived from the cartilage in the joint.

In the present study, we clearly showed that both Th1 and Th2 cells infiltrate the synovium of patients with OA[11]. The number of Th1 cells correlated with the serum C-reactive protein value, disease activity score, and the degree of synovial lining hyperplasia[8]. In addition, consistent with the data obtained from the synovium of patients with RA[8], Th1-type cells were strongly detected in the synovium of patients with OA, although the number of T-cells that infiltrated the synovium was lower than that of patients with RA. In the synovium of patients with OA, infiltrating T-cells were mainly localized in the sublining layer, suggesting that these cells were derived from vessels11. The pattern of positive staining of IFN-γ and IL-4 in the synovium of patients with OA also suggests that T-cells may have infiltrated the synovium from vessels[11].

It is generally accepted that an imbalance of Th1/Th2-type cytokines plays a crucial role in autoimmune diseases such as diabetes in non-obese mice and SLE[12,13]. Th1-type cytokines predominantly activate macrophages and cell-mediated cytotoxicity, as mentioned in the introduction, which are thought to be among the features of the mechanism of activation of an autoreactive immune response. Using an immunohistochemical method, we directly detected INF-γ and IL-4 proteins and clearly showed that IFN-γ^+ cells were more higher in number than IL-4^+ cells in the synovium of patients with OA. We also examined the expression of IL-2, a Th1-type cytokine, and IL-10, a Th2-type cytokine; these were rarely detected in the synovium of patients with OA. As previously reported regarding the expression of IL-2-, IFN-γ-, IL4- and IL-10-type

cytokines as determined by RT-PCR analysis[2,10], the sensitivity of detection by immunohistochemistry is lower than that by RT-PCR analysis. Recently, it was found that chemokines and chemokine receptors are expressed in the synovium of patients with OA and they induce cartilage destruction[14]. Because IFN-γ produced by Th1 cells that infiltrated the synovium induces macrophage activation, Th1 cells may be highly implicated in cartilage destruction via chemokine production[14].

In this study, we directly detected Th1/Th2-type cytokines in the synovium of patients with OA by immunohistochemical analysis, which supported previous findings that T-cells that infiltrated the synovium play important roles in inflammation and cartilage destruction in OA patients. Although immunomodulative or immunosuppressive drugs are rarely used for the treatment of OA, our study suggests that in order to arrest the progression of inflammation and cartilage destruction, modulation of the immune response is necessary for OA patients.

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