

Osteoarthritis and rheumatoid arthritis pannus have similar qualitative metabolic characteristics and pro-inflammatory cytokine response

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Abstract

Objective

Pannus in osteoarthritis (OA) has only recently been characterized. Little is known, however, regarding the behavior of OA pannus in vitro compared to rheumatoid arthritis (RA) pannus. The purpose of our study was to compare OA with RA pannus.

Methods

Pannus and synovial tissue co-cultures from 5 patients with OA and 5 patients with RA obtained during arthroplasty were studied. Pannus was defined as the microscopic invasive granulation tissue covering the articular surface. Tissues were cultured for 7 days and stained with Alcian Blue technique. Interleukin-1 β (IL-1 β), IL-8, IL-10, IL-12, tumor necrosis factor- α (TNF- α), and interferon gamma (IFN- γ) were also determined in supernatants by ELISA. Cartilage oligomeric matrix protein (COMP), type II collagen, TNF- α , IL-10 and Ki-67 expression were also detected by immunohistochemistry.

Results

All patients had vascular or fibrous pannus. Synovial proliferation, inflammatory infiltrates and a decrease of extracellular matrix proteins were observed in all tissue samples. Chondrocyte proliferation was lower in OA than RA cartilage. OA synovial tissue expressed lower levels of proteoglycans than RA synovium. Type II collagen levels were lower in OA than in RA cartilage. Significantly higher levels of IL-1 β were found in the supernatants of RA pannus compared to OA pannus ($p < 0.05$). High but similar levels of TNF- α , IL-8 and TIMP-1 were detected in OA and RA pannus supernatants. IL-10, IL-12 and IFN- γ were undetectable.

Conclusion

RA and OA pannus had similar pro-inflammatory and anti-inflammatory cytokine profile expression. OA cartilage, synovial tissue and pannus had lower production of proteoglycans, type II collagen and IL-1 β . It remains to be elucidated why OA pannus invades the cartilage surface but does not cause the marginal erosions typically seen in RA.

Key words

Osteoarthritis, rheumatoid arthritis, pannus, synovial tissue, cartilage, cytokines.

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Introduction

Osteoarthritis (OA), a chronic inflammatory disease characterized by progressive deterioration of articular cartilage in synovial joints (1-4), is the most common rheumatic disorder and the leading cause of disability in adults over age 45 years (5). The most prominent histological change in OA is the partial or total loss of hyaline cartilage, accompanied by new bone formation (osteophyte), softening, fibrillation and abrasion of cartilage that leads to denudation of the underlying bone (6). In addition, a remodeling process characterized by foci and plaques of new non-osseous tissue has often been seen on the articular surface of OA joints (2). The histological appearance of this tissue has been termed pannus-like fibrous tissue, chondroid, fibrocartilage or a form of hyaline cartilage (6, 7). This cloth-like soft tissue, first described in rheumatoid arthritis (RA) as an invasive granulation tissue covering the articular cartilage, gave rise to the word "pannus". The microscopic observation has shown that pannus is composed of aggressive macrophage- and fibroblast-like mesenchymal cells (8), macrophage-like cells and other inflammatory cells (8-10) arising from the junction between synovial tissue and cartilage (bare area). These contribute to cartilage destruction by the release of collagenolytic enzymes (10, 11). In fact, the soft tissue covering the articular surface of OA, often found during joint surgery and in articular specimens (6, 7), unlike RA pannus, does not typically cause marginal erosions. Contrary to RA pannus, little attention has been given to the non-cartilaginous tissue covering OA cartilage. For instance, Shibakawa *et al.* recently reported that 70-80% of articular samples from patients with advanced OA had pannus-like tissue (6). In another study, consistent with previous work from Söder *et al.* (11), Shibakawa *et al.* also reported that pannus-like tissue spontaneously express several metalloproteinases suggesting that these are involved in cartilage degradation in OA (7).

In this study, we cultured pannus, synovial and cartilage tissues from OA and RA patients to investigate their morphological characteristics, metabolic activity and inflammatory cell responses.

Methods

Patients

We studied pannus, synovium and cartilage randomly obtained during arthroplasty from 5 patients who fulfilled the 1986 American College of Rheumatology criteria for the classification of OA (12). We also obtained pannus, synovium and cartilage from 5 patients with RA that fulfilled the ACR criteria for the classification of RA (13). All patients gave their informed consent after protocol approval by the Institutional Review Board of our Institution. Pertinent clinical and demographic characteristics of patients are shown in Table I.

Pannus and tissue cultures

OA and RA pannus, defined as the microscopic invasive granulation tissue covering the articular cartilage, synovial and cartilage tissues were separated from fat and bone, and were cut in small pieces of approximately 5 mm³. Two explants that microscopically contained pannus tissue, cartilage or synovial membrane explants were cultured in duplicate wells on polycarbonate membranes (Nucleopore Costar, Cambridge, MA) in sterile 24-well plates (Costar, Cambridge, MA, USA) and were covered with one ml of RPMI-1640 medium (Gaithersburg, Md) containing 10% FCS (GIBCO BRL), 100 U of penicillin, 0.1 mg of streptomycin and 0.25 µg of amphotericin B/ml (SIGMA Chemical Co., St. Louis, MO). Plates were incubated at 37°C in a 5% CO₂/95% air incubator with a humidified atmosphere. Medium was changed daily with fresh medium. All changes were discarded except on days 1 and 7 when media and explants were harvested. Supernatants and tissue samples were stored at -70°C until assayed.

Histological analysis

To determine the extracellular matrix composition (elastin, collagen, sulphated proteoglycans and hyaluronic acid), tissues were stained with haematoxylin and Alcian Blue staining techniques. The percentage of total tissue that stained in blue (proteoglycans) or pink (non-proteoglycans) were quantified in duplicate by the Program Image-Pro

Competing interests: none declared.

Table I. Clinical and demographic characteristics of patients.

| Feature | OA | RA |
|-------------------------------------|--------------------------|---------------------|
| Age (years) (range) | 74 ± 6 (68-71) | 55 ± 22 (25-75) |
| Disease duration (years) (range) | 11 ± 9.9 (3-25) | 20 ± 13 (5-39) |
| Gender (F/M) | 3/2 | 5/0 |
| Medications at time of study | NSAIDs and acetaminophen | NSAIDs and DMARDs* |
| Rheumatoid factor (+) | 0/5 | 5/5 |
| Arthroplasty (n) | Hip (5) | Hip (3) Knee (2) |

*Methotrexate (n=3); chloroquine (n=1); azathioprine (n=1). No patient has received anti-TNF- α therapy.

Plus version 5.1. Pannus was classified as vascular or fibrous type according to Shibakawa *et al.* (6).

Immunoperoxidase staining

We used the following antibodies: mouse anti-human Ki-67 (10 μ g/ml), IL-10 monoclonal IgG (10 μ g/ml), rabbit polyclonal TNF- α (10 μ g/ml), goat anti-human cartilage oligomeric protein (COMP/thrombospondin-5) (Riesen R, 2001) polyclonal IgG (10 μ g/ml) or a goat anti-human type II collagen polyclonal IgG (20 μ g/ml, Santa Cruz, CA). A 1:100 dilution of normal human serum was used as negative control. The reactive blank was incubated with phosphate buffer saline-egg albumin (SIGMA) instead of the primary antibody. Both controls exclude non specific staining or endogenous enzymatic activities. At least two different sections were examined for each tissue in a double blind fashion. Ki-67, extracellular matrix protein and cytokine expression was assessed by estimating positively stained cells in stromal cells throughout the whole tissue. Results are expressed as the median percentage \pm SEM of positive vs. negative immunoreactive cells quantified by the Program Image-Pro Plus 5.1.

Measurement of cytokines and TIMP-1

Interleukin-1 β , TNF- α , IL-8, IL-10, IL-12, IFN- γ and TIMP-1 were determined in supernatants of OA and RA pannus, synovial and cartilage co-cultures with an enzyme-linked immuno-

sorbent assay (ELISA) kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Results were normalized by total protein concentration evaluated by the Folin-Löwry micromethod.

Statistics

All experiments were analyzed in triplicate. Comparison of continuous variables was done by the Mann-Whitney rank sum test. Data are expressed as the median \pm SD. A p -value ≤ 0.05 was considered significant.

Results

Histological findings in OA and RA pannus

Pannus tissue, found in all five OA joints, could be classified mainly as vascular type. It was characterized by hypervascularity, hypercellularity and a distinct border between cartilage and pannus tissue (Figs. 1 and 2A-D). Similarly, vascular pannus was found in 3 RA patients and fibrous type in 2 patients. The latter was avascular, hypocellular with an indistinct junction. Fibroblasts were scantily arranged but no vessels were seen (*data not shown*).

Extracellular matrix composition of OA and RA cartilage

The cartilage proteoglycan content in OA and RA tissues was evaluated by morphometric analysis. It showed that OA pannus had less mononuclear cell infiltration and blood vessel formation than RA pannus (Figs. 2A-D and 3A).

The relative proportions of proteoglycans were evaluated by an image analyzer (Image-Pro Plus 5.1). The area stained in blue (proteoglycans) and the area stained in pink (non-proteoglycans) were quantified and expressed as relative percentage. These results are shown in Figure 3B. As seen, OA and RA cartilage tissues had similar proteoglycan content throughout the study period. In contrast, a statistically significant decrement of proteoglycans was detected in OA synovium compared to its basal levels and to RA in the 7th culture day (Fig. 3B). Figure 3A (lower panel) depicts the COMP and type II collagen immunoreactive chondrocytes. Figure 3C shows the synthesis of specific matrix cartilage proteins by OA and RA tissues detected by immunoreactivity in duplicate cultures. It shows that the amount of COMP-positive chondrocytes is similar in RA and OA cartilages both at baseline and 7th day cultures. Figure 3C also demonstrates that although the baseline amount of type II collagen immunoreactive cells in OA and RA cartilages were similar at baseline, while RA type II collagen-positive chondrocytes had an almost four-fold increment at 7th days of culture compared to OA (25.0 \pm 2.5 vs. 6.5 \pm 1.4; $p < 0.05$). The amount of type II collagen-positive OA chondrocytes at 7th culture day was also lower than baseline cultures ($p = 0.05$). No differences in the elastic fibers content between OA and RA were found (*data not shown*).

Proliferation of OA and RA pannus tissues

To evaluate the cell proliferation, we measured the antigen Ki-67, a nuclear protein in proliferating cells expressed in all phases of the cell cycle except G₀. The results of these experiments are shown in Figure 4A. It shows similar amounts of Ki-67-positive OA and RA chondrocytes at baseline and a 3.6-fold decrease of Ki-67-positive OA cells at the 7th culture day compared to RA (1.2 \pm 0.5 vs. 0.3 \pm 0.3, $p < 0.05$). We found no statistically significant differences in the proliferation of OA synovial tissue compared to RA throughout the study period (Fig. 4A).

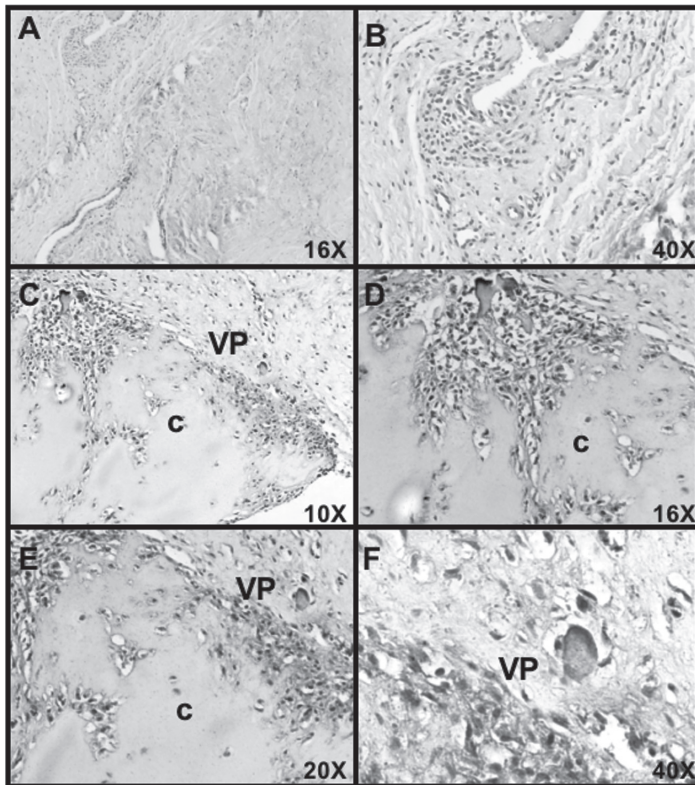


Fig 1. Photomicrographs of cartilage from OA patients stained with the Alcian Blue technique **A** and **B**. The predominant extracellular matrix macromolecules in cartilage are non-proteoglycan components stained in pink while proteoglycans are stained in blue. Photomicrographs of cartilage stained with Hematoxylin **C, D, E, F**. VP: vascular pannus; C: cartilage.

Cytokine profile in OA and RA pannus tissues

A large number of cytokines (pro- and anti-inflammatory), antagonists and growth factors are pathophysiologically involved in RA and OA. Pro-inflammatory cytokines have been demonstrated to play a pivotal role in the development of the disease process. In particular IL-1 β and TNF- α seem prominent and of major importance in cartilage destruction. As seen in Figure 4B, the levels of IL-1 β in the supernatants from RA pannus after 7 days of culture were five-fold higher than OA supernatants (269.0 ± 148.6 vs. 54.5 ± 49.1 pg/ng of protein; $p < 0.05$; Fig. 4B). Figure 4B also shows that RA and OA pannus synthesized similar amounts of TNF- α and IL-8. IFN- γ and IL-12 were undetectable. Figure 4B shows that the TIMP-1 levels did not differ in supernatants from RA and OA pannus.

Discussion

It is now clear that pannus formation is not a specific feature of RA (14). Rhodes *et al.* studied 20 OA patients with MRI and found that all had pannus formation in their knees (14). In an earlier work, the same group demonstrated pannus in patients with spondyloarthropathies (15). Others have shown that pannus is a morphologic feature of an experimental model of septic arthritis (16). More recently, Shibakawa reported that OA pannus-like tissue could be classified into vascular and fibrous types, that it is found preferentially in the marginal articular zones not causing erosions and that IL-1 β and MMP-3 expressing cells are the most predominant cells in pannus-like tissues (6). Later, the same group reported that 15/21 patients with OA (71.4%) that required joint arthroplasty had pannus-like tissue and that OA pannus cells were positive for type I and II collagen and vimentin (7). Finally, these authors found that OA pannus cells secreted several MMP, but only MMP-3 levels were higher (7). Interestingly, these findings are similar to Aniola's group who previously reported high IL-1 β -mediated expression of MMP-3 in RA pannus (17). To the best of our knowledge, this is the first work that compares OA and

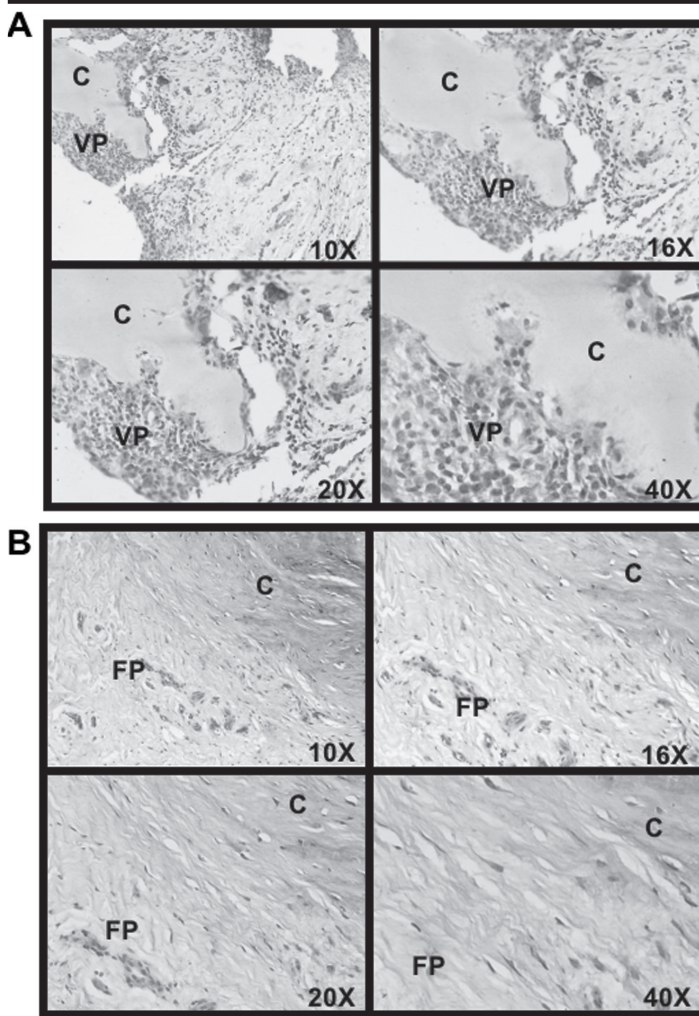


Fig 2. Photomicrographs of pannus from two OA patients (A-B) stained with the Alcian Blue technique. VP: Vascular pannus; FP: Fibrous pannus; C: cartilage.

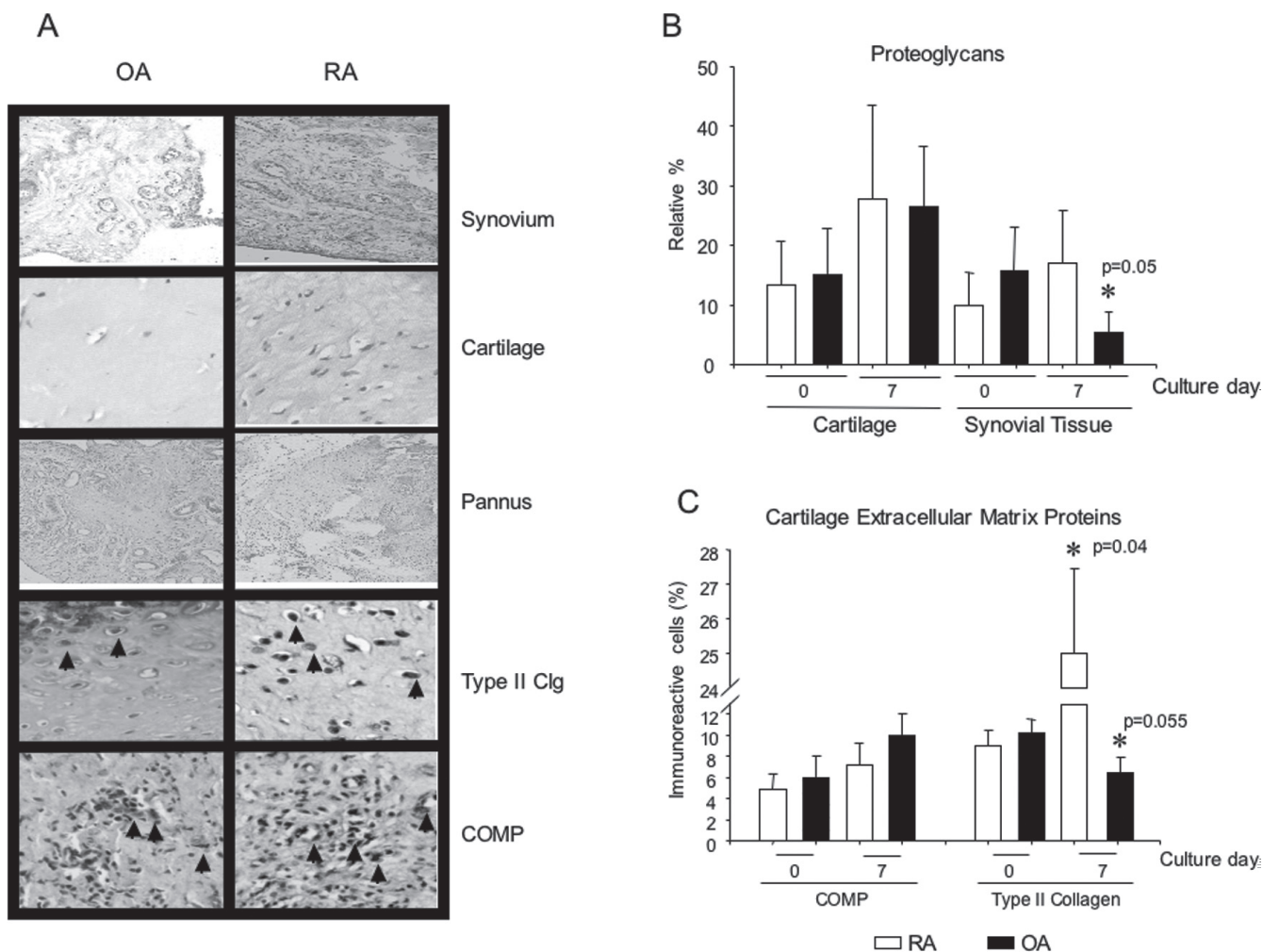
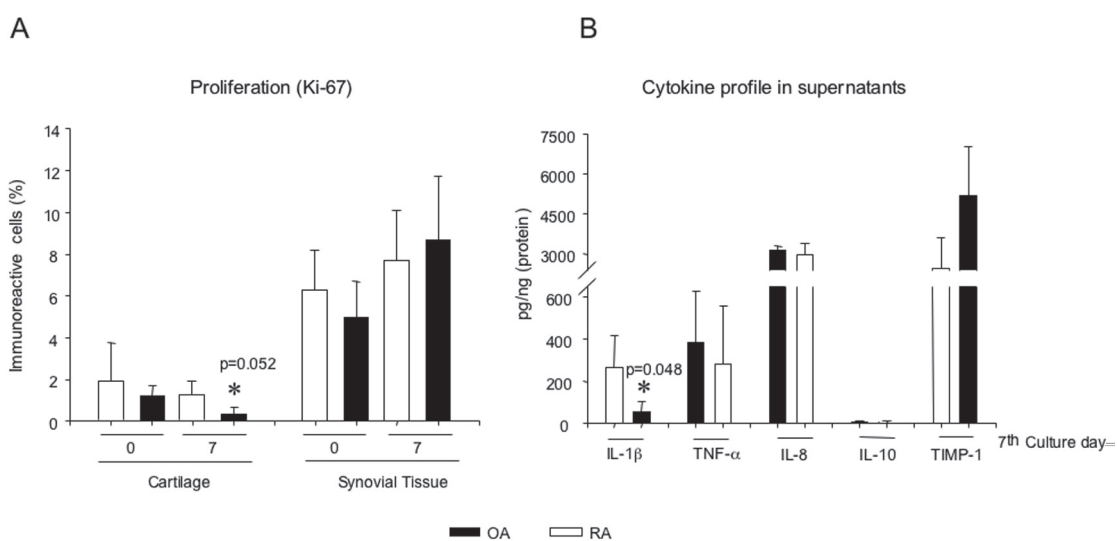


Fig 3. Extracellular matrix proteins in OA and RA tissues. **(A)** Photomicrographs of OA (left panel) or RA (right panel) synovial, cartilage and pannus tissues stained with the Alcian Blue technique or immunohistochemistry of type II collagen (type II Clg) or COMP. Arrows indicate immunoreactive chondrocytes. **(B)** The areas stained in pink or blue were quantified by the Program Image-Pro Plus 5.1. Results are expressed as relative percentage. Graph shows statistically significant lower levels of proteoglycans in synovial tissue cultures from RA versus OA on the 7th culture day ($p=0.05$). **(C)** Results show the percentage of COMP or type II collagen immunoreactive cells determined by immunohistochemistry in co-cultures from 5 OA (black bars) and 5 RA (white bars) patients each performed in triplicate where at least two sections per tissue were evaluated. Relative percentage of type II collagen-immunoreactive RA producing cells is higher compared with OA 7th day cultures ($p<0.05$). Results represent median \pm SD.

Fig 4. Proliferation and cytokine expression in OA (black bars) or RA pannus cultures (white bars). **(A)** Ki-67 immunoreactive cells in cartilage or synovial tissue co-cultures ($*p<0.05$ vs. baseline cultures). **(B)** IL-1 β ; TNF- α ; IL-8; IL-10 and TIMP-1 concentration in supernatants. IL-1 β ($*p=0.05$, RA vs. OA). Results represent median \pm SD of supernatants from 5 OA and 5 RA patients ran in triplicate.



RA pannus *in vitro*. We showed that RA and OA pannus had similar pro-inflammatory and anti-inflammatory cytokine profile expression. We found that synovial tissues from patients with advanced OA had lower production of proteoglycans compared to RA *in vitro*, while their COMP synthesis was similar. All OA and 3 RA patients had vascular type pannus and 2 RA patients had fibrous type pannus; but due to the small size of our groups, at present we cannot ascertain if our findings were in any manner influenced by the stage of the pannus tissue. Our work also demonstrated a higher production of IL-1 β by RA pannus and increased amount of type II collagen immunoreactive RA chondrocytes compared to OA cells, in spite of the fact that the latter cells had lower proliferative capacity than their RA counterparts. OA and RA chondrocytes had equal type II collagen synthesis at baseline, but this changed substantially on the 7th culture day when OA chondrocytes decreased its type II collagen synthesis and RA chondrocytes almost tripled their type II collagen production. We interpret these data to mean that these differences are most probably a metabolic characteristic of OA and RA chondrocytes.

Ours is an observational study that compared a small number of RA and OA patients; similarly, we studied OA and RA patients that required total joint replacements, thus, our findings should be interpreted with these limitations and may only be applicable to patients with advanced diseases. It is known, however, that synovial membrane biopsies guided by MRI taken from patients with early OA have increased thickening and mild chronic inflammation (18). It is also known that the degree of synovial thickening on MRI from patients with various degrees of OA, including early disease, significantly correlated with microscopic features such as thickening of synovial lining cells, surface fibrin deposition, fibrosis, edema, congestion, and infiltration (19). Similarly, the increased thickening of the lining layer, vascularity, and inflammatory cell infiltration in OA synovial membranes correlate well with an abnormal production of

proinflammatory cytokines, such as IL-1 α , IL-1 β and TNF- α , irrespective of disease severity (20). Finally, Benito *et al.* showed that synovial tissue from patients with early OA has significantly greater CD4⁺ and CD68⁺ cell infiltration, blood vessel formation, vascular endothelial growth factor, intercellular adhesion molecule-1 expression and TNF- α and IL-1 β producing cells, compared to advanced OA (4). With the limitations stated above, it appears then that our results are consistent with the notion that differences in the metabolic properties of RA and OA tissues *in vitro*, particularly with respect to the cytokine production, are only quantitative rather than qualitative (1, 6, 7, 18, 19, 21-24).

Our work confirmed that the phenotypic expression of pannus is driven by a variety of pathogenetic factors (biomechanical, infectious and immunological) across the spectrum of rheumatic diseases, rather than being a specific feature of RA (14). More studies are needed to compare OA with RA pannus from patients with early diseases to further understand the differences and similarities, including why OA pannus invades the cartilage surface, but does not typically cause the marginal erosions characteristic of RA.

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