4.

The Synovium and Its Role in Osteoarthritis

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4.1 Introduction

Osteoarthritis (OA) has long been considered to be a disease almost exclusively of the cartilage. However, in recent years studies in humans and animals have led to increasing evidence that the synovium also is involved in the OA process, and that its role starts relatively early in the disease. This chapter discusses the involvement of the synovium in OA-related pathology. Although we still are uncertain about the exact mechanism that evokes synovial activation and the mediators that induce and perpetuate its active participation, the role of the synovium should not be underestimated. The first part of this chapter describes the normal function of the synovium in a synovial joint and compares the synovium in two separate diseases: rheumatoid arthritis (RA) and OA. Subsequently, the involvement of synovial macrophages in experimental OA pathology is discussed, followed by an overview of the mediators that may cause or regulate this pathology, the possible mechanisms involved in synovial activation, and the possibility of gene therapy targeting the synovium. Unraveling the mechanisms behind this synovial involvement may help find a suitable therapeutic approach to fight this crippling condition.

4.2 Normal Synovium: Morphology and Function

The synovium in a normal joint is a thin, weak layer of tissue only a few cell layers thick that lines the noncartilaginous surfaces within articular joints. The synovium acts to control the environment within the joint. It does this in two ways: first, it acts as a loose membrane to determine what can pass into the joint space and what stays outside, such as nutrients for chondrocytes on the one hand and pathogens on the other; second, the cells within the synovium produce substances such as hyaluronan and lubricin, important components of joint fluid that give joint fluid its mechanical properties. The synovium can be divided into two compartments—the synovial lining, or intima, and the sublining. The lining layer consists of two different cell types—type A and type B synoviocytes. Intermediate types have been described, but their existence is not generally accepted. The two cell types execute different functions. Type A synoviocytes are macrophage-like cells that function to clear all excess material and potential pathogens from the joint. They can migrate through the synovium and into the synovial cavity and express major histocompatibility complex II molecules and Ia antigen, which

play key roles in the antigen presentation in the initial stages of the immune response. These cells can produce and secrete a number of enzymes and cytokines/chemokines that mediate tissue damage and inflammation. In pathologic conditions, macrophages stimulate B synoviocytes to produce matrix degrading enzymes. Type B synoviocytes are fibroblast-like cells that are thought to produce the main component of synovial fluid—hyaluronan. In addition, they function as a physical barrier that, combined with the water retention by hyaluronan, traps the synovial fluid in the joint capsule. The synovial fluid not only is important for lubrication of the joint, but also functions to transport nutrient and oxygen to the cartilage. In a normal joint, type A and B synoviocytes act to maintain a healthy environment, so that all tissues can function properly. This steady state can be disrupted by stimuli from the outside, for example, microorganisms, immune complexes, components leaking from a damaged cartilage matrix, or by an intrinsic derailment of synoviocytes.

Apart from the production of proinflammatory and matrix degrading mediators, synoviocytes also produce a plethora of protective mediators, including transforming growth factor-β (TGF-β), tissue inhibitors of metalloproteinase (TIMP), and interleukin-10 (IL-10). An elegant approach to restore the steady state would be to make use of this potential. This cytokine balance is further discussed below.

4.3 Comparison of Rheumatoid Arthritis and Osteoarthritis synovium: Lessons from Rheumatoid Arthritis Synovium

In RA, the processes that occur in the synovium have been studied and described extensively, even though the exact mechanisms are not

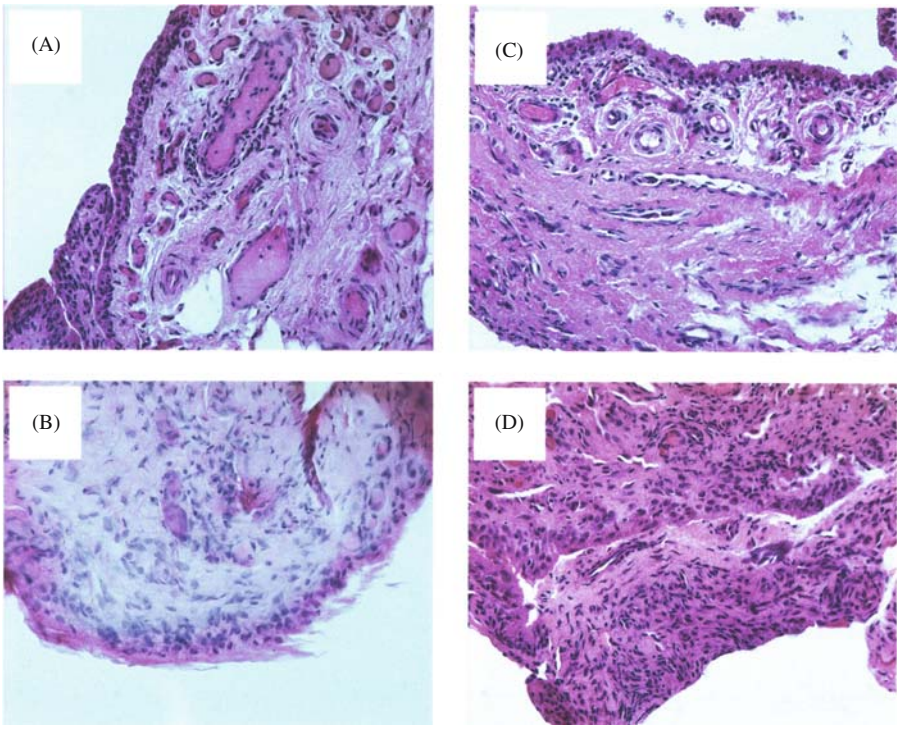

Figure 4.1. Histology of early osteoarthritis (OA) (A,B) and early rheumatoid arthritis (RA) (C,D) samples. Note the thickened synovial lining layer in both conditions. In the deeper layers of some RA synovial samples (D), more inflammatory cells are present. Original magnification 100×.

yet fully understood. Nevertheless, increased insight into the mechanisms behind RA have led to useful therapeutic strategies, such as inhibition of tumor necrosis factor-α (TNF-α) and other cytokines, or T- and B-cell–directed therapies that are in development. In RA the synovium is the target tissue for treatment, and its dysfunction has always been considered the main factor that causes pathology. A wide variety of cell types that occur in varying amounts can be identified, including polymorphonuclear neutrophils (PMNs), monocytes, T cells, B cells, plasma cells, and mast cells. In addition, the number of macrophages in the synovium, especially in the synovial lining, is greatly increased. The presence of these macrophages in the synovium correlates well with disease activity and progression of cartilage damage [12,24]. Lately, a number of studies have reported macrophage accumulation in the OA synovium as well [14]. In many cases early OA synovial biopsies resemble RA biopsies morphologically (Fig. 4.1). It is particularly the lining layer that is thickened in the two synovial conditions. The RA synovium generally contains larger amounts of adaptive immunity-related inflammatory cell types (e.g., T and B cells), although the cells occur also in many OA synovia. The synovial fluid of patients with active RA effusion contains substantial amounts of PMN, which are not found in OA. This indicates differences in the nature of synovitis in the two rheumatic disorders. However, the presence of a thickened synovial lining and a greater number of macrophages in the joints of many OA patients suggest that the OA synovium is activated and may contribute to the symptoms and disease progression.

4.4 Evidence for Involvement of Synovial Macrophages in Osteophyte Formation

One of the main pathologic changes in an osteoarthritic joint is the formation of osteophytes, that is newly formed ectopic bone at the margins of the joint (Fig. 4.2). This may be a side effect of the locally increased levels of growth factor, such as TGF-β, constituting an attempt at cartilage repair. It may also be a reaction of the tissue to changed biomechanics and an attempt to enhance the articular surface. Although the exact function of osteophytes and the role they play in clinical symptoms is not known, one can easily imagine that osteophytes in certain locations will narrow the range of movement of the joint and will cause pain by hindering free movement of soft tissues in the joint. Synovial macrophages are crucial in the induction of osteophytes [5,20]. Macrophages mediate the generation of osteophytes in two models, one induced by a growth factor, TGF-β, and the other via induction of murine OA. In the first study, osteophytes are induced in murine knee joints by intraarticular injection or overexpression of TGF-β [20]. Local TGF-β application strongly induces osteophytes [4], and blocking TGF-β

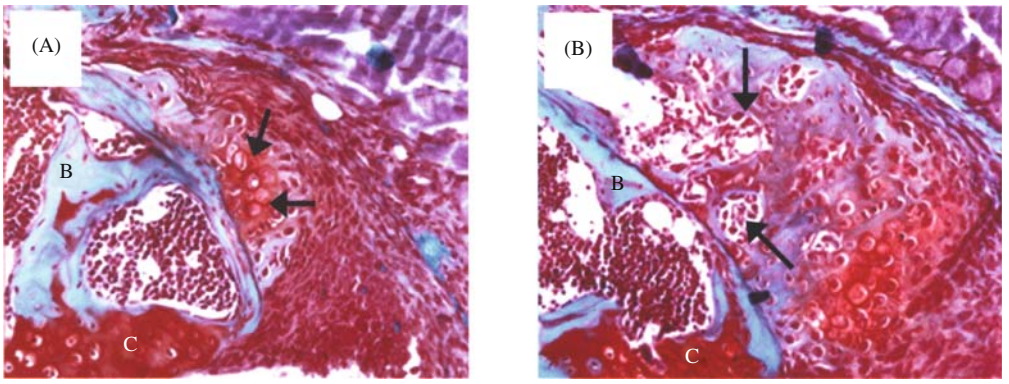

Figure 4.2. Early osteophyte formation. Osteophyte formation (arrows) starts with chondrogenesis (A). The chondroid tissue thereafter is replaced by bony structures (B). The formation of bone marrow cavities is shown by arrows. B, bone, C, cartilage. Original magnification 200×.

using a soluble receptor prevents osteophyte formation [31], thus identifying TGF-β as a key factor. Osteophytes often develop at the cartilage/bone junction, are thought to originate from mesenchymal cells in the periosteum, and start as chondrogenic outgrowths with subsequent transformation to bony structures. Whether TGF-β acts directly on these mesenchymal cells, or whether other, secondary mediators produced by synovial cells are necessary for proliferation and differentiation of these cells was largely unknown until studies of local macrophage depletion with toxic liposomes provided further insight.

After intraarticular injections of clodronate containing liposomes, these vesicles are selectively internalized by macrophages, the clodronate is released in the macrophage, and the intracellular clodronate causes cell death through apoptosis [26]. Apoptotic cells are removed from the synovium with a minimum of inflammation. Free clodronate, if released in the joint, diffuses quickly out of the joint, because the cells cannot take up free clodronate. This treatment results in a complete removal of synovial macrophages within 1 week of intraarticular injection of the liposomes. When synovial macrophages are depleted from the synovial compartment prior to either viral overexpression or injection of TGF-β intraarticularly, the formation of osteophytes is largely prevented (Table 4.1). This is not due to decreased TGF-β levels in macrophage depleted joints, as was demonstrated by enzyme-linked immunosorbent assay (ELISA) of synovial washouts. An earlier study [1] led to similar results using adenoviral overexpression of TGF-β. Immunohistochemical analysis demonstrated a lower expression of bone morphogenetic proteins (BMP-2 and BMP-4) after macrophage depletion. This indicates that macrophage involvement in osteophyte formation may be mediated by secondary growth factors, like BMPs. When injected intraarticularly [4], BMPs induce osteophytes.

Table 4.1. The effect of TGF-β on osteophyte formation by causing depletion of the lining

TGF-β		Surface area (μm^2) Patella	Femur
20 ng	Normal	662 ± 363	435 ± 278
	Depleted	0*	38 ± 72*
200 ng	Normal	2111 ± 932	2235 ± 1159
	Depleted	203 ± 513*	671 ± 627

Note: The mean surface area of osteophytes is measured at four different locations in the joint. Depletion of macrophages on the mean surface area indicates a significant difference between normal and macrophage-depleted joints ($p<0.05$). Adapted from van Lent et al [20].

In addition to these in vivo findings, in vitro data also support the involvement of macrophages in chondrogenesis. As already mentioned, osteophytes are believed to develop from mesenchymal cells in the synovium or periosteum. Mesenchymal cells, like the C3H10T1/2 cell line, undergo chondrogenesis once they are stimulated with TGF-β or certain other growth factors. Chondrogenesis is an early step in the process of osteophyte formation. Interestingly, when these C3H10T1/2 cells are co-cultured in a transwell system with a macrophage cell line (RAW 264.7), substantially less TGF-β is needed to induce chondrogenesis (Fig. 4.3). TGF-β is required to induce chondrogenesis, because co-culture of both cell types without TGF-β did not lead to chondroneogenesis. When, as a control, clodronate liposomes are added in this system, the macrophage effect disappears, even though TGF-β itself can still generate chondrogenesis. This demonstrates the selectivity of the clodronate liposomes for macrophages, without their touching mesenchymal cells. Together these findings show that macrophages produce an additional factor after stimulation with TGF-β that, together with TGF-β itself, induces chondrogenesis. TGF-β levels in the culture supernatant are at a higher concentration than the range of added concentrations. This indicates that TGF-β addition leads to autoinduction of TGF-β. Surprisingly TGF-β levels are the same in cultures with or without macrophages. Therefore, the extra factor produced by macrophages to generate osteophytes is not TGF-β itself.

There can be no doubt that macrophages are involved in TGF-β–induced osteophyte formation. However, this does not mean that macrophages are also involved in osteophyte formation in experimental OA. In follow-up studies macrophages were depleted from the synovium prior to induction of experimental OA by intraarticular injection of collagenase.

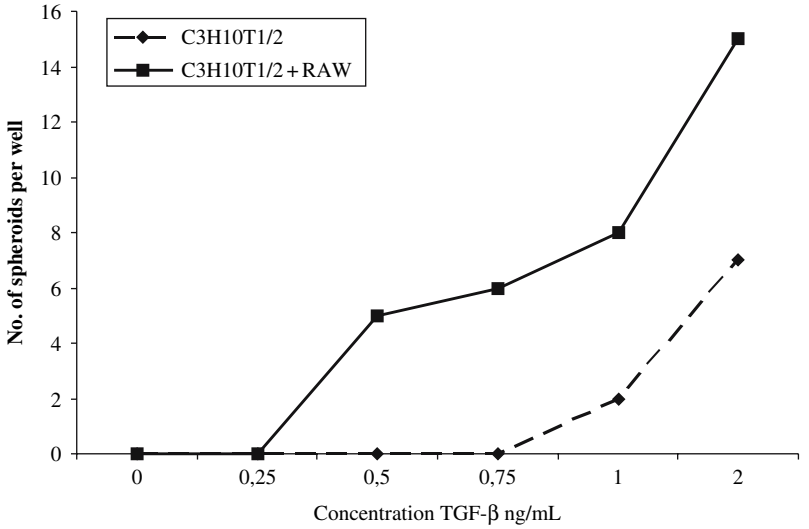

Figure 4.3. The effect of co-culture of macrophages (RAW 264.7) with C3H10T1/2 cells on TGF-β–induced chondrogenesis. As can be seen, the presence of macrophages significantly lowers the level of TGF-β to induce chondrogenesis, as evaluated by the number of spheroids that are formed.

The collagenase damaged the ligaments that normally assure joint stability, with the result that the joint became unstable. The first signs of pathology including osteophyte formation already develop after 7 days. After 6 weeks the knee joint exhibits full blown OA-like pathology. Here macrophage depletion also results in significantly fewer and smaller osteophytes in the early phase [5].

These findings indicate an important role for synoviocytes in osteophyte formation. Apparently synovial macrophages produce an additional growth factor that, combined with TGF-β, is responsible for osteophyte formation. During collagenase induced OA, significant inflammation rarely occurs, apart from the first 3 days. However, macrophage activation is evident, as reflected by the expression of the activation marker myeloid-related protein-14(MRP14) at days 7 and 14 after OA induction. Activation occurs both in the lining and in the deeper layer of the synovium. But because this layer is no longer there, due to the depletion of macrophages, the expression of growth factors such as BMPs in the synovial lining is greatly diminished. BMP expression in the deeper layers of the synovium, however, does not differ between macrophage-depleted and nondepleted animals. This makes it unlikely that the differences in osteophyte formation are due to differential BMP expression. No changes in expression of other growth factors, like platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), have been reported. So far it is not known what specific macrophage derived factors are involved in osteophyte formation. Nevertheless macrophages are clearly key players in OA-related joint pathology.

4.5 Synovial Macrophages Are Involved in Early Changes in the Cartilage During Experimental Osteoarthritis

Because macrophages are important for osteophyte formation in experimental OA, the question arises whether they are also involved in other OA-related pathology and in irreversible cartilage destruction, in particular. The limitations of the aforementioned technique for synovial macrophage elimination make it difficult to study the long-term involvement of macrophages in an experimental OA model. Normally it takes about 6 weeks for full blown OA cartilage pathology, like cartilage lesions, to develop. Macrophages start to repopulate the lining in a normal joint approximately 3 weeks

after depletion; this may occur even more quickly in a diseased joint [19]. This complicates the study of macrophage involvement in cartilage damage, inasmuch as the erosions typical of experimental OA do not usually arise within this time frame. Instead early cartilage changes were explored by quantifying the appearance of specific neoepitopes with the amino acid sequence VDIPEN, compounds that are indicative of matrix metalloproteinase (MMP) activity in the cartilage matrix. The VDIPEN neoepitope is a specific amino acid sequence that is exposed when certain enzymes (mostly MMPs) cleave aggrecan between the first and second globular domain (Fig. 4.4). Although aggrecan is an essential element of the cartilage, cleavage of this enzyme may not seriously or irreversibly affect cartilage integrity, but may only constitute a sign of enzyme activity. Many enzymes capable of generating the VDIPEN neoepitope also cleave other cartilage proteins.

The VDIPEN neoepitope staining is performed after OA induction in murine knee joints with or without synovial macrophage depletion. Some expression of VDIPEN is already found in the cartilage of mice 7 days after OA induction, but is only present marginally. There is no significant difference in expression in comparison with when macrophages have been depleted prior to induction. Between days 7 and 14 after OA induction, however, VDIPEN expression in macrophage depleted joints does not further increase, whereas VDIPEN expression is about doubled in the joints where macrophages are still present (Fig. 4.5). This indicates that synovial macrophages are involved in the induction of enzyme activity in the cartilage during early OA. The undepleted joints do not exhibit clear signs of inflammation, although synovial macrophage activation is observed when MRP14 staining is used as an activation marker. The question then arises whether the increased induction of MMP activity is caused by enzymes in the synovium, or whether soluble factors from the synovial macrophages induce MMP production by chondrocytes. Analysis of synovial and cartilage samples from synovial tissue and cartilage reveals that MMP-2,-3, and-9 are indeed induced both in synovium and in cartilage when murine OA is induced by collagenase. However, the increased production by chondrocytes is not affected by removal of

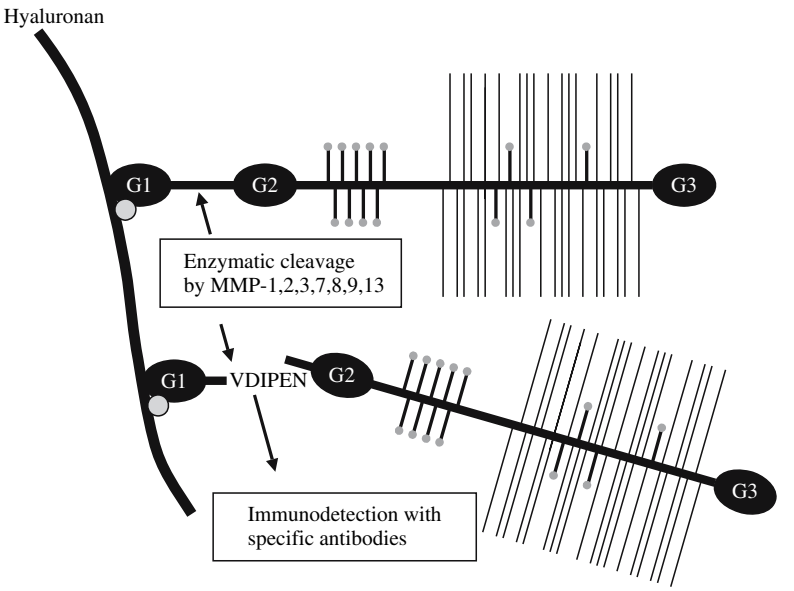

Figure 4.4. Generation of the VDIPEN neoepitope. Some MMPs can cleave the aggrecan molecule between the first and second globular domain. The larger fragment diffuses from the cartilage, whereas the shorter fragment remains in the cartilage, attached to hyaluronan. The latter has an exposed VDIPEN sequence that can be detected with a specific antibody. The amount of VDIPEN staining is a measure of the amount of active MMPs in the cartilage.

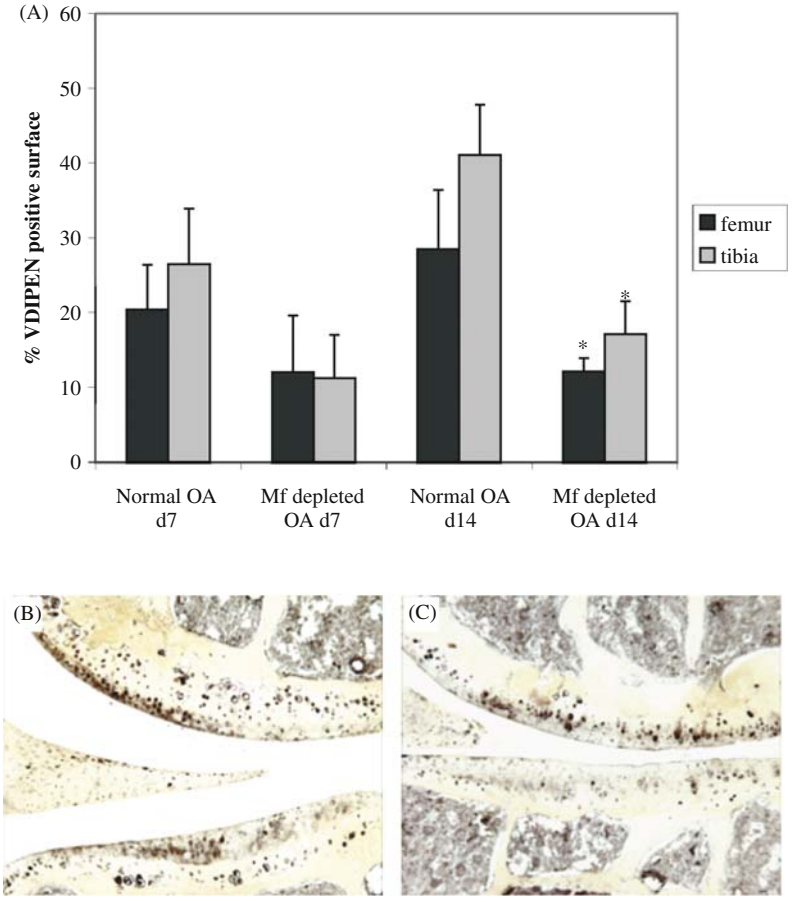

Figure 4.5. The effect of macrophage depletion on VDIPEN neoepitope generation in experimental OA. VDIPEN expression is significantly decreased at day 14 after OA induction in the cartilage of joints from which macrophages are depleted prior to OA induction (A,C), compared to OA joints with an intact synovium (A,B). Mf, *microphage*.

synovial lining macrophages, whereas expression in the synovium is changed. This indicates that macrophages either contribute directly to cartilage damage by the production of MMPs, or do so indirectly by activating chondrocyte-derived MMPs with enzymes that originate in the synovial macrophage. There is no evidence for enzyme induction in chondrocytes by the macrophages. This indicates that the increased expression of MMPs by chondrocytes in experimental or human OA is not under macrophage regulation, but may be caused by matrix fragments that stimulate the chondrocytes. Nevertheless, synovial macrophages are partly responsible for early MMP-mediated cartilage damage by causing direct production of MMPs and their subsequent diffusion to the cartilage matrix. To understand the long-term involvement of macrophages requires further research because, as mentioned earlier, the macrophage depletion technique using clodronate liposomes only allows for studies of up to 2 to 3 weeks.

Macrophages play a major role in OA-related pathology, but that may also require communication between synovial macrophages and fibroblasts as occurs in a normal joint and therefore probably also in the OA joint. Moreover fibroblasts are potent MMP producers and may in fact be the principal producers of MMPs in the synovium, probably regulated by the synovial lining macrophages. It is as yet unclear whether the observed effects are mainly macrophage related, or whether they are the result of crosstalk between macrophages and fibroblasts. Crosstalk may involve direct cell-to-cell contact, or cytokine signaling.

4.6 Expression of Cytokines in the Osteoarthritic Synovium and Implications for Their Role in Pathology

Cytokines that are produced in OA synovium and are implicated in OA pathology can be roughly divided into three categories: (1) destructive cytokines, (2) regulatory cytokines, and (3) anabolic factors. Obviously, the balance between cytokines from these three categories will determine the nature of the synovial and cartilage environment.

4.6.1 Catabolic Cytokines

IL-1 is one of the best known member of the destructive cytokines. It is found in large quantities in OA synovium, especially during early OA [2], and has a pronounced suppressive effect on chondrocyte proteoglycan and collagen type II synthesis. In addition, it stimulates the chondrocytes and synovial cells to release destructive proteases, such as MMPs, which can mediate cartilage breakdown. The net effect of IL-1 is therefore fast depletion of proteoglycans from the cartilage matrix, followed by destruction of the collagen network. Synovial macrophages are major producers of IL-1 in the diseased joint. Other factors in OA joints that in some ways act like IL-1, but with less potency, are TNF-α, IL-17, and IL-18. In both human and experimental OA, IL-1 production in the synovium is increased, as is the production of TNF-α, IL-17, and IL-18. These cytokines also have strong proinflammatory properties in addition to their direct effect on the cartilage. This means that once the synovium is activated to produce cytokines, activation is enhanced by the cytokines it produces. Inhibition of these cytokines is a feasible and potentially interesting approach to treat OA patients, especially in patients with a pronounced inflammatory phenotype. It should be mentioned that apart from higher cytokine levels in the synovium, increased levels of TNF receptors and IL-17 receptors are found in chondrocytes of human OA cartilage. Their very presence acts to enhance sensitivity of OA cartilage to these catabolic cytokines [15]. Of note, IL-1 and IL-18 are both produced in pro-forms and need similar converting enzymes to become active. Recent studies with an IL-1β converting enzyme (ICE) inhibitor in two murine OA models showed significant efficacy [27]. In severe OA, where cartilage damage is significant, patients may develop autoimmune T-cell responses to cartilage breakdown products and this may contribute to the OA pathology [28]. Under these conditions the T-cell–derived cytokine IL-17 is likely to assume pathologic significance.

4.6.2 Regulatory Cytokines

The regulatory cytokines include mediators such as IL-4, IL-6, IL-10, and IL-13. These cytokines inhibit synovial macrophages or chondrocytes from producing destructive cytokines, such as IL-1 and TNF-α. In addition, they upregulate natural inhibitors of those cytokines, such as IL-1ra and soluble receptors of both IL-1 and TNF-α. For a long time, it was believed that IL-4 was produced exclusively by T cells, but more recently this protein has been considered important for the maintenance of normal cartilage integrity. It also inhibits IL-1 and TNF-α production by OA synovium in vitro, and, when locally expressed, also induces inflammation. Whether this occurs in an OA joint is doubtful, because only minimal amounts leak from the cartilage. Interleukin-4 is found in T cells that are encountered in a subgroup of OA patients [17]. Both IL-10 and IL-13 share regulatory properties with IL-4 and are found in the synovial membrane of OA patients [29]. Another interesting regulatory mediator is IL-6, a cytokine that enhances expression of TIMPs, the naturally occurring tissue inhibitors of MMPs. Recent studies of an OA model that simulates aging have called attention to the fact that mice lacking IL-6 develop more severe OA than controls. This suggests that IL-6 acts to prevent of cartilage breakdown during OA. However, IL-6 has a proinflammatory component that excludes direct administration of the cytokine to the affected joint. Regulatory cytokines and their role in OA need further attention.

4.6.3 Anabolic Cytokines

The final category of cytokines involved in OA cartilage pathology is made up of growth factors.

These cytokines do not regulate production or action of destructive cytokines like IL-1 and TNF-α, but counteract their effects. For instance, IL-1 causes depletion of proteoglycans from cartilage by enhanced breakdown and inhibited synthesis, whereas the growth factor TGF-β enhances synthesis of proteoglycans and other cartilage matrix components [3]. It is the balance of these factors that determines whether the net effect is synthesis enhanced or inhibited. Other growth factors that can stimulate chondrocyte synthetic activity include insulin-like growth factor-1 (IGF-1) (32,38), BMPs, bFGF/fibroblast growth factor-2 (FGF-2), and PDGF. Some of these are also involved in catabolic processes in the cartilage [13,39]. In certain subsets of OA patients, decreased levels of IGF-1 are found in the synovium [25]. However, other studies report increased levels of synovial growth factor production in OA patients. This again underlines the heterogeneity of the disease. Many of these factors in addition to their beneficial effects also display unwanted side effects in other tissues, a major concern in planning therapy. When growth factors are administered therapeutically, the synthesis of matrix proteins is enhanced. This indicates possible regeneration of the cartilage matrix. Intriguingly, BMP-7 and TGF-β potently counteract IL-1 and may therefore become tools to induce cartilage repair [7]. However, BMPs have a role in endochondral ossification and stimulate terminal differentiation of chondrocytes. Cartilage calcification can be a serious drawback in the application of BMPs to cartilage repair. Also, TGF-β has many adverse effects. It induces fibroblast proliferation (fibrosis) in the synovium, attracts leukocytes to the synovium, and, as discussed earlier, induces osteophyte formation, phenomena that are also often observed in OA synovium. If these adverse effects can be overcome by maintaining the chondrocyte stimulatory capacity of the cartilage, TGF-β may become a valuable, highly potent therapeutic agent.

Cartilage pathology results from overproduction of destructive cytokines in the synovium, but will also result when anabolic stimulation is insufficient or when cytokines exert insignificant control. Both synovium and cartilage contribute to the total cytokine milieu. The relative balance of the various mediators probably plays a greater role in net cartilage destruction than does the absolute level of destructive mediators. It is tempting to speculate that the course of the illness is determined by the balance of individual cytokines or by defective receptors in the joints of OA patients. The appropriate therapeutic approach depends on which of the two possibilities prevails.

4.7 Expression of Matrix Metalloproteinases in the Osteoarthritis Synovium

An important effect of the catabolic cytokines, like IL-1, is induction of MMP production. As already discussed, MMP-mediated cartilage damage can be overcome partly by MMPs produced by synovial cells, rather than by MMP expression induced in the chondrocytes. Also, as shown above, many cytokines found in OA induce or somehow modulate MMP expression. The MMPs are released from the cell in an inactive pro-form. Once activated, most MMPs directly degrade cartilage matrix proteins, thus causing structural damage to the cartilage. They are also capable of activating other pro-MMPs, thereby increasing the damage. The TIMPs are the naturally occurring inhibitors for MMPs and the balance between MMPs and TIMPs in the joint is likely to be crucial as to whether cartilage is broken down enzymatically.

4.7.1 Synovial Expression of Catabolic Enzymes

A wide array of MMPs is produced in the osteoarthritic synovium, with MMP-13 perhaps the most extensively studied enzyme expressed in OA cartilage. When overexpressed in mouse cartilage, an OA phenotype results. Marini et al [23] reported that MMP-13 expression is enhanced in the synovium of OA patients and that a correlation exists between the synovial MMP-2 and MMP-13 and the arthroscopically observed cartilage damage. We, in turn, have demonstrated increased expression of MMP-13, both in cartilage and synovium, in early as well as late collagen-induced OA (Fig. 4.6). Levels of MMP-13 are upregulated to a larger extent in synovial than in cartilage specimens.

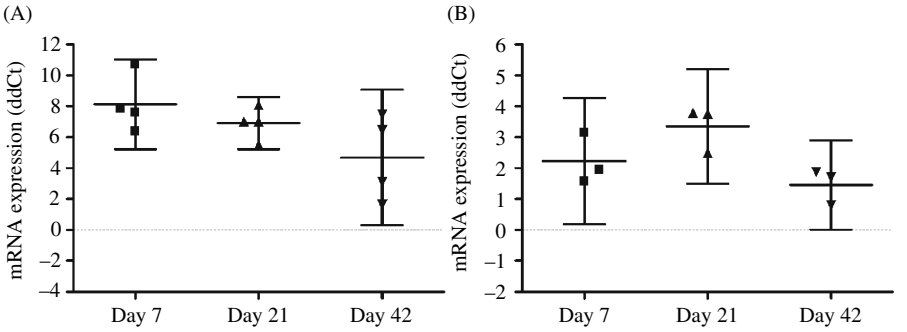

Figure 4.6. MMP-13 messenger RNA (mRNA) levels in synovium and cartilage in experimental OA. MMP-13 expression is significantly elevated in synovium (A) and cartilage (B) of joints in which OA was induced, compared to the naive contralateral joints. MMP-13 expression is already increased 1 week after induction and remains high up to day 42, when full-blown OA pathology is present. Note that expression in the synovium is increased much more (up to 250-fold) than in cartilage (up to eight-fold). Results are expressed, after correction for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expression in the contralateral knee joint, as ∇, ΔCt with a 95% confidence interval.

Large quantities of pro-MMP-1 and pro-MMP-3 are found and probably produced in the synovial fluid of OA patients [37]. Although there is no increase in enzyme activity in OA synovial fluid, it contains a large quantity of potential matrix-degrading enzymes that, upon local activation, degrade the cartilage matrix and activate other enzymes. Ex vivo synovial cultures also contain many MMPs, for example, MMP-2, MMP-9, and MT1-MMP (MMP-14) [16], both as mRNAs and as proteins. Recently, Dreier et al [8] demonstrated that pro-MMP-9 is produced by synovial OA macrophages and activated by MMPs derived from the cartilage. MMP-9 in turn activates other MMPs. Thus a whole cascade of MMP activation is induced, originating from the cartilage, but strongly enhanced by the synovium. It is apparant the role of the synovium in cartilage degradation is enhanced by the MMPs derived from it.

4.7.2 Enzyme Inhibitors in Osteoarthritis Synovium

So far, four different TIMPs have been described. Increased levels of TIMP1 are found in the synovial fluid of OA patients, but this has not decreased MMP activity in OA synovial fluid [37]. The same report shows that in acute joint injury, which predisposes to OA development, there is a rise in the molar ratio of MMP-3 to MMP-1, on one hand, and TIMP1, on the other. This increase induces a catabolic environment. TIMP-1 and TIMP-2 are found in the synovial membrane in OA patients, but what this means is not clear.

4.8 Synovial Receptors for Cartilage Components

The above findings indicate an active role for synovial cells, especially macrophages, in OA pathology. It is also clear that vast amounts of mediators are produced in the synovium of OA patients. The mechanism that causes macrophages and other synovial cells to function in the OA process is as yet speculative. For synovial cells to get drawn into the OA process, they have to be activated. The activation stimulus probably originates in cartilage, because that is the primary tissue that is damaged, with cartilage protein or cartilage protein fragments leaking to the synovial membrane. These fragments subsequently stimulate synovial cells through as-yet-unknown receptors. In response the macrophages produce mediators that aggravate the process by production of cytokines and matrix-degrading enzymes (Fig. 4.7). Candidate receptors are several of the Toll-like receptors (TLRs), especially TLR4, the receptor for advanced glycation end products (RAGE), and the hyaluronan receptor CD44.

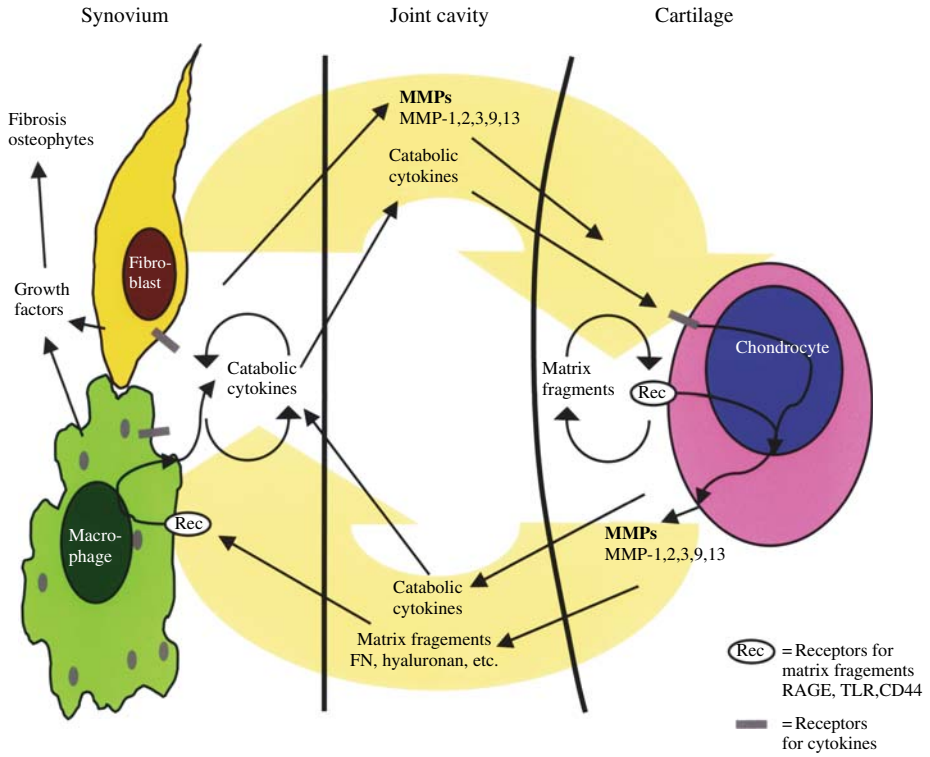

Figure 4.7. A simplified scheme of the vicious circle of macrophage activation in OA. Macrophage activation leads to induction of cartilage damage through the generation and release of MMPs and catabolic cytokines. Cartilage damage results in the release of matrix fragments, such as fibronectin or proteoglycan fragments. The fragments are released from the matrix and stimulate macrophages and other cells in the synovium via, among others, TLRs, RAGE, or CD44 to produce more catabolic cytokines and MMPs; this results in more cartilage damage. Cartilage damage also leads to cytokine production by chondrocytes. Note that self-enhancing loops can occur within the synovium and cartilage. Activation of cytokine receptors leads to more cytokine production. In the cartilage, MMP production and activation cause cartilage degradation. The resulting matrix fragments also stimulate the chondrocyte via receptors like TLRs or RAGE.

4.8.1 Toll-Like Receptors

The TLRs are expressed on many different cell types, including macrophages and fibroblasts. Notwithstanding great interest in the role played by TLRs in RA, their role in OA has not been studied extensively. The TLRs are part of the first line of host defense. They recognize many pathogenic microorganisms. Numerous endogenous ligands can also stimulate these cells via their receptors. Macrophages can be activated by biglycan to produce mediators like TNF-α. TLR2 and TLR4 on macrophages mediate this activation [30].

Biglycan is a leucine-rich proteoglycan that is present in articular cartilage, especially in the pericellular matrix. When structural cartilage damage occurs, biglycan will diffuse from the cartilage and reach the synovial macrophages. Increased levels of biglycan have been found in the cartilage of OA patients. This would suggest that OA cartilage tends to activate synovial cells via biglycan [18]. Other endogenous ligands for TLR2 and TLR4 are heat shock protein (HSP)-70, certain fibronectin (FN) fragments, oligosaccharides of hyaluronic acid, and polysaccharide fragments of heparan sulfate, all likely to be present in an osteoarthritic joint. The FN fragments in particular have been described as causing (experimental) OA. However, so far it has not been possible to assign a specific role to these receptors in human OA. Experiments using specific TLR knockout mice have not provided evidence to substantiate TLR involvement.

4.8.2 Receptor for Advanced Glycation End Products

Advanced glycation end products (AGE) have long been implicated in OA. The presence of these products in cartilage increases with age, and their effect on chondrocytes has been studied. Chondrocytes express the receptor for AGE (RAGE), and evidence is increasing that these receptors play a role in causing cartilage damage [6,21]. However, the expression of RAGE on synoviocytes and the role of this receptor in synovial tissue is less well established. Recently, with the aid of double staining for the CD68 antigen [9], RAGE has been shown to be expressed in synovial tissue of OA patients, with a preference for the synovial lining macrophages. Moreover, activation of RAGE on synoviocytes and chondrocytes in OA patients has led to the activation of these cells [33]. Taniguchi et al [35] have shown that high-mobility group box chromosomal protein 1 (HMGB1) was expressed in OA synovium and induced TNF-α expression via RAGE on macrophages, blocking the signal with soluble RAGE. Although S100A12 (ENRAGE), like some other S-100 proteins, binds to RAGE, it occurs in inflamed RA synovium, but apparently not in OA synovium. The S-100A9 proteins occur in an experimental model for OA [5]. The presence of RAGE in synovium and of its ligands in cartilage, which increase with age, makes this receptor a candidate that helps activate the synovium.

4.8.3 CD44

CD44 is a transmembrane glycoprotein to which several extracellular matrix components such as hyaluronan, fibronectin, and collagens I and IV can bind. It is implicated in processes such as cell-cell adhesion, cell migration, cell adhesion to the extracellular matrix, and lymphocyte activation. It occurs in several splice variants. CD44v6 is increased in OA chondrocytes [36]. Upregulation of CD44 is associated with local loss of hyaluronan. In contrast, CD44 has also been described as a protective receptor, since binding can result in a decreased expression of MMP1 and, if regulated on activation, in normal T expression and secretion (RANTES/CCL5) [34]. Synovial fibroblasts also express CD44, and its expression is increased in OA synovium [11]. Whether this increase in CD44 expression adds to OA pathology is not known. It is difficult to imagine that ubiquitous proteins like hyaluronan, or biglycan in the case of TLR-stimulation, are pathogenic. Alternatively, differential expression of (one of) the less frequently expressed isoforms of CD44 could constitute an additional mechanism, for instance when differences in affinity or effector mechanisms are involved.

There is no conclusive evidence so far that the genes mentioned above are involved in OA; they have been merely mentioned as possible candidates. However, they may mediate synovial activation in OA. More research is needed, and these and other candidates need to be evaluated more extensively to find mechanisms for synovial activation. This knowledge could then be used to develop new therapies to inhibit synovial activation. This, in turn, will affect parameters such as osteophyte formation, cartilage degradation, and pain caused by synovitis. Gene therapy is a promising therapeutic approach to target synovium.

4.9 Gene Therapy Targeting the Osteoarthritis Synovium

Since OA often appears in only one or two joints of OA patients, OA is a disease in which local therapy is indicated. Gene therapy is an obvious approach to achieve high and constant levels of a therapeutic agent with a minimum of systemic side effects. Because there are strong indications that the synovium is an active player in OA pathology, the synovium itself is an obvious therapeutic target. Gene therapy can be used to modulate processes in the synovium or to target the cartilage by soluble factors that diffuse to the cartilage. In the latter case, the synovium is basically a production unit for the therapeutic agent. There is increasing agreement that IL-1 is an important mediator in the etiology of this disease. When inflammation is part of the disease, IL-1 and TNF-α are likely to play an important role, and blocking these mediators may prove beneficial, inasmuch as animal studies have already demonstrated that gene therapy targeting IL-1 is a feasible approach [10,40]. Once it becomes clear which receptors are involved in the activation of the synovium, it may be possible to silence them

by RNA interference (RNAi), as in the case of TLRs and RAGE. In the types of OA that do not involve the synovium and where impaired cartilage anabolism is the major problem, compartmentalized TGF-β supplementation may be a good therapeutic alternative. It is clearly important to discriminate between the several forms of OA before undertaking targeted therapy.

Due to their size, virus particles cannot penetrate cartilage, although recent studies using adeno-associated viruses (AAVs) do claim they can. Targeting chondrocytes directly would not only reduce side effects, but also may bring about a more sustained expression of the transgene, because chondrocytes have a low turnover rate. At present, the cartilage can only be targeted indirectly, through transduction of synovial cells and subsequent diffusion of soluble factors. Another important development will be engineering viral gene constructs that carry promoters for inducible expression. Such a vector would sense local activity and generate IL-1ra production only after synovial activation, as indicated by IL-1 production. We have utilized an inducible IL-1/IL-6 promoter in RA models [22]. To apply this approach in OA requires identifying suitable promoters of mediators or markers of the OA process. Possible promoters are those of certain MMPs, such as MMP-13, which occur in large amounts in OA synovium and cartilage.

4.10 Conclusion

More research is needed to clarify the contribution made by the synovium to OA pathology, a role that has long been underestimated. However, with research focused on the importance of synovium, synovial involvement is clearly seen to be substantial. Synovial macrophages are important in mediating OA-related pathology, such as osteophyte formation and MMP-mediated cartilage matrix breakdown. From a histologic standpoint, synovial OA is a very heterogeneous tissue. In many instances inflammation is substantial; in others the synovial lining appears only thickened, and in others the synovium appears normal. This contrasts with RA synovia that appear more homogeneous with inflammation a common feature. Osteoarthritis, therefore, may not be a single disease, but rather be the outcome of different pathologic processes. In one case, OA may be caused by a process restricted to the cartilage, in another synovial involvement may be more important, with inflammation apparent. However, even when there is no clear synovial inflammation, as in collagenase-induced OA, the synovial cells may still play an important role, as was shown by synovial macrophage depletion in this model. It is therefore important to generate tools that discriminate between the different forms of OA. In some, anti-inflammatory therapy may be satisfactory. In others, therapy may be targeted principally to the cartilage, whereas in others yet, specific targeting and silencing the synovial macrophage may be the optimal approach. However, this kind of customized therapy depends on research that takes into account the many tissue and cell types that together ensure homeostasis of the joint.

References

1. Bakker AC, van de Loo FA, van Beuningen HM, Sime P, van Lent PL, van der Kraan PM, Richards CD, van den Berg WB (2001) Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation. Osteoarthritis Cartilage *9:*128–136.
2. Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B (2005) Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis *64:*1263–1267.
3. van Beuningen HM, van der Kraan PM, Arntz OJ, van den Berg WB (1993) Protection from interleukin 1 induced destruction of articular cartilage by transforming growth factor beta: studies in anatomically intact cartilage in vitro and in vivo. Ann Rheum Dis *52:*185–191.
4. van Beuningen HM, Glansbeek HL, van der Kraan PM, van den Berg WB (1998) Differential effects of local application of BMP-2 or TGF-beta 1 on both articular cartilage composition and osteophyte formation. Osteoarthritis Cartilage *6:*306–317.
5. Blom AB, van Lent PL, Holthuysen AE, van der Kraan PM, Roth J, van Rooijen N, van den Berg WB (2004) Synovial lining macrophages mediate osteophyte formation during experimental osteoarthritis. Osteoarthritis Cartilage *12:*627–635.
6. Cecil DL, Johnson K, Rediske J, Lotz M, Schmidt AM, Terkeltaub R (2005) Inflammation-induced chondrocyte hypertrophy is driven by receptor for advanced glycation end products. J Immunol *175:* 8296–8302.
7. Chubinskaya S, Kuettner KE (2003) Regulation of osteogenic proteins by chondrocytes. Int J Biochem Cell Biol *35:*1323–1340.

8. Dreier R, Grassel S, Fuchs S, Schaumburger J, Bruckner P (2004) Pro-MMP-9 is a specific macrophage product and is activated by osteoarthritic chondrocytes via MMP-3 or a MT1-MMP/MMP-13 cascade. Exp Cell Res *297:*303–312.
9. Drinda S, Franke S, Ruster M, Petrow P, Pullig O, Stein G, Hein G (2005) Identification of the receptor for advanced glycation end products in synovial tissue of patients with rheumatoid arthritis. Rheumatol Int *25:*411–413.
10. Frisbie DD, Ghivizzani SC, Robbins PD, Evans CH, McIlwraith CW (2002) Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene. Gene Ther *9:*12–20.
11. Fuchs S, Dankbar B, Wildenau G, Goetz W, Lohmann CH, Tibesku CO (2004) Expression of the CD44 variant isoform 5 in the human osteoarthritic knee joint: correlation with radiological, histomorphological, and biochemical parameters. J Orthop Res *22:*774–780.
12. Haringman JJ, Gerlag DM, Zwinderman AH, Smeets TJ, Kraan MC, Baeten D, McInnes IB, Bresnihan B, Tak PP (2005) Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. Ann Rheum Dis *64:*834–838.
13. Harvey AK, Stack ST, Chandrasekhar S (1993) Differential modulation of degradative and repair responses of interleukin-1-treated chondrocytes by platelet-derived growth factor. Biochem J *292:*129–136.
14. Haywood L, McWilliams DF, Pearson CI, Gill SE, Ganesan A, Wilson D, Walsh DA (2003) Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum *48:*2173–2177.
15. Honorati MC, Meliconi R, Pulsatelli L, Cane S, Frizziero L, Facchini A (2001) High in vivo expression of interleukin-17 receptor in synovial endothelial cells and chondrocytes from arthritis patients. Rheumatology (Oxford) *40:*522–527.
16. Hsieh YS, Yang SF, Chu SC, Chen PN, Chou MC, Hsu MC, Lu KH (2004) Expression changes of gelatinases in human osteoarthritic knees and arthroscopic debridement. Arthroscopy *20:*482–488.
17. Ishii H, Tanaka H, Katoh K, Nakamura H, Nagashima M, Yoshino S (2002) Characterization of infiltrating T cells and Th1/Th2-type cytokines in the synovium of patients with osteoarthritis. Osteoarthritis Cartilage *10:*277–281.
18. Knudson CB, Knudson W (2001) Cartilage proteoglycans. Semin Cell Dev Biol *12:*69–78.
19. van Lent PL, van den Bersselaar L, van den Hoek AE, van de Ende M, Dijkstra CD, van Rooijen N, van de Putte LB, van den Berg WB (1993) Reversible depletion of synovial lining cells after intra-articular treatment with liposome-encapsulated dichloromethylene diphosphonate. Rheumatol Int *13:*21–30.
20. van Lent PL, Blom AB, van der Kraan P, Holthuysen AE, Vitters E, van Rooijen N, Smeets RL, Nabbe KC, van den Berg WB (2004) Crucial role of synovial lining macrophages in the promotion of transforming growth factor beta-mediated osteophyte formation. Arthritis Rheum *50:*103–111.
21. Loeser RF, Yammani RR, Carlson CS, Chen H, Cole A, Im HJ, Bursch LS, Yan SD (2005) Articular chondrocytes express the receptor for advanced glycation end products: potential role in osteoarthritis. Arthritis Rheum *52:*2376–2385.
22. van de Loo FA (2004) Inflammation-responsive promoters for fine-tuned gene therapy in rheumatoid arthritis. Curr Opin Mol Ther *6:*537–545.
23. Marini S, Fasciglione GF, Monteleone G, Maiotti M, Tarantino U, Coletta M (2003) A correlation between knee cartilage degradation observed by arthroscopy and synovial proteinases activities. Clin Biochem *36:*295–304.
24. Mulherin D, Fitzgerald O, Bresnihan B (1996) Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum *39:*115–124.
25. Pagura SM, Thomas SG, Woodhouse LJ, Ezzat S, Marks P (2005) Circulating and synovial levels of IGF-I, cytokines, physical function and anthropometry differ in women awaiting total knee arthroplasty when compared to men. J Orthop Res *23:*397–405.
26. van Rooijen N, Sanders A, van den Berg TK (1996) Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine. J Immunol Methods *193:*93–99.
27. Rudolphi K, Gerwin N, Verzijl N, van der Kraan P, van den Berg W (2003) Pralnacasan, an inhibitor of interleukin-1beta converting enzyme, reduces joint damage in two murine models of osteoarthritis. Osteoarthritis Cartilage *11:*738–746.
28. Saito I, Koshino T, Nakashima K, Uesugi M, Saito T (2002) Increased cellular infiltrate in inflammatory synovia of osteoarthritic knees. Osteoarthritis Cartilage *10:*156–162.
29. Sakkas LI, Scanzello C, Johanson N, Burkholder J, Mitra A, Salgame P, Katsetos CD, Platsoucas CD (1998) T cells and T-cell cytokine transcripts in the synovial membrane in patients with osteoarthritis. Clin Diagn Lab Immunol *5:*430–437.
30. Schaefer L, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, Gotte M, Malle E, Schaefer RM, Grone HJ (2005) The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. J Clin Invest *115:*2223–2233.
31. Scharstuhl A, Vitters EL, van der Kraan PM, van den Berg WB (2003) Reduction of osteophyte formation and synovial thickening by adenoviral overexpression of transforming growth factor beta/bone morphogenetic protein inhibitors during experimental osteoarthritis. Arthritis Rheum *48:*3442–3451.
32. Schmidt MB, Chen EH, Lynch SE (2006) A review of the effects of insulin-like growth factor and platelet-derived growth factor on in vivo cartilage healing and repair. Osteoarthritis Cartilage *14:*403–412.
33. Steenvoorden MM, Huizinga TW, Verzijl N, Bank RA, Ronday HK, Luning HA, Lafeber FP, Toes RE, DeGroot J (2006) Activation of receptor for advanced glycation end products in osteoarthritis leads to increased stimulation of chondrocytes and synoviocytes. Arthritis Rheum *54:*253–263.
34. Tanaka M, Masuko-Hongo K, Kato T, Nishioka K, Nakamura H (2006) Suppressive effects of hyaluronan on MMP-1 and RANTES production from chondrocytes. Rheumatol Int *26:*185–190.
35. Taniguchi N, Kawahara K, Yone K, Hashiguchi T, Yamakuchi M, Goto M, Inoue K, Yamada S, Ijiri K,

Matsunaga S, Nakajima T, Komiya S, Maruyama I (2003) High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. Arthritis Rheum *48:*971–981.

36. Tibesku CO, Szuwart T, Ocken SA, Skwara A, Fuchs S (2005) Increase in the expression of the transmembrane surface receptor CD44v6 on chondrocytes in animals with osteoarthritis. Arthritis Rheum *52*:810–817.
37. Tchetverikov I, Lohmander LS, Verzijl N, Huizinga TW, TeKoppele JM, Hanemaaijer R, DeGroot J (2005) MMP protein and activity levels in synovial fluid from patients with joint injury, inflammatory arthritis, and osteoarthritis. Ann Rheum Dis *64:*694–698.
38. van den Berg WB, van der Kraan PM, Scharstuhl A, van Beunargen HM (2001) Growth factors and cartilage repair. Clin Orthop Relat Res 391 (suppl): S244–S250.
39. Yamanishi Y, Boyle DL, Clark M, Maki RA, Tortorella MD, Arner EC, Firestein GS (2002) Expression and regulation of aggrecanase in arthritis: the role of TGF-beta. J Immunol *168:*1405–1412.
40. Zhang X, Mao Z, Yu C (2004) Suppression of early experimental osteoarthritis by gene transfer of interleukin-1 receptor antagonist and interleukin-10. J Orthop Res *22:*742–750.