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REVIEW

The Role of Synovial Macrophages and Macrophage-Produced Mediators in Driving Inflammatory and Destructive Responses in Osteoarthritis

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Introduction

Osteoarthritis (OA), one of the most common diseases among mammals, can be considered to be part of the aging process. It is characterized pathologically by focal areas of damage on articular cartilage centered on load-bearing areas in association with the formation of new bone at the joint margins, changes in subchondral bone, and synovitis. Mechanical factors, such as obesity or a history of joint trauma, are recognized risk factors for OA, as are certain endogenous factors, such as type II collagen mutations and acetabular dysplasia. OA is the world's leading cause of chronic disability not only for the elderly but also for individuals of working age. Given the huge economic and personal burdens of OA and the fact that this disease is the major cause of the increasing demand for joint replacements, there is an urgent need for disease-modifying treatments to stop or slow the development and progression of OA. For this to be possible, however, additional knowledge is needed about the pathogenesis of disease initiation and progression in OA.

Today, it is accepted that both inflammatory and destructive features of rheumatoid arthritis (RA) are driven through synovitis. The RA synovium has a plentiful infiltration of activated macrophages, producing tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and other proinflammatory cytokines (1). Because there is a cytokine cascade with TNFα driving other proinflammatory mediators, this cytokine has become a key therapeutic target in RA, with several anti-TNFα drugs being used with considerable success (for review, see ref. 2).

In contrast, OA has long been considered a degenerative disease, mainly involving cartilage and bone. The concept of synovial inflammation contributing to OA pathology was introduced in the 1990s and has been gaining strength ever since (3–5). This concept has considerable importance for the potential development of disease-modifying anti-OA drugs (DMOADs). Here, we will review and comment on some recent work, involving both in vitro studies of human OA synovium and studies of OA pathology in animal models, which have strongly suggested that the inflamed synovium and activated synovial macrophages are important in promoting OA pathology. In particular, we will provide an overview of the role of synovial macrophages in promoting inflammatory and destructive responses in OA and the potential role of therapeutic strategies directed against macrophages or macrophage-produced cytokines as remission-inducing agents in this disease.

The role of synovitis in OA

Clinically, patients with OA have a variable degree of synovitis. In some of them, quite aggressive inflammatory OA of the knee or hip joint may develop,

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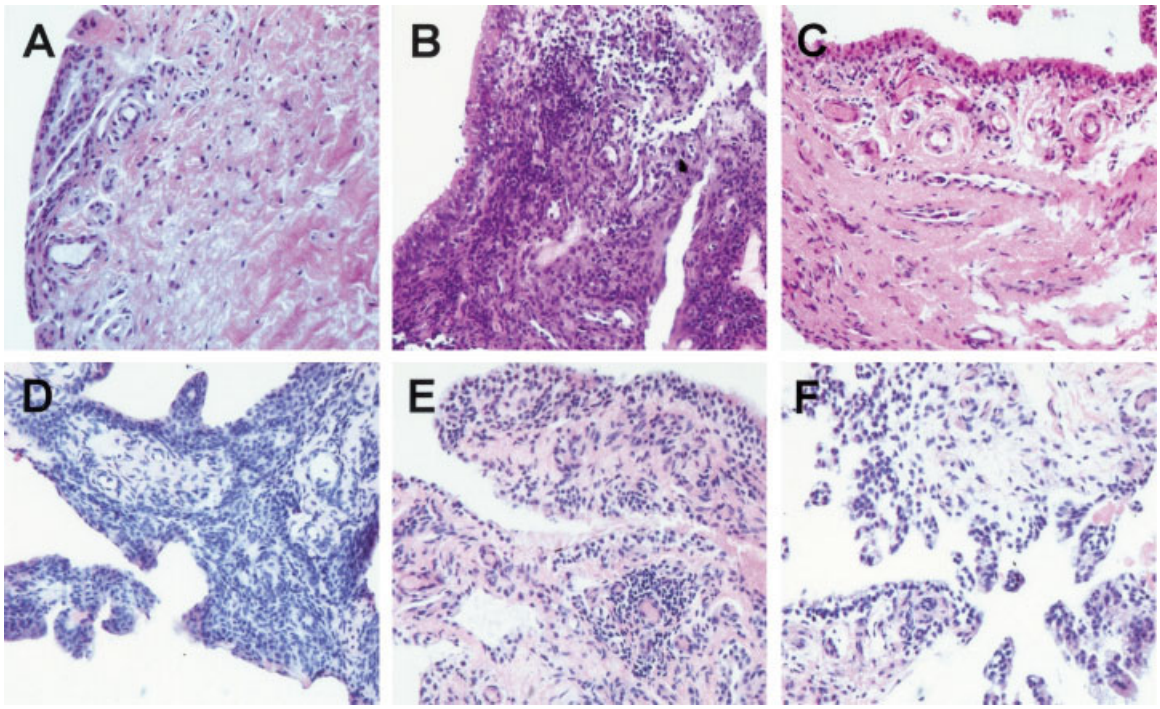

Figure 1. Photomicrographs of synovial biopsy specimens obtained from 3 patients with early inflammatory osteoarthritis (**A–C**, respectively) and 3 patients with rheumatoid arthritis (**D–F**, respectively), showing thickened synovium and infiltration of inflammatory cells. (Original magnification × 200.)

sometimes with marked exudation, which can be treated by arthrocentesis and locally injected steroids. Other patients, particularly those in whom obesity or other mechanical factors predispose to OA, have a much lesser degree of clinically obvious synovitis.

Synovial inflammation has been implicated in many of the signs and symptoms of OA, including joint swelling and effusion (5,6). It is likely to contribute to disease progression, as judged by the correlation between biologic markers of inflammation, such as C-reactive protein and cartilage oligomeric matrix protein, with the progression of structural changes in OA (7,8). Histologically, OA synovium shows hyperplasia, with an increased number of lining cells and a mixed inflammatory infiltrate mainly consisting of macrophages (6,9). In many cases, synovial biopsy specimens obtained from patients with early inflammatory OA resemble RA biopsy specimens morphologically (Figure 1), although the percentage of macrophages is lower, and the percentage of T cells and B cells is much lower (10–12).

A variety of cytokines and other mediators are involved in OA synovitis (13,14). Although the levels of proinflammatory cytokines are generally lower than those observed in RA, TNFα and IL-1 have been suggested as key players in OA pathogenesis, both in synovial inflammation and in the activation of chondrocytes and synovial fibroblasts (13–16). These cytokines can stimulate their own production and induce synovial cells and chondrocytes to produce IL-6, IL-8, and leukocyte inhibitory factor, as well as stimulate protease and prostaglandin production (13,15–17). The hypothe-

Table 1. Role of synovial macrophages and their mediators in RA and OA*

Similarities
Synovial macrophages play an important part in driving pathology.
Macrophages drive the production of several proinflammatory cytokines (e.g., IL-6, IL-8).
Macrophages drive the production of several MMPs.
Dissimilarities
There are fewer macrophages in OA synovium.
IL-1 production is driven by TNFα in RA but not in OA.
IL-1 is NF-κB dependent in RA but not in OA.
TNFα is more strongly NF-κB dependent in RA.
There is differential expression of FAK family kinases in RA and OA synovium.
Osteophyte formation is specific for OA.
In experimental OA, osteophyte formation is mediated by macrophages.

* RA = rheumatoid arthritis; OA = osteoarthritis; IL-6 = interleukin-6; MMPs = matrix metalloproteinases; TNFα = tumor necrosis factor α; FAK = focal adhesion kinase.

sis that TNFα and IL-1 are key mediators of inflammation and articular cartilage destruction has raised the possibility of anticytokine therapy in OA or the design of specific DMOADs (18–21).

Macrophage function in OA synovium

If it is accepted that synovial inflammation and the production of proinflammatory and destructive mediators from OA synovium are of importance for the symptoms and progression of OA, a key question is which cell type in OA synovium is responsible for maintaining synovial inflammation. In RA, in which the macrophage is the main promoter of disease activity, macrophage-produced TNFα is a major therapeutic target (for review, see ref. 2). Much less is known about macrophage biology in OA, however, although histologic studies have demonstrated that OA synovial macrophages exhibit an activated phenotype, as demonstrated by the production of both proinflammatory cytokines and vascular endothelial growth factor (6,9,22).

In a model of cultures of synovial cells from digested RA or OA synovium, the cells have the advantage of spontaneously producing a variety of proinflammatory and antiinflammatory cytokines, including TNFα, IL-1, and IL-10, as well as the major matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (10,11). Less TNFα and IL-10 are produced from OA samples, but the levels are still easily detectable by enzyme-linked immunosorbent assay (ELISA) (11). It is possible to use adenoviral gene transfer in this model without causing apoptosis or disrupting intracellular signaling pathways. All cell types are effectively infected, including synovial macrophages. An adenovirus effectively transferring the inhibitory subunit IκBα can be used to selectively inhibit the transcription factor NF-κB in synovial cocultures from patients with RA or patients with OA (10,11,23).

It was observed that although macrophage-produced TNFα and IL-1β were very strongly dependent on NF-κB in RA synovium, adenoviral transfer of IκBα did not affect IL-1β production and had only a partial effect on TNFα in OA synovial cells (Figure 2). The effects on other cytokines were similar in RA and OA synovium, with both IL-6 and IL-8 and the p75 soluble TNF receptor being NF-κB dependent, whereas IL-10 and IL-1 receptor antagonist (IL-1Ra) were both NF-κB independent. In addition, MMP-1, MMP-3, and MMP-13 were strongly NF-κB dependent in both RA and OA, but their main inhibitor, TIMP-1, was not (10,11).

The differential effect of NF-κB down-regulation on the spontaneous production of TNFα and IL-1β in RA and OA would indicate that the regulation of at least 1 key intracellular pathway differs fundamentally between these diseases. It is known that both TNFα and IL-1β have functional NF-κB elements on their promoters, and that in various macrophage models there are both NF-κB–dependent and NF-κB–independent methods of inducing TNFα and IL-1β. Some stimuli, such as lipopolysaccharide and phorbol ester, act via NF-κB, whereas others, such as zymosan and CD45 ligation, do not (24,25).

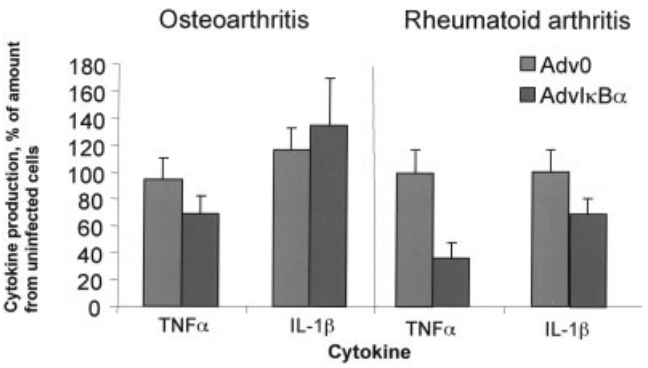

Figure 2. Production of tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) by osteoarthritis synovial cells or rheumatoid arthritis synovial cells that were left uninfected or were infected (30:1) with either an adenovirus without an insert (Adv0) or an adenovirus transferring the inhibitory subunit IκBα (AdvIκBα). Production was measured by enzyme-linked immunosorbent assay and is expressed as the mean and SEM percent of the amount from uninfected cells.

These results hint that there may be differences between RA and OA in the regulation of macrophage-produced TNFα and IL-1β, with cytokine levels being higher and NF-κB playing a more important role in RA (10,11,26,27). There is a scarcity of studies of the differences between RA and OA in the regulation of other signaling pathways, although a recent study demonstrated differences between RA and OA in the phosphorylation of the Pyk-2 and Src family kinases (belonging to the focal adhesion kinase family) (28). In order to investigate these potential differences in intracellular signaling, there is a need to compare early-stage as well as late-stage OA and RA specimens and also to correlate the results with synovial histology and the degree of macrophage infiltration. An interesting hypothesis to test would be that the higher degree of NF-κB dependence for TNFα and IL-1β induction in RA synovium, as compared with OA synovium, is related to a lesser degree of macrophage activation in OA or, alternatively, is correlated with differential expression of surface activation markers.

The role of activated synovial macrophages and their cytokines in OA

In the model of cultures of OA synovial cells described above, specific depletion of synovial macrophages could be achieved using anti-CD14–conjugated magnetic beads (29). These CD14+-depleted cultures of synovial cells no longer produced significant amounts of macrophage-derived cytokines such as TNFα and IL-1β. Interestingly, there was also significant inhibition (40–70%) of several cytokines produced mainly by synovial fibroblasts, such as IL-6 and IL-8, and also significant down-regulation of MMP-1 and MMP-3 (Figure 3A). This finding would indicate that OA synovial macrophages play an important role in activating the fibroblasts in these densely plated cultures of synovial cells and in perpetuating the production of proinflammatory cytokines and destructive enzymes. The finding that regulation is not tighter than that observed is probably attributable to the fact that fibroblasts have an activated phenotype when put into culture, with considerable spontaneous production of cytokines and other mediators. Once macrophages are removed, however, synovial fibroblasts gradually down-regulate their production of both proinflammatory cytokines and destructive MMPs.

To investigate the mechanisms involved in this macrophage-driven stimulation of inflammatory and degradative pathways in OA synovium, specific neutralization of the endogenous production of TNFα and/or IL-1β was used in these cultures of OA synovial cells (29). OA synovial cell cultures were either left untreated, incubated with etanercept (a recombinant human TNF receptor [p75]–Fc fusion protein), incubated with a neutralizing anti–IL-1β antibody, or incubated with a combination of entanercept and anti–IL-1β. As could be expected, TNFα production was effectively neutralized by etanercept treatment, and IL-1β production was neutralized by treatment with the neutralizing anti–IL-1β antibody (Figure 3B). Etanercept had no effect on IL-1β production, nor did the neutralizing anti–IL-1β antibody affect the production of TNFα (Figure 3B). This is in marked contrast to the situation in RA, in which IL-1β is strongly TNFα dependent in these cultures of synovial cells (30). This finding indicates another possible difference in macrophage cytokine biology between RA and OA: whereas TNFα is the "boss" cytokine in RA synovium, regulating the production of IL-1β, there appears to be a redundancy between these 2 cytokines in OA synovium. To evaluate this potentially important finding further, however, there is a need to also take into consideration the disease stage (early OA versus early RA and late OA versus late RA),

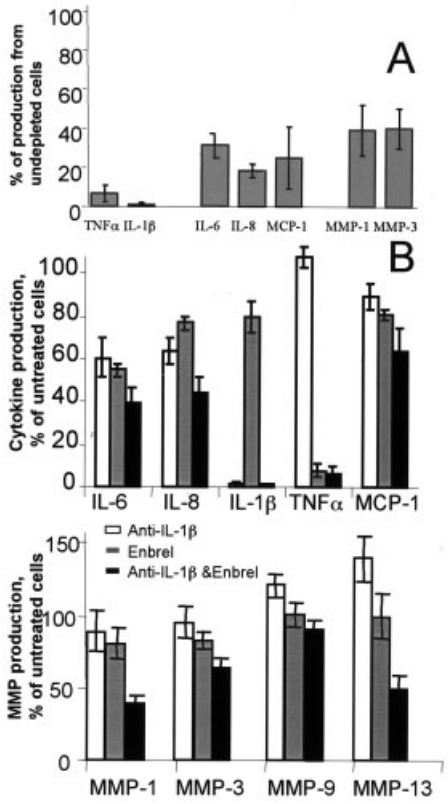

Figure 3. A, Effect of macrophage depletion on cytokine and matrix metalloproteinase (MMP) production in osteoarthritis (OA) synovial cells. Cultures of synovial cells were left intact or were macrophage depleted. Cells were left to adhere for 24 hours before the supernatants were removed for enzyme-linked immunosorbent assay (ELISA) of cytokines and MMPs. **B,** Effect of neutralization of tumor necrosis factor α (TNFα) and/or interleukin-1 (IL-1) on cytokine and MMP production in OA synovial cells. In these experiments, 2×10^6 cells/well were plated into 4 wells on a 24-well plate in 1 ml RPMI 1640 supplemented with 10% fetal calf serum. The cells in these 4 wells were either left untreated or were incubated with etanercept (Enbrel), a neutralizing anti-TNFα antibody, or a combination of etanercept and anti–IL-1β. After incubation for 48 hours, the supernatants were removed for ELISA of various cytokines and MMPs. MCP-1 = monocyte chemotactic protein 1. Bars show the mean ± SEM.

the degree of synovitis, macrophage infiltration, and the degree of macrophage activation, using histologic controls and up-to-date techniques.

Both etanercept and the neutralizing anti–IL-1β antibody inhibited production of IL-6 and IL-8, with 60% inhibition achieved when both IL-1β and TNFα were neutralized (Figure 3B). The production of monocyte chemotactic protein 1 (MCP-1) was not affected by the neutralizing anti–IL-1β antibody, but it was significantly decreased by etanercept and by the combination of the 2 treatments. There is the potential that other macrophage-produced cytokines, such as oncostatin M

and IL-6, may also play a role in stimulating synovial fibroblasts, thus explaining the lower degree of IL-8 and MCP-1 inhibition shown in Figure 3B as compared with Figure 3A. It was also possible to study the effect of neutralizing IL-1β and/or TNFα on messenger RNA (mRNA) expression and protein production of the major MMPs and aggrecanases, using reverse transcription–polymerase chain reaction and ELISA in parallel. The results indicated that although neither etanercept nor the neutralizing anti–IL-1β antibody had an impressive effect on the important collagenases MMP-1 and MMP-13, the combination of the 2 treatments led to significant inhibition on both the mRNA and protein levels (Figure 4A). These findings indicate that in OA synovium, macrophages potently regulate the production of several important fibroblast-produced cytokines and MMPs, via a combined effect of IL-1β and TNFα.

Neither etanercept nor the neutralizing anti–IL-1β antibody had an effect on ADAMTS-5 expression, nor was ADAMTS-5 expression affected by a combination of these treatments (Figure 4B). Thus, ADAMTS-5 appears to be constitutive in OA synovial cells, at least with regard to its potential regulation by TNFα and/or IL-1. In contrast, ADAMTS-4 was significantly ($P < 0.05$) inhibited by etanercept and was more potently ($P < 0.01$) inhibited by a combination of etanercept and the neutralizing anti–IL-1β antibody (Figure 4B). This would indicate that in human OA synovium, up-regulation of ADAMTS-4 is dependent on the TNFα and IL-1 produced by synovial macrophages, whereas the level of ADAMTS-5 is not changed by these cytokines (29,31).

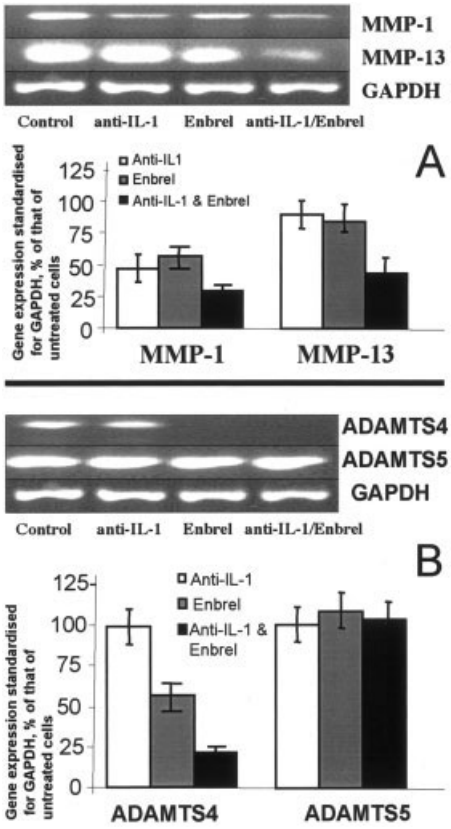

Figure 4. Effect of neutralizing tumor necrosis factor α and/or interleukin-1 (IL-1) on the expression of matrix metalloproteinase 1 (MMP-1) and MMP-13 (**A**) and ADAMTS-4 and ADAMTS-5 (**B**) in osteoarthritis synovial cell cultures. Cells (2×10^6/well) were plated into 4 wells on a 24-well plate and were either left untreated, incubated with etanercept (Enbrel), incubated with a neutralizing anti–IL-1β antibody, or incubated with a combination of etanercept and anti–IL-1β. After incubation for 48 hours, the cells were washed with phosphate buffered saline, and the RNA was extracted with TRI Reagent. Reverse transcription–polymerase chain reaction analysis was performed, using oligonucleotide primers specific for MMP-1 and MMP-13 (**A**) and for ADAMTS-4 and ADAMTS-5 (**B**). MMP-1, MMP-13, ADAMTS-4, and ADAMTS-5 mRNA levels are expressed as the percentage of the gene expression in untreated cells, as standardized to GAPDH. Bars show the mean ± SEM results from 4 individual experiments.

Animal studies of the role of macrophages in OA

Until recently, there were very few data regarding the role of macrophages in animal models of OA. However, an important series of studies using injections of liposome-encapsulated clodronate to induce depletion of synovial lining macrophages have provided some intriguing new information about the role of macrophages in driving degenerative changes in a mouse model of experimental OA induced by injection of collagenase. The collagenase injection causes weakening of ligaments, leading to the gradual onset of OA pathology within 6 weeks of induction, without any direct collagenase-induced cartilage damage being observed (32), because the appearance of MMP-induced neoepitopes does not occur until day 14 after the collagenase injection (Figures 5A and B). Due to the size and physical properties of the collagenase injection, collagenase is expected to be cleared rapidly from the joint, making it highly unlikely that MMP-mediated damage between day 7 and day 14 is induced by injected collagenase. In addition, the specific neoepitope itself cannot be generated by the bacterial collagenase that was injected. If macrophage depletion was achieved prior to the induction of experimental OA, there was a potent reduction of both fibrosis and osteophyte formation (33,34) (Figures 5C and D). This would indicate that

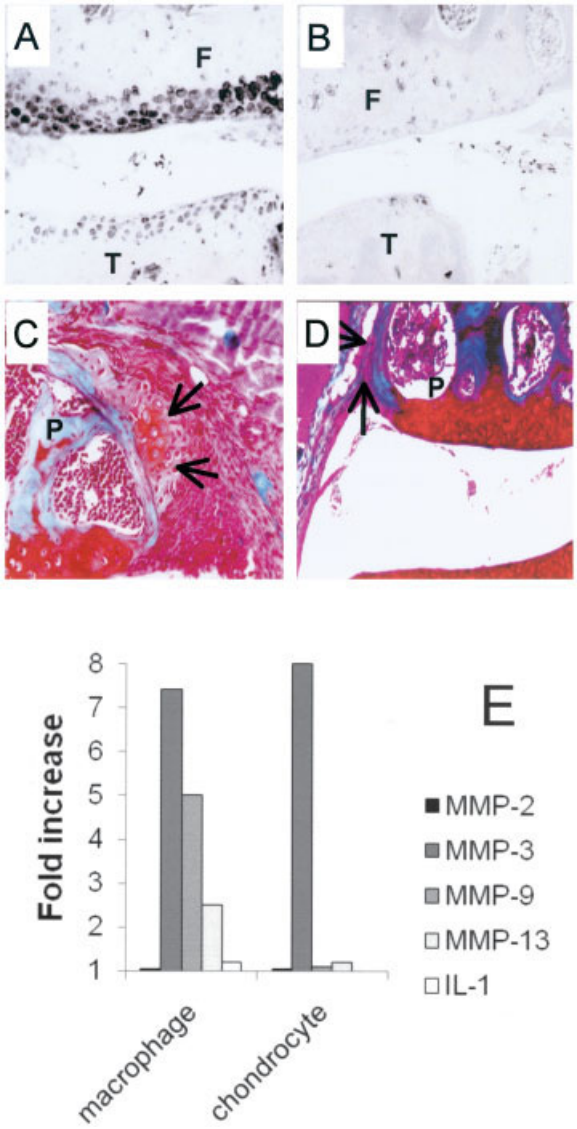

Figure 5. A and **B,** Expression of VDIPEN, a marker for matrix metalloproteinase (MMP) activity, 14 days after induction of instability-induced osteoarthritis (OA) in control knee joints (**A**) and in knee joints depleted of synovial macrophages (**B**). **C** and **D,** Osteophyte formation (**arrows**), which is reduced in macrophage-depleted knee joints (**D**) compared with OA knee joints with an intact synovial lining (**C**). **E,** Regulation of MMPs and interleukin-1 (IL-1) by stimulation of cells for 24 hours with recombinant Wnt-induced signaling protein 1 (WISP-1). WISP-1 stimulation was performed with 1 μg/ml protein (except 500 ng/ml for MMP-3), and cells were lysed in TRIzol 24 hours after stimulation. Murine macrophages were RAW264.7, and murine chondrocytes were the H4 cell line. **P** = patella; **F** = femur; **T** = tibia. (Original magnification $\times$ 200.)

synovial macrophages control the production of growth factors.

Locally produced transforming growth factor β (TGFβ) seems a likely candidate to be controlled by macrophages. Inhibition of TGFβ using soluble TGFβ receptors or adenoviral overexpression of the intracellular inhibitor Smad7, specifically in the synovium, markedly reduced both fibrosis and osteophyte formation (35). Moreover, TGFβ overexpression mimics OA-like osteophyte formation, and this is mediated by synovial macrophages (34). Although TGFβ is also anabolic, and impaired TGFβ signaling leads to chondrocyte hypertrophy and OA cartilage pathology (36,37), more evidence is accumulating that anabolic and catabolic pathways run through different receptors, and that the pathogenic activin receptor–like kinase 1 pathway dominates in OA (38,39).

In this mouse model of collagen-induced OA, it was also possible to monitor the effect of macrophage depletion on formation of the VDIPEN neoepitope that indicates MMP-induced cleavage of aggrecan (12,40). Some marginal VDIPEN expression could be observed already on day 7 after induction of collagen-induced arthritis, but such expression was decreased only slightly in macrophage-depleted joints. Between day 7 and day 14, however, VDIPEN expression more than doubled in nondepleted joints, whereas it remained unchanged in those that were macrophage depleted (Figures 5A and B). This would indicate that, in agreement with the data from human OA synovium discussed above, the production of MMPs in this murine model of OA is macrophage dependent.

Analysis of synovium and cartilage specimens from mouse OA joints in this model demonstrated that MMP-2, MMP-3, and MMP-9 were induced in both of these tissues when murine OA was induced by collagenase. However, whereas the MMP levels in cartilage were unaffected by macrophage depletion, those in the synovium were inhibited, suggesting that the removal of macrophages would down-regulate the production of MMPs from synovial fibroblasts, and that the gradual decrease in diffusion of these MMPs to the cartilage would prevent aggrecanolysis, as evidenced by the reduction in VDIPEN expression. Moreover, because the activated MMPs that generate the VDIPEN neoepitope are also capable of cleaving type II collagen and/or gelatin, this strongly suggests that macrophages are involved in the structural, irreversible cartilage damage in OA.

In the same model of murine OA, it was demonstrated that MMP-3–knockout mice showed a 67% reduction in the occurrence of severe cartilage damage with a concomitant decrease in VDIPEN expression, indicating the involvement of this MMP in the OA disease process (40). Other studies, however, have indi-

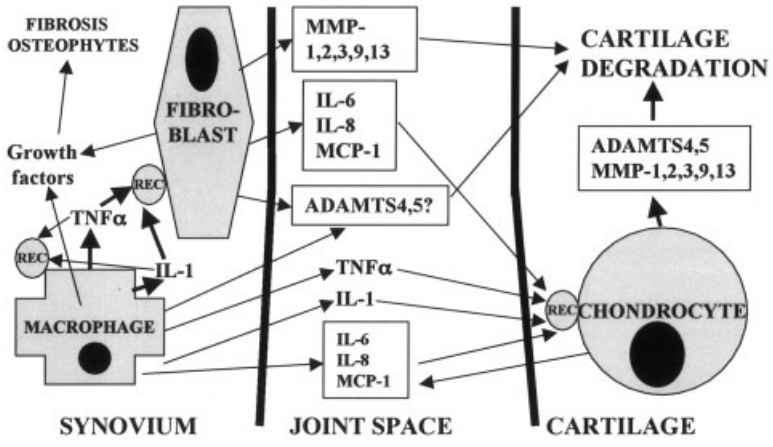

Figure 6. Schematic of the role of osteoarthritis synovial macrophages in activating synovial fibroblasts and driving inflammatory and destructive responses. In this diagram, "REC" signifies all types of cell surface–related receptors. It remains unproven, although it is not unlikely, that ADAMTS-4 and/or ADAMTS-5 produced by synovial cells can be secreted into synovial fluid to influence cartilage degradation, nor is it entirely clear that synovial macrophages produce ADAMTS-4, although some preliminary data hint that this is possible. MMP = matrix metalloproteinase; IL-6 = interleukin-6; MCP-1 = monocyte chemotactic protein 1; TNFα = tumor necrosis factor α.

cated an important role for the collagenase MMP-13 in OA. This enzyme is strongly up-regulated in OA synovium (12,41), and there is a correlation between MMP-13 levels and cartilage damage in human OA, as demonstrated by arthroscopy (41). It is possible that several MMPs contribute to the OA disease process, with an intricate network between proMMPs and their activators. Considering that other mouse studies have pointed out an important role for the ADAMTS aggrecanases and ADAMTS-5 in particular (42–44), it would also be necessary to investigate whether the activity of these enzymes is macrophage driven in animal models of OA. The data from the human OA synovium discussed above indicate that ADAMTS-4 is up-regulated by macrophage-produced cytokines, whereas ADAMTS-5 is constitutive; however, it should be noted that previous human and murine studies have shown discrepancies with regard to both the relative role of ADAMTS-4 and ADAMTS-5 in OA pathogenesis and the regulation of these enzymes (31).

Is macrophage involvement in OA partly independent of IL-1?

The in vitro data discussed above make a strong case for the involvement of synovial macrophages in OA pathology, with TNFα and IL-1 acting as important mediators driving the production of MMPs and ADAMTS-4 (Figure 6). The in vivo role of IL-1 has been explored in various animal models of OA, demonstrating decreased cartilage damage after anti–IL-1 antibody therapy or gene therapy with IL-1Ra (45,46). However, the results of more recent studies in IL-1–deficient mice were less convincing or even showed the opposite (47). In yet another study, instability-induced OA was reduced >50% in IL-1–deficient mice (48). In our own studies in IL-1$\alpha\beta$–deficient mice (16), we observed a moderate reduction in pathology, using an instability-induced model of OA. In contrast, spontaneous OA-like cartilage damage in aging mice was aggravated. The latter finding is consistent with a role for IL-1 in normal cartilage homeostasis (16,47). In these studies, it is impossible to discriminate between the impact of IL-1 in synovium and that in cartilage. It may be argued that a destructive role of IL-1 is dominant only in inflammatory types of OA. Because anti–IL-1 treatment provided, at best, a partial reduction in the amount of damage, and because the experience with anti–IL-1 agents in human OA has been disappointing so far, other pathways that act independently of IL-1 must be involved in OA pathology.

A likely candidate for such an alternative pathway is the Wnt signaling pathway. Canonical Wnt signaling is important in many cellular processes that occur during synovial joint formation (49,50) and leads to

intracellular β-catenin accumulation and the transcription of a plethora of proteins, among which are several MMPs (51). Recent studies focusing on ankylosing spondylitis have demonstrated that the Wnt signaling pathways are involved in inducing bone formation and joint fusion (52,53). Polymorphisms in genes from this signaling pathway have been shown to be associated with OA (54,55). Most research focuses on the role of this signaling pathway in determining cell fate, phenotype, and proliferation in cartilage. However, recent data suggest a role for Wnt-induced signaling protein 1 (WISP-1; a Wnt-induced secreted protein) in the synovium during OA (56). During experimental OA, Wnt signaling is occurring not only in cartilage but also in synovium, as was demonstrated by β-catenin staining. The WISP-1 gene, in which a polymorphism was shown to be associated with spinal OA (57), was strongly up-regulated in synovium in 2 models of OA. Further investigation indicated that WISP-1 is a potent inducer of MMPs in macrophages, whereas the short-term effect on chondrocytes is less pronounced (Figure 5E). In addition, overexpression of WISP-1, specifically in synovium, induced the MMP- and aggrecanase-mediated neoepitopes VDIPEN and NITEGE in cartilage, indicating that WISP-1 expression in synovial cells leads to cartilage degradation. Interestingly, these effects were independent of IL-1, because WISP-1 did not induce IL-1 production in macrophages, nor was cartilage damage decreased in IL-1–deficient mice after synovial WISP-1 overexpression. Blocking studies are needed in order to substantiate this role for WISP-1 in (experimental) OA.

Macrophages and macrophage-produced cytokines as therapeutic targets in OA

Due to the obvious risks involved, no attempts have been made to use strategies of systemic induction of macrophage cell death or apoptosis in human arthritis. To be at all feasible, such antimacrophage strategies must act strictly locally in OA synovium and not lead to any risk of septic arthritis or local infection. In RA, intraarticular injection of clodronate-containing liposomes was successful in reducing the number of macrophages (58), but there have been no similar studies in OA. Because OA is often a polyarticular disease, and because macrophages are key players in protecting tissue against infectious agents, major obstacles must be overcome before the use of such antimacrophage strategies can become feasible.

Considerable interest has been devoted to investigating the role of various signaling pathways leading to proinflammatory cytokine production from OA synovial macrophages. The mitogen-activated protein kinases (MAPKs) have been implicated in driving TNFα and IL-1β production, as has the aforementioned transcription factor NF-κB. But in spite of the development of small-molecule inhibitors of the p38 or JNK MAPKs, or alternatively, inhibitors of NF-κB and its regulatory kinase inducible IKK-2, systemic inhibition of these ubiquitous intracellular signaling pathways is unlikely to satisfy safety concerns (59). Local delivery of NF-κB inhibitors via adenoviral gene transfer is a more appealing prospect, but toxicity concerns for the viral vectors used remain an issue (60,61).

After the success of targeted biologic therapy in RA, there has been a good deal of interest in investigating anticytokine strategies also in OA (16). In RA, TNFα has become the major therapeutic target, whereas strategies targeting IL-1 have met with only moderate success; from the clinical data available, the same appears to be true for psoriasis, psoriatic arthritis, and ankylosing spondylitis. In chronic juvenile arthritis, strategies directed against either TNFα or the IL-1Ra have been successful (62,63). Such success may indicate that there are subtle differences in cytokine biology between these inflammatory arthritides, with IL-1 having a relatively more prominent role in juvenile chronic arthritis and in adult Still's disease (62,63). Some of the potential small-molecule DMOADs, such as pralnacasan and diacerein, seem to act at least in part as inhibitors of IL-1 (64,65).

The experimental data described above would hint that unlike the situation in RA, there is redundancy between TNFα and IL-1 in OA synovium. Both of these cytokines appear to play important roles in driving the production of other proinflammatory cytokines, as well as MMPs and aggrecanases (29). In a patient with inflammatory knee OA, with synovitis visible on a magnetic resonance imaging scan, an anti-TNF drug had marked benefit on pain and walking distance, as well as synovitis, synovial effusion, and bone marrow edema (66). In a pilot study involving 12 patients with inflammatory hand OA, the anti-TNF antibody adalimumab had no significant effect, although some patients experienced improvement (67). Another pilot study involving 10 patients indicated that intraarticular injection of the anti-TNF antibody infliximab caused significant symptomatic relief compared with placebo, although there was no significant difference in the radiographic progression scores after 12 months (68). Interestingly, another study of the radiographic progression of inter-

phalangeal OA in a large cohort of RA patients treated with various disease-modifying drugs or with infliximab showed that OA progression was significantly reduced in the patients receiving infliximab (69). An early study in 13 patients with knee OA indicated that intraarticular administration of the recombinant IL-1Ra anakinra had some degree of analgesic effect (70,71). Disappointingly, however, a recent double-blind, placebo-controlled study could demonstrate no improvement in knee OA symptoms after intraarticular injection of anakinra (72).

Conclusions

Although inhibition of macrophage-produced cytokines in OA remains an appealing concept, early results of this strategy have not been greatly impressive. As would be expected, the immediate effect of anti-TNF biologic agents in RA, with regard to inflammation, pain, and fatigue, has not been reproduced in OA. As pointed out earlier in this review, there may be differences between RA and OA macrophages in the regulation of key intracellular pathways (Figure 2), as well as potential differences in cytokine biology (Figure 3). The suggestion that there is redundancy between TNFα and IL-1 in OA synovium, whereas TNFα drives IL-1 in RA, may have some importance for the potential of anticytokine biologic treatment of OA (Figures 3 and 6). To investigate these potential differences in intracellular signaling, there is a need to compare proinflammatory cytokine induction and regulation in early-stage as well as late-stage OA and RA and also to correlate the results with synovial histology and the degree of macrophage infiltration. Although it remains most likely that the fundamental difference between OA and RA is found at the level of cartilage and bone rather than in the synovium, the above findings would certainly stimulate interest from academia and industry to investigate intracellular signaling and cytokine biology in OA synovium, in the search for potential therapeutic targets.

More importantly, the concept of OA as a heterogeneous disease would seem to be crucial for the application of anti-TNFα and/or anti–IL-1 strategies in this disease: in a patient with synovitis, exudation, and bone marrow edema, these strategies are likely to be more successful than in a patient with "dry" OA secondary to obesity or noninflammatory OA of the distal interphalangeal joints. Further clinical trials are needed, with larger numbers of patients, perhaps also to compare large-joint (knee/hip) with small-joint (hand) OA, as well as correlating the results of targeted cytokine inhibition with the clinical amount of synovitis and exudation. It would seem likely that inhibition of either TNFα or IL-1 would be much more efficacious in patients with significant inflammatory OA, as evidenced by active synovitis. Because a combination of the anti-TNF biologic etanercept and the recombinant IL-1Ra anakinra provided no added benefit and increased risk of infection and other side effects, such combination therapy is not recommended in RA (73). In OA, however, such a combination could potentially be more attractive (due to evidence that there is redundancy between TNFα and IL-1 in OA synovium), if there is a way to solve the obvious safety concerns. In addition, therapy should be focused on novel pathways and mediators involved in OA pathology, including pathogenic growth factors such as TGFβ and elements of the Wnt pathway. These factors play a role in both synovium and cartilage and may be more dominant in patients with a less inflammatory phenotype.

A major obstacle for anticytokine therapy in OA will be the difficulty in recruiting patients with early disease. One approach might be using imaging techniques, involving either scintigraphy with technetium (74) or a tracer binding to the macrophage folate receptor β (75–77). These methods have the potential to identify a subgroup of patients with a higher degree of macrophage infiltration, or alternatively, to correlate the success of anticytokine approaches with the number of macrophages detected.

In patients who already have significant irreversible bone and cartilage damage, the effect of these biologic agents would be less impressive. As with all potential disease-modifying strategies in OA, a major obstacle will be the difficulty in recruiting patients with early inflammatory OA, before gross osteophyte formation and cartilage loss are obvious on radiographs and by clinical examination.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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