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

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1. Introduction

Osteoarthritis (OA), one of the most common diseases among humans, is characterised pathologically by focal areas of damage on articular cartilage centred on load-bearing areas, associated with formation of new bone at the joint margins and changes in subchondral bone. Given the huge economic and personal burden of OA, and the fact that this disease is the major cause for the increasing demand for joint replacements, there is urgent need for disease modifying treatments to stop or at least slow the development and progression of OA.

But for this to be possible, we need further knowledge about the pathogenesis of disease initiation and progression in OA. The great success of targeted biologic therapy against rheumatoid arthritis (RA) in recent years has meant that much research has been devoted to investigating the pathophysiology of osteoarthritis (OA), in the hope of defining novel therapeutic targets. In contrast to RA, with its pannus and erosions, OA has long been thought of as a degenerative disease of cartilage, with secondary bony damage and osteophytes. In recent years, the importance of the synovium, and in particular the synovial macrophages, in OA, has been highlighted in both in vitro and in vivo studies. This article will give an overview of some important recent findings concerning the ability of macrophages to drive inflammatory and destructive disease mechanisms in OA, the role of their proinflammatory cytokines in doing so, and the potential for macrophages and macrophage-produced cytokines to be used as therapeutic targets for the development of disease-modifying anti-ostroarthritic drugs (DMOADs). There is also an abundance of potential downstream therapeutic targets in OA, including the matrix metalloproteinases, the aggrecanases, the inducible nitric oxide synthetase, and elements of the Wnt pathway.

2. Synovial macrophages and macrophage-produced mediators in driving inflammatory and destructive responses in osteoarthritis

2.1 Macrophage biology in RA and OA

In rheumatoid arthritis (RA), it is today accepted that both inflammatory and destructive features of the disease are driven through synovitis. The RA synovium has a plentiful infiltrate of activated macrophages, particularly at the cartilage-pannus junction (Kinne et al., 2007). These macrophages produce tumour necrosis factor α (TNFα), interleukin (IL)-1β and other proinflammatory cytokines. Since there is a 'cytokine cascade' with TNFα driving the other inflammatory mediators, this cytokine has become a key therapeutic target in RA, with several anti-TNFα biologic agents being used with considerable success (Feldmann & Maini, 2008). Although biologics with anti-B cell and anti-T cell co-stimulation properties have since been introduced, the anti-TNFα agents remain a mainstay of RA therapy. They have shown long-term sustained efficacy and safety, and are used all over the world with excellent results.

Clinically, RA and OA are usually easy to differentiate. X-rays of affected joints show erosions and periarticular osteoporosis in RA; in OA, they show reduction of joint space as a sign of cartilage degradation, and in later stages of the disease bony sclerosis and osteophytes. The joint pattern differs, with early RA affecting the proximal interphalangeal, metacarpophalangeal and metatarsophalangeal joints, and OA usually affecting the large joints, like the hips and knees, and also the distal (and sometimes proximal) interphalangeal joints. The typical RA patient has an elevated erythrocyte sedimentation rate, C-reactive protein and IL-6, the vast majority of OA patients do not. In RA patients, synovitis is a major feature of the disease, causing joint swelling and exudation, and driving cartilage degradation and the formation of pannus and erosive changes. In OA, there is much less joint swelling and exudation, and no pannus or erosions.

Many OA patients also have a variable degree of synovitis. Synovial inflammation is likely to contribute to disease progression in OA, as judged by the correlation between biological markers of inflammation and the progression of structural changes in OA (Clark et al., 1999; Sowers et al., 2002). Histologically, the OA synovium shows hyperplasia with an increased number of lining cells and a mixed inflammatory infiltrate mainly consisting of macrophages [Benito et al., 2005; Farahat et al., 1993]. Synovial biopsies from patients with early inflammatory OA may even resemble RA biopsies morphologically, although the percentage of macrophages is lower (1-3% as compared with 5-20%) and the percentages of T and B cells much lower [Bondeson et al., 1999a; Amos et al., 2006; Blom & van den Berg, 2007]. The synovial fluid of patients with active RA synovitis contains numerous polymorphonuclear leucocytes, something that is not the case in OA; another indicator that there is difference in pathophysiology between RA and OA synovitis.

In RA, it is today accepted that the synovitis is cytokine driven, through an disequilibrium between proinflammatory (TNFα, IL-1) and anti-inflammatory (IL-10, the IL-1 receptor antagonist, soluble TNF receptors). These proinflammatory cytokines are largely produced from a considerable infiltrate of synovial macrophages, which are particularly numerous and highly activated at the cartilage-pannus junction. Since macrophage-produced TNFα is the main mediator of disease, driving the other proinflammatory cytokines through a cytokine cascade, neutralisation of this one cytokine can reverse both synovitis and progression of joint damage (Brennan & McInnes, 2008).

Until recently, very little was known about the pathophysiology of synovitis in OA, or its role in promoting cartilage degradation, osteophytes and other features of the disease. The marked

differences in cell percentages in the inflammatory infiltrate between RA and OA would speak in favour of differences also in the cytokine interdependence in these two diseases. For example, the great scarcity of T cells in the OA synovium would tend to rule them (and their cytokines) out as potential drivers of synovitis in this disease. Instead, it has been proposed that this OA synovitis is cytokine driven, possibly through macrophage-produced TNFα and/or IL-1β, although the levels of proinflammatory cytokines are lower than in RA. 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 (Fernandes et al., 2002). The hypothesis that TNFα and IL-1 are key mediators of inflammation and articular cartilage destruction has raised the possibility of anti-cytokine therapy in OA, or the design of specific disease-modifying osteoarthritic drugs (Abramson & Yazici, 2006; Pelletier & Martel-Pelletier, 2005; Berenbaum, 2007; Qvist et al., 2008).

If it is accepted that synovial inflammation, and the production of proinflammatory and destructive mediators from the OA synovium, are of importance for the symptoms and progression of osteoarthritis, it is a key question which cell type in the OA synovium is responsible for maintaining synovial inflammation. In RA, where the macrophage is the main promoter of disease activity, macrophage-produced TNFα is a major therapeutic target. Much less is known about macrophage biology in OA, however, although histological studies have demonstrated that OA synovial macrophages exhibit an activated phenotype, and that they produce both proinflammatory cytokines and vascular endothelial growth factor (Benito et al., 2005; Haywood et al., 2003).

The spontaneous production of a variety of pro- and anti-inflammatory cytokines, including TNFα, IL-1β and IL-10, is one of the characteristics of synovial cell cultures derived from digested RA or OA synovium. In addition, the major MMPs and TIMPs are spontaneously produced by these cell cultures (Foxwell et al., 1998; Bondeson et al., 1999a; Amos et al., 2006). Less TNFα and IL-10 is produced from OA samples but the levels are still easily detectable by ELISA (Amos et al., 2006). It is possible to use effective adenoviral gene transfer in this model without causing apoptosis or disrupting intracellular signalling pathways. Using an adenovirus effectively transferring the inhibitory subunit IκBα, it was possible to selectively inhibit the transcription factor NFκB in synovial cocultures from RA or OA patients. Macrophage-produced TNFα and IL-1β was very strongly NFκB dependent in the RA synovium, but in OA synovium, adenoviral transfer of IκBα did not affect IL-1β production and had only a partial effect on TNFα. Effects on other cytokines were similar in RA and OA synovium, with IL-6 and IL-8 both being NFκB dependent, as well as the p75 soluble TNF receptor, whereas IL-10 and the IL-1 receptor antagonist were both NFκB independent. In addition, the matrix metalloproteinases (MMP) 1,3, and 13 were strongly NFκB dependent in both RA and OA, whereas their main inhibitor, tissue inhibitor of metalloproteinases (TIMP)-1 was not (Bondeson et al., 1999a; Amos et al., 2006).

The differential effect of NFκB downregulation on the spontaneous production of TNFα and IL-1β on RA and in OA would indicate that the regulation of at least one key intracellular pathway differs fundamentally between these diseases. It is known that both TNFα and IL-1β have functional NFκB elements on their promoters and that in various macrophage models, there are both NFκB dependent and NFκB independent ways of inducing TNFα and IL-1β (Bondeson et al., 1999b; Hayes et al., 1999). It would seem as if there are fundamental differences in the regulation of macrophage-produced TNFα and IL-1β

between RA and OA, with cytokine levels being higher and NFκB playing a more important role in RA (Bondeson et al., 1999a; Amos et al., 2006; Brennan et al., 2002; Andreakos et al., 2003). The differential effect of NFκB downregulation on the spontaneous production of TNFα and IL-1β on RA and in OA would indicate that the regulation of at least one key intracellular pathway differs fundamentally between these diseases. There are not many other studies comparing RA and OA intracellular signalling, although a recent study demonstrated differences in the phosphorylation of the Pyk2 and Src kinases, belonging to the focal adhesion kinase family, between RA and OA (Shahrara et al., 2007).

2.2 Macrophages drive both inflammatory and destructive responses in the OA synovium

In cultures of osteoarthritis synovial cells, specific depletion of synovial macrophages could be achieved using incubation of the cells with anti-CD14-conjugated magnetic beads (Bondeson et al., 2006). These CD14+-depleted cultures of synovial cells no longer produced significant amounts of macrophage-derived cytokines like TNFα and IL-1β. Interestingly, there was also significant inhibition (40-70%) of several cytokines produced mainly by synovial fibroblasts, like IL-6 and IL-8, and also significant downregulation of MMP-1 and MMP-3 (Figure 1). This would indicate that OA synovial macrophages play an important role in activating fibroblasts in these densely plated cultures of synovial cells, and in perpetuating the production of proinflammatory cytokines and destructive enzymes (Bondeson et al., 2006). That the regulation is not tighter than observed is probably because the fibroblasts have an activated phenotype when put into culture, with considerable spontaneous production of cytokines and other mediators. It can be speculated that once the macrophages are removed, the synovial fibroblasts change their phenotype and downregulate their production of both proinflammatory cytokines and destructive MMPs.
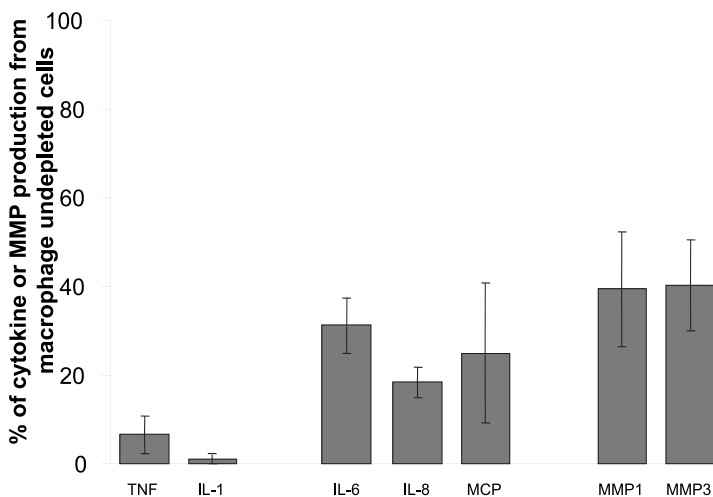

Fig. 1. OA cultures of synovial cells were either left intact or macrophage depleted. Cells were left to adhere for 24 h before the supernatants were removed for ELISA analysis of cytokines and MMPs, with data expressed as the percentage of cytokine/MMP production in the depleted culture as compared with the undepleted one, with the SEM given. Adapted from [23].

An important series of papers, using injections of liposome-encapsulated clodronate to induce depletion of synovial lining macrophages, has 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 (Blom et al., 2004, 2007a). The collagenase injection causes weakening of ligaments leading to gradual onset of OA pathology within six weeks of induction, without any direct collagenase-induced cartilage damage being observed. If macrophage depletion had been achieved prior to the elicitation of experimental OA, there was potent reduction of both fibrosis and osteophyte formation (Blom et al., 2004, 2007a; van Lent et al, 2004). This would indicate that in this murine model of OA, synovial macrophages control the production of the growth factors that promote fibrosis and osteophyte formation, both key pathophysiological events in OA.

In this model of murine experimental OA, it was also possible to monitor the effect of macrophage depletion on the formation of the VDIPEN neoepitope that indicates MMP-induced cleavage of aggrecan (Blom et al., 2007). Between day 7 and day 14, however, VDIPEN expression more than doubled in non-depleted joints, whereas it remained unchanged in depleted ones. 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 samples of synovium and cartilage from the murine OA joints in this model demonstrated that MMP-2,3 and 9 were induced in both these tissues when murine OA was induced by collagenase. But whereas the MMP levels in the cartilage were unaffected by macrophage depletion, those in the synovium were inhibited, suggesting that removal of the macrophages would downregulate the production of MMPs from the synovial fibroblasts, and that the gradual decrease in the diffusion of these MMPs to the cartilage would prevent aggrecanolysis, as evidenced by the reduction in VDIPEN expression.

2.3 Macrophage-produced cytokines as therapeutic targets in OA

To investigate the mechanisms involved in this macrophage driven stimulation of inflammatory and degradative pathways in the OA synovium, specific neutralisation of the endogenous production of TNFα and/or IL-1β could be used in the cultures of OA synovial cell (Bondeson et al., 2006). OA synovial cell cultures were either left untreated, incubated with the p75 TNF soluble receptor Ig fusion protein etanercept (Enbrel), incubated with a neutralizing anti-IL-1β antibody, or incubated with a combination of Enbrel and anti-IL-1β. As could be expected, TNFα production was effectively neutralised by Enbrel treatment, and IL-1β by treatment with the neutralizing anti-IL-1β antibody (Figure 2). There was no effect of Enbrel on IL-1β production, nor did the neutralizing anti-IL-1β antibody affect the production of TNFα. This is in marked contrast to the situation in RA, where IL-1β is strongly TNFα dependent in these cultures of synovial cells (Brennan et al., 1989). This finding would seem to indicate yet another difference in macrophage cytokine biology between RA and OA: whereas TNFα is the 'boss cytokine' in the RA synovium, regulating the production of IL-1β, there is a redundancy between these two cytokines in the OA synovium, with neither TNFα nor IL-1β regulating the production of the other (Figure 2).

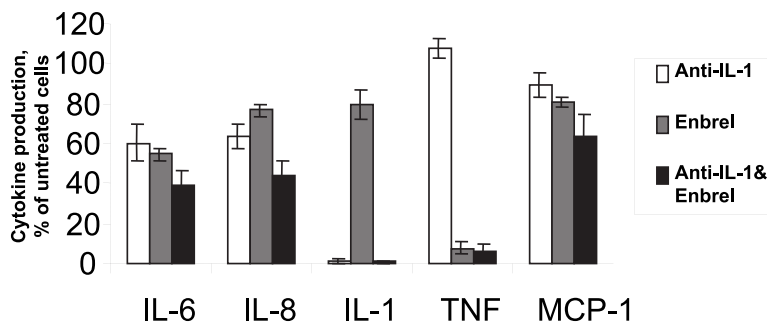Fig. 2. Effect of neutralisation of TNFα and/or IL-1β on cytokine production in OA synovial cells. In these experiments, 2 x 10^6 cells per well were plated into 4 wells on a 24 well plate in 1 ml RPMI 1640 supplemented with 10% FCS. The cells in these 4 wells were either left untreated, incubated with the p75 TNF soluble receptor Ig fusion protein 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 h the supernatants were removed for ELISA analysis of various cytokines. The data is expressed as percentage of the production of untreated cells, with the SEM given.

Both Enbrel and the neutralizing anti-IL-1β antibody inhibited IL-6 and IL-8, with 60% inhibition achieved when both IL-1β and TNFα were neutralized (Figure 2). The production of MCP-1 was not affected by the neutralizing anti-IL-1β antibody, but it was significantly decreased by Enbrel and by the combination of the two. It was also possible to study the effect of neutralizing IL-1β and/or TNFα on the mRNA expression and protein production of the major MMPs and aggrecanases, using RT-PCR and ELISA analysis in parallel (Bondeson et al., 2006, 2008). The results indicate that although neither Enbrel nor the neutralizing anti-IL-1β antibody had an impressive effect on the important collagenases MMP-1 and MMP-13, combination of the two led to significant inhibition both on the mRNA and protein levels (Figure 3). These findings indicate that in the OA synovium, the macrophages potently regulate the production of several important fibroblast-produced cytokines and MMPs, via a combined effect of IL-1β and TNFα.

There was no effect of either Enbrel or the neutralizing anti-IL-1β antibody on ADAMTS5 expression, nor was it at all affected by a combination of these treatments (Figure 3). Thus ADAMTS5 appears to be constitutive in OA synovial cells. In contrast, ADAMTS4 was significantly ($p<0.05$) inhibited by Enbrel, and more potently ($p<0.01$) inhibited by a combination of Enbrel and the neutralizing anti-IL-1β antibody (Figure 3). This would indicate that in the human OA synovium, the upregulation of ADAMTS4 is dependent on TNFα and IL-1 produced by the synovial macrophages, whereas ADAMTS5 is constitutive, and not changed by these cytokines (Bondeson et al., 2006, 2008).

After the success of targeted biological therapy in RA, there has been a good deal of interest in investigating anti-cytokine strategies also in OA (Malemud, 2004; Blom et al., 2007b). 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, ankylosing spondylitis and juvenile chronic arthritis. In juvenile chronic arthritis, strategies directed against either TNFα or the IL-1 receptor antagonist have been successful (Burger et al., 2006; Kalliolas & Liossis, 2008). This 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. Some of the potential small molecule disease-modifying anti-osteoarthritic drugs, like pralnacasan and diacerein, would appear to act at least in part as inhibitors of interleukin-1 (Rudolphi et al., 2003; Pavelka et al., 2007; Qvist et al., 2008).
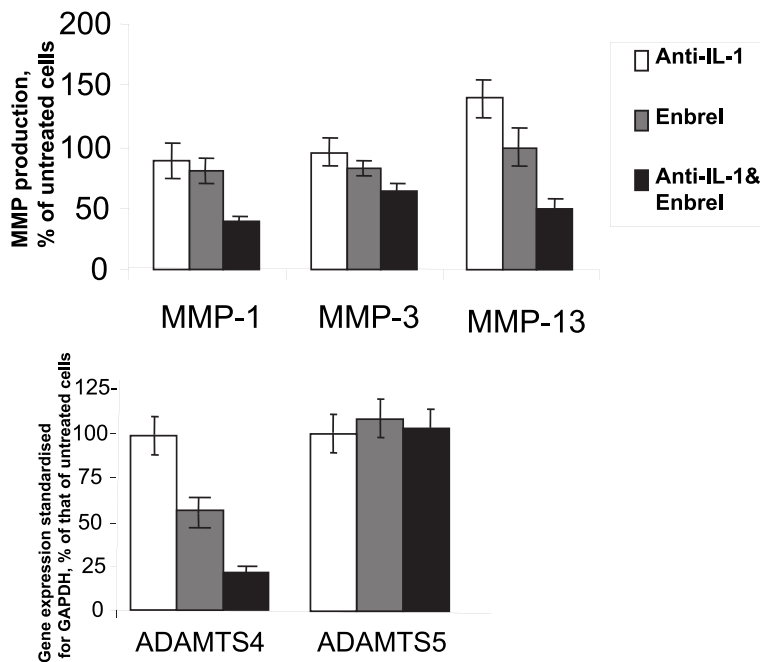

Fig. 3. Effect of neutralisation of TNFα and/or IL-1β on MMP production and ADAMTS gene expression in OA synovial cells. Experimental conditions were as in the Legend to Figure 2. After incubation for 48 h the supernatants were removed for ELISA analysis of MMPs. The cells were washed with PBS and the RNA extracted using Tri-reagent for RT-PCR analysis using oligonucleotide primers specific for ADAMTS4 and ADAMTS5. Analyis of GAPDH was used for comparison of gene expression, and in the right panel. ADAMTS4 and ADAMTS5 mRNA levels, expressed as percentage of the gene expression in untreated cells, as standardised for GAPDH, are given (n=4).

The experimental data described above would hint that unlike the situation in RA, there is redundancy between TNFα and IL-1 in the OA synovium. Both these cytokines appear to play important roles in driving the production of other proinflammatory cytokines, as well as MMPs and aggrecanases, however (Bondeson et al., 2006, 2010). In a patient with inflammatory knee OA, with synovitis visible on an MRI scan, an anti-TNF drug had marked benefit on pain and walking distance, as well as synovitis, synovial effusion and bone marrow oedema (Grunke & Schulze-Koops, 2006). In a pilot study involving 12 patients with inflammatory hand OA, the anti-TNFα antibody adalimumab had no significant effect (Magnano et al., 2007). Another pilot study involving 10 patients indicated that intra-articular injection of the anti-TNFα antibody infliximab caused

significant symptomatic relief compared with placebo, although there was no significant difference in the radiological progression score after 12 months (Fioravanti et al., 2009). Interestingly, another study looked at the radiological progression of interphalangeal OA in a large cohort of RA patients treated with various disease-modifying drugs or with the anti-TNF antibody infliximab found that OA progression was significantly reduced in the patients receiving infliximab (Güler-Yüksel et al., 2008, 2010). An early study in 13 patients with knee OA indicated that intra-articular administration of the interleukin-1 receptor antagonist anakinra had some degree of analgesic effect (Chevalier et al., 2005; Goupille et al., 2007). Disappointingly, a later double-blind, placebo-controlled study could demonstrate no improvement in knee OA symptoms after intra-articular injection of anakinra, however (Chevalier et al., 2009). This may well be related to the short half-life of the drug, and the invention of an effective sustained-release system, or another alternative anti-IL-1 strategy that works intra-articularly, might still be worth trying (Martel-Pelletier & Pelletier, 2009).

There is a need for further clinical trials, with larger numbers of patients, to compare the effect of anti-cytokine strategies in 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 macrophage infiltration. 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 joint exudation and active synovitis. In patients who already have significant irreversible bone and cartilage damage, the effect of these biologics would be less impressive. Since a combination of the anti-TNF biologic etanercept and the recombinant IL-1 receptor antagonist anakinra provided no added benefit and increased risk of infection and other side effects, such combination therapy is not recommended in RA (Genovese et al., 2004). In OA, however, such a combination could potentially be more attractive, due to the evidence that there is redundancy between TNFα and IL-1 in the OA synovium, if there is a way to solve the obvious safety concerns. As with all potential disease-modifying strategies in OA, a major obstacle for anti-cytokine therapy in OA will be the difficulty of recruiting patients with early inflammatory OA, before gross bone and cartilage loss is obvious on X-rays and clinical examination. In recent years, some exciting molecular imaging techniques, involving a tracer binding to the macrophage peripheral benzodiazepine receptor, or alternatively folate receptor β, have been invented (van der Laken et al., 2008; van der Heijden et al., 2009). Although hitherto published only for RA, there is no reason these methods could not be used also in OA, with the potential to identify a sub-group of patients with a higher degree of macrophage infiltration, or alternatively to correlate success with anti-cytokine approaches with the amount of macrophages detected.

2.4 Some potential downstream therapeutic targets in OA

Nitric oxide (NO) has been demonstrated to be a pathogenic mediator in OA. NO and its metabolites plays a role in the cyclooxygenase-2 activation leading to prostaglandin production, in activation of MMPs, in DNA damage, lipid peroxidation, chondrocyte apoptosis, and reduction of proteoglycan synthesis. NO production is regulated by the enzyme inducible NO synthetase (iNOS), which is in turn driven by proinflammatory cytokines and other pathologic stresses. In animal models of OA, the presence of iNOS and NO production was correlated with a higher rate of chondrocyte apoptosis and meniscal degeneration (Hashimoto et al., 1998; Hellio le Graverand et al., 2000). iNOS is

overexpressed in human OA synovium and cartilage, and the levels of 3-nitrotyrosine and other NO metabolites is elevated in OA patients. Sustained high levels of NO leads to the formation of various harmful NO-derived metabolites, of which the radical peroxynitrite is an inducer of cytotoxicity and tissue damage.

Due to its many harmful effects on joint integrity, iNOS has long been of interest in both inflammatory and degenerative arthritis. It was defined as a potential therapeutic target in OA after a study in a murine model of joint instability-induced experimental OA showed that iNOS-deficient mice developed significantly less OA than wild-type animals, with about 50% reduction of both osteophytes and cartilage lesions (van den Berg et al., 1999). In a canine model of joint instability-induced experimental OA, treatment with a small-molecule iNOS inhibitor led to impressive inhibition of 3-nitrotyrosine formation, and significant (nearly 50%) reduction of OA lesions. A two-year Phase IIb/III clinical trial (Pfizer) is ongoing to evaluate the safety and efficacy of a selective iNOS inhibitor in the treatment of obese or overweight patients with knee OA (Hellio le Graverand-Gastineau, 2010).

An interesting and novel therapeutic target in OA is osteogenic protein-1 (OP-1), which exhibits potent anabolic activity in models of cartilage homeostasis and repair. This growth factor also has anti-catabolic actions, including MMP and aggrecanase inhibition (Badlani et al., 2008). It has been investigated in various animal models of OA with positive results: injected intra-articularly, it inhibited cartilage degeneration and the progression of OA (Sekiya et al., 2009). A Phase I clinical trial of intra-articular recombinant OP-1 (Stryker), assessing safety and effect on signs and symptoms, with dose escalation over 24 weeks, has been completed (Hellio le Graverand-Gastineau, 2010).

Another potential therapeutic target in OA is fibroblast growth factor (FGF)-18, which plays a role in chondrogenesis and osteogenesis during skeletal development and growth. In a rat model of meniscal tear-induced OA, bi-weekly intra-articular injections of recombinant FGF-18 induced a significant, dose-dependent reduction in cartilage degeneration, as well as an increased in chondrocyte size and subchondral bone remodelling (Moore et al., 2005). Intra-articular, recombinant FGF-18 (Merck Serono) has undergone a 12-month Phase II clinical trial in knee OA, and another Phase II study in acute cartilage injury is under way (Hellio le Graverand-Gastineau, 2010).

In recent years, the Wnt signalling pathways has been implicated in the pathophysiology of OA. The Wnts are a complex family of lipid modified, secreted glycoproteins that play a role in synovial joint formation, and are involved in the transcription of many proteins. Wnt signalling occurs through at least three pathways. Best known is the canonical Wnt pathway that induces β-catenin, but there is also a Wnt-Ca^{2+} pathway and a planar cell polarity pathway. It is canonical Wnt that is thought to play a role in OA, however. In this pathway, Wnt binds to the Frizzled receptor, and through several signalling steps this leads to accumulation of cytoplasmic β-catenin, which translocates to the nucleus and binds to TCF/LEF transcription factors, converting them from repressors to activators of the transcription of a great variety of genes, important MMPs, growth factors and chondrocyte hypertrophy markers among them (Blom et al., 2010). Whereas low levels of β-catenin are of importance to prevent chondrocyte apoptosis, intracellular accumulation of β-catenin appears to induce OA-like changes. In particular, increased levels of β-catenin are observed in areas of cartilage degeneration.

Recent data suggest a role for wnt-1 induced signalling protein 1 (WISP-1), a wnt-induced secreted protein, in the synovium during OA (Blom et al., 2009). During experimental OA

wnt-signalling is not only occurring in the cartilage but also in the synovium, as was found by β-catenin staining. WISP-1, a gene in which a polymorphism was shown to be associated with spinal OA (Urano et al., 2007) was strongly upregulated in the synovium of two models for 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. In addition, overexpression of WISP-1 specifically in the synovium induced MMP and aggrecanase mediated neoepitopes VDIPEN and NITEGE in the cartilage, indicating that WISP-1 expression in synovial cells leads to cartilage degradation. Interestingly, these effects were independent of IL-1, since WISP1 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.

The targeting of Wnt signalling in OA drug discovery is likely to be impaired by the lack of knowledge concerning the normal function of these signalling pathways. For example, the direct targeting of β-catenin is likely to be hazardous, considering its importance for normal chondrocyte physiology, and its role in carcinogenesis. Whereas intracellular accumulation of β-catenin is linked to the induction of OA-like pathology, conditional knockdown of β-catenin signalling is equally harmful, since it induces chondrocyte apoptosis (Blom et al., 2010). Importantly, there is an increasing amount of data concerning the change of expression of certain Wnt protein and their inhibitors in the OA synovium. For example, Wnt16 is strongly upregulated in the synovium in a model of experimental OA, and also as a result of cartilage injury. Although both WISP-1 and Wnt16 have promise, more knowledge of Wnt signalling in health and disease is needed before any member of this family of protein can be defined as a therapeutic target in OA.

2.5 Matrix metalloproteinases as potential therapeutic targets in OA

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are synthesized as inactive proenzymes and activated extracellularly through cleavage of their prodomains by other proteases. Since MMPs cleave many of the structural components of the extracellular matrix, they have long been known to play a part in both inflammatory and degenerative arthritis. Inhibition of their activity through various broad-spectrum MMP inhibitors was effective in both mouse and guinea-pig models of osteoarthritis, but in humans these nonspecific MMP inhibitors caused musculoskeletal side effects, with painful joint stiffening and adhesive capsulitis (Hutchinson et al., 1998). Since no specific MMP has been pointed out as being involved in this musculoskeletal syndrome, the lack of selectivity of these broad-spectrum MMP inhibitors has been blamed for this side effect.

Since there is evidence that MMP-13 may well be the dominant collagenase in OA cartilage, with higher activity against type II collagen than any of the others, this MMP has been a main target for drug discovery. In a mouse model, overexpression of MMP-13 via an inducible transgene caused an OA-like phenotype (Neuhold et al., 2001). Recently, a group of compounds with a high degree of potency against MMP-13, as well as selectivity against other MMPs, were presented as a novel class of MMP-13 inhibitors. In the rat medial meniscal tear model of OA, one of these compounds protected articular cartilage as effectively as a broad-spectrum MMP inhibitor (Baragi et al., 2009). Numerous MMP-13 inhibitors are in early phase clinical development as DMOADs.

In osteoarthritis, aggrecan degradation, caused by increased activity of proteolytic enzymes that degrade macromolecules in the cartilage extracellular matrix, is followed by irreversible collagen degradation. The degradation of aggrecan is mediated by various matrix proteinases, mainly the aggrecanases, multidomain metalloproteinases belonging to the ADAMTS family. There has been much interest in the possible role of these aggrecanases, mainly ADAMTS4 and ADAMTS5, as therapeutic targets in osteoarthritis. It has long been debated which of the ADAMTSs is the main aggrecanase in human OA. Due to observations of ADAMTS4 mRNA being inducible through interleukin (IL)-1 and other stimuli in human OA chondrocytes and synovial fibroblasts, this enzyme attracted a good deal of attention (Bondeson et al., 2008).

But in models of murine OA induced by antigen or surgical joint destabilisation, ADAMTS5 is the pathologically induced aggrecanase. ADAMTS4 deficient mice develop normally and develop surgically induced degenerative arthritis in a similar manner to wild-type mice, but deletion of ADAMTS5 protects mice from developing arthritis (Glasson et al., 2004, 2005; Stanton et al., 2005). These results suggest that at least in murine models of OA, ADAMTS5 is the major aggrecanase. The only caveat to this conclusion is that there is a discrepancy between human and murine cells with regard to the regulation of ADAMTS5: the murine, but not the human, ADAMTS5 gene responds to IL-1 stimulation. Furthermore, a study using a small interfering RNA approach could demonstrate that both ADAMTS4 and ADAMTS5 contribute to the aggrecanase activity in human cartilage explants (Song et al., 2007). The search for the primary aggrecanase in human OA is still ongoing (Bondeson et al., 2008; Tortorella & Malfait, 2008).

The available data on ADAMTS5 gene promoters would suggest that this enzyme is the antithesis of ADAMTS4, with regard to its regulation. ADAMTS5 activity is reduced by C-terminal processing, whereas ADAMTS4 activity is enhanced (Gendron et al., 2007; Fosang et al., 2008). Then, in human cartilage and synovium, ADAMTS5 is constitutive whereas ADAMTS4 is the inducible aggrecanase, responding to IL-1 and TNFα in an NFκB dependent manner (Bondeson et al., 2008). The contrast between murine and human chondrocyte studies indicates that the situation may well be profoundly different in mice, something that of course would affect the validity of OA animal studies using these animals.

Several pharmaceutical companies (Wyeth/Pfizer, Schering-Plough, Rottapharm SpA, Alantos Pharm, Japan Tobacco) have patented small-molecule inhibitors of ADAMTS4 and ADAMTS5, developed mainly as potential DMOADs. Some of these compounds are claimed to be specific, whereas others have effect against both enzymes, against other ADAMTS members, or even against MMPs (Wittwer et al., 2007; Tortorella et al., 2009). The Wyeth/Pfizer compound was recently used in a phase I clinical trial in osteoarthritis (Hellio le Graverand-Gastineau, 2010; Gilbert et al., 2011).

The main endogenous inhibitor of ADAMTS4 and ADAMTS5 is tissue inhibitor of metalloproteinases (TIMP)-3, with K_i values in the subnanomolar range (Kashiwagi et al., 2001). This inhibition may well be modulated by interactions between aggrecan and the C-terminal domain of ADAMTS4 (Wayne et al., 2007). Interestingly, reactive-site mutants of the N-terminal inhibitory domain of TIMP-3, also inhibit ADAMTS4 (Lim et al., 2010). TIMP-3 knockout mice spontaneously lose their articular cartilage (Sahebjam et al., 2007). There is a good deal of interest in recombinant full-length or N-terminal TIMP-3 as a potential DMOAD, to be delivered intra-articularly, although it is yet to enter clinical trials.

3. Conclusions

In a recent editorial about the development of biologic therapy in RA, its distinguished authors pointed out that two of the greatest impediment for drug discovery were preconceived ideas about disease mechanisms and vested interests among those responsible for investigating potential therapeutic targets (Maini & Feldmann, 2007). In the early 1990s, it was generally accepted that 'autoimmune' diseases like RA were T cell driven. The synovial T cells were driving both inflammatory and destructive pathways, it was presumed, and although immunosuppression with drugs like cyclosporine or azathioprine could ameliorate symptoms, the disease remained incurable. Although this concept was successively undermined by the demonstration of low levels of lymphokines in RA synovial tissue and exudates, and later by the failure of anti-CD4 therapy in RA, it was adhered to with a rigidity that today seems quite inexplicable. Another both unconstructive and nihilistic notion popular at this time was that there was redundancy between proinflammatory cytokines and other inflammatory mediators, meaning that the targeting of an individual cytokine would be pointless. The notion of TNFα as a therapeutic target was initially greeted with incredulity, leading to a significant delay in the clinical development of these strategies (Feldmann, 2009). An idea originating in Britain was overlooked by the biotech and pharmaceutical industry of that country, and later commercialized in the United States with remarkable success.

The discovery that the neutralization of a single cytokine could lead to lasting remission in RA, with regard to both inflammation and development of erosions, had several beneficial effects for medical research. Firstly, it inspired further research into the disease mechanisms of other forms of chronic inflammation, leading to the establishment of anti-TNFα biologic therapy also in inflammatory bowel disease, psoriatic arthritis, ankylosing spondylitis, juvenile chronic arthritis and psoriasis. Secondly, it blew aside the concept that RA was an incurable 'autoimmune' disease, and inspired intensive research into RA pathophysiology, with the aim to find other targets for directed biologic therapy. This research has been rewarded with considerable success, with effective anti-CD20 and anti-T cell costimulation biologics now being available for use in RA, and many other biologics on their way in clinical development. Thirdly, the successes for biologic therapy of RA opened the door for more energetic work to identify potential therapeutic targets also in other chronic diseases. Even OA, the 'ugly sister' of rheumatology, received considerable attention, since here was a disease with immense unmet need and no disease modifying strategies on the market.

It is of course important that the lessons learnt from the successful drug development for RA and other forms of inflammatory arthritis are implemented in the search for therapeutic targets in OA. First to go should be the counterproductive notion of OA as the incurable result of 'wear and tear'. Although mechanical trauma and strain definitely play a part in the pathogenesis of OA, it remains a multifactorial disease. Many sick and obese people never develop OA; some fit and healthy ones do. It would also be beneficial if the concept of OA an primarily a disease of cartilage was challenged. A more promising approach, conducive to the definition of potential therapeutic targets, would be to consider the pathophysiological contributions of both synovium and cartilage (Figure 4). Activated synovial macrophages stimulate synovial fibroblasts, leading to the production of proinflammatory cytokines and that will have the ability to activate chondrocytes into producing further degradative enzymes. Furthermore, the production of MMPs, and quite possible aggrecanases, from the synovium, would also have a pathophysiological potential.

Since OA is a heterogenous disease, with variable degree of synovitis and macrophage infiltration, this simplified diagram of inter-cell and inter-tissue signalling (Figure 4) is likely to differ between patients: some have a higher degree of macrophage activation, synovitis and joint exudation, whereas others have 'dry' OA.
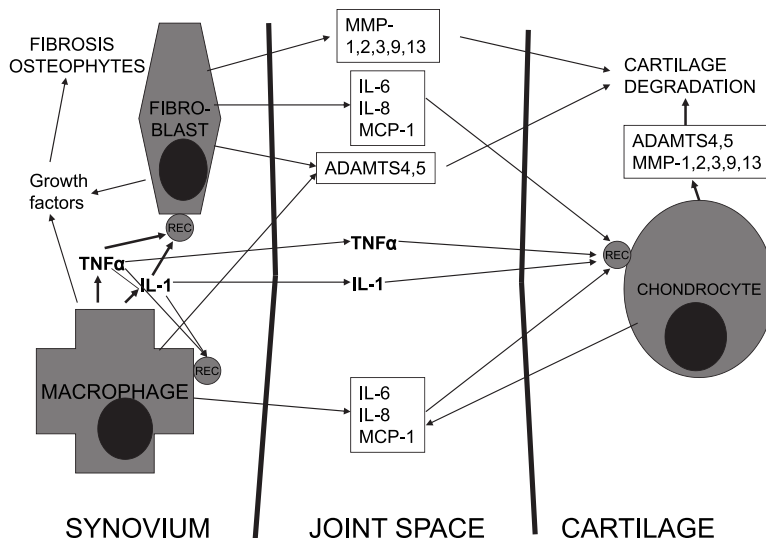

Fig. 4. A simplified view of the role of synovial macrophages in OA, in activating synovial fibroblasts and driving inflammatory and destructive responses. In this figure, 'REC' signifies all kinds of cell surface-related receptors. It remains unproven, although not unlikely, that ADAMTS4 and/or ADAMTS5 produced by synovial cells can be secreted into the synovial fluid, to influence cartilage degradation. Nor is it entirely clear that synovial macrophages produce ADAMTS4, although some preliminary data hints that this is possible.

Both in vitro and in vivo data point out the synovial macrophages and their main proinflammatory cytokines as potential therapeutic targets in OA. Macrophages drive the production of IL-6 and IL-8, the main MMPs (1,3,9,13) and ADAMTS4 from the synovial fibroblasts, and they are also crucial for the development of OA-related pathology, such as osteophyte formation and MMP-mediated cartilage breakdown (Bondeson et al., 2006, 2010; Blom et al., 2004, 2007). It should be remembered that the biology of OA synovitis is quite dissimilar from that in RA, with different cell composition, fewer macrophages, less synovial proliferation and synovial cell transformation, and no pannus or erosions. There are also differences in the regulation of key intracellular pathways between RA and OA macrophages (Amos et al., 2006) and important differences in cytokine biology (Bondeson et al., 2006), indicating that on the molecular as well as the clinical and histopathological levels, RA and OA are quite different diseases. The finding that there is redundancy between TNFα and IL-1 in the OA synovium, whereas TNFα drives IL-1 in RA, may well have some importance for the potential of anti-cytokine biologic treatment of OA. The concept of OA as a heterogenous 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 oedema, these strategies are likely to be more successful than in a patient with 'dry' OA secondary to obesity, or non-inflammatory OA of the distal interphalangeal joints. Had the clinical trials concerning anti-IL-1 and anti-TNFα strategies in OA selected patients with inflammatory knee OA verified by MRI, instead of patients with OA of the distal interphalangeal joints, the results may well have been different.

Disappointingly, there are currently no approved disease-modifying therapeutic strategies for OA. The three main impediments of drug development in OA have been the inadequacy of animal models of the disease, the difficulty in defining endpoints, finding validated biomarkers, and conducting worthwhile clinical trials in a disease that is so very slowly progressive, and the selection of patients for these clinical trials. Early OA is often asymptomatic, and the denudation of articular cartilage in advanced OA is likely to be an irreversible process. Many patients are likely to have some degree of denudation of cartilage, and exposure of subchondral bone, already at the time they exhibit radiographically obvious OA. Due to the slow progression of the disease, OA clinical trials need to take between one and three years, and use large numbers of patients. It would have been important to recruit patients with early disease, and a high risk of rapid progression, but the criteria currently used to define inclusion into clinical studies, and the lack of reliable predictors of disease progression, renders this very difficult. Furthermore, the commonly used measurement of OA progression, joint space narrowing on plain radiographs, is something of a blunt instrument, due to the slow progression of the disease.

The first criterium a DMOAD must fulfil is that its safety profile must be impeccable. Various drug companies and research organizations have performed clinical trials with existing drugs of proven safety, like the bisphosphonate drug Risedronate and the antibiotic Doxycycline but with unimpressive results. Nor has Diacerein, a compound with some degree of interleukin-1β inhibitory effect in vitro, or Licofelone, supposed to act as a combined cyclooxygenase and 5-lipoxygenase inhibitor, any obvious disease modifying potential in OA (see review by Hellio le Graverand-Gastineau, 2010). There is also a good deal of data concerning the widely available over-the-counter 'nutraceuticals' glucosamine and chondroitin sulphate. Although some early studies indicated that these substances at least had an analgesic effect, a recent meta-analysis found no evidence of them affecting neither joint pain, nor joint space narrowing, in OA (Wandel et al., 2010). Worryingly, there was also a discrepancy between industry sponsored and industry independent clinical trials, the latter indicating that the substances were close to worthless.

The situation appears to be that the existing drugs can provide little help to OA drug discovery: not only are they ineffective, but they provide no worthwhile clues as to future therapeutic targets in this disease. For some of them, their mechanisms are unknown, whereas others have been introduced from elements of serendipity rather than from understanding of the basic principles of OA pathophysiology. For OA drug discovery to move in the right direction, new ideas are required. Three compounds in phase III or phase IV development, namely calcitonin, vitamin D3 and avocado-soybean unsaponifiable; it would be an agreeable surprise if either of them has success as a DMOAD. Some more promising candidates are currently in phase II clinical trials. The Pfizer iNOS inhibitor benefits from quite solid preclinical data, and there is nothing to suggest it would be unsafe. Even a DMOAD leading to 20-30% slowing of the

progression of OA would be in a strong market position, due to the absence of any competitor. Both OP-1 and FGF-18 are also in Phase II clinical trials. Albeit showing enormous future as a potential therapeutic target in OA, the canonical Wnt pathway is currently insufficiently understood, due to its complexity and its role in maintaining physiological function.

Non-selective MMP inhibitors are unlikely to have any future as potential DMOADs, but the selective MMP-13 inhibitors may well feature, although they do not appear to have progressed into clinical trials. Theoretically, the aggrecanase inhibitors have considerable promise as DMOADs. In mice, ADAMTS5 is clearly the dominant aggrecanase with regard to the development of OA in mice, but the dominant aggrecanase in human OA has not yet been identified. It is an important task for OA drug development that this is achieved, due to the need for an aggrecanase inhibitor used as a DMOAD to be as selective as possible. Another problem is that the normal function of ADAMTS4 and ADAMTS5 has not been elucidated. Both ADAMTS4 and ADAMTS5 knockout mice are fertile and phenotypically normal, speaking against these enzymes influencing the natural murine skeletal or joint development (Glasson et al., 2004, 2005). However, a recent study has indicated that ADAMTS5-deficient mice in fact had reduced apoptosis and decreased versican cleavage in the interdigital webs (McCulloch et al., 2010).

The search for a future DMOAD is reaching a critical time. There is a need for one of the abovementioned strategies to show definite promise in clinical trials, for the pharmaceutical industry to become convinced that the enormous effort and very considerable dollar costs to conduct preclinical and clinical OA research can be worth the effort. Some companies have already has OA projects, or even entire OA research departments, axed due to disappointing results in spite of vast financial spending. The window of opportunity for the search for therapeutic targets in OA, opened by the success of the anti-TNFs and other biologics for use in RA and other forms of inflammatory diseases, might be in danger of closing fast if some of the potential DMOADs in clinical development would join the long list of compounds used in 'failed' clinical studies in OA.

4. Acknowledgements

5. Abbreviations

ADAMTS, A Disintegrin And Metalloproteinase with Transspondin Motives.
DMOAD, Disease-modifying anti-osteoarthritis drug
IL, Interleukin
MMP, Matrix metalloprotease.
NFκB, Nuclear factor κB.
OA, Osteoarthritis.
RA, Rheumatoid arthritis.
TIMP, Tissue inhibitor of metalloproteases.
TNF, Tumour necrosis factor.

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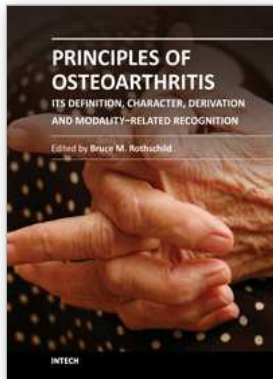
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