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REVIEW ARTICLE

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Rheumatoid arthritis: new insights into the role of synovial inflammation in joint destruction

Abstract Rheumatoid arthritis (RA) is characterized by inflammation and proliferation of synovial tissue, leading to degradation of articular cartilage and bone with functional impairment as a result. It has recently become clear that early suppression of synovial inflammation is essential in preventing progressive joint destruction, although inflammation and destruction are in part uncoupled. New insights into the role of matrix metalloproteinases (MMPs), aggrecanase, granzyme B, receptor activator of nuclear factor κB (RANK)–receptor activator of nuclear factor κB ligand (RANKL) interaction, and other factors involved in joint destruction may lead to the development of novel therapies aimed at specific inhibition of cartilage and bone degradation.

Key words Aggrecanase · Granzyme · Matrix metalloproteinases (MMPs) · Osteoprotegerin (OPG) · Receptor activator of nuclear factor κB ligand (RANKL) · Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is characterized by inflammation of the synovial membrane, leading to invasion of synovial tissue into the adjacent cartilage matrix with degradation of articular cartilage and bone as a consequence.[1] This results in erosion of bone, which is often observed as marginal joint erosions radiographically, and which is predictive of a poorer prognosis.[2] Although the pathophysiologic mechanisms for cartilage and bone destruction in RA are yet to be completely understood, it is known that matrix metalloproteinases (MMPs), cathepsins, mast cell proteinases, and other mediators can contribute to cartilage and bone destruction in RA. Some of the novel factors involved in joint damage will be discussed in this review. We will focus here on MMPs, aggrecanase, granzyme B, and osteoclasts, and we refer to recent reviews for a detailed description of the role of fibroblast-like synoviocytes, cytokines, transcription factors, and other mechanisms.[1,3–7]

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Joint destruction and MMPs

Irreparable degradation of the extracellular matrix in RA is at least partly mediated by the MMPs.[8–11] MMPs are a family of proteolytic enzymes that, between them, can degrade all components of the extracellular matrix and are responsible for both normal connective tissue remodeling and pathological tissue destruction in inflammatory diseases such as RA.[12] MMPs are classified into four groups according to their substrate specificities: the collagenases, gelatinases, stromelysins, and membrane-type (MT) MMPs.[13–15] There are natural inhibitors of MMPs called tissue inhibitors of metalloproteinases (TIMPs), which are produced in the RA synovial membrane.[9,12] It has been suggested that cartilage loss and joint destruction in RA may be due to a local imbalance between activated MMP and TIMP,[12,16,17] and that the development of joint damage in RA may be determined by the balance between MMPs and TIMPs at the synovial membrane level.[10,16,17] Although several MMPs and TIMPs have been demonstrated in RA serum, synovial fluid, and synovial tissue, probably the best studied are MMP-1 and MMP-3. These have been found in higher levels in RA than in osteoarthritis (OA) and normal controls.[18] Overexpression of various MMPs has been demonstrated in synovial tissue at the pannus–cartilage junction as well as in other areas in the joint.[19] The detection of MMPs in synovial tissue soon after the onset of symptoms[20,21] and the positive correlation between MMP-1 synovial tissue expression and the development of erosive

disease[21] highlights the potential for early joint destruction in patients with RA. It is interesting that two metalloproteinases [MMP-13 (collagenase 3) and MMP-15 (MT2-MMP)], appear to be exclusively expressed in RA.[13]

Recent clinical studies have suggested that glucocorticoids may reduce the development and the progression of joint erosions,[22] possibly related to the in vitro ability of glucocorticoids to inhibit MMP-1 and MMP-3 production by fibroblast-like synoviocytes. In addition, an in vivo study in RA demonstrated a reduction in MMP-1 and TIMP-1 mRNA expression in the synovial intimal lining layer of three patients treated with intraarticular glucocorticoids.[23] Consistent with these data, we have recently demonstrated that intravenous methylprednisolone succinate, given as "pulse" therapy, reduces MMP-1 and TIMP-1 but not MMP-3 expression in the synovial membrane of patients with active RA.[24] Both leflunomide and methotrexate treatment decrease RA synovial tissue expression of MMP-1 and TIMP-1, with greater reduction in MMP-1 levels, resulting in a reduced MMP-1:TIMP-1 ratio following treatment.[25] Therapy with biologicals may also inhibit MMP production at the site of inflammation. For example, a statistically significant reduction in the synovial tissue expression of MMP-1 was observed after interferon β treatment.[26] It is likely that tumor necrosis factor (TNF)-α blockade, which protects against joint destruction, also inhibits MMP production, although this has not been shown yet.

Previous work has also revealed that macrophage infiltration in the intimal lining layer and synovial sublining is significantly reduced in patients who achieved disease remission and in whom there was no radiological progression.[27,28] A significant observation of these studies was the reduction in MMP-1 and MMP-3 expression in both the lining and sublining layers in RA patients who achieved remission when compared with those who did not achieve remission after disease-modifying antirheumatic drug (DMARD) therapy (Table 1). Furthermore, in patients in whom there was no radiological progression, there was a reduction in MMP-1 and MMP-3, but no change in TIMP expression. In contrast, no reduction in MMP expression was observed in patients in whom there was radiological progression.[28] Macrophages are the major producers of MMPs[1,5] in the synovium and, therefore, the reduction in MMP expression was most likely due to a reduction in macrophage numbers in the synovial membrane.

Taken together, these results suggest a major role for MMPs in joint tissue destruction in RA and there may be some therapeutic potential in specifically targeting MMPs in the synovial membrane.[29]

Role of aggrecanase in cartilage destruction in RA

Aggrecanase is a member of the "a disintegrin and metalloprotease with thrombospondin motifs" (ADAMTS) family of proteases, which is important in the specific cleavage of aggrecan, an integral component of articular cartilage, at a specific site in the interglobular domain between glutamine at position 373 and alanine at 374.[30–32] This results in early depletion of aggrecan from articular cartilage, with a resultant loss of joint function. There are, currently, two characterized aggrecanases, aggrecanase-1 (ADAMTS4) and aggrecanase-2 (ADAMTS5), both being implicated in the degradation of aggrecan in articular cartilage in arthritis.[30,32] Aggrecanase activity is usually detected in RA and OA cartilage and synovial fluid by the detection of neoepitopes generated as a result of enzymatic cleavage of aggrecan.[31]

However, recently both aggrecanases have been cloned and the expression of both has been detected in RA and OA fibroblast-like synoviocytes.[33,34] Aggrecanase-1 and -2 are constitutively expressed in the intimal lining layer and around blood vessels.[30,32] Aggrecanase-1 but not aggrecanase-2 expression by fibroblast-like synoviocytes is induced by cytokines, especially transforming growth

Table 1. Expression of MMPs and TIMPs in the intimal lining layer and synovial sublining of rheumatoid arthritis patients who improved after disease-modifying antirheumatic drug (DMARD) therapy (remission patients or patients who fulfilled at least the 50% ACR criteria for improvement) compared with those who did not achieve remission (ACR <20% patients)

Protein	Remission patients IOD mean (sem)		*P* value	ACR50%–70% patients IOD mean (sem)		*P* value	ACR <20% patients IOD mean (sem)		*P* value
	Pretreatment	Posttreatment		Pretreatment	Posttreatment		Pretreatment	Posttreatment	
MMP-1									
Lining	17598 (3036)	8227 (1436)	0.003	6449 (1953)	6483 (2369)	0.989	4574 (1269)	12585 (5761)	0.208
Sublining	13424 (3329)	5468 (870)	0.033	4454 (1858)	4461 (2315)	0.998	1975 (658)	6432 (3359)	0.2
MMP-3									
Lining	7647 (2481)	1817 (381)	0.03	4284 (2498)	2370 (753)	0.391	2670 (925)	5816 (1121)	0.04
Sublining	4894 (1713)	1390 (487)	0.045	5706 (4314)	3023 (1666)	0.39	2556 (1413)	4474 (682)	0.134
TIMP-1									
Lining	12985 (3672)	10588 (2687)	0.624	7435 (2675)	7699 (2250)	0.912	4891 (742)	10031 (4241)	0.292
Sublining	10868 (4223)	9591 (2701)	0.798	7414 (3468)	6485 (1957)	0.857	4897 (2119)	11213 (4676)	0.239
TIMP-2									
Lining	11777 (2885)	11679 (3316)	0.982	15229 (4018)	15960 (1286)	0.892	10430 (3180)	12861 (3819)	0.669
Sublining	10916 (2502)	6123 (2042)	0.163	10401 (2369)	8917 (2877)	0.79	6080 (1417)	7678 (2080)	0.566

IOD, integrated optical density; sem, standard error of the mean; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; ACR, American College of Rheumatology

factor-β.[35] A recent in vitro study suggested that cyclosporin A may inhibit interleukin (IL)-1-stimulated, aggrecanase-mediated proteoglycan catabolism in articular cartilage.[36] So far, there have been no in vivo studies reporting the effect of drug treatment on aggrecanase activity in RA, but targeting aggrecanase-1 activity in particular in RA would appear to be a suitable therapeutic goal in RA.

Granzymes and destruction of extracellular matrix

Granzymes are cytotoxic enzymes, which are produced by cytotoxic T cells and natural killer cells. Granzyme-positive cells are present in both the intimal lining layer and in the synovial sublining. Consistent with the specific increase in the number of granzyme-positive cells in rheumatoid synovial tissue,[37–39] markedly elevated levels of soluble granzymes have been reported in the synovial fluid and plasma of RA patients.[40] It has been suggested that granzyme B may enhance cartilage loss by degrading resident aggrecan.[41] Moreover, granzyme B expression has been detected at the pannus–cartilage junction.[19,42] Recently, we further explored the capacity of granzyme B to degrade articular cartilage matrix in vitro. Purified human granzyme B was used to directly investigate its capacity to degrade an organized newly synthesized cartilage matrix, produced during 4 weeks of chondrocyte culture, and to degrade explants of intact bovine articular cartilage.[42] Incubation with granzyme B resulted in the loss of proteoglycans from the newly synthesized cartilage matrix. In addition, granzyme B was able to mediate the release of glycosaminoglycans from whole articular cartilage explants, suggesting the digestion of proteoglycans to protein fragments that are small enough to diffuse out of the tissue. However, granzyme B had no collagenolytic activity in these models. Taken together, these data suggest that granzyme B is capable of degrading the proteoglycan component of cartilage and may be involved in the destruction of articular cartilage in RA. This notion is supported by a recent study in early arthritis patients, showing that granzyme B levels are specifically increased in destructive RA patients.[43] Of importance, elevated granzyme B levels were strongly predictive of early erosions in these patients. There are as yet no studies showing the effects of inhibition of granzyme activity in arthritis models or RA patients.

Osteoclastogenesis and joint destruction in RA

Although MMPs and other enzymes undoubtedly have an important role in cartilage destruction in RA in particular it is clear that osteoclast formation and activation at the cartilage–pannus junction is an essential step in the destruction of bone matrix in RA patients.[44–47] A number of inflammatory cytokines found in the RA synovial tissue [including IL-1α and β, IL-6, TNF-α, and macrophage colony-stimulating factor (M-CSF)] have the potential to promote osteoclast formation and bone resorption.[48] However, recent evidence indicates that the interaction between receptor activator of nuclear factor κB ligand (RANKL) [also known as osteoclast differentiation factor, TNF-related activation-induced cytokine (TRANCE), and osteoprotegerin ligand] has an essential role in osteoclastogenesis.[44,45,48] RANKL is expressed on osteoblasts, fibroblast-like synoviocytes, and T cells,[49] whereas receptor activator of nuclear factor κB (RANK) is mainly expressed on preosteoclasts, possibly of macrophage lineage. A recent study has demonstrated that activated T cells in the RA synovial membrane play an important role in osteoclastogenesis and bone destruction in RA.[50] Another member of the TNF family is TNF-related apoptosis-inducing ligand (TRAIL), which shares homology with RANK and RANKL, which are also members of the TNF family of proteins. TRAIL has also been shown to bind to osteoprotegerin (OPG).[51] This is a naturally occurring inhibitor of the RANKL interaction with RANK, which binds RANKL with high affinity, preventing RANKL from interacting with RANK.[52,53] The biological relevance of OPG as a regulator of RANKL–RANK interaction and osteoclast formation and activation is clearly demonstrated by the development of osteopetrosis in OPG transgenic mice and severe osteoporosis in OPG knockout mice.[54,55]

The cartilage–pannus junction in RA contains many types of cells, which produce inflammatory cytokines reported to stimulate osteoclast differentiation and bone resorption. These include IL-1α and β, IL-6, IL-11, and TNF-α.[1] However, it is now clear that M-CSF and RANKL are essential factors required for the development of osteoclasts.[47] One of the end results of the production of inflammatory cytokines, such as IL-1β and TNF-α, in the inflamed joint is likely to be the upregulation of RANKL (produced by T cells, fibroblast-like synoviocytes, and

Table 2. RANKL and OPG synovial tissue expression in inflammatory arthritis and osteoarthritis patients and normal controls

	RANKL IOD[a]	OPG (vessels) IOD[a]	OPG (lining) IOD[a]	RANKL:OPG (vessels) ratio[b]	RANKL:OPG (lining) ratio[b]
Active RA	2426.8 (6524.2)	720 (336)	61.1 (92.8)	4.1 (0–50)	1478 (0–28 471)
Inactive RA	35.7 (34.6)**	2104 (724)*	6094.8 (4741.2)*	0.02 (0–0.05)	0.012 (0–0.035)
Spondyloarthropathy	702.8 (903.3)	5782.1 (2086.6)*	5980 (6399.5)*	0.22 (0–1.7)	0.39 (0–0.16)
Osteoarthritis	52.9 (57.7)**	892.5 (405.8)	7648.4 (2651.5)*	0.08 (0–0.34)	0.008 (0–0.024)
Normal	55.6 (73)**	556.9 (226.2)	5276 (4716.4)*	0.13 (0.002–0.98)	0.01 (0–0.03)

RANKL, receptor activator of nuclear factor κB ligand; OPG, osteoprotegerin; IOD, integrated optical density; RA, rheumatoid arthritis
* $P < 0.05$; ** $P < 0.005$
[a] Mean (Standard deviation)
[b] Mean (range)

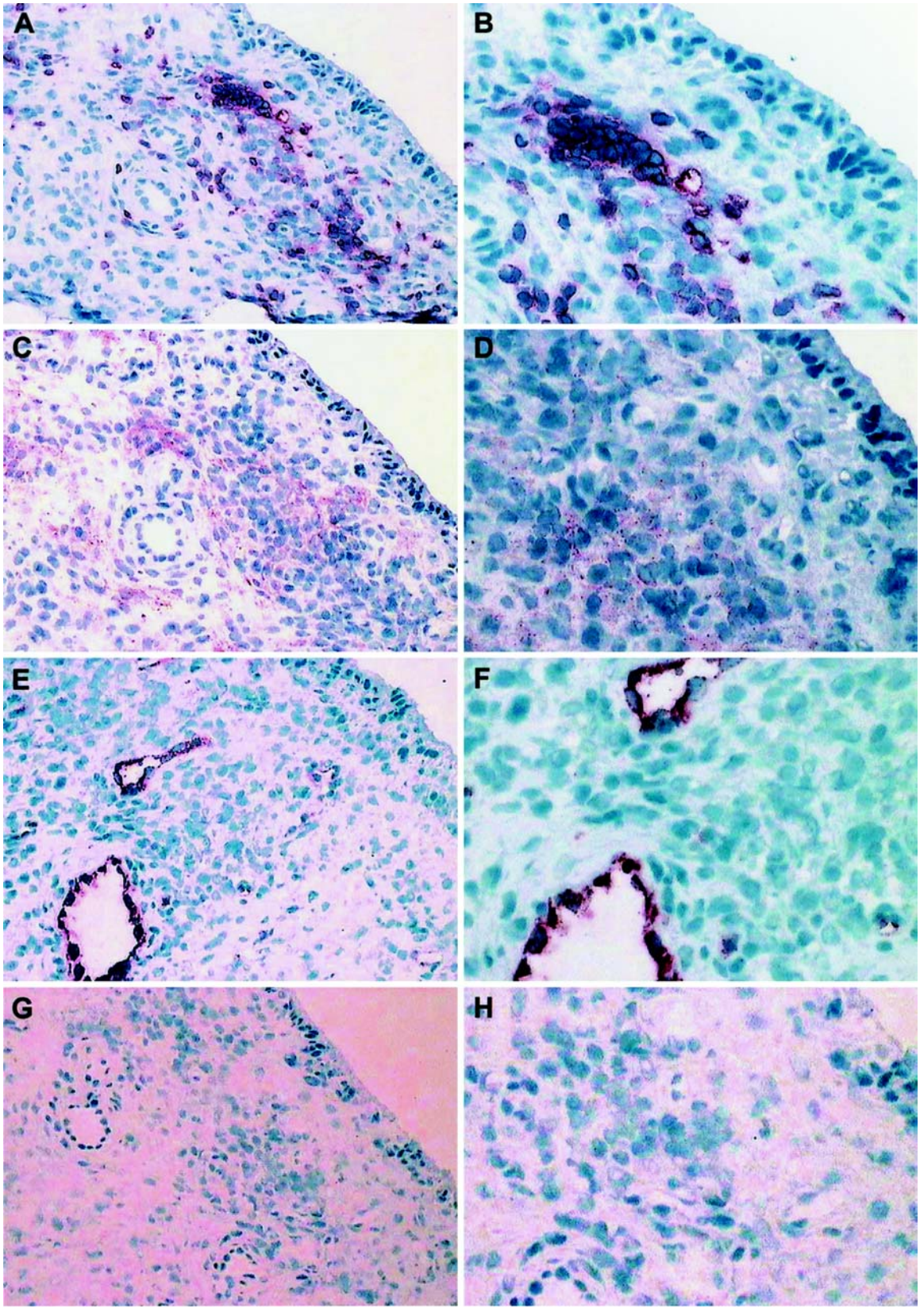

Fig. 1. Synovial tissue from a patient with active rheumatoid arthritis. Panels A and B show staining with anti-CD3 antibody, panels C and D staining with anti-activator of nuclear factor κB ligand antibody, panels E and F staining with one anti-osteoprotegerin (OPG) antibody (Mab 805, R and D Systems, Minneapolis, MN, USA), and panels G and H staining with another anti-OPG antibody (Mab 8051, R and D Systems). Panels A, C, E, and G: ×200; panels B, D, F, and H: ×400

osteoblasts) and RANK (expressed by preosteoclasts, T cells, and dendritic cells).[48,56] Recently, it has been suggested that TNF-α may have an additional major role in regulating osteoclast formation, through the TNF receptor type I.[57,58] In addition to their pivotal role in osteoclast formation, RANKL and RANK have a role in immune cell differentiation and T cell–dendritic cell interactions.[48,50]

We have recently demonstrated that OPG expression is present in the synovial tissue of normal subjects and both inflammatory and degenerative arthritides with the notable exception of active RA patients and that OPG may have an important role in regulating osteoclast formation within the joint. However, RA patients with inactive disease did express OPG in the synovial membrane, suggesting that successful treatment of the disease state in RA may upregulate OPG expression and possibly inhibit bone erosion at the joint margins (Table 2). This hypothesis needs to be tested by studying sequential synovial biopsies from patients undergoing DMARD treatment for RA and correlating the expression of OPG with radiological outcome measures in hand and feet x-rays. We have also shown that there are two different staining patterns for OPG within the joint, one of which is predominantly found in intimal macrophages found in the intimal lining layer (Fig. 1), whereas the other is exclusively endothelial (DR Haynes, EC Barg, TN Crotti, H Weedon, GJ Atkins, A Zannatino, et al.). In view of its ability to block RANKL–RANK interactions and inhibit osteoclast formation, OPG may have a role in normal homeostasis within the joint and has therapeutic potential in the treatment of conditions such as RA, where bone destruction is a major sequel of chronic inflammation. Of interest, a recent study has shown, in the collagen-induced arthritis model of RA, that IL-4 gene therapy can suppress synovial membrane IL-17 and RANKL expression and prevent bone erosion.[59] These results have yet to be duplicated in a human system, but suggest that IL-4 has promise as a therapeutic intervention to limit bone destruction in RA. In addition, systemic OPG administration to block the RANK–RANKL interaction was able to protect against bone and cartilage destruction in rats with adjuvant arthritis,[60] and the degree of bone erosion in TRANCE–RANKL knockout mice with arthritis was significantly reduced.[61] Thus, these studies suggest that targeting the RANK–RANKL interaction might be a new therapeutic approach in RA patients. Other strategies aimed at suppression of osteoclast function, for example, by regulation of the activity of Src family members of tyrosine kinases,[62] may also hold promise for the future.

Conclusion

It has recently become clear that early suppression of synovial inflammation is pivotal in preventing progressive joint destruction and preserving daily activity of RA patients. New insights into the factors that are involved in the process of joint destruction may lead to the development of novel therapies aimed at specific inhibition of cartilage and bone degradation.

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