[Frontiers in Bioscience 12, 536-550, January 1, 2007]

Collagenase gene regulation by pro-inflammatory cytokines in cartilage

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1. ABSTRACT

The essentially irreversible degradation of articular cartilage collagen represents a key, rate-limiting process in arthritic diseases. This process is typically initiated as a consequence of an inflammatory response, and if left unchecked ultimately leads to loss of joint function, pain, disability and a need for joint replacement surgery. Although we have identified the enzymes capable of effecting such destructive proteolysis, and considerable evidence indicates that tumour necrosis factor alpha and interleukin-1 are major pro-inflammatory mediators in joint destruction, we still know relatively little about how these mediators regulate collagenase gene expression in chondrocytes. Inflammatory arthritis has long been considered to be synovium-driven but compelling data now also implicate the chondrocyte, the sole cell type present in cartilage, as an active player in the destructive process. An understanding of how different cytokines interact, and how the pathways they activate cross-talk will not only provide important new insight into the mechanisms of joint destruction but also identify new targets for therapeutic intervention.

2. INTRODUCTION

Destructive joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) affect about 15% of the population of the United Kingdom, and represent a significant burden to both patients and healthcare services in many countries. A key research aim is to discover the mechanisms by which cartilage degradation occurs in arthritis in order to develop new therapies that effectively prevent tissue destruction. It is well established that cartilage collagenolysis is of critical importance in the arthritides since this leads to essentially irreversible damage (1). Although a very small subset of proteinases, specifically belonging to the matrix metalloproteinase (MMP) family, are known to be able to effect such collagenolysis, the identity of the collagenase(s) that mediates pathological cartilage catabolism remains the subject of much debate. Indeed, only recent work has begun to shed light on the mechanism by which collagenases actually hydrolyse triple helical collagen (2). It is highly probable that a combination of these proteinases actually mediate the destruction which will vary dependent on the resorptive situation. A detailed understanding of the

factors that influence their expression and catalytic activity are paramount before we can specifically prevent their action without undue adverse effects; an important consideration here is that MMPs effect many normal physiological processes (3), and 'non-specific' inhibition of MMP activity has been the downfall of some highly promising anti-arthritis drugs (4). The complexity of the MMP family is evidently another confounding issue, but data are now emerging that point to clear differences in how some of these proteinases are regulated. These, combined with our current understanding of the molecular events that mediate gene expression, have provided the impetus to begin to address issues important in the mechanisms that drive cartilage destruction. It is clear that cartilage destruction in the arthritides is a complex process that can arise from interactions between cartilage, synovium and bone depending on the disease state. This review specifically aims to outline some of the key findings pertaining to the collagenolytic MMPs (MMP-1, -2, -8, -13 and -14) (5-9) in the context of cartilage and the pro-inflammatory cytokines thought to mediate cartilage degradation.

3. COLLAGENASE EXPRESSION AND CARTILAGE HOMEOSTASIS

3.1. The metalloproteinases

It is widely recognised that of the five different classes of proteinases (10), the metalloproteinase family are most closely associated with the proteolysis of the components of extracellular matrices (ECM) such as cartilage. Specifically, the metzincins which include the MMPs and the related ADAM (a disintegrin and metalloproteinase) and ADAMTS (ADAM with thrombospondin motifs) enzymes are thought to be collectively responsible for the vast majority of proteolytic events that occur during tissue development and normal homeostasis as well as the pathological tissue degradation prevalent in arthritis (see [MMP review (11); ADAM review (12); ADAMTS review (13)] for further details). These proteinases are all active at the physiologically neutral pH of the extracellular environment, require calcium and zinc ions and have several functionally similar domains within an overall common structure although some have distinct, additional domains (reviewed in (3)).

An important control mechanism for the potent proteolytic activity of these metalloproteinases is that they are synthesised as inactive precursors (proenzymes) that require the proteolytic removal of an N-terminal polypeptide (the pro-peptide) that then allows catalysis to occur. The proteolytic cascades for this activation remain relatively poorly understood, although both serine and other metalloproteinases have been implicated (14-18). However, many metalloproteinases such as the membrane-bound MMPs and ADAMTS proteinases have furin recognition motifs that enable intracellular activation to occur within the trans Golgi network leading to the secretion of an active proteinase. This mechanism ideally localises these proteinases to effect proteolysis close to the cell, an event thought to occur in arthritic disease (19, 20). A family of endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) potently inhibit the MMPs although there are some differences in specificity between the four TIMP members. TIMP-3 also inhibits several ADAM/ADAMTS proteinases and is important for cartilage homeostasis since it associates with components of the ECM (21) (Figure 1).

3.2. Cartilage structure and function

The cartilage ECM covers the ends of bones to provide smooth, friction-less joint articulation. This unique tissue is avascular, aneural and alymphatic, deriving nutrients from the synovial fluid produced by the synovial lining of the joint cavity. Cartilage is sparsely populated by chondrocytes; the only cell-type present in this specialised ECM which is often viewed as being comprised primarily of collagens and proteoglycans. Specifically, these are type II collagen which provides tensile strength, and aggrecan which draws water into the tissue due to its highly negatively charged glycosaminoglycan side chains and provides tissue compressibility. This over-simplistic view has helped focus attention on aggrecan- and collagenolysis as the major degradative steps in cartilage breakdown. Some ADAMTS proteinases have been implicated in aggrecanolysis (13, 22); whilst the collagenolytic MMPs effect collagenolysis (3, 11). However, it is evident that the cartilage ECM is complex, with many "minor components" such as collagen types VI, IX, X and XI (23), biglycan, decorin, laminin, tenascin and fibromodulin (24); all assist in maintaining tissue integrity (25). Collectively these maintain links between chondrocytes and the ECM that are important for homeostatic maintenance and tissue functions such as relaying shear-stress-induced mechanotransduction signals following loading of the tissue (26). The diversity of ECM molecules helps to explain the number and repertoire of metalloproteinases in mammals in that it is highly likely that proteolysis of such a complex ECM will involve numerous proteolytic activities which will differ dependent upon the resorptive circumstance (e.g. cytokines, growth factors, trauma etc).

3.3. Cartilage destruction in arthritis

Collagen is the most abundant protein in the body, and provides cartilage with many of its structural properties. Fibrillar collagens form a triple helical structure which under physiological conditions is very resistant to proteolytic attack. Indeed, only a small subset of the MMP family are collagenolytic at neutral pH and hydrolyse each collagen strand in turn at a defined single peptide bond approximately 3/4 from the N-terminus (2). These fragments then become susceptible to further proteolysis by other metalloproteinases.

The 'classical' collagenases (MMP-1, -8 and -13) (5, 7, 8, 27-29) plus MMP-14 (9) and MMP-2 (6) specifically degrade collagen in cartilage destruction (30, 31). Indeed, an increase in collagenase-specific collagen cleavage occurs in pathological cartilage destruction (32, 33) and cartilage collagenolysis is considered an essentially irreversible stage in disease pathogenesis (1). The distribution of collagenolytic MMPs differs between synovium and cartilage, and in OA and RA (7, 8, 29, 30, 34-45). Although all these proteinases cleave collagen, it is

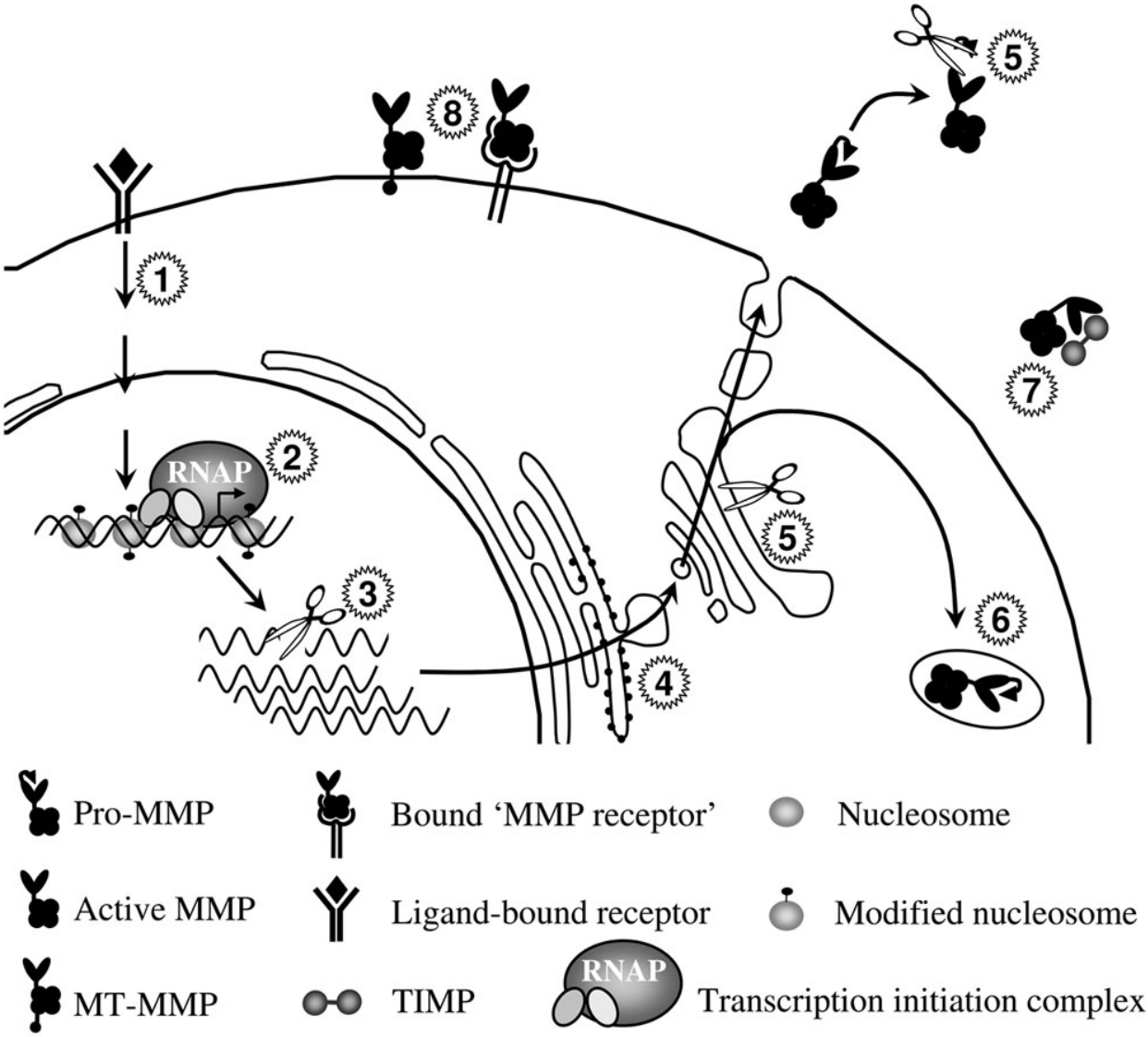

Figure 1. Control levels for MMP activity. Cytokines and growth factors can stimulate different intracellular signalling pathways which are able to combine (1) to activate or suppress MMP transcription. MMP transcription is regulated by the binding of sequence-specific transcription factors, such as c-Fos, and the subsequent modification of the surrounding chromatin allowing recruitment of the RNA polymerase (RNAP) and transcription initiation (2). MMP mRNA can be unstable and rapidly processed (3). Data suggest that ADAMTS expression can be controlled at the level of translation, implying similar phenomena may regulate overall MMP activity (4). ProMMPs can be activated intracellularly by furin (5) or after they have left the cell (5). Some MMPs are stored in granules within the cell (6) prior to secretion. All active MMPs can be inhibited by TIMPs (7). Secreted MMPs can be expressed on the cell surface, bound to cell surface receptor proteins or sequestered by ECM proteins (8). Other control mechanisms include secretion to specific regions of the plasma membrane, proteolytic processing and inactivation of MMPs and endocytosis and lysosomal breakdown.

likely the relative importance of individual collagenases will differ in different diseases and at different disease stages. For example, current dogma suggests MMP-1 is the major collagenase in RA whilst MMP-13 is more associated with OA. MMP-8 is only a minor gene product of human articular chondrocytes (46) but is thought to be important in septic arthritis since it is primarily produced by neutrophils. However, although serum MMP-8 levels correlate with inflammatory markers they do not correlate with disease activity scores (47). Despite mice not representing an ideal model for investigating arthritic disease (e.g. mice differ with respect to load distribution across joints and MMP-1 orthologues are not expressed in cartilage (48, 49)), gene deletion studies indicate MMP activity is not always associated with pathology. For example, MMP-2 or -14 deficiencies give rise to severe

connective tissue defects and the development of arthritis (50-52), evidence that implies these collagenases are not strongly involved in pathological tissue turnover but may have important homeostatic roles. Conversely, inhibitor studies indicate that gelatinases may also be involved in cartilage destruction (53), and MMP-2 has been suggested to be responsible for soft tissue destruction as it is expressed by pannocytes in invasive pannus tissues at the cartilage/bone interface (54), and is elevated in OA cartilage (55). MMP-2 has also been linked with erosions in early synovitis via increased synovial MMP-14 expression (43), although synovial MMP-14 expression levels have been reported to be unaltered in either RA or OA compared to control (56), data indicating that both tissue and stimulus-specific factors influence MMP-14 expression which has been implicated in human growth plate remodelling (57).

Thus, cartilage proteolysis is complex, involving multiple metalloproteinases. It is widely accepted that aggrecanolysis is a rapid and early event in cartilage resorption whilst collagenolysis is a much slower process that occurs in the latter stages of cartilage dissolution (58-60). This concept makes the collagenases attractive targets for therapeutic intervention, especially MMP-1 and/or MMP-13. The failure of broad-spectrum MMP inhibitors in clinical trials for arthritis (4) highlights the importance of specifically targeting appropriate MMPs or developing therapeutics that regulate the expression of specific collagenases.

4. COLLAGENASE GENE REGULATION IN CARTILAGE

Cytokines and growth factors have classically been divided into either 'pro-inflammatory' or 'anti-inflammatory'; a classification that originally had a wide application in most settings. A simplistic view has been that catabolic events are mediated by pro-inflammatory agents whilst anabolism is initiated by the anti-inflammatory agents. However, we are now aware of examples where certain mediators can exhibit both properties dependent on the situation and/or cell-type. For example, interleukin-4 (IL-4) is a well characterized pro-inflammatory mediator in asthma (61) but a protective factor in the context of cartilage biology (62, 63). Transforming growth factor beta (TGF-beta) has been reported to be both anabolic (reviewed in (64)) as well as catabolic for cartilage (65, 66); its high abundance in cartilage (67) suggests it is for initiating repair responses, but this may well involve localised collagenolysis concomitant with new ECM biosynthesis. It is therefore important to consider the context in which a given cytokine or growth factor acts since this may alter its impact on a given cell.

4.1. Inflammatory mediators of cartilage collagenase expression

Albeit with a few exceptions, collagenase gene expression is typically induced by pro-inflammatory mediators. Several major cytokines have been described since the pioneering discovery of catabolin (IL-1) (68) and its effects on cartilage (69). In the context of arthritic disease, IL-1, IL-17 and tumour necrosis factor alpha (TNF-alpha) are considered the major pro-inflammatory mediators (70-72), although more recent work also suggests that IL-15 and IL-18 as well as agonists for toll-like receptors (TLRs) may also be important mediators in the inflammatory arthritides (73-77). Both IL-1 and TNF-alpha have been prime targets as treatments for RA (78, 79); although OA has been traditionally viewed more as a non-inflammatory arthropathy, there is increasing evidence that many of these pro-inflammatory mediators may well also play important roles in OA pathogenesis (80-82). These mediators are present in RA and OA synovial fluids (e.g. (60, 81, 83-85)), and many have been shown to induce cartilage collagenolysis (e.g. (86)). Evidence in the literature, however, indicates that this is somewhat variable with relatively high levels of cytokine (that may exceed pathophysiological relevance) often required to induce significant resorption. Much of the early work was with single cytokines in line with the concept of a cytokine hierarchy whereby these major pro-inflammatory mediators induce the production of other mediators. Although this clearly occurs in the inflammatory arthropathies, the lack of complete efficacy for any specific anti-cytokine treatment supports the belief that other mediators participate actively in disease pathogenesis. This has been clearly highlighted by observations of cytokine synergy (87), whereby cytokines known to be present in diseased joints interact synergistically to promote marked cartilage collagenolysis with a concomitant synergistic induction of collagenases (87-90). These observations add yet another level of complexity which must be considered in experimental design if we are to fully elucidate disease mechanisms through laboratory experimentation.

Important 'effector' molecules in cartilage catabolism are IL-6 and oncostatin M (OSM) which both belong to the glycoprotein 130 (gp130)-binding cytokine family (91). When in combination with IL-1, IL-17 or TNF-alpha, a synergistic induction of MMP-1 and -13 is seen with marked collagenolysis (89, 90, 92-95). Considerable controversy existed for IL-6 which had originally been viewed as a chondro-protective agent since it is known to induce TIMP-1 in chondrocytes (96). Indeed, OSM also induces TIMPs, and factors that induce TIMP expression would appear to be anabolic for cartilage. However, the pro-inflammatory nature of IL-6 and OSM are now well established (97): anti-OSM blocks experimental arthritis (98) whilst anti-IL-6 therapy is used clinically (78, 99).

Thus, collagenase gene expression can be induced by a wide variety of pro-inflammatory mediators, many of which are known to be present in disease (78, 81, 82, 100), whilst several anti-inflammatory mediators down-regulate collagenase expression or prevent its activity by preventing pro-enzyme activation.

4.2. Signalling cascades in inflammation

Cytokines and growth factors mediate their effects on cells by binding to specific cell surface receptors. This 'signal' is then transduced to the nucleus via specific intracellular signal transduction pathways which culminate

in the activation or repression of target genes. Interest in the signal transduction of arthritis has increased dramatically over the past few years (101-103). Signalling in inflammation is complex (e.g. (104-107)), and multiple signalling cascades are often activated by a given cytokine in different cell-types. A further level of complexity is added in that interactions between these different signalling pathways can occur, and it is this 'cross-talk' that most probably accounts for the observed synergy between various cytokines (87, 93, 94). A series of major signalling pathways exist that have been proposed to participate in the inflammation associated with arthritic diseases. These include the mitogen-activated protein kinases (MAPKs) (108, 109), nuclear factor kappa B (NF-kappaB) (110, 111), activator protein-1 (AP-1) (89, 112-115), the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (91, 116) and the phosphatidylinositol 3-kinase (PI3K) pathway (117). Most of these pathways are activated by IL-1, IL-17, TLRs and TNFα, except JAK/STAT signalling which is the predominant pathway of the gp130-binding cytokines (the IL-6 family) although this family also signal through a variety of other pathways (91). A common feature of many of these pathways is their rapid, but rather transient activation through a series of phosphorylation events (typically mediated via serine, threonine and/or tyrosine kinase activity) before a return to basal levels. The transient nature of such signalling is presumably a regulatory mechanism, and it may be that deregulated signalling occurs in disease that allows inflammatory signals to persist.

The inherent specificity that some signalling pathways exhibit for different cytokines and growth factors has provided a major impetus to identify the pathways that help drive disease processes so as to help develop the next generation of highly specific and more efficacious drugs. We are now beginning to make inroads into this new field in the context of arthritic diseases, and some compounds that block specific signal transduction pathways are at advanced stages of clinical testing (e.g. (118-121) and see below).

Little is known about the signalling pathways that regulate metalloproteinase expression in chondrocytes although much more is known in other cell types (e.g. fibroblasts). Although it is reasonable to assume that aspects of these studies will be relevant to chondrocytes, cell-specific phenomena are commonplace in signal transduction biology. For arthritis, the relative dearth of literature on chondrocyte signalling probably reflects the prevailing paradigm of inflammatory disease being synovium-driven. Recent data indicating that chondrocytes are also very active players in the destructive processes of cartilage degradation (60, 93, 122-126) challenge this concept, and it is well known that therapies that simply target inflammation (i.e. reduce synovitis) do not prevent cartilage destruction (127). Thus, a better appreciation of the processes that regulate metalloproteinase production in chondrocytes will be highly informative in our understanding of disease pathogenesis in the arthritides.

4.2.1. Anti-arthritic effects of signalling pathway inhibition

Both synthetic and natural inhibitors, along with biologics, to the main pathways involved in the inflammatory response (NF-kappaB, MAPK, PI3K and JAK/STAT) have been developed and tested both *in vitro* and *in vivo* with varying degrees of success (128). SP600125, a pharmacological inhibitor of the c-Jun N-terminal kinase (JNK) MAPK pathway decreases joint destruction in a rat adjuvant arthritis model, in part by diminishing the production of MMP-1 (119). Furthermore, JNK-deficient murine synoviocytes show an almost complete loss of IL-1-induced MMP-13 expression (119). Inhibition of the p38 MAPK pathway resulted in anti-arthritic activity in rat and mouse models of arthritis (129). The same inhibitor, as well as a specific JNK MAPK inhibitor and a specific extracellular signal-regulated kinase (ERK) inhibitor, block collagenase induction by the pro-inflammatory cytokines IL-1, IL-17 and TNF-alpha in human and/or bovine articular chondrocytes (113, 114, 130) suggesting activation of similar signalling pathways by these pro-inflammatory cytokines. p38 is necessary for IL-1-induced MMP-1 expression, and p38 blockade results in a loss of MMP-1 and prevention of IL-1-induced bovine cartilage collagenolysis (131). The mechanism of action on MMP expression by these signalling pathways is currently unclear. The JNK MAPK pathway activates c-Jun and NF-kappaB, both of which can transactivate the expression of MMPs (see below), while the ERK and p38 MAPK pathways can activate STATs. The p38 pathway can alter the stability of MMP mRNA (discussed in 5) although this has yet to be demonstrated in articular chondrocytes. IL-1 and OSM signal via the NF-kappaB and JAK/STAT pathways respectively, a cytokine combination that *in vivo* causes a RA-like phenotype and rapid joint destruction in mice concomitant with an up-regulation of MMPs (122). Gene therapy using inhibitors of both these pathways appear efficacious in rodent arthritis models (132, 133) and represent excellent potential methodologies to prevent the induction of the degradative MMPs.

The success of anti-TNF-alpha therapy in some RA patients may well be attributable, in part, to a reduction in signalling effects as a consequence of reduced TNF-alpha levels. This will indirectly reduce the extent to which these pro-inflammatory signalling cascades are activated, and provide added impetus to ongoing clinical trials.

4.3. Transcriptional activation of collagenase genes in chondrocytes

As mentioned previously, relatively little is known about the regulation of MMP-1, or indeed many other metalloproteinases, in chondrocytes although much more is known in other cell types (e.g. fibroblasts). When cartilage or chondrocytes are treated with IL-1+OSM, MMP-1 is up-regulated first followed by MMP-13, then MMP-14 and later MMP-8 (34). Indeed, there is a cluster of MMP genes on chromosome 11q22 that can be co-regulated dependent on the stimulus and includes most of the collagenases. However, this example of temporal differences in expression suggests that differences in the mechanism of transcriptional regulation may exist for the

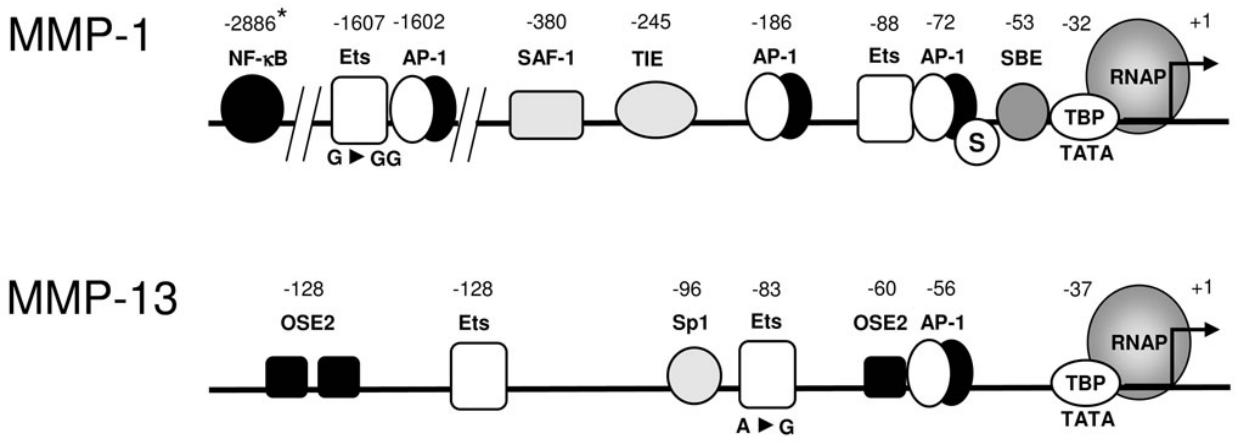

Figure 2. Schematic of MMP-1 and MMP-13 promoter *cis*-elements. Defined functional consensus binding sites with approximate nucleotide position relative to the transcription start site (indicated by the arrows at +1) that regulate MMP-1 or -13 gene transcription. Key to the binding sites; SBE, STAT-binding element; TIE, TGF-beta inhibitory element; S, Smad-binding site; SAF-1, serum amyloid A-activating factor-1; OSE2, osteoblast-specific element 2 (Cbfa1/Runt binding site); TBP, TATA binding protein; RNAP, RNA polymerase II complex. *NF-kappaB-like binding site. MMP-1 -1607 Ets binding site is generated by a G to GG promoter polymorphism while the -83 Ets binding site of MMP-13 is lost by an A to G transition.

collagenases. Figure 2 contains a schematic representation of the known regulatory transcription factor binding motifs found in the MMP-1 and MMP-13 promoters, as summarised below.

4.3.1. Collagenase gene transcription in chondrocytes

4.3.1.1. Activator Protein-1 (AP-1)

Activator protein-1 (AP-1) complexes are heterodimers of proteins of the two proto-oncogene families, Jun and Fos. It is widely accepted that AP-1 DNA binding elements within the MMP-1 and MMP-13 promoters that are important for fibroblasts are also important for gene transcription in chondrocytes (134-140). The MMP-1 promoter is activated by Jun complexes as well as Fos/Jun heterodimers, while MMP-13 up-regulation by IL-1 or IL-17 appears to require c-Fos and FosB activation respectively (138). Interestingly, c-Fos expression has been demonstrated in mid- and deep-zone cartilage from inflammatory arthritis (141). Other data suggests JunB maybe a negative regulator of MMP expression in chondrocytes (138). Vascular endothelial growth factor (VEGF) potently induces MMP-1 and MMP-13 through VEGF receptor kinase activity and subsequent phosphorylation of ERK1/2 leading to sustained AP-1 formation (142). TGF-beta1 exerts repressive effects on MMP-1 gene transcription via Smad binding close to the -72bp AP-1 (143, 144) in fibroblasts and it is likely that a similar mechanism occurs in chondrocytes. These repressive effects further support the high significance of AP-1 elements for MMP-1 gene transcription, as do inhibition of AP-1 activation and DNA binding (115). Interestingly, it is reported that TGF-beta induces MMP-13 expression in OA chondrocytes via Smad proteins and co-operation between promoter proximal AP-1 and polyomavirus enhancer A (PEA-3) binding sites (65). Interactions between AP-1 and PEA-3 (which bind Ets factors) have been reported for both basal and induced expression of numerous MMPs and, somewhat paradoxically, also for the TIMPs (137, 145).

4.3.1.2. Nuclear Factor-kappaB (NF-kappaB)

NF-kappaB is a ubiquitously expressed family of transcription factors with five known members, p50 (NF-kappaB1), p52 (NF-kappaB2), p65 (RelA), c-Rel and RelB, which can homo or heterodimerise to provide differential biological affects. Interestingly, although the human MMP-1 promoter has a NF-kappaB-like site (-2886bp) there is only limited evidence for a role for NF-kappaB-dependent activation of MMP-1 in chondrocytes (89, 111, 146), however, considerable data exist in other cell types (e.g. (111, 147)). Bcl-3, a member of the inhibitor of NF-kappaB (IkappaB) family, is an IL-1-responsive gene that activates MMP-1 transcription via co-operation with the NF-kappaB subunit p50; although proposed for chondrocytes this has only been demonstrated in fibroblasts (148). TNF-alpha-induced p50/p65 heterodimers have been shown to be responsible for MMP-1 and -3 induction in chondrocytes, and proteosomal inhibitors that prevent IkappaBalpha and IkappaBbeta degradation and thus retain NF-kappaB in the cytoplasm, block this induction (149). Proteosomal inhibitors along with the more specific NF-kappaB inhibitors curcumin and BAY-11-7085 can also inhibit MMP-13 induction in chondrocytes in response to TNF-alpha or IL-1, however this induction was also ablated by MAPK inhibitors (113, 114).

4.3.1.3. Signal Transducers and Activators of Transcription (STATs)

The MMP-1 promoter contains a STAT binding element located at approximately -58bp close to the essential AP-1 element. In response to OSM, this STAT element can be bound by STAT-1 and -3 in fibroblasts and astrocytes (150). It has been proposed that the AP-1 and STAT sites comprise an OSM response element for the induction of MMP-1 by OSM. However, in chondrocytes conflicting data exist about the induction of collagenases by OSM and STATs. Catterall *et al* (89) could not identify OSM (or IL-6) induction of MMP-1 mRNA or STAT

binding to the proposed OSM response element, but did demonstrate induction of a MMP-1 promoter reporter construct. This was probably due to activated STAT-1 and -3, since over-expression of protein inhibitor of STAT-3 (PIAS-3) suppressed MMP-1 transcription, but this is via the induction of the immediate-early oncogene c-Fos, an important regulator for AP-1 transactivation. Meanwhile, Li et al (151) showed that OSM induced MMP-1 and -13 via the activation of the JAK-STAT pathway in bovine and human chondrocytes. An upstream inhibitor of JAK3 (but not a JAK2 inhibitor) inhibited STAT-1 phosphorylation (and DNA binding) and blocked MMP-1 and -13 induction by OSM. IL-6 (in combination with the soluble IL-6 receptor) can activate STATs and ERK to induce MMP-1 and -13 expression in bovine chondrocytes, and cross-talk between the two pathways is required to maximally induce both genes (152). It is unclear in either of these reports whether the effects seen with OSM on MMP expression are direct or via the induction of an intermediary such as c-Fos.

4.3.1.4. Other Pathways

Several other elements have also been implicated in MMP-1 transcription. There are two functional binding sites (-386 to -354bp) for the inflammation-responsive transcription factor serum amyloid A-activating factor-1, which is constitutively expressed in OA chondrocytes (153). The nuclear hormone receptor, peroxisome proliferator-activated receptor gamma (PPARgamma) is constitutively expressed in chondrocytes and acts repressively by binding in the region -83 to -71bp. PPARgamma and AP-1 binding are mutually exclusive but competitive, and such PPARgamma-response elements overlap AP-1 sites in other MMP promoters (154).

Elevated levels of Runx2 (Cbfa1) have been demonstrated in OA chondrocytes, and over-expression of this transcription factor increased MMP-13 promoter activity in the presence of fibroblast growth factor 2 (155). This affect was via ERK (155) and Runx2 has also been shown to be important for IL-1 induction of MMP-13 via interacting with P38 MAPK (139).

Fibronectin fragments including the 110 kDa fragment that binds alpha5beta1 integrin stimulates MMP-13 expression via the proline-rich tyrosine kinase-2 (PYK-2). Over-expression of this kinase also induces MMP-13. Inhibitors of ERK, P38 and JNK MAPKS, as well as protein kinase C (PKC) all blocked fibronectin fragment-induced MMP-13 promoter activity (156).

4.5. Control of collagenase expression by acetylation and chromatin modifications

Nucleosomes, around which genes are packaged, are repressive to transcription by preventing access of the transcriptional machinery (157). To facilitate transcription, chromatin structure is altered by the enzymatic modification of histones via acetylation, methylation or phosphorylation that allows the recruitment of transcriptional regulators specifically to that nucleosome-modified locus (158-160). ATP-dependent nucleosome remodellers which slide nucleosomes to expose previously inaccessible DNA sequences or transcription start sites are yet another mechanism (161, 162).

The acetylation status of specific lysyl residues on the N-terminal tails of histones is balanced by the activities of the histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzyme families (157, 163). Recently, non-histone substrates of HATs and HDACs have been described which include signalling molecules and transcription factors, many of which are key regulators of MMP-1 expression such as NF-kappaB, STAT-3 and the AP-1 component c-Jun (164-169). This suggests acetylation may represent a wide-ranging biological modification (160).

Only a limited number of reports have examined potential chromatin-modifications in the regulation of collagenase gene expression, with only a single report focussing on the chondrocyte (95). A co-ordinated cascade of histone modifications, transcription factor recruitment and nucleosome remodelling at the MMP-1 promoter occurs during phorbol ester or serum induction of the gene (170). Upon stimulation the nucleosome encompassing the MMP-1 transcription start is first methylated on histone H3 lysine 4, which corresponds with the binding of the SET9 methyltransferase and the assembly of a complex containing c-Jun, c-Fos, TATA binding protein and RNA polymerase II. This is followed by the assembly of a pre-initiation complex and concomitant histone acetylation and phosphorylation. Only after these events have occurred can the induction of the *MMP-1* gene be measured. Although performed in T98G human glioblastoma cells it is intriguing to speculate that a similar mechanism may occur in the induction of MMP-1 and possibly other collagenases in chondrocytes by various stimuli.

Small molecule HDAC inhibitors (HDACi) are suggested as a treatment for inflammatory disease (171) as they can modulate gene expression in synovial cells *in vivo* (172, 173) by blocking proliferation and inhibiting TNF-alpha expression, thus abrogating cartilage destruction, possibly by MMP-1 repression (173). In cartilage, HDACi can block induced cartilage resorption *in vitro* due to not only a decrease of collagenase activity but also a dramatic repression in the expression of all the induced collagenases (MMP-1, -8, -13 and -14) (95). Interestingly, HDAC4 gene disruption in mice causes abnormal skeletal development due to early onset chondrocyte hypertrophy resulting in premature ossification of developing bones, mimicking a Runx2 loss-of-function phenotype. HDAC4 normally associates with Runx2, inhibiting its ability to bind and transactivate target promoters (174). Human HDAC4 is located on chromosome 2q37, a region linked to Albright's hereditary osteodystrophy-like syndrome (175). It therefore appears that HDAC inhibition could be beneficial to the maintenance of cartilage integrity, whilst HDACs (HDAC4 at least) are essential for correct tissue development. Although appearing paradoxical, such an observation is not without precedent. Knockout mice for HDAC5 or -9 genes develop markedly hypertrophic hearts (174, 176) while HDACi actually prevent cardiac hypertrophy (177, 178). The eleven HDACs can be divided into different classes: class I HDACs repress the expression of anti-hypertrophic genes (177-179) while class II HDACs (which includes HDAC5, -9 and interestingly HDAC4) suppress cardiac

hypertrophy by blocking MEF2 activation/activity which is a well characterised promoter of cardiac hypertrophy (180). Why in the wild-type normal animal the anti-hypertrophic program overrides the pro-hypertrophic program when both pathways involve HDACs is as yet unknown (181) but it is interesting to speculate that a similar phenomenon may occur in cartilage. Currently, the biological roles of each HDAC are unknown but it is evident that elucidation of the HDACs involved in pro-inflammatory cytokine signalling and the development of specific inhibitors to these enzymes could well be of therapeutic benefit for the arthritides.

A full review of the role of chromatin modifications in the regulation of metalloproteinase gene expression is available in an accompanying article in this issue of Frontiers in Biosciences (Clark, Swingler and Young).

5. POST-TRANSCRIPTIONAL COLLAGENASE REGULATION

Inhibition of p38 MAPK activity (with SB203580) can prevent IL-1-induced cartilage collagenolysis *in vitro* (131) and *in vivo* (129, 182). p38 MAPK is required for the expression of many MMPs, and transcriptionally it controls NF-kappaB transactivation, histone phosphorylation/acetylation, phosphorylation of RNA polymerase-binding proteins and c-Jun expression (183, 184). The p38 pathway contains four related kinases (alpha, beta, gamma and delta), and is crucial for post-transcriptional stabilization of inflammatory mRNAs (185) which is achieved by promoting gene translation through AU-rich elements (AREs) in the 3' untranslated region (3'UTR) of the mRNAs; repeats of the motif AUUUA facilitate this effect. Several MMP mRNAs (e.g. MMP-1, -3 and -13 (185-187)) contain such elements and are regulated by p38 MAPKs. The role of such 3'UTRs in chondrocyte regulation of MMPs has yet to be studied, but it is interesting to note that immediate early genes such as c-Fos that are known to regulate MMP expression also contain well-characterized AREs in their 3'UTRs (188).

6. SUMMARY AND PERSPECTIVE

Emerging data are now beginning to shed important light on the regulatory mechanisms that govern collagenase gene expression. The success of anti-cytokine treatments in arthritis combined with considerable *in vitro* data clearly indicate that the signalling cascades activated by the pro-inflammatory cytokines are an essential event in the destructive phases of cartilage breakdown. We now more fully appreciate that arthritis cartilage destruction is a complex process. Just as more than one collagenase is probably involved, the same applies for the signalling pathways that give rise to the expression of these potent proteinases. The growing interest in signal transduction within arthritis, and how this regulates collagenase expression, represents an exciting challenge for research that will have wide applications to many connective tissue disorders. Although we must remain cautious about therapies that may suppress inflammation per se, elucidation of the intracellular mechanisms that drive collagenase gene expression within an inflammatory setting herald a new dawn in the search for new therapies that more effectively block collagenase-mediated cartilage destruction.

7. ACKNOWLEDGEMENTS

ADR would like to acknowledge the support of the Arthritis Research Campaign, the Wellcome Trust and the Oliver Bird Fund. DAY is funded by the JGWP Trust.

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Key Words: Collagenase, Matrix Metalloproteinase, Cartilage, Chondrocyte, Gene Expression, Gene Regulation, Pro-Inflammatory Cytokines, Synergy, Signalling Cascades, Review

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