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Metalloproteinase production from macrophages – a perfect storm leading to atherosclerotic plaque rupture and myocardial infarction

Andrew C Newby, University of Bristol, School of Clinical Sciences and Bristol Heart Institute, Bristol, UK

Running title: Metalloproteinases from macrophages

Address for correspondence:
Prof Andrew C Newby
Bristol Heart Institute
Research and Teaching Floor Level 7
Bristol Royal Infirmary
Bristol BS2 8HW, UK
Tel +441173423583
Fax +441179299737
Email a.newby@bris.ac.uk

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Key words

Macrophage, metalloproteinase, transcription, inflammation, atherosclerosis, plaque rupture, myocardial infarction

Abstract

Matrix metalloproteinases (MMPs) produced from macrophages contribute to plaque rupture, atherothrombosis and myocardial infarction. New treatments could emerge from defining the mediators and underlying mechanisms. In human monocytes, prostaglandinE2 (PGE2) stimulates MMP production and inflammatory mediators such as TNFα, IL-1 and toll-like receptor ligands can act either through or independently of PGE2. Differentiation of human monocytes to non-foamy macrophages increases constitutive expression of MMPs-7, -8, -9, -14 and -19 and TIMPs-1 to -3 through unknown, PGE2-independent mechanisms. Human macrophages express more MMPs-1, -7, -9 and TIMP-3 and less MMP-12 and -13 than mouse macrophages. Inflammatory mediators working through AP-1 and NF-κB transcription factor pathways upregulate MMPs-1, -3, -10, -12 and -14 in human macrophages (MMP-9, -12 and -13 in mice) and studies with plaque tissue sections and isolated foam cells confirm this conclusion *in vivo*. Classical activation with GM-CSF upregulates MMP-12, whereas IFNγ upregulates MMPs-12, -14 and -25 and downregulates TIMP-3 in human but not mouse macrophages. Alternative activation with IL-4 markedly stimulates the expression of only MMP-12 in humans and MMP-19 in mice. Anti-inflammatory cytokines, IL-10 and TGFβ, decrease production of several MMPs. Epigenetic upregulation of MMP-14 during foam cell formation or by GM-CSF occurs by decreasing miRNA-24. A 'perfect storm' caused by a combination of these mechanisms most likely promotes MMP-mediated macrophage invasion, tissue destruction and atherosclerotic plaque rupture.

Introduction

Production of matrix metalloproteinases (MMPs) from macrophages contributes to destruction of the extracellular matrix (ECM) in a broad range of chronic inflammatory diseases. Atherosclerosis is a special case because lipoprotein particles trapped in the artery wall recruit monocytes that convert to foam-cell macrophages by engorging oxidised and other modified forms of these lipoproteins (Williams & Tabas, 1995). In advanced atherosclerosis, plaques consisting of amorphous lipid deposits with overlying, expanded connective tissue can obstruct the coronary and other conduit arteries, leading to stable ischaemic syndromes, including angina pectoris. Moreover, depletion of collagen and other ECM molecules from the core and fibrous cap overlying plaques can lead to loss of mechanical competence, culminating in rupture of the cap, thrombus formation on the exposed thrombogenic core and partial or complete occlusion of the lumen (Libby, 2013). Plaque rupture underlies the majority of myocardial infarctions (MIs) and strokes (Virmani *et al.*, 2006), which together constitute the principal cause of death in many advanced societies. Inhibiting MMP activity (Newby, 2012; Newby, 2015), or the mechanisms responsible for production of MMPs from macrophages (reviewed here), therefore represent viable targets for therapies to prevent MIs and strokes. The earlier literature relating to this topic was previously discussed exhaustively (Newby, 2008), and hence this article seeks to provide an update by emphasizing findings during the last seven years. These new insights suggest that multiple inflammatory mediators need to act in concert to raise a 'perfect storm' that provokes net destruction of the ECM leading to MIs and strokes.

Involvement of matrix metalloproteinases in atherosclerosis

There are at least 23 MMP enzymes, most of which are secreted, except the six membrane-type MMPs that are inserted into or attached to the external membrane surface. The catalytic sites of MMPs may be blocked by all or at least some of the four tissue inhibitors of MMPs (TIMPs) (reviewed in detail elsewhere (Nagase *et al.*, 2006)). A structurally-similar active catalytic domain occurs also in some members of the disintegrin metalloproteinases (ADAMs) and in the ADAMs with thrombospondin domains (ADAM-TSs). MMPs have the ability to degrade a variety of ECM

components but also many other cell surface, secreted or ECM-sequestered substrates many of which regulate inflammation (Khokha *et al.*, 2013).

As summarized previously (Newby, 2012; Newby, 2015), the evidence that MMPs play pathological roles in atherosclerosis comes partly from rabbit and especially mouse models. However, the expression pattern of MMP mRNAs in human blood and mouse bone marrow macrophages isolated and classically activated under very similar conditions is quite divergent, with far more MMPs-1, -7, -9 and TIMP-3 in human macrophages but much less MMP-12 and -13 compared to mouse (Newby, 2015). This conclusion is reinforced by other studies of mouse bone marrow macrophages and Raw264.7 cells (Hald *et al.*, 2012; Murray *et al.*, 2013). Moreover, a comparison of unstimulated mouse and human blood monocytes and macrophages shows the same similarities and differences (Fig. 1). Differentiation of blood monocytes to macrophages greatly increases expression of MMPs in both mice and humans (Fig. 1) but levels of MMPs-1, -7, and -9 and TIMPs-1 and -3 are much higher in man (Huang *et al.*, 2012), whereas MMPs-12, -13 and -23 are much higher in mice (Tsaousi *et al.*, 2016). The high levels of MMP-12 and -13 expression in mice macrophages correspond with dramatic effects on atherosclerosis (Johnson *et al.*, 2011; Quillard *et al.*, 2011). However, MMP-12 (Scholtes *et al.*, 2012) and MMP-13 (Molloy *et al.*, 2004) have restricted expression in human atherosclerotic plaques, which invites caution over the clinical translation of the mouse studies. In the case of MMP-12 there are genome wide association studies (GWAS) studies supporting a causative role in strokes (Traylor *et al.*, 2014) but this is not the case for MMP-13. Conversely, MMP-7 is hardly expressed in mouse macrophages and has a modest impact on atherosclerosis (Johnson *et al.*, 2005) but could be more important in man (Fig. 1). Most recently, MMP-28 was shown to affect macrophage functions in mice (Ma *et al.*, 2013) but MMP-28 is not expressed actively in human monocytes or macrophages (Bar-Or *et al.*, 2003). Furthermore, the profound morphological differences and the need for high level transfer of fully active forms of MMPs to provoke plaque rupture in mice (Gough *et al.*, 2006; Liang *et al.*, 2006) discourage extrapolation to the human disease.

GWAS provides convincing evidence of a pathogenic role for MMP-12 (Traylor *et al.*, 2014) and the distantly-related ADAMTS-7 (Reilly *et al.*, 2011). For other MMPs and TIMPs only correlative evidence is available so far. For example, many MMPs and TIMPs are overexpressed in human atherosclerotic plaques compared to normal tissues (reviewed in detail elsewhere (Newby, 2005)). More persuasively, MMP-8, -9, -12 and -14 have been shown in biobank studies to associate with plaque morphologies suggesting vulnerability to rupture, whereas MMP-2 and TIMP-3 show negative association (Sluijter *et al.*, 2006; Peeters *et al.*, 2011; Scholtes *et al.*, 2012; Johnson *et al.*, 2014). Furthermore, at least MMP-8 and MMP-12 levels in plaques are risk factors for subsequent adverse cardiovascular events (Peeters *et al.*, 2011; Scholtes *et al.*, 2012). In future it may be possible to combine biochemical and genetic analyses for example in Mendelian Randomisation studies or by the identification of rare null mutations. In the meantime a causative role for MMPs in human plaque rupture is highly plausible but still a hypothesis.

Monocyte and macrophage diversity in atherosclerosis

Production of monocytes and macrophages from myeloid precursors relies on the trophic effects of colony stimulating factor (CSF-1). Deletion of CSF-1 or blocking its receptor in mice prone to atherosclerosis greatly reduces plaque formation (Di Gregoli & Johnson, 2012). Similarly, depletion of monocytes and macrophages in the early stages of mouse atherosclerosis abolishes foam cell formation and reveals the accumulation of lipoprotein deposits in susceptible sites (Paulson *et al.*, 2010). These experiments establish that macrophages derived from circulating monocytes are

required to clear lipoprotein deposits retained in the ECM and that this leads to foam cell formation. Some contribution from macrophage proliferation (Robbins *et al.*, 2013) or from expansion of resident stem cell populations has also been debated (Nguyen *et al.*, 2012); and additional foam cells can be generated by transdifferentiation of resident vascular smooth muscle cells (VSMCs) (Shankman *et al.*, 2015). As reviewed previously (Newby, 2005), both macrophages and VSMCs elaborate MMPs and TIMPs. Moreover, MMPs and other proteases promote VSMC migration and proliferation so as to establish the fibrous cap of plaques. On the other hand, the high levels of many MMPs produced by macrophages (see below) may provoke destruction of the ECM causing plaque rupture.

At least two phenotypes of monocytes (Ly6Chi, CCR2hi, and Ly6CloCXCR3hi) and three phenotypes of human monocytes (CD14hiCD16lo, CD14dimCD16lo and CD14dimCD16hi) have been characterised (Ziegler-Heitbrock *et al.*, 2010). Despite performing different functions in relation to acute inflammation and patrolling behaviour, both monocyte phenotypes appear to contribute to atherosclerosis in mouse models (Combadiere *et al.*, 2008). Moreover they do not seem to give rise to different macrophage populations in plaques (Tacke *et al.*, 2007).

Differentiated macrophages adopt a host of different phenotypes. These were initially divided into pro-inflammatory, so-called classically activated or M1 type, or anti-inflammatory, so-called alternatively activated or M2 type. However, the M1/M2 dichotomy has more recently been replaced with more nuanced descriptions of phenotypes (Murray *et al.*, 2014) based on the activating mediators and their related signalling pathways some of which are illustrated in Fig. 2.

Regulation of MMP and TIMP production from monocytes and macrophages

Binding of transcription factors of the activator protein-1 (AP-1) family to regulatory elements in the proximal promoters of many MMPs appears to be of central importance for their transcriptional regulation, and certainly contributes to their increased production during inflammation (Clark *et al.*, 2008). However, not all MMP promoters contain proximal AP-1 sites or even a TATA box, which is necessary for induced transcription of most genes (Clark *et al.*, 2008). Moreover, a plethora of other proximal transcription factors binding sites, including for specificity protein-1 (SP-1), nuclear factor-κB (NF-κB) and signal transducer and activator of transcription-1 (STAT-1), mediate inflammatory activation of several MMPs (Clark *et al.*, 2008). Synergy between activation of AP-1 and NF-κB is responsible for induction of several MMPs in a variety of cell types (reviewed in (Newby, 2005)), including macrophages. This may depend on a signalosome that brings together widely separated transcriptional activators including distal enhancer or suppressor elements (Glass & Natoli, 2015).

In order to influence MMP and TIMP expression therapeutically in plaques and other inflammatory foci, it would be valuable to identify the key mediators and also the underlying mechanisms. This review will attempt to synthesise the available information, in part by providing a searchable databases for monocytes (Supplementary Table 1) and macrophages (Supplementary Table 2).

ProstaglandinE2 and the cAMP pathway

ProstaglandinE2 (PGE2) mediates upregulation of at least MMP-1, -7, -9, -10 and -14, as well as TIMP-1 in undifferentiated human monocytes, as previously reviewed (Newby, 2008). PGE2-dependent MMP up-regulation has also been observed in human alveolar macrophages, mouse peritoneal macrophages and RAW264 cells (see Supplementary Tables 1 and 2). As shown in Fig. 2, action of inflammatory mediators or integrin-mediated binding to various ECM components activates phospholipase C, which releases arachidonic acid. This is transformed by the sequential

activity of cyclooxygenase (COX) and PGE2 synthase (PGES-1) to PGE2. COX-1 is constitutively expressed in human monocytes and COX-2 is rapidly upregulated by adherence or LPS (Reel *et al.*, 2011) or by TNFα together with GM-CSF (Zhang & Wahl, 2015). PGE2 acts specifically on EP4 receptors to stimulate cAMP formation, which then activates transcription through direct binding of cAMP response element binding protein (CREB) to the MMP-1 promoter or by enhancing the binding NF-κB to the MMP-9 promoter (Lai *et al.*, 2003). Work from other cell types also identifies cross-talk with the mitogen activated protein kinases (MAPKs) (Gerits *et al.*, 2008) that could promote AP-1 binding (Fig. 2). Other activators of PGE2-dependent MMP production include extracellular MMP-1 or MMP-3, which can cleave active TNFα from the surface of mouse peritoneal macrophages, leading to MMP-9 secretion (Steenport *et al.*, 2009). Furthermore, TNFα generated in this way upregulates early growth response protein 1 (EGR-1), which induces mPGES-1 expression (Khan *et al.*, 2012). Exposure to Mycobacterium tuberculosis infection can also upregulate MMP-1 but not MMP-7 in a PGE2-dependent manner (Rand *et al.*, 2009).

Differentiation of monocytes to macrophages

MMPs-2, -7, -9, -11, -12 and -14 and TIMPs-2 and -3 are selectively upregulated in human MCSF-differentiated macrophages, independently of COX, MAP kinases or NF-κB (Reel *et al.*, 2011), and MMP-8, -13, -19, -23 and -25 are also increased in mouse macrophages (Tsaousi *et al.*, 2016). Increased expression of MMP-14 has been ascribed to upregulation of the SAF-1 transcription factor (reviewed in (Newby, 2008)) but the other mechanisms remain to be clarified.

Foam cell formation

The properties of foam cells formed from differentiated macrophages *in vitro* depends on the source of lipid (e.g. platelets, acetylated LDL, minimally or extensively oxidised LDL). Hence widely different results have been obtained suggesting stimulation, inhibition or little effect on levels of MMPs (Supplementary tables 1 and 2). Stimulation could result from (weak) action on toll like receptors (Lundberg & Yan, 2011), whereas inhibition of MMP-9 expression in U937 cells (Supplementary Table 1) resulted from formation of peroxisome proliferation activator receptorγ (PPARγ) ligands. We found no effect of oxidised LDL on mRNA levels of MMPs and TIMPs but foam cells expressed more MMP-14 and less TIMP-3 protein, which implicated epigenetic mechanisms in part mediated by microRNA24 (Johnson *et al.*, 2014). *In vivo* results are also divergent. Rabbit granuloma foam cells showed increased MMP-1, -3, -12, -14 and decreased TIMP-3 expression (Supplementary Table 2), although mouse granuloma foam cells showed no changes (Thomas *et al.*, 2015) and peritoneal foam cells had decreased MMP-13 expression, owing to activation of the LXR nuclear receptor by the cholesterol pathway intermediate desmosterol (Spann *et al.*, 2012). So far only the rabbit studies investigated protein levels, which might vary despite similar mRNA expression if epigenetic mechanisms intervene (Johnson *et al.*, 2014).

Classical macrophage activators and the AP-1/NF-κB pathway

The pro-inflammatory mediators TNFα, IL-1β, CD40L and pathogen associated molecular patterns that act at several toll-like receptors have been observed to stimulate MMP expression in both monocytes and macrophages (Supplementary Tables 1 and 2). Moreover, activation of TLR-2 was implicated directly in MMP-1 and MMP-3 production from isolated human plaque-derived cells examined *ex vivo* (Monaco *et al.*, 2009). These inflammatory mediators share the ability to activate the MAP kinases, extracellular related kinases 1/2 (ERKs1/2), p38 MAP kinase and c-jun N-terminal kinase (JNK), as well as phophoinositide-3 kinase (PI3 kinase) and the inhibitor of κB kinase2 (IKK2) that leads to activation of NF-κB (Fig. 2). Not surprisingly, therefore inhibitors of one or more these kinases generally reverse the effects of this broad class of inflammatory mediators (Supplementary Tables 1 and 2). However, the precise identity of the activating kinases seems to depend on the

MMP and the source of cells (Supplementary Tables 1 and 2). One complicating factor is the participation to greater or lesser degree of PGE2 derived from COX-2 (Fig. 2), induction of which requires all these kinases (Huang *et al.*, 2012). For example, inhibition of p38 reduced MMP-1 expression in a PGE2 dependent manner in human monocytes, whereas inhibition of ERKs1/2 decreased both MMP-1 and MMP-9 expression independently of PGE2 (Zhang & Wahl, 2015). In the absence of PGE2, upregulation of MMP-1 and -10 in human blood derived monocytes depended on ERKs1/2, JNK and IKK2 but not p38 MAP kinase (Reel *et al.*, 2011). The same was true for LPS induction of MMP-1, -3, -10, -12 and -14 in human macrophages but induction of MMP-25 required p38 (Huang *et al.*, 2012). Furthermore, specificity for the various activating kinases may depend on the specific inflammatory signal. For example, induction of MMP-1 in human alveolar macrophages by *M. tuberculosus* depends selectively on p38 MAP kinase (Rand *et al.*, 2009). Both basal and induced expression of many MMPs, especially MMPs-1, -3, -10 and -13 and TIMP-3 is reduced by inhibitors of PI3 kinase in human macrophages (Huang *et al.*, 2012). However, the basis for these effects is still not clarified. Other inflammatory mediators such as clusterin (Shim *et al.*, 2011) or complement component C5a (Speidl *et al.*, 2011), or homophylic interactions of CD147 (EMMPRIN) or with its ligand cyclophylin A (Yang *et al.*, 2008) also employ MAP kinases, PI3 kinase, IKK2 and the resultant activation of AP-1/NF-κB signalling to upregulate MMPs (Fig. 2). To confirm that these signalling pathways contribute to increased MMP expression in human atherosclerotic plaques, we demonstrated co-localisation of activated NF-κB with MMP-1 and MMP-10 (Huang *et al.*, 2012).

Interferons and the JAK/STAT pathway

Earlier studies demonstrated profound inhibitory effects of IFNγ on MMPs-1, -3, -9, and -12 and TIMP-1 production from human monocytes and macrophages (Supplementary Tables 1 and 2). On the other hand, IFNγ acting through the JAK/STAT pathway can upregulate MMP-12, -14 and -25 and suppresses TIMP-3 mRNA expression in human macrophages (Huang *et al.*, 2012). The effect on MMP-25 may be especially interesting in view of its ability to modulate the activity of several chemokines (Marco *et al.*, 2013). Given that many human plaques contain IFNγ, induction of MMP-14 and suppression of TIMP-3 could promote the invasive and destructive $MMP14^{+}TIMP\text{-}3^{-}$ macrophage phenotype that we detected in rabbit and human foam cells (Johnson *et al.*, 2008). Effects of IFNγ appear to be very different in human and mouse macrophages (Hayes *et al.*, 2014), which complicates the interpretation of the mouse models. Nevertheless, inhibition of mouse macrophage MMP-9 production by IFNγ correlated with slower ECM degradation and thrombus resolution in wild type compared to IFNγ knockout mice in a model of deep vein thrombosis (Nosaka *et al.*, 2011). Deletion of TGFβ receptors in T-lymphocytes, which promotes polarization to the Thelper1 phenotype that releases IFNγ also decreased MMP-9 expression in atherosclerotic mouse aortas (Ovchinnikova *et al.*, 2009). On the other hand, MMP-13 was increased, suggesting that IFNγ from Thelper1 cells can promote as well as inhibit expression of different MMPs. In other experiments, deletion of all T and B cells (Hayes *et al.*, 2014) or just Thelper1 cells (Tsaousi *et al.*, 2016) did not affect MMP or TIMP expression in mouse foam cells from subcutaneous granulomas or in atherosclerotic plaques. Consequently, the evidence for stimulatory effects of IFNγ on MMP expression is stronger in humans than mice.

IL-6 and GM-CSF

As illustrated in Fig. 2, IL-6 activates JAK1 and STAT-3, MAP kinases and PI3 kinases (Schaper & Rose-John, 2015), which may account for its upregulation of MMPs (Supplementary Tables 1 and 2). Interestingly, induction of MMP-9 in mouse macrophages by IL-6 is independent of COX-2 (Kothari *et al.*, 2014).

GM-CSF signals through the CSFR2 complex to activate JAK2 and STAT-5 as well as MAP kinases and PI3 kinases (Broughton *et al.*, 2012). Hence the transcriptional programme initiated by GM-CSF is unique, although it replicates some aspects of both the classical and alternative paradigms. GM-CSF is especially associated with upregulation of MMP-12 (Supplementary Table 1), which occurs through activation of the proximal AP-1 site. Why this direct action of GM-CSF is selective for MMP-12 over other MMPs with proximal AP-1 sites is unclear. GM-CSF can also induce TNFα secretion leading to the upregulation of other MMPs (Zhang *et al.*, 1998). Given this and the fact that GM-CSF can be upregulated by oxidised LDL and several inflammatory mediators (Di Gregoli & Johnson, 2012), GM-CSF-stimulated and classically-activated macrophage phenotypes are probably an overlapping *in vivo.* Despite this, in mice exposed to cigarette smoke, neutralisation of GM-CSF selectively decreases MMP-12 but not MMP-9 activity in lung macrophages (Vlahos *et al.*, 2010). GM-CSF increases MMP-14 protein expression and activity independently of changes in mRNA expression but because micro-RNA24 is decreased, which relieves an inhibitory effect on protein translation (Di Gregoli *et al.*, 2014). These observations are particularly interesting because there is evidence for distinct populations of M-CSF and GM-CSF macrophages in human plaques that may make different contributions to plaque stability (Di Gregoli & Johnson, 2012). Indeed, GM-CSF action might also account for the harmful $MMP14^{+}TIMP\text{-}3^{-}$ macrophage phenotype (Johnson *et al.*, 2008)

Hypoxia

Most macrophages in atherosclerotic plaques are in a chronic state of hypoxia (Sluimer *et al.*, 2008). Hypoxia increases expression of MMP-7 (Supplementary Table 2). Transcriptomic data from hypoxic macrophages indicates that, MMPs-1, -3, -10 and -12 are also significantly upregulated, perhaps secondarily to increased production of IL-1α,β (Fang *et al.*, 2009). Pathways through hypoxia inducible factor-1α (HIF-1α) (Lee *et al.*, 2012), HIF-2α (Yang *et al.*, 2010) and JAK2/STAT-3 have been implicated (Gao *et al.*, 2015).

Anti-inflammatory pathways

Priming with IL-4 inhibitors expression of MMP-1, MMP-9 and TIMP-1 in monocytes and macrophages (Supplementary Tables 1, 2), perhaps owing to overexpression of suppressor of cytokine signalling (SOCS) proteins. However, consistent with previous work in mouse macrophages (Supplementary Table 2), we found that IL-4 selectively increases MMP-12 in human monocyte derived macrophages (Huang *et al.*, 2012). MMP-25 and TIMP-3 were also up-regulated (Huang *et al.*, 2012) but the mechanisms responsible remain unclear. IL-10 also antagonises the upregulation of MMP-1 and MMP-9, but unlike IL-4 it increases expression of TIMP-1 (Supplementary Tables 1 and 2). Again the intermediary action of SOCS proteins appears reasonable but remains to be fully documented. IL-10, in particular, is abundant in atherosclerotic plaques and therefore most likely exerts a physiological dampening effect on MMP activity. TGFβ inhibits MMP-12 production in human monocytes (Supplementary Table 1). However, TGFβ can both stimulate and inhibit MMP-2 and MMP-9 secretion from mouse peritoneal macrophages (Ogawa *et al.*, 2011). Upregulation of MMP-9 by TGFβ has been recently ascribed to stimulation of PI3K leading to activation of AP-1 transcription factors (Haidar *et al.*, 2015). Activation of several anti-inflammatory nuclear hormone receptors inhibits MMP production (see Supplementary Tables 1 and 2). For example, PPARα selectively inhibits IL-1β induced MMP-12 production by direct binding to components of the AP-1 complex (Souissi *et al.*, 2008), whereas both PPARα and PPARγ inhibit MMP-9 secretion from human macrophages (Supplementary Table 2). PPARγ agonists protect against the macrovascular complications of diabetes (Dormandy *et al.*, 2005) and inhibition of MMP activity could play an important part in this action. Statins, the mainstay of atherosclerosis prevention, have also been

shown to inhibit the expression of a broad range of MMPs by both transcriptional and post-transcriptional mechanisms (reviewed in (Newby, 2008)).

Conclusions: the combined action of multiple mediators causes MMP up-regulation and plaque rupture

Animal and human data supports the concept that an excess of MMP over TIMP production from macrophages and foam cells contributes to atherosclerotic plaque growth and rupture. In rabbit and mouse models, several MMPs promote plaque progression and affect plaque morphology in ways consistent with greater vulnerability to rupture. Furthermore, foam cell macrophages in subcutaneous granulomas or atherosclerotic plaques actively express several MMPs that are also secreted by non-foamy macrophages. Adaptive immunity seems to have little impact on macrophage polarization and increasing levels of MMPs in mice, implying a more prominent role for innate immune mechanisms, including the production of CSFs, inflammatory cytokines and toll-like receptor ligands. Even so MMP activity must be tightly regulated because overexpression of high levels of fully activated MMPs is needed to provoke plaque disruption in mice. The importance of specific MMPs may be over or under emphasized in mice, where they are more or less abundant, compared to man (see Fig. 1). Hence studies in human cells and tissues should be given primary importance, especially if genetic approaches at a population level (such as that for MMP-12) can be developed to give clearer indications of causality.

Longitudinal imaging studies lead to the striking conclusion that most vulnerable plaques go on to heal rather than rupture (Van Mieghem *et al.*, 2006). Hence ulceration of human plaques is a relatively rare outcome that, just like any other accident, most probably occurs because of an unusual combination of adverse circumstances. Plaque rupture most likely results from a 'perfect storm' caused by the synergistic local effects of multiple inflammatory mediators acting together in a hypoxic environment combined with the loss of inhibitory signals from nuclear hormone receptors, TGFβ and IL-10. The potential mediators of MMP overproduction include IL-1β, which can be produced in plaques in response to oxidised LDL (Williams & Tabas, 1995) and as a result of inflammasome activation by cholesterol crystals (Duewell *et al.*, 2010; Rajamaki *et al.*, 2010). The ongoing CANTOS clinical trial will examine the causal role of IL-1 in unstable coronary disease (Dinarello *et al.*, 2012). Other pro-inflammatory mediators, including TNFα, GM-CSF and IL-6, which stimulate macrophages through different signalling pathways (Fig. 2) have the potential to induce MMPs synergistically. Suppression of TIMP-3 expression by foam cell formation, INFγ or GM-CSF could be a further significant factor. Toll-like receptor ligands, the most effective stimulators of MMP production *in vitro*, are also present in the atherosclerotic plaques (Lundberg & Yan, 2011). Conversely, anti-inflammatory treatments including, importantly, the use of statins currently provide the best approach to reducing MMP activity in plaques and therefore preventing plaque rupture. In future it is likely that more selective treatments will be developed. These should be aimed at inhibiting excess production of specific MMPs, especially the collagenases MMP-1 (Libby, 2013) and MMP-8 (Ye, 2015) and MMP-12 (Traylor *et al.*, 2014), whilst preserving the activity of those MMPs, including MMP-9, that are primarily involved in vascular repair (Newby, 2005). The widely different regulation of different MMPs in human macrophages that recent studies have so clearly emphasized (Huang *et al.*, 2012) provide strong encouragement for such an approach.

References

Bar-Or A, Nuttall RK, Duddy M, Alter A, Kim HJ, Ifergan I, Pennington CJ, Bourgoin P, Edwards DR & Yong VW (2003). Analyses of all matrix metalloproteinase members in leukocytes emphasize monocytes as major inflammatory mediators in multiple sclerosis. *Brain* **126,** 2738-2749.

Broughton SE, Dhagat U, Hercus TR, Nero TL, Grimbaldeston MA, Bonder CS, Lopez AF & Parker MW (2012). The GM-CSF/IL-3/IL-5 cytokine receptor family: from ligand recognition to initiation of signaling. *Immunological Reviews* **250,** 277-302.

Clark IM, Swingler TE, Sampieri CL & Edwards DR (2008). The regulation of matrix metalloproteinases and their inhibitors. *Int J Biochem Cell Biol* **40,** 1362-1378.

Combadiere C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A & Mallat Z (2008). Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation* **117,** 1649-1657.

Di Gregoli K, Jenkins N, Salter R, White S, Newby AC & Johnson JL (2014). MicroRNA-24 Regulates Macrophage Behavior and Retards Atherosclerosis. *Arterioscler Thromb Vasc Biol* **34,** 1990-2000.

Di Gregoli K & Johnson JL (2012). Role of colony-stimulating factors in atherosclerosis. *Current Opinion Lipidol* **23,** 412-421.

Dinarello CA, Simon A & van der Meer JWM (2012). Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov* **11,** 633-652.

Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefebvre PJ & Murray GD (2005). Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* **366,** 1279-1289.

Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V & Latz E (2010). NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* **464,** 1357-1361.

Fang HY, Hughes R, Murdoch C, Coffelt SB, Biswas SK, Harris AL, Johnson RS, Imityaz HZ, Simon MC, Fredlund E, Greten FR, Rius J & Lewis CE (2009). Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. *Blood* **114,** 844-859.

Gao W, McCormick J, Connolly M, Balogh E, Veale DJ & Fearon U (2015). Hypoxia and STAT3 signalling interactions regulate pro-inflammatory pathways in rheumatoid arthritis. *Annals of the Rheumatic Diseases* **74,** 1275-1283.

Gerits N, Kostenko S, Shiryaev A, Johannessen M & Moens U (2008). Relations between the mitogen-activated protein kinase and the cAMP-dependent protein kinase pathways: Comradeship and hostility. *Cellular Signalling* **20,** 1592-1607.

Glass CK & Natoli G (2015). Molecular control of activation and priming in macrophages. *Nat Immunol* **17,** 26-33.

Gough PJ, Gomez IG, Wille PT & Raines EW (2006). Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice. *J Clin Invest* **116,** 59-69.

Haidar M, Whitworth J, Noe G, Liu WQ, Vidal M & Langsley G (2015). TGF-beta 2 induces Grb2 to recruit PI3-K to TGF-RII that activates JNK/AP-1-signaling and augments invasiveness of Theileria-transformed macrophages. *Sci Rep* **5**.

Hald A, Rono B, Lund LR & Egerod KL (2012). LPS counter regulates RNA expression of extracellular proteases and their inhibitors in murine macrophages. *Mediators Inflamm* **2012,** 157894.

Hayes EM, Tsaousi A, Di Gregoli K, Jenkinson SR, Bond AR, Johnson JL, Bevan L, Thomas AC & Newby AC (2014). Classical and alternative activation and metalloproteinase expression occurs in foam cell macrophages in male and female ApoE null mice in the absence of T- and B-lymphocytes. *Frontiers in Immunology* **5**.

Huang WC, Sala-Newby GB, Susana A, Johnson JL & Newby AC (2012). Classical Macrophage Activation Up-Regulates Several Matrix Metalloproteinases through Mitogen Activated Protein Kinases and Nuclear Factor-kappaB. *PLoS One* **7,** e42507.

Johnson JL, Devel L, Czarny B, George SJ, Jackson CL, Rogakos V, Beau F, Yiotakis A, Newby AC & Dive V (2011). A Selective Matrix Metalloproteinase-12 Inhibitor Retards Atherosclerotic Plaque Development in Apolipoprotein E-Knockout Mice. *Arterioscler Thromb Vasc Biol* **31,** 528-535.

Johnson JL, George SJ, Newby AC & Jackson CL (2005). Divergent effects of matrix metalloproteinases 3, 7, 9, and 12 on atherosclerotic plaque stability in mouse brachiocephalic arteries. *Proc Natl Acad Sci USA* **102,** 15575-15580.

Johnson JL, Jenkins NP, Huang WC, Di Gregoli K, Sala-Newby GB, Scholtes VP, Moll FL, Pasterkamp G & Newby AC (2014). Relationship of MMP-14 and TIMP-3 expression with macrophage activation and human atherosclerotic plaque vulnerability. *Mediators Inflamm* **2014,** 276457.

Johnson JL, Sala-Newby GB, Ismail Y, Aguilera CM & Newby AC (2008). Low tissue inhibitor of metalloproteinases 3 and high matrix metalloproteinase 14 levels defines a subpopulation of highly invasive foam-cell macrophages. *Arterioscler Thromb Vasc Biol* **28,** 1647-1653.

Khan KMF, Kothari P, Du BH, Dannenberg AJ & Falcone DJ (2012). Matrix Metalloproteinase-Dependent Microsomal Prostaglandin E Synthase-1 Expression in Macrophages: Role of TNF-alpha and the EP4 Prostanoid Receptor. *Journal of Immunology* **188,** 1970-1980.

Khokha R, Murthy A & Weiss A (2013). Metalloproteinases and their natural inhibitors in inflammation and immunity. *Nature Reviews Immunology* **13,** 649-665.

Kothari P, Pestana R, Mesraoua R, Elchaki R, Khan KMF, Dannenberg AJ & Falcone DJ (2014). IL-6-Mediated Induction of Matrix Metalloproteinase-9 Is Modulated by JAK-Dependent IL-10 Expression in Macrophages. *Journal of Immunology* **192,** 349-357.

Lai WC, Zhou M, Shankavaram U, Peng G & Wahl LM (2003). Differential regulation of lipopolysaccharide-induced monocyte matrix metalloproteinase (MMP)-1 and MMP-9 by p38 and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases. *Journal of Immunology* **170,** 6244-6249.

Lee YA, Choi HM, Lee SH, Hong SJ, Yang HI, Yoo MC & Kim KS (2012). Hypoxia differentially affects IL-1beta-stimulated MMP-1 and MMP-13 expression of fibroblast-like synoviocytes in an HIF-1alpha-dependent manner. *Rheumatology (Oxford)* **51,** 443-450.

Liang J, Liu E, Yu Y, Kitajima S, Koike T, Jin Y, Morimoto M, Hatakeyama K, Asada Y, Watanabe T, Sasaguri Y, Watanabe S & Fan J (2006). Macrophage metalloelastase accelerates the progression of atherosclerosis in transgenic rabbits. *Circulation* **113,** 1993-2001.

Libby P (2013). Mechanisms of acute coronary syndromes and their implications for therapy. *N Engl J Med* **368,** 2004-2013.

Lundberg AM & Yan Z-q (2011). Innate immune recognition receptors and damage-associated molecular patterns in plaque inflammation. *Current Opinion in Lipidology* **22,** 343-349 310.1097/MOL.1090b1013e32834ada32880.

Ma Y, Halade GV, Zhang J, Ramirez TA, Levin D, Voorhees A, Jin YF, Han HC, Manicone AM & Lindsey ML (2013). Matrix metalloproteinase-28 deletion exacerbates cardiac dysfunction and rupture after myocardial infarction in mice by inhibiting M2 macrophage activation. *Circ Res* **112,** 675-688.

Marco M, Fortin C & Fulop T (2013). Membrane-type matrix metalloproteinases: key mediators of leukocyte function. *Journal of Leukocyte Biology* **94,** 237-246.

Molloy KJ, Thompson MM, Jones JL, Schwalbe EC, Bell PRF, Naylor AR & Loftus IM (2004). Unstable Carotid Plaques Exhibit Raised Matrix Metalloproteinase-8 Activity. *Circulation* **110,** 337-343.

Monaco C, Gregan SM, Navin TJ, Foxwell BM, Davies AH & Feldmann M (2009). Toll-like receptor-2 mediates inflammation and matrix degradation in human atherosclerosis. *Circulation* **120,** 2462-2469.

Murray MY, Birkland TP, Howe JD, Rowan AD, Fidock M, Parks WC & Gavrilovic J (2013). Macrophage Migration and Invasion Is Regulated by MMP10 Expression. *PLoS One* **8,** 12.

Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN & Wynn TA (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* **41,** 14-20.

Nagase H, Visse R & Murphy G (2006). Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* **69,** 562-573.

Newby AC (2005). Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. *Physiol Rev* **85,** 1-31.

Newby AC (2008). Metalloproteinase expression in monocytes and macrophages and its relationship to atherosclerotic plaque instability. *Arterioscler Thromb Vasc Biol* **28,** 2108-2114.

Newby AC (2012). Matrix metalloproteinase inhibition therapy for vascular diseases. *Vasc Pharmacol* **56,** 232-244.

Newby AC (2015). Metalloproteinases promote plaque rupture and myocardial infarction: A persuasive concept waiting for clinical translation. *Matrix Biol* **44-46,** 157-166.

Nguyen AT, Gomez D, Bell RD, Campbell JH, Clowes AW, Gabbiani G, Giachelli CM, Parmacek MS, Raines EW, Rusch NJ, Speer MY, Sturek M, Thyberg J, Towler DA, Weiser-Evans MC, Yan C, Miano JM & Owens GK (2012). Smooth Muscle Cell Plasticity: Fact or Fiction? *Circ Res* **112,** 17-22.

Nosaka M, Ishida Y, Kimura A, Kuninaka Y, Inui M, Mukaida N & Kondo T (2011). Absence of IFN-gamma accelerates thrombus resolution through enhanced MMP-9 and VEGF expression in mice. *Journal of Clinical Investigation* **121,** 2911-2920.

Ogawa K, Funaba M & Tsujimoto M (2011). The effects of TGF-beta 1 on the expression of type IV collagenases in mouse peritoneal macrophages. *Mol Biol Rep* **38,** 1451-1456.

Ovchinnikova O, Robertson AK, Wagsater D, Folco EJ, Hyry M, Myllyharju J, Eriksson P, Libby P & Hansson GK (2009). T-cell activation leads to reduced collagen maturation in atherosclerotic plaques of Apoe(-/-) mice. *Am J Pathol* **174,** 693-700.

Paulson KE, Zhu SN, Chen M, Nurmohamed S, Jongstra-Bilen J & Cybulsky MI (2010). Resident intimal dendritic cells accumulate lipid and contribute to the initiation of atherosclerosis. *Circ Res* **106,** 383-390.

Peeters W, Moll FL, Vink A, van der Spek PJ, de Kleijn DP, de Vries JP, Verheijen JH, Newby AC & Pasterkamp G (2011). Collagenase matrix metalloproteinase-8 expressed in atherosclerotic carotid plaques is associated with systemic cardiovascular outcome. *Eur Heart J* **32,** 2314-2325.

Quillard T, Tesmenitsky Y, Croce K, Travers R, Shvartz E, Koskinas KC, Sukhova GK, Aikawa E, Aikawa M & Libby P (2011). Selective inhibition of matrix metalloproteinase-13 increases collagen content of established mouse atherosclerosis. *Arterioscler Thromb Vasc Biol* **31,** 2464-2472.

Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT & Eklund KK (2010). Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One* **5,** e11765.

Rand L, Green JA, Saraiva L, Friedland JS & Elkington PTG (2009). Matrix Metalloproteinase-1 Is Regulated in Tuberculosis by a p38 MAPK-Dependent, p-Aminosalicylic Acid-Sensitive Signaling Cascade. *Journal of Immunology* **182,** 5865-5872.

Reel B, Sala-Newby GB, Huang W-C & Newby AC (2011). Diverse patterns of cyclooxygenase-independent metalloproteinase gene regulation in human monocytes. *Br J Pharmacol* **163,** 1679-1690.

Reilly MP, Li M, He J, Ferguson JF, Stylianou IM, Mehta NN, Burnett MS, Devaney JM, Knouff CW, Thompson JR, Horne BD, Stewart AF, Assimes TL, Wild PS, Allayee H, Nitschke PL, Patel RS, Myocardial Infarction Genetics C, Wellcome Trust Case Control C, Martinelli N, Girelli D, Quyyumi AA, Anderson JL, Erdmann J, Hall AS, Schunkert H, Quertermous T, Blankenberg S, Hazen SL, Roberts R, Kathiresan S, Samani NJ, Epstein SE & Rader DJ (2011). Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. *Lancet* **377,** 383-392.

Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, Gorbatov R, Sukhova GK, Gerhardt LM, Smyth D, Zavitz CC, Shikatani EA, Parsons M, van Rooijen N, Lin HY, Husain M, Libby P, Nahrendorf M, Weissleder R & Swirski FK (2013). Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat Med* **19,** 1166-1172.

Schaper F & Rose-John S (2015). Interleukin-6: Biology, signaling and strategies of blockade. *Cytokine Growth Factor Rev* **26,** 475-487.

Scholtes VPW, Johnson JL, Jenkins N, Sala-Newby GB, de Vries J-PPM, Borst GJd, de Kleijn DPV, Moll FL, Pasterkamp G & Newby AC (2012). Carotid Atherosclerotic Plaque Matrix Metalloproteinase-12–Positive Macrophage Subpopulation Predicts Adverse Outcome After Endarterectomy. *Journal of the American Heart Association* **1,** e001040.

Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, Swiatlowska P, Newman AA, Greene ES, Straub AC, Isakson B, Randolph GJ & Owens GK (2015). KLF4-

dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. *Nat Med* **21,** 628-637.

Shim YJ, Kang BH, Jeon HS, Park IS, Lee KU, Lee IK, Park GH, Lee KM, Schedin P & Min BH (2011). Clusterin induces matrix metalloproteinase-9 expression via ERK1/2 and PI3K/Akt/NF-kappa B pathways in monocytes/macrophages. *Journal of Leukocyte Biology* **90,** 761-769.

Sluijter JP, Pulskens WP, Schoneveld AH, Velema E, Strijder CF, Moll F, de Vries JP, Verheijen J, Hanemaaijer R, de Kleijn DP & Pasterkamp G (2006). Matrix metalloproteinase 2 is associated with stable and matrix metalloproteinases 8 and 9 with vulnerable carotid atherosclerotic lesions: a study in human endarterectomy specimen pointing to a role for different extracellular matrix metalloproteinase inducer glycosylation forms. *Stroke* **37,** 235-239.

Sluimer JC, Gasc JM, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, Cleutjens JP, van den Akker LH, Corvol P, Wouters BG, Daemen MJ & Bijnens AP (2008). Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. *J Am Coll Cardiol* **51,** 1258-1265.

Souissi IJ, Billiet L, Cuaz-Perolin C, Slimane MN & Rouis M (2008). Matrix metalloproteinase-12 gene regulation by a PPAR alpha agonist in human monocyte-derived macrophages. *Experimental Cell Research* **314,** 3405-3414.

Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, Reichart D, Fox JN, Shaked I, Heudobler D, Raetz CR, Wang EW, Kelly SL, Sullards MC, Murphy RC, Merrill AH, Jr., Brown HA, Dennis EA, Li AC, Ley K, Tsimikas S, Fahy E, Subramaniam S, Quehenberger O, Russell DW & Glass CK (2012). Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. *Cell* **151,** 138-152.

Speidl WS, Kastl SP, Hutter R, Katsaros KM, Kaun C, Bauriedel G, Maurer G, Huber K, Badimon JJ & Wojta J (2011). The complement component C5a is present in human coronary lesions in vivo and induces the expression of MMP-1 and MMP-9 in human macrophages in vitro. *Faseb Journal* **25,** 35-44.

Steenport M, Khan KMF, Du BH, Barnhard SE, Dannenberg AJ & Falcone DJ (2009). Matrix Metalloproteinase (MMP)-1 and MMP-3 Induce Macrophage MMP-9: Evidence for the Role of TNF-alpha and Cyclooxygenase-2. *Journal of Immunology* **183,** 8119-8127.

Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, Garin A, Liu J, Mack M, van Rooijen N, Lira SA, Habenicht AJ & Randolph GJ (2007). Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest* **117,** 185-194.

Thomas AC, Eijgelaar WJ, Daemen MJ & Newby AC (2015). Foam Cell Formation In Vivo Converts Macrophages to a Pro-Fibrotic Phenotype. *PLoS One* **10,** e0128163.

Traylor M, Makela KM, Kilarski LL, Holliday EG, Devan WJ, Nalls MA, Wiggins KL, Zhao W, Cheng YC, Achterberg S, Malik R, Sudlow C, Bevan S, Raitoharju E, Metastroke ISGCWTCC, Oksala N, Thijs V, Lemmens R, Lindgren A, Slowik A, Maguire JM, Walters M, Algra A, Sharma P, Attia JR, Boncoraglio GB, Rothwell PM, de Bakker PI, Bis JC, Saleheen D, Kittner SJ, Mitchell BD, Rosand J, Meschia JF, Levi C, Dichgans M, Lehtimaki T, Lewis CM & Markus HS (2014). A novel MMP12 locus is associated with large artery atherosclerotic stroke using a genome-wide age-at-onset informed approach. *PLoS Genet* **10,** e1004469.

Tsaousi A, Hayes EM, Di Gregoli K, Bond AR, Bevan L, Thomas AC & Newby AC (2016). Plaque Size Is Decreased but M1 Macrophage Polarization and Rupture Related Metalloproteinase Expression Are Maintained after Deleting T-Bet in ApoE Null Mice. *PLoS One* **11,** e0148873.

Van Mieghem CA, McFadden EP, de Feyter PJ, Bruining N, Schaar JA, Mollet NR, Cademartiri F, Goedhart D, de Winter S, Granillo GR, Valgimigli M, Mastik F, van der Steen AF, van der Giessen WJ, Sianos G, Backx B, Morel MA, van Es GA, Zalewski A & Serruys PW (2006). Noninvasive detection of subclinical coronary atherosclerosis coupled with assessment of changes in plaque characteristics using novel invasive imaging modalities: the Integrated Biomarker and Imaging Study (IBIS). *J Am Coll Cardiol* **47,** 1134-1142.

Virmani R, Burke AP, Farb A & Kolodgie FD (2006). Pathology of the vulnerable plaque. *J Am Coll Cardiol* **47,** C13-C18.

Vlahos R, Bozinovski S, Chan SPJ, Ivanov S, Linden A, Hamilton JA & Anderson GP (2010). Neutralizing Granulocyte/Macrophage Colony-Stimulating Factor Inhibits Cigarette Smoke-induced Lung Inflammation. *Am J Respir Crit Care Med* **182,** 34-40.

Williams KJ & Tabas I (1995). The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol* **15,** 551-561.

Yang S, Kim J, Ryu JH, Oh H, Chun CH, Kim BJ, Min BH & Chun JS (2010). Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med* **16,** 687-693.

Yang Y, Lu N, Zhou J, Chen ZN & Zhu P (2008). Cyclophilin A up-regulates MMP-9 expression and adhesion of monocytes/macrophages via CD147 signalling pathway in rheumatoid arthritis. *Rheumatology (Oxford)* **47,** 1299-1310.

Ye S (2015). Putative targeting of matrix metalloproteinase-8 in atherosclerosis. *Pharmacology & Therapeutics* **147,** 111-122.

Zhang Y, McCluskey K, Fujii K & Wahl LM (1998). Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-alpha, granulocyte-macrophage CSF, and IL-1 beta through prostaglandin-dependent and -independent mechanisms. *J Immunol* **161,** 3071-3076.

Zhang YH & Wahl LM (2015). Cytokine-induced monocyte MMP-1 is negatively regulated by GSK-3 through a p38 MAPK-mediated decrease in ERK1/2 MAPK activation. *Journal of Leukocyte Biology* **97,** 921-927.

Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM & Lutz MB (2010). Nomenclature of monocytes and dendritic cells in blood. *Blood* **116,** e74-80.

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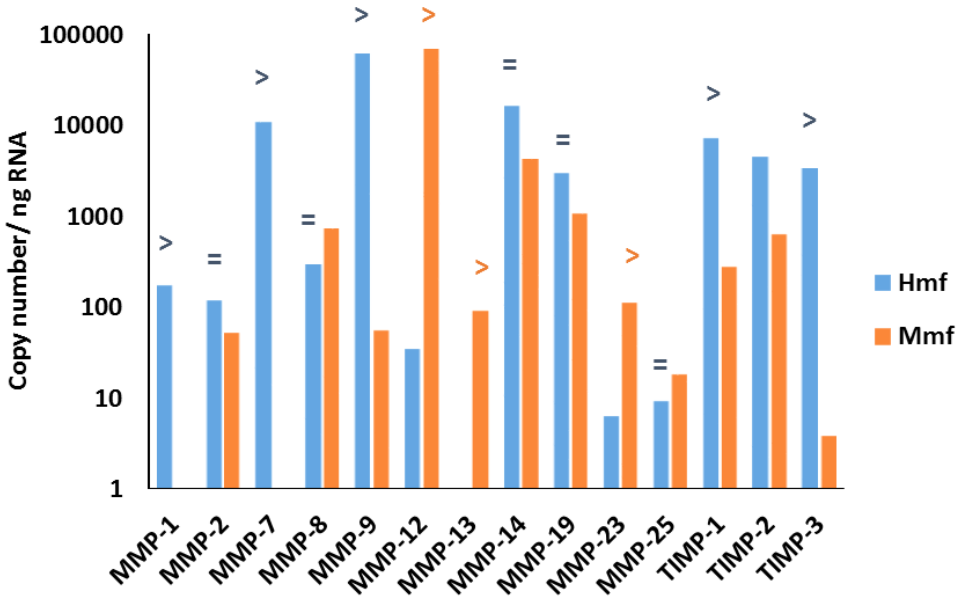
Legends to Figures

Figure 1 Comparison of MMP and TIMP expression in human and mouse macrophages

Human (Huang *et al.*, 2012) and mouse (Tsaousi *et al.*, 2016) blood monocytes were differentiated to macrophages for 10-14 days in the presence of M-CSF. Total RNA was extracted and mRNA levels were measured by quantitative reverse transcription polymerase chain reaction using standards to derive copy numbers of transcripts per ng RNA. Differences greater than 100 fold are noted with arrows.

Figure 2 Simplified pathways of MMP and TIMP induction.

Binding of ligands to integrins (INT), toll-like receptors (TLR) and receptors for interferons (IFN), IL-1 (IL1R), TNF (TNFR), PGE2 (EP4), IL-6 (GP130), GM-CSF (CSFR2) provide the initial signals. These interact with signal transduction pathways (shown in outline only). An integrative network activates the phophoinositide-3 kinase (PI3K), extracellular related kinases 1/2 (ERKs), p38 MAP kinase and c-jun N-terminal kinase (JNK), as well as the inhibitor of κB kinase2 (IKK2). These lead together to activation of the activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) transcription factors that directly induce several MMPs and TIMP-1. Insulin response factors (IRFs) are also induced by this pathway and also through activation of janus kinase 2 (JAK2), which can cause release of IFNα,β, leading to autocrine actions. Other autocrine pathways are triggered by the production of arachidonic acid (AA) from the action of phospholipaseA2 (PLA2). This is converted to prostaglandin E2 (PGE2) by the consecutive action of cyclooxygenase-2 (COX-2) and prostaglandin E synthetase-1 (PGES1). Autocrine action on EP4 receptors triggers cAMP production and activation of the cAMP response element binding protein (CREB) transcription factor, which induces MMPs further. Activation of janus kinases JAKs at the GP130 and CSFR2 receptors leads to nuclear translocation of signal transducer and activator of transcription-3 (STAT-3) and STAT-5, respectively. Production of TNFα through these pathways provides addition possibilities for autocrine feedback.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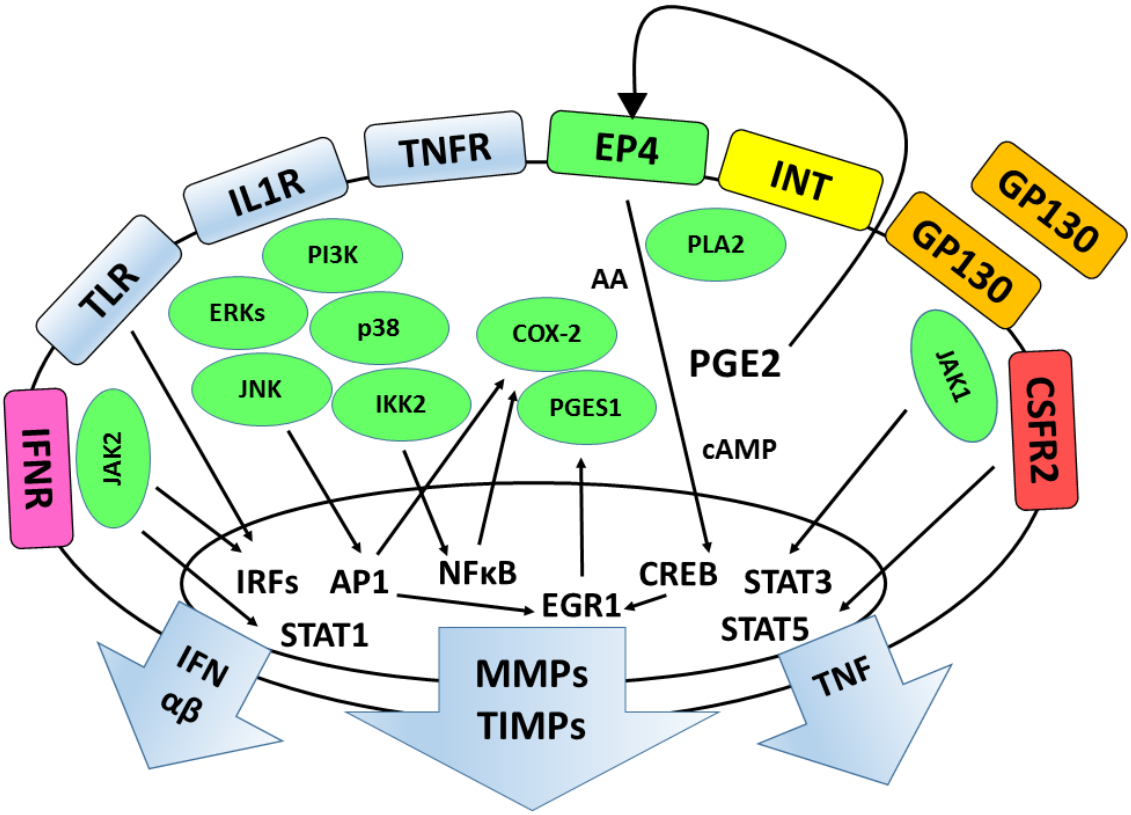
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