The dual personalities of matrix metalloproteinases in inflammation

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1. ABSTRACT

Collagen, gelatin, elastin, fibronectin, proteoglycans and vitronectin are just a few proteins which form the "mesh" that holds a multicellular organism together. The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade the extracellular matrix. Over several decades it has been clearly established that MMPs are the key molecules associated with matrix remodeling. The remodeling of this matrix is important for physiological and pathological processes such as pregnancy, wound repair, cancer and arthritis. The identification of new non-matrix MMP substrates involved in inflammation, highlights the diverse role of MMPs. These enzymes can enhance leukocyte invasion and regulate the inflammatory activity of serine proteases, cytokines and chemokines. Interestingly, the MMP family appears to have a "dual personality" in that several MMPs such as MMP-2 and -9 can favour either anti- or pro- inflammatory action, respectively. The extent of this dual functionality of MMPs is yet to be realized. Elucidating these processes may assist in the development of drugs for the treatment of inflammatory diseases such as arthritis, cancer and chronic wounds.

2. INTRODUCTION

The breakdown of the extracellular matrix (ECM) is an essential component of multiple biological processes such as wound healing, immune cell infiltration, pregnancy, skeletal development, angiogenesis and embryogenesis. Matrix metalloproteinases (MMPs) are a family of enzymes that play an important role in tissue modeling through their ability to hydrolyze the protein components of the ECM. Enzymes within this family can be classified into groups based on their substrate specificity (ie collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs) or their MMP number assigned in temporal order of discovery. For example, gelatinase A and B are also known as MMP-2 and -9, respectively. At present the MMP family in vertebrates consists of twenty-five secreted or membrane bound proteins which share a number of structural and functional similarities (Table 1 and Figure 1). It should be noted that the current MMP classification extends to MMP-28 for only 25 MMPs. This numerical discrepancy is due to the fact that MMP-4, -5 and -6 represent duplicate MMPs and thus have been removed from the classification.
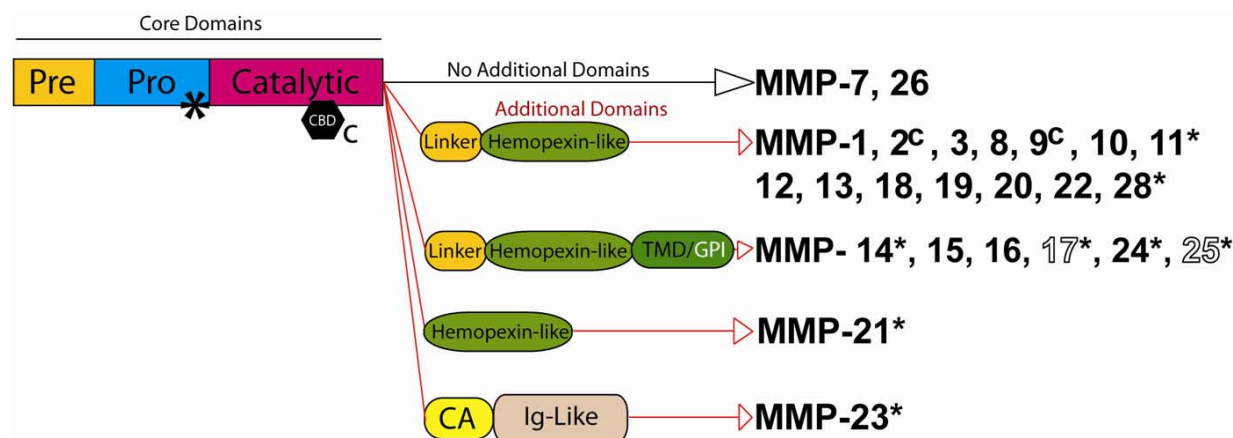
Figure 1. Domain structures of all matrix metalloproteinases (MMPs). All MMPs contain a predomain (Pre), prodomain (Pro) and catalytic domain (CAT). These core domains are the only motifs present for MMP-7 and 26. MMPs with a furin recognition site are indicated on the diagram with an asterisk (*****) and its relative location on the core domains. All other MMPs with the exception of MMP-7, -21 and -26 have a linker/hemopexin-like motif adjacent to the catalytic domain. MMP-21 possesses a hemopexin motif without a linker sequence. In addition to the core domain, the gelatinase family of MMPs (MMP-2 and -9) have a collagen binding domain (CBD; represented as a ^{C}) or fibronectin type II-like domains repeats located within its catalitic motif which is followed by the linker/PEX domains. The membrane-bound MMPs, MMP-14, -15, -16, -17, -24 and -25, are attached to the plasma membrane with either a type I transmembrane domain (TMD; indicated as black text) or a glycosylphosphatidylinisotol (GPI; indicated as outlined text) anchor (MMP-17 and -25) at their C-terminus. MMP-23 is a membrane-bound MMP with no TMD or GPI domains but instead encodes a MMP type II transmembrane protein with a cysteine array and immunoglobulin-like motif (Ig-Like). *See text for details.*

Traditionally it was thought that the peptidase activities of MMPs defined their function as ECM remodeling proteins, but new evidence suggests that these molecules may be central in the control of the inflammation. Loss of control of MMP activity is associated with numerous inflammatory diseases such as cancer, asthma, atherosclerosis and arthritis. This review discusses recent knowledge on the structure and regulation of MMPs and their role in inflammation.

2.1. Structure of MMPs
2.1.1. The three M's: motifs of MMPs
All MMPs share three common domains; the predomain (PRE) for protein secretion, prodomain (PRO) responsible for regulating the molecule's function and the zinc-dependent catalytic endopeptidase (CAT) motif, which is necessary for substrate processing. MMPs are expressed as inactive proenzymes that can be activated by the proteolytic removal of the propeptide to reveal its active site. The cleavage of the propeptide and activation of all MMPs with the exception MMP-23 (1) is thought to be mediated by a cysteine residual or switch found in the molecule's prodomains (2). Extracellular proteinases such as plasmin or other MMPs within the tissue microenvironment may cleave and activate MMPs at this cysteine amino acid in a process termed "cysteine switching". Alternatively, MMPs with a furin recognition site between the pro and catalytic domains (MMP-11, -21, -23, -28 and the membrane bound MMPs, MMP-14, -17, -24 and -25) may be activated by proprotein convertases (PCs) or furin/paired basic amino-acid-cleaving enzymes (PACEs) (3, 4). The predomain, prodomain and catalytic domains form the core motifs of MMPs and in the case of

MMP-7 and -26 are the only motifs present (Figure 1). The addition of other subunits following the catalytic domain confers further functionality.

2.1.2. Hemopexin-like (PEX) domain
All MMPs except MMP-7, -23 and -26 contain a PEX domain which is bound to the catalytic domain via a linker or hinge motif (Figure 1). In the case of MMP-21 the PEX domain links directly to the catalytic domain (5, 6) (Figure 1). Depending on the MMP, the function of the PEX domain varies. For MMP-1, -3, -8, -13 the PEX motif mediates the binding of collagen for subsequent peptide cleavage (7) and for MMP-9 it assists the binding of gelatin (8). Using gene deletion, Itoh *et al* (9) demonstrated that the PEX domain is required for the homodimerization of MMP-14 and the activation of MMP-2. Another interesting function of the PEX motif involves the binding of MMPs to cell surface integrins and suggests the involvement of these molecules in cellular migration (10-12). In particular, MMP-1 and MMP-2 bind the alpha$_2$beta$_1$ and alpha$_v$beta$_3$ integrins, respectively, through their linker and PEX domains (10-12).

2.1.3. Other domains
The membrane bound MMPs including MMP-14, -16, -17, -24, and -25 have either a type I transmembrane domain (TMD) or glycosylphosphatidylinisotol (GPI) anchor immediately after the PEX motif, responsible for the attachment of these molecules to the plasma membrane (Fig 1). MMP-23 is the only MMP with a type II transmembrane MMP containing a cysteine array and an immunoglobulin-like motif in place of the TMD or GPI anchor (1, 13) (Figure 1). The gelatinase subfamily of

MMPs (MMP-2 and -9) have a collagen-binding domain consisting of fibronectin type II-like repeats within the catalytic motif, which facilitates binding to collagen and gelatin for subsequent peptidase degradation (14, 15). Xu *et al* (16), showed that the collagen-binding domain of MMP-2 is required for full-length collagen alpha-chain degradation.

2.2. Regulation of MMPs

MMPs are tightly controlled at multiple levels including gene activation and transcription, translation and secretion of latent enzyme, proenzyme activation and inactivation by endogeneous inhibitors. There are several transcription factors which can initiate the expression of various MMPs such as activator protein-1 (AP-1), nuclear factor kappaB (NF-kappaB) and serum amyloid A-activating factor (SAF-1) following exposure to pro-inflammatory cytokines. The cytokine tumor necrosis factor-alpha (TNF-alpha) can initiate the transcription of MMP-1, -3 (17) and -9 (18) through the transcription factor NF-kappaB. Other pro-inflammatory cytokines associated with NF-kappaB-mediated MMP-1 and MMP-9 induction are interleukin-1 (IL-1) and transforming growth factor-beta (TGF-beta) (18, 19). The transcription factor AP-1 may be an important molecule for the effective transactivation of MMPs. In fact, both NF-kappaB and AP-1 are coordinately involved in increasing MMP-9 (20) and MMP-1 (19). AP-1 may act as a cis-acting element responsible for the recruitment of transcription factors (21) such as NF-kappaB to encourage transactivation.

Active MMPs can also be inhibited at the protein level by circulating alpha2-macroglobulin (22) or more specifically, the tissue inhibitors of MMPs (TIMPs). There are currently four proteins which form the TIMP family of molecules designated TIMP-1, -2, -3 and -4 (23-26) which tightly bind to the catalytic subunit of MMPs to inhibit their ECM remodeling function. Despite sharing a 40-50% homology with the other TIMPs, TIMP-1 is the only exception and shows weak binding and inhibition of MMP-14, -15 and -19 (27-29). Mutational studies show that threonine 98 may be an important residual in defining TIMP-1 interactions with membrane type (MT)1-MMP (30) as substitution of threonine 98 with leucine transforms TIMP-1 into a MT1-MMP binding protein (30).

2.3. MMPS as regulators and effectors of inflammation

Inflammation may result from the healing response to damage of living tissues by a number of triggers including physical trauma, pathological infection or auto-immune defects. The inflammatory response involves a cascade of molecular signals including the secretion of molecules such as cytokines, chemokines, histamine, eicosanoids and proteases by inflammatory cells such as mast cells, neutrophils and macrophages (31, 32) which are the sentinels responsible for detecting tissue damage or infection. This triggers a series of events that encourages further invasion of leukocytes through blood vessels into the inflamed site (33), one of the hallmarks for inflammation. The adhesion and transendothelial migration of leukocytes from blood vessels into the site of inflammation may be regulated by MMP activity. In fact,

MMPs have a unique role in the inflammatory process by regulating the biological activity of cytokines, chemokines and serine proteases involved in the inflammatory process.

2.3.1. Leukocyte invasion

During the early stages of invasion leukocytes adhere to endothelial cells via selectins present on both cell types in a tethering or rolling process (34). The firm adhesion of cells is largely dependent on the interactions between leukocyte integrins to their endothelial cell ligands (34). The passage of leukocytes from the peripheral circulation through the endothelial monolayer to the site of inflammation requires degradation of the vascular basement membrane. The major MMPs responsible for degradation of this ECM are thought to be MMP-2 and MMP-9. Neutrophils do not express MMP-2 (35) but store MMP-9 in granules primed for rapid release by endogenous stimuli (36). In monocytes, the expression of MMP-9 and TIMP-1 is induced by inflammatory stimuli (37). MMP-9 is also required for the *in vitro* migration of resting T lymphocytes across a basal lamina equivalent (38). Knockout mice studies have provided further evidence of the importance of MMP-9 in leukocyte invasion. The infiltration of leukocytes into the blood brain barrier is markedly reduced in MMP-9 deficient mice compared to the wildtype (39) and the ability of leukocytes to interact or adhere with endothelial cells is adversely affected in the MMP-9 knockout mice (39). In contrast, Allport *et al* (40) demonstrated that neutrophils from MMP-9-deficient mice show no defect in transendothelial migration under flow *in vitro*.

MMPs can also indirectly affect leukocyte infiltration by regulating the signaling pathways responsible for the expression of adhesion molecules. Fernandez-Patron and colleagues (41) have demonstrated that MMP-2 cleaves big endothelin-1 (ET-1) to yield a novel 32 amino acid peptide, ET-1(1-32). This peptide binds the endothelin A receptor to signal the down-regulation of L-selectin and up-regulation of integrin CD11b/CD18 on the surfaces of neutrophils via the MAPK kinase pathway (41) (Fig 2A). Integrins are involved in facilitating the arrest of leukocyte rolling and promotion of transendothelial migration. Thus, MMP-2 cleavage of big ET-1 to yield ET-1(1–32)promotes leukocyte-endothelial cell adhesion and neutrophil trafficking into inflamed tissues.(Fig 2A). ET-1(1-32) can also stimulate the release of MMP-9 normally stored in granules to further encourage the conversion of big ET-1 to ET(1-32)to form a positive feedback loop and encourage further leukocyte migration (41).

2.3.2. Serine proteases

A prominent feature of inflammation involves the release of proteolytic enzymes from inflammatory cells. For example, neutrophils release serine elastase, cathepsin G and proteinase 3 in order to initiate tissue destruction and remodeling during inflammation (for review see (42)). The regulation of these serine proteinases is largely dependent on a group of serine proteinase inhibitors (serpins). Balancing the expression of these two groups of molecules is central in the inflammatory response. As a consequence

Table 1. MMPs and the chemokines that can induce their expression

Class	Common Name	MMP Number	Induction by Chemokines (Reference Articles)	Chemokine Substrate Reference articles)
Collagenases	Collagenase 1 Collagenase 2 Collagenase 3 Collagenase 4	MMP-1 MMP-8 MMP-13 MMP-18	TECK, SDF-1alpha (124, 125) TECK (125)	MCP-1, -2, -3, -4 (75) MCP-3(75)
Gelatinases	Gelatinase A Gelatinase B	MMP-2 MMP-9	TECK, SDF-1alpha (124, 125) IL-8, MIP-3alpha, SDF-1alpha (71, 124, 126)	MCP-3 (72) IL-8, GCP-2, ENA-78, CTAP-III, PF-4, GRO-alpha (69, 71)
Stromelysins/ Matrilysins	Stromelysin 1 Stromelysin 2 Stromelysin 3 Matrilysin Matrilysin-2	MMP-3 MMP-10 MMP-11 MMP-7 MMP-26	MCP-1, MCP-2, SDF-1α(127) TECK (125) TECK (125)	MCP-2, -3 (75)
Membrane-type MMPs	MT1-MMP MT2-MMP MT3-MMP MT4-MMP MT5-MMP MT6-MMP	MMP-14 MMP-15 MMP-16 MMP-17 MMP-24 MMP-25	IL-8, MCP-1 (51, 128) SDF-1alpha (129)	MCP-3(75)
Others	Macrophage Metalloelastase Enamelysin MMP-23a MMP 23b Epilysin	MMP-12 MMP-19 MMP-20 MMP-21 MMP-22 MMP-23 MMP-27 MMP-28		

of tissue damage or infection, the expression of serine proteinases dominates and the activity of the serpins is suppressed.

Liu *et al.* (43) used neutrophil serine elastase and MMP-9 knockout mice to study the inflammatory skin disorder bullous pemphigoid. Both MMP-9 and serine elastase deficient mice were resistant to blister formation. Subsequent experimentation showed that MMP-9 is the upstream molecule which governs the development of the disease through its ability to cleave and inactivate the effects of the serine elastase inhibitor, serpin α1-proteinase. In addition, cleaved serpin α1-proteinase acts as a potent chemoattractant for polymorphonuclear leukocytes (44). Molecular modeling has determined that other MMPs, including MMP-1, -2 and -3, can also potentially inactivate serpins (45).

2.3.3. Cytokines

MMPs can cleave and activate pro-inflammatory cytokines. Tumor necrosis factor-alpha (TNF-alpha) is a pleiotropic cytokine with diverse biological actions, including major pro-inflammatory actions (46). It binds to the TNF receptor (TNFR) and mediates signal transduction to stimulate the expression of cytokines, chemokines, MMPs and adhesion molecules (Fig 2B). TNF-alpha is mainly expressed by monocytes/macrophages as an inactive pro-transmembrane type II protein of 26 kDa (47) which requires proteolytic cleavage on the cell surface for activation and release (Fig 2B). MMPs capable of cleaving and activating TNFalpha include human MMP-1, (48, 49), MMP-7 (48-50), MMP-9 (48, 49), MMP-14 (51) and MMP-17 (52). The binding affinity of TNF-alpha to it's receptor, TNFR, can be manipulated to inhibit inflammation and MMPs may be central to this effect since the release of soluble TNFR (sTNFR) from the cell surface can inhibit the effects associated with TNF-alpha (53). The TNFalpha inhibitor, etanercept, is a recombinant form of sTNFR used to treat inflammatory arthritis (54). Although the exact cleavage molecules responsible for the release of sTNFR are unknown, incubation of cells with a MMP inhibitor BB-

275 prevents the release of the TNFR, suggesting that MMPs are involved (53). Another pro-inflammatory cytokine with similar functions to TNFalpha, interleukin-1beta (IL-beta), can be activated by MMP-1, -2, -3 and -9 (55, 56). Transforming growth factor-beta (TGF-beta), a multifunctional cytokine that controls cellular proliferation, apoptosis, wound healing and inflammation (57, 58), is secreted as an inactive form or stored as a non-functional homodimer (59) and requires processing by MMPs, such as MMP-3, -9 or -14, for activation (59-63).

2.3.4. Chemokines

Chemokines are a family of proteins that facilitate leukocyte chemotaxis (64) by creating a gradient that acts as a beacon for leukocyte migration (65). They have four conserved cysteine residues (66) which are critical for their tertiary structure (67) and are classified based on the first two cysteine amino acids on the N-terminus (66). Chemokines with no amino acids between the cysteine residues form the "CC" subfamily (66) and those with one or three amino acids separating the cysteine residues are classed as CXC and CX3C, respectively. Molecules within these subfamilies are numbered in chronological order of discovery (68) and also have common names. In addition, the subfamilies can be separated into either ligands or receptors e.g. TECK is classed as CCL25 with its respective CCR9 receptor (68). The chemotactic potential of chemokines can be altered following MMP cleavage to either potentiate or inhibit inflammation.

2.3.5. MMP cleavage of chemokines to potentiate inflammation

Van den Steen and colleagues (69) demonstrated that MMP-9 can truncate the CXC chemokine, interleukin-8 (IL-8), resulting in a 10-27 fold increase in its chemotactic ability (69). Interestingly, cleavage of IL-8 up-regulates the release of MMP-9 from neutrophil granules via chemokine receptor 1 (CXCR2) to create a positive feedback loop (69, 70). The activation of chemokines can have a positive effect on a number of other MMPs, summarized in Table 1. Other pro-inflammatory chemokines processed by MMP-9 include

granulocyte chemotactic protein-2 (GCP-2) and epithelial cell derived neutrophil activating peptide-78 (ENA-78) (71). Similar to IL-8, the chemotactic activity of GCP-2 may be enhanced as a consequence of MMP-9 processing (71). The effects of MMP-9 on ENA-78 are different to IL-8 or GCP-2 in that its cleavage initially discourages chemotaxis although further processing of ENA-78 into smaller fragments eventually promotes leukocyte migration (71). MMP-8 appears to be more selective than MMP-9 in that it can enhance GCP-2 activity, but not the other chemokines (71).

2.3.6. MMP cleavage of chemokines to inhibit inflammation

Anti-inflammatory effects of MMPs have also been associated with the cleavage of chemokines, particularly the monocyte chemoattractant proteins (MCPs). Cleavage of MCP-3 at glycine-4/isoleucine-5 by MMP-2 creates a truncated N-terminus protein which reduces leukocyte migration (72). Normally the binding of full length MCP-3 to its receptors CCR-1, -2 or -3 result in leukocyte activation and migration (73, 74). However, the MMP-2 cleaved MCP-3 antagonist protein cannot induce the migration of the THP-1 monocyte cell line despite being able to bind the CCR-1 and CCR-3 receptors (72). Subsequent studies demonstrated that MMP-1, -3, -13 and -14 also cleave the N-terminus of MCP-3 to form its antagonist (75) although the anti-inflammatory effect of this product is less pronounced. Interestingly, MMP-7, -8 and -9 are unable to cleave MCP-3 (75) which suggests that the anti-inflammatory effects of various MMPs may not be related to their respective ECM degradation activities. The molecular basis for these differences requires further investigation.

Other members of the MCP family examined for MMP cleavage include MCP-1, -2 and -4. MCP-1 is a substrate for MMP-1, MMP-3 and to a much lesser extent MMP-8 (75). MMP-1 and MMP-3 have activity for MCP-2, whereas, MCP-4 truncation is mediated by MMP-1 only. Cleavage of the MCP-1, -2 and -4 by their respective MMPs, results in the generation a protein which inhibits chemotaxis of leukocytes. Other chemotactic molecules inhibited by MMP-9 cleavage include CTAP-III, PF-4 and GRO-α (69). However, it is unclear whether cleavage of these molecules generates a chemokine antagonist or inhibits leukocyte invasion by an alternative mechanism.

2.4. MMPs in inflammatory diseases
2.4.1. Rheumatoid arthritis (RA)

RA is a chronic inflammatory disease characterized by the destruction of joints and is a typical example of poor MMP regulation. Excessive MMP activity is thought to play an important role in the pathogenesis of RA by directly degrading cartilage and bone and indirectly stimulating angiogenesis and inflammation. MMPs that are elevated in serum and joints of RA patients include MMP-1, -2, -3, -9, -13 and -14. MMP-9 in particular is dramatically elevated and its levels correlate with the severity of RA (76) MMP-9 from macrophages and neutrophils is thought to play a key role in promoting invasion of these cells in RA. *In vivo* studies using antibody-induced arthritis have shown that MMP-9 knockout mice display mild arthritis compared to controls indicating a pivotal role of MMP-9 in the development of inflammatory joint disease (77).

In contrast to MMP-9, MMP-2 null mice exhibit severe antibody-induced arthritis compared to wild-type mice, indicating that MMP-2 suppresses inflammatory joint disease (77). Although MMP-2 is elevated in the arthritic joint, Itoh *et al* (77) suggest that its matrix-degrading activities appear to have little effect on development of arthritis but instead MMP-2 prevents inflammation by cleaving pro-inflammatory factors, such as MCP-3.

The development of septic arthritis induced by *S. aureus* is less severe in MMP-7-deficient mice when compared with their wild-type counterparts (78). These mice showed decreased synovitis and joint destruction to suggest that MMP-7 contributes to the development of arthritis. During the early stages of inflammation, the migration of leukocytes and monocytes are significantly reduced in the MMP-7 knockout mice which confirms the pro-inflammatory role of MMP-7 forming a chemotactic gradient for neutrophil migration (79).

There has been considerable interest in prevention of MMP activity to treat RA. Theurapeutic approaches include inhibition of MMP production, blocking the active site of MMPs, increasing endogenous production of TIMPs or preventing the activation of MMPs. Extensive research has focused on blocking the active site of MMPs. The broad spectrum inhibitor, Marimastat, was the first orally available MMP inhibitor tested for use against cancer in clinical trials (80). Trocade™ (Ro 32-3555) (Roche) has been evaluated in clinical trials as a treatment for RA (81) but showed poor efficacy (82, 83). There are a number of possible reasons why MMP inhibitors appear to be unsuccessful including limited bioavailability in synovial joints (82). To further complicate events, the broad spectrum inhibitory effects of these molecules may block not only the pro-inflammatory actions but also the anti-inflammatory effects of MMPs. As a consequence of the clinical trials, the development of novel MMP inhibitors has slowed considerably-but are we missing an important therapeutic opportunity? A possible therapeutic approach in RA would involve targeting selected pro-inflammatory MMPs of which MMP-7 or -9 would appear to be suitable candidates.

2.4.2. Cancer

Although the observation that cancers occur at sites of chronic inflammation was made as early as the 18th century (84) the relationship between inflammation and tumour development is yet to be understood. A number of reviews discuss the role of inflammation as a co-conspirator in tumour development (85-88) and considerable evidence suggests that inflammatory cells may contribute to malignant cell progression. One theory known as the "landscaper theory" suggests that the persistence of a chronic inflammatory response encourages a defective microenvironment which can lead to malignant transformation (89). The release of pro- and anti-
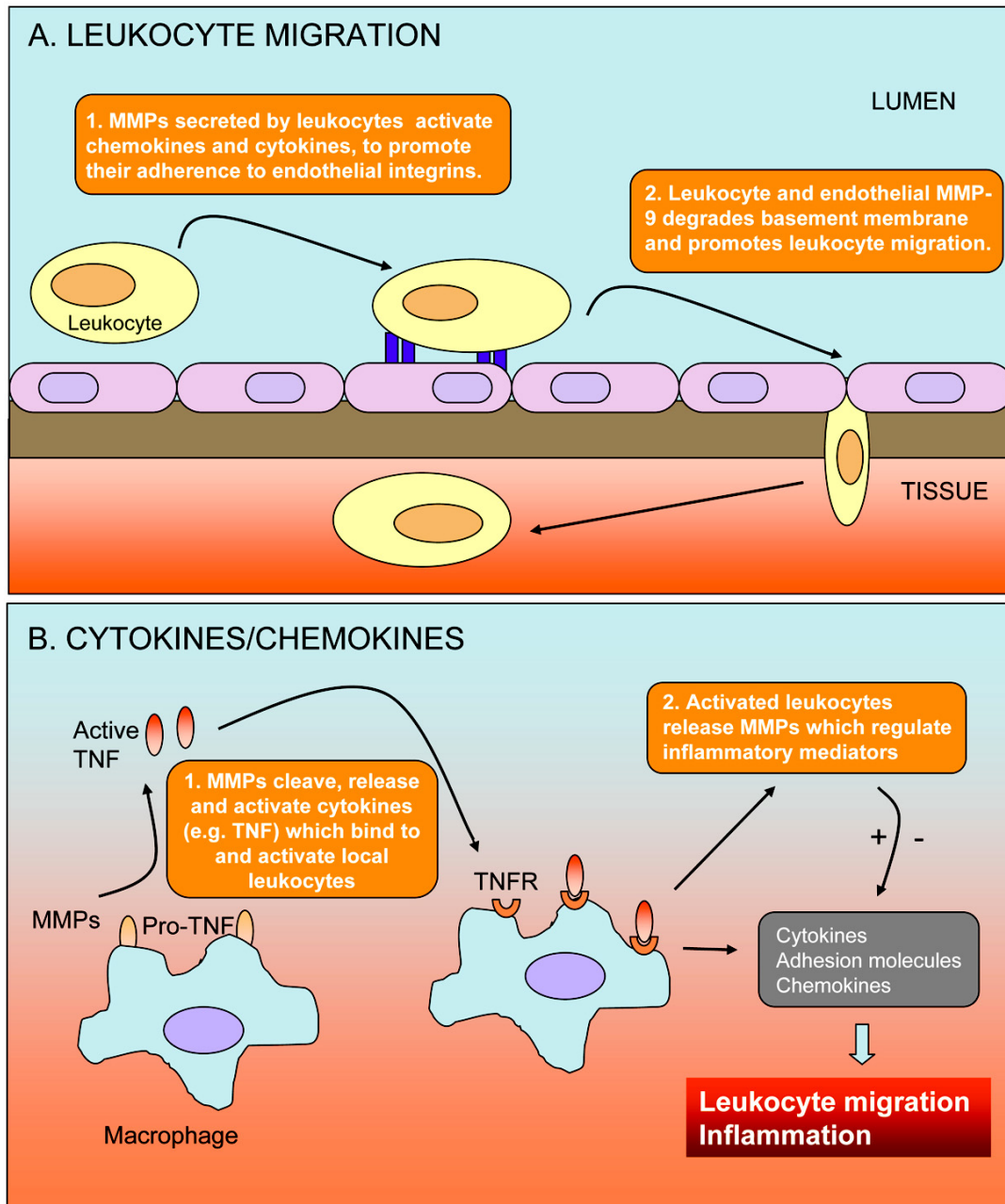
Figure 2. Simplified model of the pro-inflammatory role of matrix metalloproteinases. (A) MMPs are required for the transendothelial migration of leukocytes. Chemokines and cytokines activated by MMP cleavage bind their respective receptor to up-regulate or change the conformation of integrins on endothelial cell. In addition, cleavage of big endothelin-1 (ET-1) by MMP-2 yields a vascular peptide ET-1(1-32)which down regulates L-selectins and up-regulates integrins on the surfaces of leukocytes. The increased expression of integrins on the surface of endothelial cells facilities adhesion and transendothelial migration of leukocytes into tissue. MMP-9 directly assists invasion by degrading the basement membrane. (B) Pro-inflammatory cytokines such as tumor necrosis factor α are expressed as inactive membrane bound proteins (pro-TNF-alpha) on the surface of mast cells and macrophages. These cells act as sentinels and are involved in the initiation and propagation of an inflammatory signal. As a result of infection or tissue damage, cells within microenvironment release MMPs to cleave and release TNF-alpha. The activated TNF-alpha then binds to the TNF-receptor (TNFR) on neighboring cells resulting in signal transduction, which up-regulates MMPs, adhesion molecules, cytokines and chemokines. Chemokines are important in leukocyte infiltration since they form a chemotactic gradient which allows these cells to correctly localize to the inflamed site. MMPs can regulate the chemotactic potential of these molecules through their endopeptidase activity. *See text for details.*

inflammatory cytokines and reactive oxygen species (ROS) to a site of inflammation can activate a cascade of events including DNA damage, inhibition of apoptosis and angiogenesis (90). The emerging evidence suggests that chronic inflammation supports the transition of a tissue from normal to malignant. It is well known that tumours produce cytokines, chemokines, interleukins and angiogenic factors which recruit different stromal cell populations to promote tumor growth (91-94). Amongst other functions, stromal cells are involved in facilitating the tumour's ability to escape into secondary sites via the degradation of the extracellular matrix. This degradation is machined by MMPs, particularly MMP-2 and MMP-9. Although many cancer cells including breast, prostate and colon, express high mRNA and protein levels of MMP-2 and MMP-9 (95), active forms of these gelatinases have been mainly associated with stromal cells *in vivo* (96). Depending on the tumour type, tumour cells are believed to be primarily involved in the regulation of stromal MMP expression (97). For example, colorectal cancer cells, but not liver metastasis cancer cells, induce MMP-9 production by fibroblasts in co-culture (98). MMP activity is also dependent on the stage of the tumour. Increased levels of MMP-9 are present in the early stages but not in the more advanced phases of melanoma (99) whereas MMP-2 activity is positively related to the invasive stage and lymph node metastasis of colorectal carcinoma (100).

The differential MMP expression and activity has been partly responsible for the failure of MMP inhibitors such as marimastat in clinical trials. Although initial animal studies using this drug showed a significant inhibitory effect on gastric carcinoma xenograft growth in mice (101), subsequent phase III trials have been unsuccessful in decreasing later stage tumour progression (102). The failure of marimastat in clinical trails may also be related to its diverse MMP blocking ability. Specifically inhibiting the activities of certain MMPs may be a more effective anti-cancer therapy. The specific inhibition of MMP-2 and MMP-9 activity using the MMP-9 PEX domain has shown some promise at inhibiting the invasion of cancer cells (8). The PEX domain of MMP-9 not only inhibited gelatinase activity of its parent molecule but also tumor cell invasion. Supporting this approach, Kessler *et al.* (103) used lentiviral system to overexpress the PEX domain of MMP-2 in endothelial cells. The invasive, angiogenic and MMP-2 potential of these cells were dramatically reduced (103). Recently, the potent anti-proliferative and anti-metastatic ability of the PEX domain was demonstrated *in vitro* with nude mice xenographs of glioma cells (104). To date the potential anti-inflammatory effects of the PEX domain has not been studied. In fact, the use of anti-inflammatory agents against cancer has not been sufficiently studied. However, a new synthetic anti-inflammatory compound known as SM-7368 has shown promising results in inhibiting the invasion of HT1080 human fibrosarcoma cell line by blocking TNF-alpha-induced MMP-9 expression (105). Further studies on the mechanisms of MMPs in inflammation and cancer progression are needed.

2.4.3. Wound Healing

Wound repair is a physiological event which occurs after tissue injury to restore structure and function of tissue. Cutaneous wound repair can be divided into a series of dynamic phases including (i) formation of fibrin clot (ii) inflammatory response (iii) granulation tissue formation incorporating re-epithelialization, angiogenesis and fibroblast invasion and (iii) matrix formation and remodeling (106). The fibrin clot serves as a reservoir of cytokines and growth factors released from activated coagulation cascade, injured cells, and platelets to initiate the inflammatory response. Neutrophils arrive at the wound site within minutes of injury to clear the contaminating bacteria and tissue debris and provide a source of pro-inflammatory cytokines to activate local fibroblasts and keratinocytes (107-109). It is important that this inflammatory response ceases after a few days to allow the granulation stage to proceed.

During wound healing, MMPs are involved in degradation of ECM, facilitation of cell migration for re-epithelialization and angiogenesis, deposition of new ECM and remodeling of scar tissue. Immediately following injury, MMPs are induced and showed strict expression patterns (110-113). For example, the expression of MMP-1 in basal keratinocytes is rapidly induced after dermal injury, persists during healing, and subsides during re-epithelialization (114). Wounds that do not heal (chronic ulcers) often become locked up in the inflammatory phase and cannot progress to produce granulation tissue. Increased MMP activity in chronic ulcers is markedly elevated and contributes to this persistent inflammation which in turn further enhances MMP activity (115-118). Elevated levels of MMPs (MMP-2, MMP-8, MMP-9 and MMP-14) (115-118)and decreased TIMP-2 (119) were found in chronic diabetic foot ulcers compared with healing wounds in normal patients. Poor healing has been correlated with elevated expression of MMP-9 in chronic venous wound biopsy specimens and an elevated ratio of MMP-9/TIMP-1 in wound fluids (117).

One treatment strategy for chronic ulcers is being directed towards down-regulation of MMP activity (119). However, since MMP activity is necessary for normal wound healing, the use of broad spectrum inhibitors is unlikely to be efficacious. Activated protein C (APC) is a natural anticoagulant that has significant anti-inflammatory properties associated with a decrease in pro-inflammatory cytokines and a reduction of leukocyte recruitment (120) which dampens excess MMP activity, Interestingly, APC specifically up-regulates and activates MMP-2 in skin fibroblasts, umbilical vein endothelial cells (121) and human keratinocytes (122), which is required for cell migration (123). In a full-thickness rat skin-healing model, a single topical application of APC enhances wound healing via a complex mechanism involving inhibition of inflammation, stimulation of angiogenesis and selective activation of MMP-2 (121). These unique properties of APC make it an attractive therapeutic agent to promote the healing of chronic wounds.

3. CONCLUSIONS

The identification of new non-matrix MMP substrates involved in inflammation, highlights the diverse role of MMPs in physiological and pathological processes. It is noteworthy that several MMPs such as MMP-2 and -9 can favour anti- or pro- inflammatory action, respectively. The extent of this dual functionality of MMPs is yet to be realized. Elucidating these processes may enable the design of anti-inflammatory drugs for the treatment of diseases such as arthritis, cancer and chronic wounds.

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