

## Membrane-type matrix metalloproteinases: key mediators of leukocyte function

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### ABSTRACT

Leukocytes are major cellular effectors of the immune response. To accomplish this task, these cells display a vast arsenal of proteinases, among which, members of the MMP family are especially important. Leukocytes express several members of the MMP family, including secreted- and membrane-anchored MT- MMPs, which synergistically orchestrate an appropriate proteolytic reaction that ultimately modulates immunological responses. The MT-MMP subfamily comprises TM- and GPI-anchored proteinases, which are targeted to well-defined membrane microdomains and exhibit different substrate specificities. Whereas much information exists on the biological roles of secreted MMPs in leukocytes, the roles of MT-MMPs remain relatively obscure. This review summarizes the current knowledge on the expression of MT-MMPs in leukocyte and their contribution to the immune responses and to pathological conditions. *J. Leukoc. Biol.* 94: 000–000; 2013.

### Introduction

Leukocytes are derived from multipotent hematopoietic stem cells in bone marrow that differentiate into different immune cell lineages depending on specific stimuli and microenvironmental cues. Each subpopulation of leukocytes has specific roles in the layout of the immune response, and many are able to transform themselves further in different ways, depending (between others) on the cytokines secreted by surrounding cells. The adaptive and innate immune responses are two tightly collaborating segments of the immune response and have many overlapping components and functions. T and B lymphocytes are the main cells that constitute the effector arms of adaptive immune responses. Other leukocytes that interact with lymphocytes play crucial roles in the presentation

of antigen and in mediating immunologic functions, essential for both arms of the immune response. These cells include DCs and the related LCs, monocyte/MΦs, NKs, PMNs, MCs, basophils, and eosinophils [1].

An important characteristic of all leukocyte types is their ability to activate proteolytic systems, which then contribute to the accomplishment of adequate immune response without causing self-damage. However, under certain conditions, the deregulated proteolysis by leukocytes can inflict irreversible tissue injury, for instance, in cases such as persistent inflammation [2, 3]. Therefore, a tight control of the proteolytic response is essential for the normal function of the immune system. Control over potentially tissue-damaging proteinases by leukocytes is accomplished at multiple levels, including transcriptional regulation, storage in specific granules or insertion into membrane microdomains, regulated release upon stimulation, latency and activation, and inhibition by specific proteinase inhibitors. In the case of MT-MMPs, insertion into the plasma membrane by defined structural domains facilitates tight control of proteinase activity and localized proteolysis at the cell-matrix interface. Thus, MT-MMPs are perfectly suited for the mediation of biological effects requiring regulated and localized proteolysis. However, as opposed to the secreted MMPs, which have been studied extensively in experimental animal models and in human diseases (for comprehensive reviews on secreted MMPs in leukocytes, see refs. [4–8]), there is a significant gap in the knowledge of the role of MT-MMPs in leukocyte functions. This is particularly noteworthy for the GPI-anchored MT-MMPs, as their function in immune cells is just starting to be elucidated. The purpose of this review is to summarize and discuss the current understanding of the MT-MMP contribution to leukocyte functions in normal and pathological processes.

### MMPs

MMPs are zinc-dependent endopeptidases that collectively have the potential to hydrolyze all protein components of the

Abbreviations: BBB=blood brain barrier, EAE=experimental autoimmune encephalomyelitis, ENC=endothelial cell, iDC=immature DC, LC=Langerhans cell, MΦ=macrophage, MBP=myelin basic protein, MC=mast cell, mDC=mature DC, MMP=matrix metalloproteinase, MS=multiple sclerosis, MT-MMP=membrane-type matrix metalloproteinase, PMN=polymorphonuclear neutrophil, RA=Rheumatoid arthritis, SDF-1=stromal cell-derived factor 1, TM=transmembrane

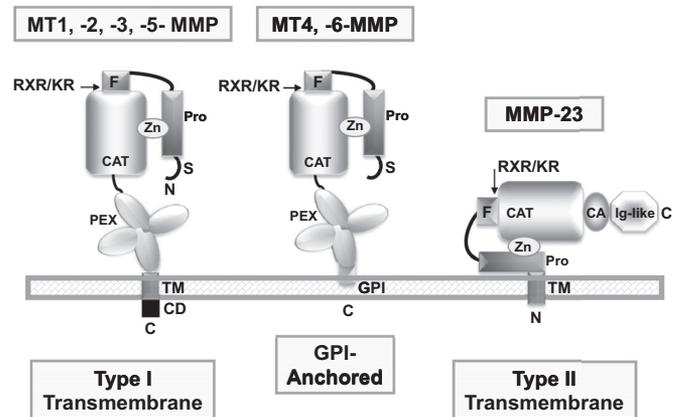
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ECM. In addition, MMPs cleave a wide range of cellular and secreted proteins known to play key roles in a variety of physiological functions and pathological conditions. In humans, the MMP family includes 24 members on the base of structural organization and substrate specificity and is organized into five groups: collagenases, gelatinases, stromelysins, matrilysins, and MT-MMPs. There are, however, a few exceptions: MMP-12 ( $\Phi$  metalloelastase), MMP-20 (Enamelysin), MMP-23 (Ig-like domain), and MMP-28 (Epilysin) are not members of these five groups. All members of the MMP family share a common domain organization that includes: (1) a pro-domain that maintains the enzyme in its latent form; (2) a catalytic domain containing the consensus sequence HEXXHXXGXXH, which binds a  $Zn^{+2}$  atom and is essential for catalysis; and (3), a carboxy-terminal hemopexin-like domain, which depending on the MMP, is involved in substrate recognition and/or interactions with the TIMPs. A hinge region links the catalytic domain with the hemopexin-like domain. Of importance for this review, the MMP family is divided further in two major subgroups: secreted and membrane-anchored proteinases (referred to as MT-MMPs). The distinction is made upon the absence or presence of membrane-anchoring domains.

To control their proteolytic activity, all MMPs are synthesized as latent proenzymes (referred to as pro-MMPs). Activation, the acquisition of catalytic activity, is achieved by various proteinases or ROS that disrupt the interaction between the active site zinc atom in the catalytic domain and a conserved cysteine within the pro-domain. Exposure of the zinc atom results in the autolytic cleavage of the prodomain, a process known as the "cysteine switch" [9]. Once activated, MMP catalytic activity is controlled, in part, by TIMPs, which act as specific proteinase inhibitors. The TIMP family comprises four homologous proteins: TIMP-1, TIMP-2, TIMP-3, and TIMP-4, which bind to the catalytic domain and inhibit enzymatic activity. Several comprehensive reviews on structure and biochemistry of MMPs and TIMP inhibitors are available [8, 10, 11].

## OVERVIEW OF MT-MMP PROPERTIES AND FUNCTIONS

Based on the structure of membrane-anchoring domain and orientation, the MT-MMP subfamily comprises four type I TM proteases, two GPI-anchored proteinases, and one type II TM proteinase (Fig. 1). The type I TM proteases include MT1 (MMP14)-, MT2 (MMP15)-, MT3 (MMP16)-, and MT5 (MMP24)-MMP, and GPI-anchored MT-MMPs include MT4 (MMP17)- and MT6 (MMP25)-MMP. MMP23 is the only known type II TM protein. Here, we will focus on the type I TM and GPI-anchored MT-MMPs and thus, refer to them herein as MT-MMPs. Structurally, the MT-MMPs contain all of the three specific domains common to MMPs. All MT-MMPs contain a conserved RR(K/R)R motif between the prodomain and the catalytic domain (Fig. 1), which is a cleavage site for proprotein convertases, such as furin. During trafficking, through the trans Golgi apparatus, convertases cleave at the RR(K/R)R motif, causing the removal of the prodomain, which results in proteinase activation. A short stalk links the



**Figure 1. Domain structure of membrane-anchored MMPs.** Most MMPs contain a propeptide domain (Pro), a catalytic domain (CAT), a linker (hinge-region), and a hemopexin (PEX) domain. All of the membrane-anchored MMPs have a basic RX(K/R)R motif at the C-terminal end of their prodomains. This motif can be cleaved inside of the cells by furin-like proteinases. Four of the six MT-MMPs are anchored to the cell membranes through a type I TM domain and the other two, through a GPI moiety. The seventh membrane-anchored MMP, MMP-23, has an N-terminal type II TM domain. The two minimal domain, MMPs and MMP-23, lack the PEX domain, and in the latter enzyme, this domain is replaced by a C-terminal cysteine array (CA) and an Ig-like (Ig) domain.

hemopexin-like domain with the membrane-anchoring apparatus, which in the case of MT1-, MT2-, MT3-, and MT5-MMP, is a TM domain and in the case of MT4- and MT6-MMP, is a GPI anchor. The type I TM MT-MMPs also contain a short cytoplasmic tail of ~20 aa, which contributes to signaling and cell/ECM communication via interactions with intracellular proteins [12–14]. As GPI-anchored proteins, MT4- and MT6-MMP are targeted specifically to the lipid raft cell fraction, and this may influence their regulation and substrate preferences [15–17].

Like all MMPs, the enzymatic activity of MT-MMPs is inhibited by TIMPs. However, these proteases exhibit selectivity with regard to sensitivity to specific TIMP family members. Indeed, whereas TM MT-MMPs are highly sensitive to TIMP-2, TIMP-3, and TIMP-4, they are insensitive to TIMP-1. In contrast, the GPI-anchored MT-MMPs are known to be equally sensitive to all TIMPs [15, 18, 19]. The differential sensitivity of MT-MMPs to TIMP inhibition can be used to discriminate between cellular activities mediated by MMPs/GPI-MT-MMPs (inhibited by TIMP-1), and TM MT-MMPs (insensitive to TIMP-1) in functional assays. In the case of MT1-MMP, binding of TIMP-2 to the catalytic domain was shown under certain conditions to regulate pericellular proteolysis positively by promoting the activation of pro-MMP-2 (gelatinase A), a secreted MMP that is a major target of MT1-MMP. The formation of a ternary complex among MT1-MMP, TIMP-2, and pro-MMP-2 leads to the generation of active MMP-2 [20]. In addition to pro-MMP-2 activation, the binding of TIMP-2 to MT1-MMP and MT3-MMP slows down the overall autocatalytic turnover of these MT-MMPs. Paradoxically, this enhances surface proteolysis

further by stabilizing the pool of active enzyme at the cell surface [21]. Thus, in MT-MMPs, TIMPs have effects on MT-MMPs that go beyond inhibition of catalysis, and under certain conditions, they may induce an increased pericellular proteolysis [22].

**MT-MMP: role in ECM degradation**

The complete spectrum of substrates cleaved by leukocyte-derived MT-MMPs has not been elucidated completely, but so far, we know that it includes ECM and non-ECM proteins (Table 1) [23]. Evidence so far points to a role for the TM MT-MMPs, in particular, MT1-MMP, as key proteases involved in the degradation of ECM components, including collagens, laminin, fibronectin, and fibrin, as well as various integrin receptors and cadherins mediating the cell–cell interactions [23–25]. As for GPI-MT-MMPs, several studies reported their ability to cleave ECM components in vitro [18, 19, 26–28]. In spite of this, a complete picture of the specific contributions of ECM proteolysis mediated by these MT-MMPs in models of leukocyte migration is lacking. Indeed, that MT1-MMP localizes to podosomes and process ECM components in MΦs was only shown recently [29–31], whereas it was studied extensively for tumor cells [32]. Regardless, there is a strong rationale to support the hypothesis that the ECM degradative action of MT-MMPs can elicit profound effects on leukocyte behavior and function. Indeed, there are evidences that degradation of ECM components by MMPs contributes to the generation of bioactive fragments that modulate immune function [33, 34].

**MT-MMP: role in immunomodulating protein cleavage**

It is suspected that cleavage and shedding of inflammatory proteins by proteinases provide another layer of modulation and regulation during inflammatory responses [35]. Accumulating evidences also suggest that members of the MT-MMP family may regulate leukocyte functions, directly or indirectly, by cleaving substrates that have a significant role in the regulation of the immune response. MT1-MMP cleaves cell surface adhesion molecules, such as CD44 [36], ICAM-1 [37], and syndecan-1 [38]; moreover, this is thought to contribute to leukocyte mobility [39, 40]. In the case of syndecan-1, its MT1-MMP-

induced shedding was also found to regulate cell proliferation in a human breast carcinoma cell line [41]. In addition, other cell surface enzymes, such as tissue transglutaminase, were shown to be cleaved by MT1-, MT2-, and MT3-MMP, with the overall effect of reducing cell adhesion and enhancing migration [42].

A novel approach involving proteomics and mass spectrometry was used to investigate the degradome of MT1-MMP. Interestingly, it was found that it had more substrates than expected: MT1-MMP cleaved different cytokines and chemokines, such as pro-TNF-α, connective tissue growth factor, IL-8, CCL5, CCL23, and CCL16 [27, 43]. Of note, MT1-MMP cleavage of chemokines yields truncated forms that are slightly different than those of non-TM MMPs, indicating a cleavage preference for MT1-MMP [27, 43]. Despite that, some of the truncated chemokines that MT1-MMP generates are almost identical to reported truncated species of IL-8 [44], CCL5 [45, 46], and CCL23 [45] that have increased agonistic activity. Moreover, the activation of latent TFG-β by MT1-MMP in osteoblast was reported [47]. Conversely, CCL7 (MCP-3) [48] and CXCL12 (SDF-1) [49], when cleaved by MT1-MMP, lose their ability to attract leukocytes. Even if MT1-MMP [27, 50] and MT4-MMP [19] have been shown to cleave pro-TNF-α in vitro, pro-TNF-α cleavage was not impaired in MT4-MMP-deficient MΦs [51]. These suggest that every in vitro target of MT-MMP may not, in fact, be cleaved in vivo. One of the many reasons could be that intracellular segregation does not allow metalloproteinases to interact with their putative substrate as they do in vitro. Nonetheless, all of these data collectively, strongly suggest that MT1-MMP plays an important role in migration of leukocytes and regulation of the inflammatory process.

Focusing on the other largely studied member of the MT-MMP family, MT6-MMP, it was shown that it degrades and inactivates the α1-protease inhibitor [52], a serine protease inhibitor that dampens proteolytic activity during inflammation. Thus, expression of MT6-MMP in leukocytes may help to further progress the inflammatory response by facilitating action of serine proteases. The degradome of MT6-MMP was investigated recently, and it was found that MT6-MMP cleaved chemokines involved in PMNs and monocyte migration [28]. Chemokine processing by MT6-MMP increased the potency of

**TABLE 1. Substrates of Human MT-MMPs Related to Leukocyte Functions**

Protease name	ECM substrates	Non-ECM substrates
MT1-MMP	Types I, II, and III collagen, gelatin, Fn, Ln-5, fibrin, proteoglycans, aggrecan, Ln	Pro-TNF-α, tissue transglutaminase, syndecan, αβ3 integrin, α1-proteinase inhibitor, CD44, δ-like 1, SDF-1, α2-macroglobulin, MCP-3, IL-8
MT2-MMP	Fn, tenascin, Ln, aggrecan, perlecan, nidogen	Pro-TNF-α, tissue transglutaminase
MT3-MMP	Type III collagen, Fn, gelatin	Tissue transglutaminase, syndecan
MT4-MMP	Fibrinogen, fibrin gelatin	Pro-TNF-α
MT5-MMP	PG	
MT6-MMP	Gelatin, collagen IV, fibrin, Fn, Ln	α1-Proteinase inhibitor CXCL2, CXCL5, CCL15, CCL23, vimentin, CXCL12, CCL2, CCL7, CCL13

Description of ECM and non-ECM biological molecule substrates, which are described for each MT-MMP, related with possible effects in immune responses. Fn, fibronectin; Ln, laminin; PG, plasminogen.

CXCL5, CCL15, and CCL23 to recruit PMNs or monocytes [28]. This could promote progression of the acute inflammatory response and underscore the importance of MT6-MMP for innate immunity responses. Conversely, MT6-MMP processing of CXCL12, CCL2, CCL7, and CCL13 leads to their inactivation [28]. Finally, MT6-MMP also cleaves vimentin, generating fragments that impair monocyte migration, but enhances phagocytosis [28]. MT6-MMP thus plays a key role at various levels of the inflammatory process.

## MT-MMP EXPRESSION AND FUNCTION IN SPECIFIC LEUKOCYTE SUBPOPULATIONS

### Mononuclear leukocytes

**Monocytes/MΦs.** Monocytes leave the bloodstream to differentiate into DCs or MΦs in tissues, where they are versatile cells playing pleiotropic roles. Their main function is to process antigen material and present it on their surface to other cells of the immune system. They also produce a wide array of powerful chemical substances, including enzymes, proteins, and regulatory factors, such as cytokines, and in consequence, act as messengers between the innate and adaptive immunity. Resting human monocytes purified from blood express mRNA for MT1-, MT4-, and MT6-MMP [53–55], and stimulation of mouse RAW 264.7 cells with LPS resulted in an increased expression of MT1-, MT2-, and MT6-MMP mRNA [56]. Recent experiments described an up-regulation of MT1- and MT6-MMPs at the mRNA levels in classically or alternatively activated human monocytes *in vitro* [55]. Moreover, MT1-MMP expression is induced by COX-dependent and -independent mechanisms in human monocytes [57, 58]. In addition, resident MΦs in human endometrium were shown to express MT1- and MT2-MMP mRNA and proteins during the menstrual cycle, with MT1-MMP levels fluctuating during the menstrual cycle, whereas those of MT2-MMP remained constant, suggesting different roles for these two MT-MMPs in this physiological process [59].

Whereas the involvement of MT1-MMP in invasive and collagenolytic activities of ECs and tumor cells seems to be well-established [60, 61], it is an entirely different situation for monocytes/MΦs. Conflicting results, however, were only reported for mouse MΦs, as it was clearly shown that MT1-MMP is critical in human monocytes for transmigration through ECs [37, 62]. Furthermore, TIMP-2 and -3 blocked the migration of human monocytes, whereas TIMP-1, which does not inhibit MT1-MMP, had no effect [37]. For mouse MΦs, two independent teams, using largely similar protocols for Matrigel and transwell assays, reported that MT1-MMP was involved in MΦ migration [63, 64] or that it was not [65]. Unfortunately, conflicting results were also reported for *in vivo* studies looking at MΦ migration to a site of inflammation [64, 66]. Therefore, the involvement of MT1-MMP in MΦ migration/invasion needs to be studied further; this would be especially relevant *in vivo*, as it is possible that the requirement for MT1-MMP in migration varies upon the different environmental clues that MΦ receive, as suggested elsewhere [67].

Moving out the problematic issue of MΦ mobility, recent evidences reveal that MT1-MMP contributes much more to MΦ function than could be expected. In addition, these do not even require its catalytic activity. For example, it was shown in mice that MT1-MMP was required for MΦ fusion during osteoclast and giant-cell formation *in vitro* and *in vivo* in a manner independent of its catalytic activity or MMP-2 activation [12]. In addition, MΦs deficient in MT1-MMP produce less ATP, a process that is independent of its proteolytic activity [63]. Finally, it was found that MT1-MMP traffics to the nucleus, where it modulates gene expression by a mechanism independent of its catalytic activity but involving the Mi-2/nucleosome remodeling and deacetylase complex, which dampens MΦs functions during immune responses [65]. In conclusion, these results suggest that MT1-MMP plays a role in a broad range of functions, as it was shown for other cell types [68, 69], and not only in migration.

**DCs and LCs.** LCs are DCs that capture foreign antigens in the skin and then migrate to regional LNs, where they present these antigens to naive T cells. A few studies investigated MT-MMP expression in these cells. MT1- and MT3-MMP protein expressions were measured *in vitro* in LCs derived from CD34+ human cord blood cells and epidermal LCs [70]. The addition of TNF- $\alpha$  increased the expression of these MT-MMPs and also induced the surface association of secreted MMPs [70]. These studies suggest that TNF- $\alpha$  is a key molecule for MMP production in LCs and that MT-MMPs, rather than soluble MMPs, are involved in LC migration [70]. The expression of MT1-MMP was reported in iDCs as well as in mDCs. Matrix invasion by iDCs was blocked by the inhibition of MT1-MMP, whereas mDCs mobility was not affected [71]. iDCs and mDCs also express MT2-MMP; however, its exact functional role in DCs remains to be established [32, 72].

**NK cells.** NK cells are large, granular lymphocytes that patrol lymph and blood vessels to dispose cytotoxic agents and virus-infected or tumor cells. To accomplish these goals, NK cells possess proteinases that facilitate their destructive activity and promote their traffic through tissues. IL-2 and IL-18 are chemokines that stimulate NK cell migration, cytotoxicity, and antitumor activity, and most studies examined their effect on MT-MMP expression. Experiments done with IL-2-activated rat NK (RNK-16, rat large, granular lymphocytic leukemia) cells showed that these cells express MT1- and MT2-MMP [73, 74]. Moreover, the ability of activated NK cells to migrate through Matrigel was reduced significantly by BB-94, a selective inhibitor of MMPs [74]. In humans, NK cell lines with different migratory capacity (NK-92 and YT) were tested for MT-MMP expression [75]. The cell line with superior migratory capacity, NK-92, was found to have higher mRNA expression of MT1-, MT3-, and MT6-MMP compared with YT cells, which are less mobile. Also, NK-92 cell migration through Matrigel was reduced significantly by GM6001, another selective inhibitor of MMPs [75]. The same group later confirmed the expression of these MT-MMPs in human NK cells [76]. Conversely, in another study in which MT6-MMP expression was not determined, human NK cells were found to express MT1- and MT2-MMPs but not MT3-MMP [77]. As for migration, human NK cells stimulated with IL-2 and IL-18 displayed an augmented

migration through Matrigel [76, 77], and at least for the case of IL-2 stimulation, this was in a MMP-dependent manner [76]. Finally, IL-18 stimulation resulted in an increased expression of MT1-MMP [77] whereas IL-2 increased the expression of MT6-MMP [76]. Thus, MT-MMPs may play an important role in NK cell trafficking and thereby, contribute to the innate immune response, but the lack of studies in which TIMPs or MT-MMP-deficient NK cells are studied precludes further conclusion.

**T and B lymphocytes.** Although T and B cells are the components of the adaptive immune response and extremely important cells in many aspects of immune responses, the contribution of MT-MMPs to their function remains unknown. It was shown, however, that T and B cells isolated from normal blood donors have a different pattern of MT-MMP expression. Indeed, MT4-MMP is expressed preferentially in unstimulated B cells, whereas the expression of MT2-MMP is higher in T cells [53]. Unstimulated T and B cells had a high expression of MT1-MMP but a low expression of MT6-MMP, which was not increased by stimulation. MT3-MMP was expressed in unstimulated T cells only, but B cell activation with anti-IgG for 8 h could induce its expression in these cells [53]. As we have highlighted in this review—the important contribution of MT-MMPs for the function of other cell types—it is thus surprising that this was never studied in lymphocytes.

### Polymorphonuclear leukocytes

**PMNs.** PMNs are the first to be recruited from the circulation to the site of infection and are mainly responsible to clear tissues from invading pathogens. PMNs are particularly enriched with MT6-MMP [78] but seem to lack expression of other MT-MMPs (in spite of a positive signal for MT1- and MT2-MMP in normal uterus tissues) [59]. In PMNs, MT6-MMP is stored in specific granules but is also detected in the lipid rafts in the plasma membrane [16, 79]. The exposure of human PMNs to phorbol ester, IL-1 $\alpha/\beta$ , or IL-8 induces the release of MT6-MMP from granules into the extracellular space by an unknown mechanism [80]. Although a number of cytokines and chemokines can regulate MT6-MMP secretion, IL-8 is the most effective [80]. In addition, MT6-MMP was shown to activate pro-MMP-2 [81, 82]. Matsuda et al. [83] showed that in PMNs, MT6-MMP copurifies with clusterin (an abundant protein in the body fluid in tissues), rendering the secreted enzyme inactive. Thus, in addition to TIMPs, clusterin may act as a natural inhibitor of MT6-MMP activity. It is thus conceivable that PMNs migrating to inflammatory sites degrade the ECM components by a mechanism involving secreted MT6-MMP.

In living PMNs, membrane-anchored MT6-MMPs may contribute to cytokine secretion and respiratory burst [16]. During PMN apoptosis, membrane-anchored MT6-MMP becomes externalized and is readily displayed on the surface of the cell [16]. As described earlier, the cleavage of vimentin *in vitro* by MT6-MMP was shown to increase M $\Phi$  phagocytosis [28], as vimentin is expressed on the surface of apoptotic PMNs [84]; this could be a mechanism by which PMNs are targeted for removal by M $\Phi$ s. As we have seen, proteomics approaches have revealed that the degradome of MT6-MMP is vaster than

expected, especially regarding chemokines [28, 43]. As such, MT6-MMP could be one of the main mediators of the effects of PMNs in the coordination of early inflammation and wound repair [28].

**Eosinophils, basophils, and MCs.** Eosinophils are multifunctional leukocytes, which mostly reside in mucosal tissues, eliciting diverse, inflammatory responses, and are major modulators of innate and adaptive immunity. Basophils and MCs are considered primary effector cells in different allergic responses, such as bronchial asthma, and possess complex and partially overlapping roles in innate immunity. MCs and basophils are implicated in the pathogenesis of a number of respiratory diseases, particularly asthma and allergic rhinitis. A limited number of studies reported the expression of MT-MMPs in these subsets of leukocytes. For instance, eosinophils isolated from human blood were shown to constitutively express MT4-MMP mRNA and protein, which were increased significantly after TNF- $\alpha$  treatment [54]. Moreover, eosinophils in nasal polyps were found to be positive for MT4-MMP [54]. Although the ability of MT4-MMP to facilitate degradation of ECM has not been studied in detail, it is possible that this proteinase may contribute to the transendothelial migration and emigration of eosinophils. Whether eosinophils express other MT-MMPs remains unclear. As for human MCs, they were shown to express MT1-MMP mRNA upon treatment with phorbol ester [85]. However, the role of this MT-MMP in these cells remains unknown. Lastly, there is no information reported on the expression of MT-MMPs in basophils.

## ROLE OF MT-MMPs IN PATHOLOGICAL CONDITIONS

### Atherosclerosis

Atherosclerosis is a gradually developing, chronic disease with potentially devastating consequences. Acute ischemic coronary syndrome is the common final pathology as a result of unstable atherosclerotic plaque rupture. Disruption of endothelial surfaces results in exposure of underlying prothrombotic vessel walls to circulating platelets and coagulation factors. Infiltration of inflammatory monocyte-M $\Phi$ , activated T cells, and MCs in the fibrous cap and adventitia and the expression of MT-MMPs can contribute to the plaque instability. Furthermore, MT-MMPs expressed in atherosclerotic plaques may contribute to plaque disruption and vascular remodeling. It was shown that MT3-MMP is expressed in M $\Phi$ s and is present within complex human atherosclerotic plaques. Furthermore, a progressive increase in the expression of MT3-MMP in cultured M $\Phi$ s in the presence of proinflammatory molecules, such as TNF- $\alpha$  and MCP-1, has been demonstrated. These results suggest a mechanism by which inflammatory molecules could influence ECM remodeling and promote its degradation by up-regulating MT-MMPs in infiltrating leukocytes and thus, contribute to plaque destabilization and rupture [86–89].

### Diabetes

The pathogenesis of type 1 diabetes begins with the activation of autoimmune CD8 T cells, followed by their homing into

pancreatic islets and subsequent tissue damage and destruction. For this process, activated T cells need to adhere to the ENC layer and subsequently, discharge proteolytic enzymes to move through the subendothelial basement membrane. The dynamic interaction that links T cell MT1-MMP and the adhesion CD44 receptor with T cell transendothelial migration and the subsequent homing to pancreatic islets was studied. Results show that MT1-MMP plays a key role in the homing of T cells to pancreatic islets in a process that is partly dependent on the cleavage of CD44, an adhesion receptor [40, 90].

## RA

RA is a chronic, autoimmune disease that results in synovial inflammation, which eventually leads to significant cartilage destruction. Local MΦs are major mediators of this chronic inflammatory condition. Human studies done with RA tissues compared with normal synovium revealed considerable differences in the expression pattern of MT1-, MT2-, MT3-, and MT4-MMP mRNA in infiltrating MΦs. Whereas MT1- and MT3-MMP mRNAs were highly expressed, MT2- and MT4-MMP were only marginally expressed in RA tissues [91]. Normal synovial samples showed only limited staining for all MT-MMPs. As MT1-MMP has the ability to activate pro-MMP-2 and pro-MMP-9 via a MT1-MMP/MMP-13 cascade [92], these findings seem to involve MT1- and MT3-MMP, not only in matrix degradation but also in the degradation of cartilage and osteoclast-mediated bone resorption [93].

## MS

MS is characterized by the infiltration of CNS parenchyma with leukocytes that migrate across the BBB. The disruption of BBB continuity results in an influx of activated T cells and monocytes that contribute to the lesions and to the functional consequences that are characteristics of this autoimmune disease. A key aspect of MS is the degradation of MBP by T cells, a process that has been ascribed, in part, to MMP activity. Indeed, several studies reported the ability of MT-MMPs to degrade MBP [94, 95]. Although all MT-MMPs can degrade MBP, the MT6-MMP expressed in bone marrow was found to be the most efficient [96]. In mice with severe EAE induced by the adoptive transfer of MBP-reactive T cells, MT6- and

MT1-MMP were found to be up-regulated in the spinal cord, whereas other MT-MMPs were down-regulated [94]. In addition, infiltrating EAE-inducing T cell clones were found to express MT1-MMP, which is known to activate MMP-2 [97]. In humans, a higher expression of MT1-MMP and MMP2 in monocytes of MS patients was reported compared with normal individuals [53]. Collectively, these studies further support a role for MT-MMPs in MS. As MT6-MMP exhibits a restricted cell/tissue expression pattern, it has been proposed to represent a promising drug target in MS [96].

This is not an extensive overview of the role played by MT-MMPs in the physiopathology of diseases, as, for example, they were found to be involved in glomerulonephritis [98] and aneurysm [66]. Further experiments *in vitro* and *in vivo* are required to expand and define the roles of MT-MMPs in pathologies. The development of refined mouse models with tissue-specific deletion of MT-MMP will help to accomplish this task.

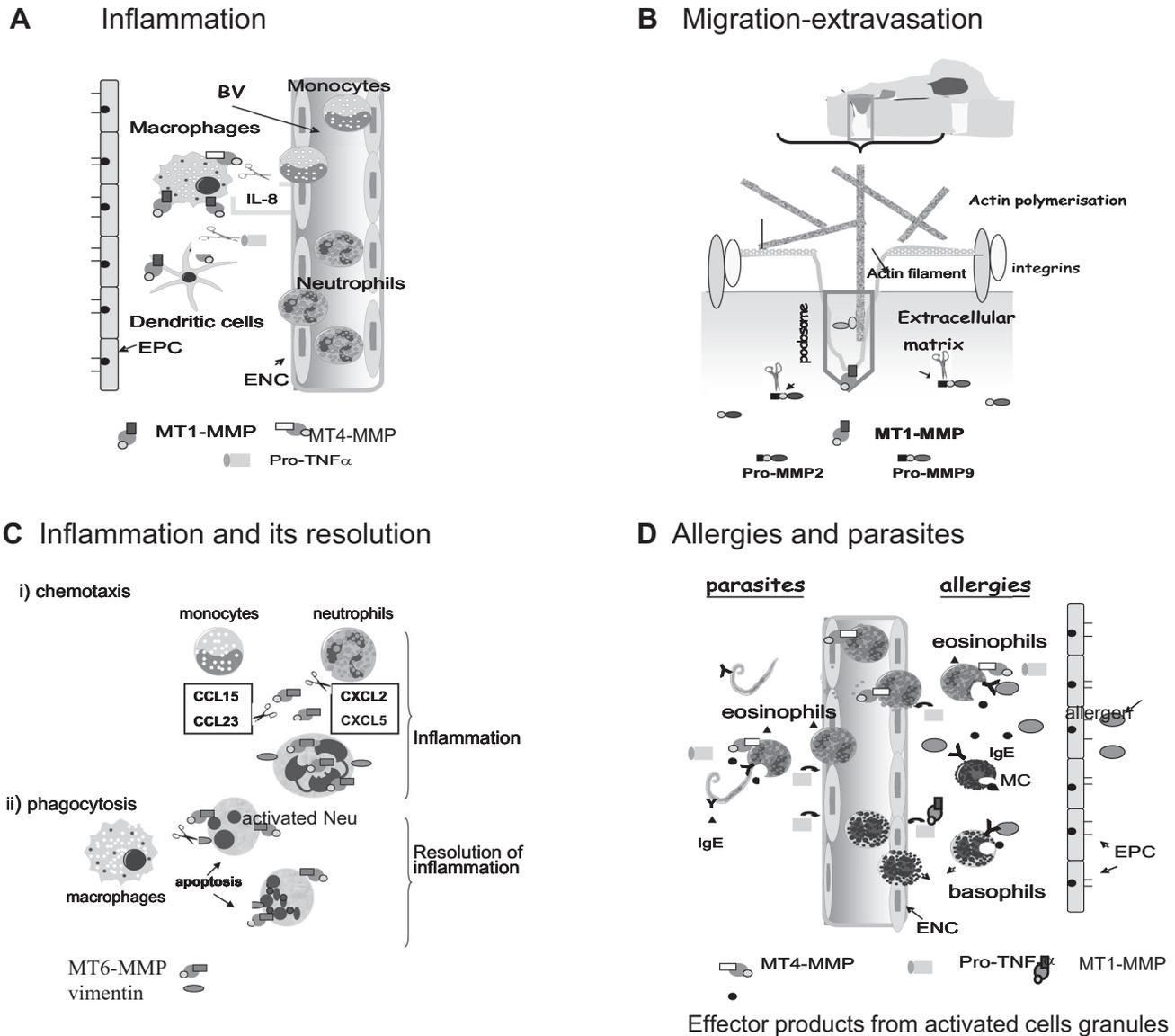
## CONCLUDING REMARKS

The members of the MT-MMP family have been reported to be expressed by different cell types of leukocytes (Table 2). In spite of this, very little information has been reported about the possible roles they could be playing. The main suggested function is related to the damage that takes place once the cells are activated and begin to perform their actions. Cells communicate with the surrounding ECM through cell-surface molecules, and therefore, pericellular degradation of the ECM influences the physiology of the cells directly, particularly those processes that depend on adhesion, followed by cell migration. MT-MMPs have been recognized as modulators of signal transfer for cell migration by degrading ECM, as well as non-ECM substrates. In this manner, the MT-MMPs are enhancing signaling for firm leukocyte adhesion and migration to inflammatory sites. Until now, the majority of the substrates reported for MT-MMPs is related to proinflammatory events and migration (Table 1). However, recent reports show that cleavage of inflammatory chemokines (CXCL12, CCL2, CCL7, and CCL13) by MT6-MMP results in fragments that are potent receptor antagonists. Moreover, the cleavage of vimentin by MT6-MMP was shown to increase the phagocytic activity MΦs, which could then clear

**TABLE 2. Expression of MT-MMPs in Leukocytes**

Cell type/protease	Mononuclear cells					Polymorphonuclear cells			
	Monocytes/MΦs	DCs	B Cells	T Cells	NKs	MCs	Neutrophils	Eosinophils	Basophils
MT1-MMP (MMP-14)	Yes <sup>a</sup> (53)	Yes <sup>a</sup> (71)	No (53)	No (53)	Yes <sup>a</sup> (74)	Yes (86)	Yes (59)	No (59)	ND
MT2-MMP (MMP-15)	Yes (59)	Yes (71)	No (53)	Yes (53)	Yes (74)	No (59)	Yes (59)	No (59)	ND
MT3-MMP (MMP-16)	No (53)	Yes (71)	No (53)	Yes (53)	Yes (76)	ND	ND	ND	ND
MT4-MMP (MMP-17)	Yes (53)	ND	Yes <sup>a</sup> (53)	No (53)	ND	ND	No (54)	Yes <sup>a</sup> (54)	ND
MT5-MMP (MMP-24)	No (53)	ND	No (53)	Yes (53)	ND	ND	ND	ND	ND
MT6-MMP (MMP-25)	Yes (53)	ND	No (53)	No (53)	Yes (76)	ND	Yes <sup>a</sup> (80)	ND	ND

YES, Positive signal; YES<sup>a</sup>, high expression; NO, negative signal; N/D, not determined or reported. Note: MCs activated by PMA express MT1-MMP; resting cells do not. NK cells activated by IL-2 express MT3 and MT6-MMP, but resting cells do not. MT3-MMP expression is reported in resident MΦs in atherosclerosis and RA, and MT1-MMP is expressed in T cells in MS.



**Figure 2. Suggested biological roles of MT-MMPs in leukocytes.** (A) Inflammation. MT1-MMP and MT4-MMP in M $\Phi$  and MT1-MMP in DCs promote inflammation by cleaving TNF- $\alpha$  and IL-8, by increasing permeabilization, and by chemotaxis of white blood cells to the site of infection. (B) Migration-extravasation. MT1-MMP present in podosomes activates gelatinases and promotes the migration of cells by digestion of ECM and endocytosis. (C) Neutrophils MT6-MMP. i) In inflammation, MT6-MMP participates in inflammation by being involved in respiratory burst and chemokine secretion. Following PMN activation by CXCL8 and IFN- $\gamma$ , MT6-MMP is secreted in an active form. MT6-MMP is able to cleave CXCL2 and CXCL5, as well as CCL23 and CCL25, to induce chemotaxis in leukocytes. ii) In the resolution of inflammation, MT6-MMP is expressed on the surface of the cells and is able to cleave vimentin. This fragment is able to direct the phagocytosis of apoptotic PMNs by M $\Phi$ s to restore homeostasis. (D) Allergies and parasites. Only MT4-MMP in eosinophils is related to the activation of TNF- $\alpha$ , whereas MT1-MMP is related to activation by PMA in MCs. No report was found correlating the presence of MT-MMPs in basophils related with the events in these pathologies. BV, Blood vessels; EPC, epithelial cells.

sites of inflammation. These show that MT6-MMP activity can cause the activation or inactivation of cytokines and other mediators, which can then modulate cellular immune responses. Despite the considerable body of literature, the roles of MT-MMPs in leukocyte functions remain largely to be uncovered (see Fig. 2 for suggested biological functions). Much work remains to be done to completely un-

cover the complex role of MT-MMP in leukocytes in vivo and ultimately, to use them as therapeutic targets.

## AUTHORSHIP

M.M., C.F., and T.F. wrote the manuscript together.

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**KEY WORDS:**  
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