

● Introduction

Activated cells of the immune system secrete various types of proteases, and many cytokines secreted by these inflammatory cells can also induce connective tissue or mesenchymal cells to express proteases (1-4). Regardless of the source, it appears that members of several classes of proteases are strongly expressed at sites of acute and chronic inflammation. Matrix metal-

consequences of inflammatory proteolysis are complex and highly relevant to the expression and regulation of dis-

conditions such as allergy and asthma. However, the importance of this expanding family of regulatory molecules suggests that their manipulation will eventually become an important means for treating these highly morbid conditions.

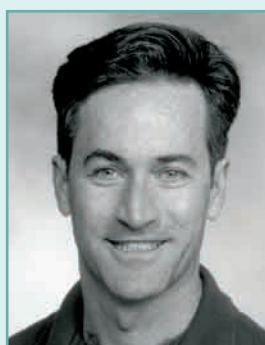


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loproteinases (MMPs) are a family of zinc dependent extracellular matrix (ECM)-degrading enzymes that are either secreted or expressed on the cell surface as latent proenzymes) which require extracellular activation and are collectively inhibited by tissue inhibitor of metalloproteinases 1-4 (TIMP1-4) (5,6). Several members of the MMP family have been found to modulate the innate and cognate immune systems through selective proteolysis of growth factors, cell adhesion molecules, cytokines, and chemokines (7-9).

Matrix metalloproteinase 2 (MMP2), also known as 72-KD gelatinase, or gelatinase A, is a protease expressed by mesenchymal cells and is secreted as a pro-proenzyme. In the pericellular and extracellular space, this zymogen is activated through formation of a trimolecular complex with MMP-14 and TIMP-2 (10). The active form of MMP2 is capable of degrading collagens I, IV, V, VII, X, gelatins, elastin, fibronectin, laminin, vitronectin, but also many other biologically active proteins such as tumor necrosis factor- α , interleukin-1 β , and growth factors (5). More recently however, MMP2 has also been shown to dampen inflammation by processing of CC chemokines to form new chemokine antagonists (8,11), or by providing a chemokine gradient to clear inflammatory cells from the lung (9). As reviewed here, the functional

eases such as asthma, and thus demand equally complex regulatory mechanisms.



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Targeted mutations in mice, combined with animal models of allergic inflammation, are also beginning to reveal functions of many members of the MMP family *in vivo*. Complicating our attempts to fully understand how MMPs function *in vivo* is the fact that new members of this already sizable family are still being discovered. In addition, the enormous degrading capability of MMPs demands a multitude of regulatory processes at pre- and post-transcriptional stages that are still only vaguely understood (12). Can we use MMP inhibitors to alter pathological outcomes during inflammation? As we will develop below, our knowledge base is currently insufficient to allow the safe targeting of these molecules with the goal of treating inflammatory

soybean trypsin inhibitor, α -1 anti-protease (α -1 anti-trypsin) for serine proteases; E-64 for cysteine proteases; pepstatin A for aspartic proteases; and EDTA, tissue inhibitor of metalloproteases (TIMPs), and 1,10-phenanthroline for metalloproteases (14). A considerable body of literature indicates that proteases, in particular serine and MMP, together with their endogenous inhibitors, take part in inflammation, wound repair, and fibrosis (15-17). However, details of how such diverse roles can be attributed to individual proteolytic enzymes are lacking.

The family of MMPs consists of over 26 secreted or membrane-bound zinc-dependent endopeptidases that have been reported in organisms ranging from protozoa to

The Role of MMPs in Allergic Airway Disease

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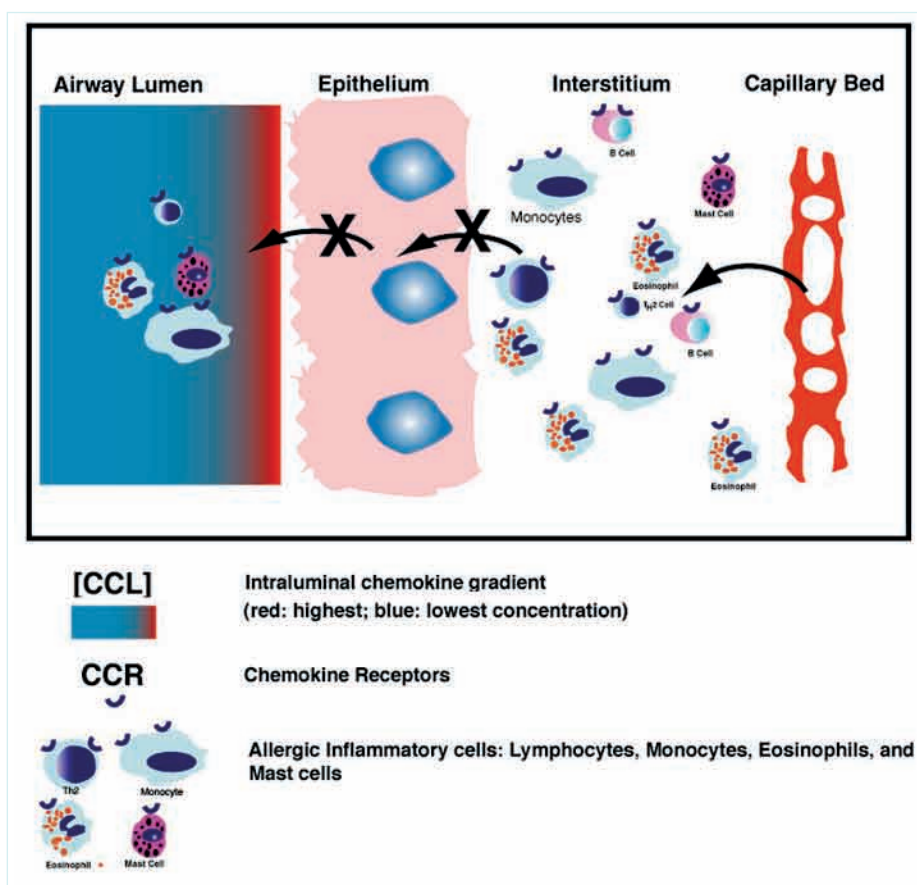


Figure 1: Schematic diagram depicting the migration (extravasation, intraparenchymal homing and transepithelial egression) of allergic inflammatory cells recruited to the lung. Cellular migration is shown progressing from right to left, with recently extravasated cells (including T cells, monocytes, eosinophils and mast cells) traversing the pulmonary interstitium and the airway epithelium to enter the airway lumen where they are cleared. Interstitial inflammatory cells are recruited to the lumen by a transepithelial chemotactic gradient in which chemokines are strongly expressed on the apical surface of epithelial cells relative to the interstitium. MMP inhibition is proposed to disrupt formation of this chemokine gradient and impair migration of cells at the points marked "X".

mammals (12). MMPs are characterized for their ability to cleave extracellular and transmembrane proteins, as well as factors that are embedded within the matrix, e.g., growth factors, chemokines, and cytokines. Several members of the MMP gene family are upregulated during allergic inflammation (18,19) and may participate in the pathogenesis of several lung diseases, especially obstructive lung diseases and asthma (9,20,21). MMPs also facilitate inflammatory cell recruitment across the endothelial basement membrane (22,23).

As part of the essential regulatory processes in the development of embryonic lung, timely expression and regulation of extracellular proteases of the MMP family are required for release of many transmembrane growth and differentiation factors, e.g., epidermal growth factors, as well as for proteolysis of ECM molecules (24,25). Such regu-

latory processes can be complex. For instance, MMP2, which is critical for alveogenesis, requires epidermal growth factor-dependent expression of MMP14 (MT1-MMP) in the epithelium and mesenchyme during lung development (25). MMP14 proteolytically activates the latent MMP2, without affecting mRNA synthesis.

While proteolysis is critical in lung development, the resurgence of proteolytic activity within the reactive stroma during chronic inflammatory processes of the mature lung is viewed as part of the irreversible tissue remodeling that can favor fibrosis (16,26,27). Thus, in order to understand lung remodeling, it is critical to understand why mediators of inflammation result in programs of gene activation that favor degradation and paradoxically fibrotic reactions.

Degrading structural proteins within the ECM, the main function of MMPs, appears quite simple and

yet the mechanism by which proteolysis alters immune cell function is decisively complex (28). For instance, elastin, a complex structural ECM in the lung that provides elasticity and resilience, when degraded by MMP2 or gelatinase B (MMP9), forms fragments that are potent chemoattractant factors for inflammatory cells (29,30). Similarly, MMPs mediate cleavage of other ECM molecules, growth factors, chemo-kines, or cytokines in inflamed tissue, which can render these factors inactive (8,11,31). However, the cleaved products from these molecules can in turn exert opposing functions (11). Thus, as we learn more about substrate and enzyme interaction *in vivo*, new and diverse functions of the MMP multigene family emerge that can affect all aspects of tissue remodeling. In the case of inflammation, there may be dual pro- and anti-inflammatory functions (11).

Most studies to date provide evidence that MMPs are present at the site of inflammation, although insight into their possible function during inflammation has proved difficult to show (32). Several interesting questions remain unanswered: What is the role of proteases in lung inflammation? What are the key proteases implicated in the pathogenesis of lung reactive stroma? Where are proteases expressed and how would we decipher their role in inflammatory reactions in the lung? The most challenging question, however, is knowledge of how MMPs could alter remodeling in chronic allergic disease when there is not yet a unified understanding of spatio-temporal regulation of MMPs under normal or pathological conditions. Specifically, several studies have detected MMPs in the epithelium of peribronchial glands and conducting airways under a variety of conditions (33-37). Others have reported expression of MMP7 (matrilysin), but not MMP1 (collagenase-1), MMP2 (gelatinase A), MMP3 (stromelysin-1), or MMP9 (gelatinase B) in the same cells (25,38,39). Furthermore, even following inflammation or acute injury, only MMP7 was upregulated in the more proximal and distal epithelial lining cells of the lung, whereas MMP1, MMP2, MMP3 and MMP9 were not upregulated)

(39). Thus, we await further research that may help to clarify some of these basic unanswered questions regarding the spatio-temporal regulation of MMPs during allergic airway inflammation.

● *ECM and other regulatory molecules as MMP substrates*

ECM degradation results in disruption and remodeling of structural barriers, which can allow inflammatory cell movement within inflamed tissue (12,40). Although this role for ECM degradation is not disputed, alternative outcomes from the proteolysis of extracellular matrix are now being entertained (28,41). Specifically, the instructive information embedded within the extracellular matrices is the target of MMP cleavage, such that release of sequestered bioactive molecules such as cytokines and chemokines, or shedding of the surface molecules such as L-selectins can alter immune cell function (13,42). MMP2, for instance, cleaves fibroblast growth factor receptor 1, a tyrosine kinase receptor, rendering it inaccessible for fibroblast growth factor binding and releasing a soluble receptor fragment of unknown biological function (43). On the other hand, MMP9 was shown to cleave the interleukin 2 (IL-2) receptor α chain on T cells, which results in downregulation of IL-2-dependent autocrine proliferation (44). The cleavage of laminin-5 by MMP2 generates new fragments that expose a normally inaccessible site to epithelial cells, resulting in their enhanced motility (31). Availability of transforming growth factor- β to carry out its biological function was also shown to depend on degradation of decorin, a small collagen-associated proteoglycan that binds and sequesters its activity (45).

Generation and inactivation of bioactive molecules during inflammation can also alter cellular function. For instance, MMPs can cleave many serine class proteases, such as plasminogen and urokinase-type plasminogen activators that are potent mitogenic factors for lung fibroblasts (46). Similarly, MMP2, MMP7 and MMP9 can cleave α 1-proteinase inhibitor, the major endogenous serine class protease inhibitor (12). This cleavage inactivates the α 1-proteinase inhibitor, and also generates a

new biologically active fragment that is a powerful neutrophil chemoattractant factor (47). Since α 1-proteinase inhibitor is a potent endogenous inhibitor of neutrophil elastase, its degradation by MMP9 may indirectly activate neutrophil elastase by removing its natural inhibitor, which results in the elastin degradation seen in inflammatory lung disease (48,49). Thus, interfering with inhibitory molecules may be a plausible mechanism by which the prominent presence of MMP9 may participate in tissue remodeling (50).

Recent screening methods have identified potential physiological substrates for MMPs. For instance, using the MMP2 C-terminal domain as bait in a yeast two-hybrid screen, monocyte chemoattractant protein-3 was identified as an MMP2 substrate (11). Furthermore, searching the protein databases for specific peptide sequences identified the prodomain of transforming growth factor β as a target for MMP cleavage (51).

● *Regulation of MMPs by Th2 cytokines*

As discussed earlier, recent studies show activated CD4⁺ T cells produce Th2 cytokines, including IL-4, IL-5, IL-10 and IL-13, in asthmatic lung (52). Despite the role of Th2 cytokines in mediating the acute asthma phenotype, the literature remains divided on their role in orchestrating a microenvironment that allows formation of a reactive stroma and progression of the sub-epithelial fibrosis that is believed to underlie chronic airway dysfunction. In contrast to the stimulation of macrophages by IL-4, IL-5, or IL-10, it has been shown that fibroblasts and chondrocytes downregulate MMPs *in vitro* (53-56). Broncho-alveolar lavage fluid from asthma patients expressing the same cytokines shows upregulated production of MMPs (57, 58). IFN- γ acts as an anti-fibrotic agent by downregulating collagen synthesis from human lung fibroblasts (59). Interestingly, clinical studies have shown an increase in IFN- γ expression following steroid treatment that corresponds with disease amelioration, although the effect on collagen synthesis was not examined (60). While only correlative, these studies nonetheless dem-

onstrate a consistent relationship between airway inflammation and airway dysfunction and suggest that cytokine manipulation may influence disease expression. However, a clear understanding of the role of MMPs and reactive stroma formation in airway hyperresponsiveness (AHR) and lung remodeling is lacking.

● *New and old functional roles of MMPs in allergic lung disease*

The diversity in the role of proteases stems from their action as part of a complex interplay between cytokines, cell surface, transmembrane, and extracellular molecules that can influence local composition of bioactive molecules (see figure). A significant advance in our understanding of how MMPs interact with ECM in the reactive stroma has come from analysis of mutational deletion of MMPs in mice. In particular, mice deficient in MMP2 when sensitized with allergens exhibited a robust allergic phenotype (9). Histopathology of lungs revealed that compared to the wild-type mice, numerous eosinophils accumulated abnormally in the lungs of MMP2^{-/-} mice concomitant with marked overexpression of mRNA for Th2 cytokines. Although excess eosinophils might account for this aberrant cytokine expression, lymphocytes produce the majority of Th2 cytokine mRNA in murine lungs following antigen challenge, and are therefore a more likely source. Thus, both activated Th2 cells and eosinophils accumulate excessively in the absence of active MMP2. MMP2-dependent cell egression may therefore be a general mechanism required for the elimination of recruited inflammatory cells. Th2 cells and eosinophils especially are strongly implicated in the pathogenesis of allergic diseases such as asthma (61,62) and their accumulation, together with their excess cytokine products, would be expected to correlate with more severe disease.

How leukocytes are cleared from lung is not fully understood, but it may proceed through one of three known routes: apoptosis and phagocytosis, lymphatic recirculation, and cell egression into the

lumen (63). Recent studies show that *in vitro* clearance of apoptotic neutrophils is mediated through phagocytosis by macrophages (64). In acute allergic airway inflammation, however, eosinophils and macrophages constitute over 80% of total recovered leukocytes (9,65).

Although allergic inflammation may be deleterious to any organ, the host is particularly susceptible to lung involvement because of the potentially lethal effects of the inflammatory exudate on gas exchange (66). In this regard, luminal clearance of inflammatory cells is a potentially important means of inflammatory cell clearance. The lung epithelial basement membrane, rather than an insurmountable barrier, is readily degraded by members of the MMP clan. Further, transmigration of IL-5-activated eosinophils through an artificial basement membrane was partially blocked by synthetic MMP inhibitors (67). Surprisingly, recent studies have demonstrated that extravasation of inflammatory cells across the endothelial basement membrane occurs independently of the MMPs required for luminal clearance. Thus, the processes of extravasation and luminal clearance, while obviously related, rely on distinct mechanisms with MMP2 participating significantly only in the latter.

● Immune-mesenchymal cross-talk: the role of MMPs in inflammation

To understand the immune-mesenchymal cross-talk that must underlie the repair phase of allergic inflammation, we investigated the role of MMP2 and its regulation in allergic airway disease. MMP2 expression in the lung is upregulated in the presence of Th2 cells and it requires the IL-4/IL-13- receptor-specific signaling chain IL-4R α (9). Furthermore, lung mesenchymal cells secrete MMP2 directly in response to stimulation by IL-13 ((9) & Corry, Kheradmand unpublished data). Mice deficient in MMP2 or treated with MMP inhibitor develop allergic lung disease and a grossly abnormal phenotype marked by the massive accumulation of inflammatory cells in the lung, a concomitant reduction in bronchoalveolar lavage

cellularity, and extreme susceptibility to lethal asphyxiation (9). This compartmental derangement of recruited inflammatory cells is accompanied by decreased chemotactic activity within the airway and reduced secretion into the airway lumen of selected chemokines. Therefore, during allergic inflammation MMP2 establishes a transepithelial chemokine gradient that is required for cellular egression into the airway lumen, a mechanism that is required to prevent the lethal effects of the inflammatory exudate on gas exchange. IL-13 and IL-4R α , although required to initiate the asthma phenotype and associated inflammation, also function to resolve allergic inflammation by directly eliciting MMP2 secretion from mesenchymal cells. In promoting the clearance of inflammatory cells through the airway lumen, MMP2 therefore serves a novel, beneficial role and represents an essential link in an IL-13/IL-4R α -dependent regulatory loop that dampens inflammation.

● Conclusions

The ongoing search for potential targets of ECM-degrading enzymes promises a better understanding of how MMPs may alter cell function. Although many MMPs share similar substrate profiles, differential expression and mechanisms that control activation of these enzymes provide clues as to their specific roles in lung inflammation and repair. Specifically, regulatory steps that ensure proteolytic activity against components of the basement membrane will also potentiate ECM remodeling by generating several different biologically active molecules, such as chemokines and fibronectin fragments that will alter immune cell function.

Among the major tasks that lie ahead is to determine the transcriptional regulation of the cytokines and chemokines that is needed as part of the inflammatory regulatory loop required for clearance of the recruited inflammatory cells. While excess production of proteases during inflammation may indeed be a necessary phase of the repair process, identification of novel, functional genetic variants in the human MMP gene family may

be a plausible explanation for genetic predisposition to over- or underproduction of MMPs. Knowledge gained from studies of animal models of allergic inflammation will provide the insight into MMP function that is required to determine the utility of proteases and protease inhibitors in the clinical management of diseases such as asthma.

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