Metalloproteinases as modulators of allergic asthma: therapeutic perspectives

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Abstract: Matrix metalloproteinases (MMPs) are proteolytic enzymes that facilitate extracellular matrix turnover; thus, these enzymes are important in both normal wound repair and pathologic processes, such as inflammation and fibrosis. In addition, MMPs regulate cell signaling through proteolytic shedding of bioactive growth factors, cytokines, and other molecules. Therefore, the expression and activation of MMPs and their endogenous inhibitors, tissue inhibitors of metalloproteinases, play roles in the development of a variety of diseases. Allergic asthma is a chronic inflammatory disease of the airways that affects millions of patients, both children and adults, worldwide. Allergen-induced airway injury and immune responses stimulate airway inflammation, hyperresponsiveness, and remodeling in these patients. The action of MMPs links these pathologic processes through a complex interaction of airway inflammatory cells and structural cells. This review explores the important roles of MMPs and tissue inhibitors of metalloproteinases in mediating the pathobiology of allergic asthma and highlights the potential for these enzymes to serve as biomarkers or therapeutic targets in allergic asthma.

Keywords: matrix metalloproteinase, MMP, asthma, allergy, airway

Introduction

Asthma is a chronic inflammatory disease that is estimated to affect 334 million adults and children worldwide, with nearly 14% of the world's children experiencing symptoms associated with asthma.[1] These symptoms include wheezing, chest tightness, and coughing, with some patients experiencing diminished lung function over time, resulting in substantially reduced quality of life.[1] The primary long-term therapy for asthma is inhaled corticosteroids, sometimes combined with long-acting beta-agonists and oral corticosteroids in the most severe asthma patients.[2] However, not all therapies are beneficial for all asthma patients. Asthma is a heterogeneous disease with different causes, manifestations, and severities. Much recent research has allowed for classification of the disease into various subphenotypes based upon clinical criteria, biomarkers, and genetic and molecular profiles.[3] Therefore, more personalized therapies tailored for specific subphenotypes of asthma are currently being developed that may provide benefits where other, more standard therapies are not efficacious.

The most common and best-understood subphenotype of asthma, allergic or type 2 asthma, is characterized by a pathologic airway immune response in atopic individuals resulting from exposure to inhaled allergens. This response involves many different cell types and a complex cascade of cell signaling events, contributing to chronic airway inflammation, airways hyperresponsiveness (AHR), and remodeling. The allergic

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cascade in asthma has been reviewed elsewhere.[4,5] To briefly summarize here, allergic asthma is characterized by an early asthmatic response (EAR), which occurs immediately upon inhaled allergen exposure and lasts 30–60 minutes, and a late asthmatic response (LAR), which manifests several hours after exposure.[6] In the EAR, repeated exposure to a sensitizing allergen triggers recognition by specific type 2 cells such as T-helper 2 lymphocytes and innate lymphoid cells, which initiate secretion of type 2 cytokines (eg, interleukins [ILs]-4, -5, and -13) and stimulates immunoglobulin (Ig) E isotype switching in B-cells.[7] Binding of allergen to specific IgE activates basophils and mast cells to release histamine, prostaglandins, leukotrienes, and other inflammatory mediators.[8,9] Allergen exposure also promotes airway inflammation in the LAR by disrupting the protective airway epithelium layer and increasing permeability between epithelial cells.[10–12] Allergen-induced damage and triggering of epithelial cell pattern recognition receptors lead to mucous secretion[13] and production of IL-33,[14] IL-25,[15] and thymic stromal lymphopoietin,[16] which activate dendritic cells and promote release of type 2 cytokines from innate lymphoid cells.[17] Type 2 cytokines direct inflammation in the airway by promoting the survival and migration of eosinophils,[18] activation of macrophages,[19] and production of inducible nitric oxide synthase by airway epithelial cells.[20,21]

Ongoing inflammation contributes to AHR in asthma. Variable or episodic AHR results from acute allergen exposure and the effects of airway inflammation.[22] IgE-mediated release of type 2 cytokines,[23–25] leukotrienes,[26,27] serine proteases,[28] histamine,[26] and complement cleavage products[29] by mast cells and other inflammatory cells in the airway has been linked to acute onset of AHR following allergen challenge in both human and animal models. In addition to variable AHR, which is related directly to allergen exposure, a second component of AHR in asthma, persistent or baseline AHR, is present in most patients with asthma and is associated with structural alterations of the airways, termed airway remodeling.[22,30]

Airway remodeling is thought to result from chronic inflammation in the airways of atopic asthmatics, which involves mucous cell metaplasia[31] and mucous hypersecretion,[23,32] thickening of the airway smooth muscle layer,[33,34] and an impaired wound healing response that results in increased airway fibroblast proliferation,[35] invasion,[36] and transformation of airway fibroblasts to myofibroblasts,[37,38] leading to subepithelial collagen deposition.[39,40] These permanent structural changes in the airway result in decreased airway luminal area[41] and irreversible airway obstruction[42] and is associated with persistent AHR.[43,44]

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that play key roles in both normal processes, such as wound healing,[45,46] and pathogenic processes, such as inflammation[47] or fibrosis.[48] Although MMPs are well known for their ability to degrade extracellular matrix (ECM), these enzymes also serve to regulate cell signaling through the cleavage and processing of bioactive molecules. MMPs are secreted by a number of cell types in the allergic asthmatic airway, including both inflammatory cells, such as macrophages[49] and leukocytes,[49–51] and airway structural cells, such as airway fibroblasts,[36,39] epithelial cells,[52,53] and smooth muscle cells.[54] Expression and activity of MMPs are tightly regulated at the transcriptional and post-translational levels and by the action of endogenous inhibitors of MMPs, the tissue inhibitors of metalloproteinase (TIMPs). Thus, the relative ratios of MMPs to TIMPs play a role in the pathobiology of various diseases.[55,56]

This review will focus on the functions of specific MMPs and TIMPs in each of the pathologic processes of allergic asthma and explore the potential of MMPs to serve as therapeutic targets or biomarkers in allergic asthma.

MMPs in airway inflammation in allergic asthma

Both acute and chronic airway inflammation play major roles in the pathobiology of allergic asthma. Upon stimulation with inhaled allergen, inflammatory cells are activated to migrate from the circulation to sites of injury in the airway wall and lumen. Much research has focused on the role of MMPs in facilitating this extravasation of inflammatory cells across the vascular and airway membranes. Cells that are key to the allergic inflammatory response secrete a variety of MMPs with diverse functions (Table 1), and these enzymes act to proteolytically degrade ECM to promote cellular migration to sites of inflammation in the airway. Ohno et al first demonstrated that *MMP9* mRNA expression was significantly increased in the submucosal regions of airway biopsy tissue of patients with asthma as compared to normal subjects. They further localized the major source of expression of *MMP9* in asthma patients' tissue to airway eosinophils,[57] although other studies have since shown that neutrophils also secrete MMP-9 following allergen challenge.[50,51] In the follow-up studies, the same group used a murine model of allergic airways disease to determine that inhaled allergen stimulated significantly increased airway secretion of gelatinases, including MMP-9, and this effect was associated with an

Table 1 Airway secretion of MMPs and their functions in asthma pathobiology

MMP	Airway cell type	Function	Model	Reference
MMP-9	Eosinophils	Cell migration; recruitment; resolution of inflammation	Murine – ovalbumin challenge	58,59
	Neutrophils	Cellular migration; recruitment; tissue fibrosis; resolution of inflammation; Activation of pro-MMP9; AHR; Overnight decrement in FEV$_1$; elastolysis	Human segmental allergen challenge; nocturnal asthma Murine – cockroach antigen challenge Murine or guinea pig ovalbumin challenge MMP9–/– mice – acute ovalbumin or *Aspergillus* challenge	51,61,66,67, 74,92,93,96
	Epithelial cells	Activation of pro-MMP-9	Murine – cockroach antigen challenge	72
	T-cells	Regulation of smooth muscle cell proliferation	Brown Norway rat – ovalbumin challenge	89
MMP-2	Mesenchymal cells	Eosinophil migration; resolution of inflammation; AHR	MMP2–/– and MMP9/2 double knockout mice with *Aspergillus* challenge	58,59,67,68
	Fibroblasts	Mediate IL-13-stimulated cellular invasion, collagen production, and suppression of elastin mRNA expression	Primary asthmatic human airway fibroblasts in culture	36,39,85,90
MMP-7	Epithelial cells	Cleavage of IL-25; induction of type 2 cytokines	Murine – *Aspergillus* challenge Human – ragweed intranasal challenge	53
MMP-8	Neutrophils	Cleavage of membrane-bound IL-13Rα2	Murine – house dust mite challenge	79
MMP-1	Smooth muscle cells	Mediate IL-13-stimulated contraction Regulate cell proliferation – cleavage of IGFBP-2, IGFBP-3	Bovine tracheal smooth muscle cells in culture Asthmatic human airway biopsy samples; rat model of ovalbumin challenge	54,81,82,88
	Fibroblasts	Mediate IL-13-stimulated suppression of elastin mRNA expression	Primary asthmatic human airway fibroblasts in culture	36,39,85
MMP-12	Macrophages	Induced by type 2 cytokines; regulates inflammatory cell influx to airway; AHR Associated with elastin fiber staining in airway tissue	Murine models of ovalbumin and cockroach antigen challenge; sheep model of *Ascaris* challenge Airway biopsy tissue from fatal asthma patients	75–77,91
MMP-25	Neutrophils	Serum concentrations associated with serum IgE levels	Atopic asthma patients	106

Abbreviations: MMP, matrix metalloproteinase; IL, interleukin; IL-13Rα2, IL-13 receptor alpha 2; FEV$_1$, forced expiratory volume in 1 second; mRNA, messenger RNA; IGFBP, insulin-like growth factor binding protein; AHR, airways hyperresponsiveness; IgE, immunoglobulin E.

accumulation of eosinophils in bronchoalveolar lavage (BAL) fluid from ovalbumin-challenged animals. Administration of recombinant TIMP-2 prior to allergen challenge significantly reduced the numbers of inflammatory cells in BAL fluid, the numbers of eosinophils in the airway lumen and wall, and the degradation of type IV collagen and other ultrastructural effects of basement membrane injury, suggesting that MMP-9 and MMP-2 were important mediators of eosinophil infiltration of the airway wall in asthma.[58,59] Schwingshackl et al[60] extended these studies by demonstrating that addition of tumor necrosis factor-alpha (TNF-α), a proinflammatory cytokine, to human peripheral blood eosinophils, cultured from atopic asthmatic patients, stimulated a 95% increase in MMP-9 activity above baseline. Similarly, studies by Kelly et al[51] and Mattos et al[61] showed that airway secretion of MMP-9 significantly increased 24–48 hours following segmental allergen challenge of allergic patients with asthma, as compared to saline treatments; however, the immunore-

activity of MMP-9 in airway cells was predominantly associated with neutrophils, not eosinophils in the Kelly et al's study.[51] Furthermore, the concentration of MMP-9 and the MMP-9/TIMP-1 ratio in induced sputum at baseline was significantly increased in severe asthma patients as compared to healthy controls and patients with mild asthma, but the levels of MMP-9 did not correlate with numbers of sputum eosinophils.[61] Similarly, a study of atopic preschool children showed that MMP-9 protein levels in BAL fluid were significantly increased in wheezers as compared to healthy controls, and this MMP-9 concentration was significantly and positively correlated with numbers of neutrophils in the BAL fluid. Follow-up studies 23 months after the initial bronchoscopy identified which of the children were persistent wheezers and determined that TIMP-1 protein levels in the initial BAL fluid were significantly elevated in the persistent wheezing children as compared to children who had outgrown wheezing.[62] In contrast to these reports, one

study found that MMP-9 protein and activity levels as well as MMP-9/TIMP-1 ratios were significantly reduced in BAL fluid collected from 34 atopic asthmatic children as compared to 35 nonatopic healthy children and that the MMP-9 levels were significantly associated with numbers of neutrophils in the BAL fluid, which were reduced in the atopic asthma patients.[63] However, the majority of studies have shown MMP-9 is released by inflammatory cells in response to allergen provocation in human asthma.

Studies of allergen-induced airway disease in *MMP9*- and *MMP2*-deficient mice have revealed a role of these gelatinases in airway inflammation in asthma. Cataldo et al[64] reported significantly decreased peribronchiolar inflammation, reduced numbers of lymphocytes, and reduced secretion of IL-13 in BAL fluid of *MMP9*-deficient mice as compared to wild type following acute ovalbumin challenge. Furthermore, allergen-challenged mice that are deficient for *TIMP1*, an endogenous inhibitor of MMP-9, MMP-2, and MMP-12, exhibited significantly enhanced peribronchiolar inflammation and increased lung type 2 cytokine expression associated with significantly increased lung MMP-9 activity, suggesting that TIMP-1 regulates allergen-induced inflammation in asthma by inhibiting MMP-9 activity.[65] In contrast, a subsequent study of *MMP9*-deficient mice using a similar model of acute ovalbumin challenge demonstrated significantly increased airway eosinophils, lymphocytes, and macrophages, as well as secretion of type 2 cytokines and eotaxin in both BAL fluid and lung tissue, and increased peribronchiolar inflammation in the *MMP9*-deficient mice compared to wild type. Further investigation showed that resolution of inflammation was impaired in the *MMP9*-deficient mice as compared to wild type, as concentrations of eotaxin in BAL fluid and numbers of T-helper 2 lymphocytes in the lungs of *MMP9*-deficient mice were still significantly elevated as compared to wild type 6 days after the last ovalbumin challenge.[66] In addition, Corry et al demonstrated that MMP-2 and MMP-9 expression and secretion in murine BAL fluid and lung tissue were significantly increased following allergen challenge and that IL-13 alone was sufficient to induce this effect. *MMP2* was expressed primarily by mesenchymal cells, and *MMP9* was expressed primarily by inflammatory cells. Challenge with *Aspergillus fumigatus* allergen revealed significantly decreased eosinophils in BAL fluid but significantly increased inflammatory cells and type 2 cytokines in the lung tissue of *MMP2*- or *MMP9*-deficient mice as compared to wild type. Measurement of chemokines in BAL fluid showed significantly reduced eotaxin, CCL7 (MARC), and CCL17 (TARC [thymus and activation related chemokine]) chemokines suggesting an abnormal

chemotactic gradient that impaired egression of inflammatory cells to the airway lumen.[67–69] Dendritic cell trafficking to the airway lumen was also impaired in *MMP9*-deficient mice.[69] The considerable accumulation of inflammatory cells in the lung tissue contributed to increased mortality of the *MMP2*-deficient mice due to asphyxiation[68] and increased numbers of dead and apoptotic cells in *MMP9*-deficient mice.[67] Likewise, ovalbumin challenge of mice with targeted overexpression of *MMP9* in tissue macrophages resulted in reduced levels of IL-13 in serum, decreased numbers of lymphocytes in BAL fluid, but increased numbers of lymphocytes in draining lymph nodes, suggesting that MMP-9 was involved in lymphocyte trafficking.[70] Study of double *MMP2/MMP9* knockout mice provided no additive effect on the *MMP9*-deficiency-alone phenotype, suggesting that MMP-9 has the primary role in regulating inflammatory cell egression from lung tissue to the airway lumen. MMP-2 has an overlapping role that is specific for eosinophilia.[67] Finally, Greenlee et al[71] identified three substrates of MMP-2 and MMP-9 in the BAL fluid of allergic mice, Ym1, S100A8, and S100A9. These proteins serve as chemokines for inflammatory cells, and cleavage of these proteins provides a potential mechanism to explain the impaired chemotactic responses observed in *MMP2*- and *MMP9*-deficient mice. Taken together, these studies, although somewhat contradictory, show that MMP-9 and MMP-2 are key mediators of inflammatory cell egression in allergic airways disease and may have an influence on type 2 cytokine expression.

Allergens with protease activity, such as tree pollen, fungi (*A. fumigatus*), cat, house dust mite, and cockroach allergens, are clinically important as allergic asthma triggers and have specific effects on activation of MMPs. Page et al[72] found that serine protease activity in German cockroach frass augmented TNF-α-induced *MMP9* expression by bronchial epithelial cells and promoted cleavage of the pro-MMP-9 into the active form of MMP-9.[73] This group further showed that German cockroach frass induced *MMP9* expression in a murine model of allergic airways disease and that release of MMP-9 from neutrophils was mediated by a toll-like receptor 2 (TLR2) ligand in frass. Also, when challenged with German cockroach frass, *MMP9*-deficient mice exhibited significantly increased airway eosinophils but reduced airway neutrophils, increased type 2 cytokines in the lung, and increased serum IgE levels as compared to saline controls and wild-type animals. These data suggested that TLR2 agonists in complex allergens may serve to protect against type 2 airway responses in asthma by mediating release of MMP-9 on neutrophils.[74] Considering the

overlapping functions of MMP-9 and MMP-2 as reported by Corry et al,[67] it is plausible to consider that MMP-2 is compensating for the lack of MMP-9 in this model to stimulate eosinophilic inflammation in response to the cockroach allergen.

Murine models of ovalbumin, *Ascaris*, or cockroach antigen-induced allergic airways disease were used to assess the role of macrophage-derived metalloelastase (MMP-12) in allergic inflammation in asthma. *MMP12* mRNA, protein, and activity were upregulated in airway tissues following allergen challenge.[52,75] Pouladi et al demonstrated reduced numbers of eosinophils in BAL fluid, but not in tissue, in *MMP12*-deficient mice as compared to wild-type mice following ovalbumin challenge. Also, *MMP12* mRNA expression and eosinophilia were significantly reduced in IL-13-deficient mice, suggesting that IL-13 stimulated *MMP12* expression in response to allergen challenge.[75] Warner et al reported that mice deficient for *MMP12* exhibited significantly reduced airway and peribronchiolar inflammatory cell infiltration and reduced BAL fluid levels of proinflammatory cytokines and chemokines, TNF-α, IL-5, and TARC, as compared to *MMP12* replete animals following repeated challenges with cockroach antigen. Levels of MMP-9 and MMP-2 in BAL fluid were significantly induced by cockroach antigen in the *MMP12*-expressing animals, but this effect was not observed in the *MMP12*-deficient mice.[76] Additionally, administration of an MMP-12-specific inhibitor (MMP408) to *Ascaris*-challenged sheep resulted in significantly fewer inflammatory cells in BAL fluid, suggesting that the inhibitor blocked the influx of inflammatory cells to the airway.[77] Thus, MMP-12 may be regulated by type 2 cytokines and act to direct inflammatory cell responses, which effects release of MMP-9 and MMP-2 in the airway.

Proteolytic allergens directly damage airway epithelium, and Goswami et al demonstrated that *Aspergillus* allergen strongly upregulated *MMP7* mRNA expression in airway epithelial cells and that MMP-7 activity cleaved IL-25, leading to enhanced type 2 responses. Mice deficient for *MMP7* exhibited significantly reduced airway eosinophilia and reduced concentrations of IL-4, IL-5, IL-13, and eotaxin in BAL fluid and reduced *IL25* mRNA in the lung tissue. Moreover, MMP-7 expression was significantly increased in airway biopsy tissue from patients with asthma as compared to healthy patients, and intranasal challenge with another proteolytic allergen, ragweed, resulted in increased MMP-7 secretion as compared to saline controls.[53] Therefore, allergen-activated MMP-7 may act to stimulate type 2 cytokine responses in asthma.

MMP-8 also plays a role in the regulation of type 2 cytokine activity in allergic asthma. Chen et al[78] demonstrated that levels of soluble IL-13 receptor alpha 2 (IL-13Rα2), a decoy receptor and a negative regulator of IL-13,[79] were increased in both serum and BAL fluid of house dust mite allergen-challenged mice. MMP-8 was found to be the enzyme that specifically cleaves IL-13Rα2 from mouse and human cells, and levels of soluble IL-13Rα2 in BAL fluid were attenuated in house dust mite allergen-challenged *MMP8*-deficient mice as compared to wild-type mice.[78] Gueders et al[80] also found increased numbers of eosinophils in airway walls and increased IL-4 concentration in BAL fluid of ovalbumin-challenged *MMP8*-deficient mice. These data suggest that MMP-8 acts to regulate type 2 cytokine signaling by shedding membrane-bound IL-13Rα2 to increase the soluble IL-13Rα2 concentration in the airway that binds to IL-13 with high affinity and sequester IL-13 from its signaling receptor subunits. Thus, allergen induction of MMPs in airway epithelium (MMP-7), neutrophils (MMP-9/MMP-8), macrophages (MMP-12), or even mesenchymal cells (MMP-2) contributes to airway inflammation in asthma, either through modulation of type 2 cytokine production and signaling or through regulation of leukocyte egress and infiltration of airway tissues.

Contribution of MMPs to AHR in allergic asthma

AHR is a hallmark feature of allergic asthma, but the development of AHR is linked to airway inflammation and remodeling, so the effects on either of the latter processes in asthma may also affect AHR. In addition, much of the current knowledge of the role that MMPs play in the development and persistence of AHR in asthma is derived from animal models of allergic airways disease, which makes overall interpretation of the role of these enzymes in human AHR difficult since these reports use models that utilize several different allergens and measure airways reactivity using different bronchoconstrictors and systems. One of the first studies to provide data on MMPs and AHR reported that administration of TIMP-2 to mice prior to ovalbumin challenge significantly attenuated methacholine-induced airways reactivity as compared to vehicle control-treated mice.[59] These data suggested that MMP-2 plays a role in AHR in asthma. Similarly, Sands et al[65] showed that methacholine-induced airways reactivity was enhanced in ovalbumin-challenged *TIMP1*-deficient mice as compared to saline control-treated mice, and these responses were accompanied by significantly increased *MMP9* expression

in lung homogenates. However, in ovalbumin-challenged mice with targeted overexpression of *MMP9* in tissue macrophages, methacholine-induced airways resistance was attenuated as compared to wild-type mice.[70] And, in ovalbumin-challenged *MMP9*-deficient mice, methacholine-induced airways reactivity was enhanced.[66] These effects of MMP-9 on AHR are likely associated with the role that MMP-9 plays in airway inflammatory cell egression and resolution of inflammation as described earlier.

The development of AHR in both *MMP8*- and *MMP7*-deficient mice is likely related to the reported effects of each of these enzymes on IL-13 production and signaling. In *A. fumigatus* allergen-challenged *MMP7*-deficient mice, IL-13 levels in BAL fluid were reduced, and acetylcholine-induced airway resistance was significantly lower than wild-type mice.[53] In contrast, house dust mite allergen-challenged *MMP8*-deficient mice exhibited increased acetylcholine-induced airway resistance as compared to wild-type mice,[79] suggesting that these enzymes modulate IL-13 signaling to regulate AHR in allergic asthma.

An *Ascaris*-challenged sheep model was used by Mukhopadhyay et al to evaluate the effectiveness of MMP408, an MMP-12 inhibitor, on airway resistance and carbachol-induced reactivity. Pretreatment of either intravenous or orally administered inhibitor prior to allergen challenge significantly abrogated both early-phase and late-phase allergic airway resistance and AHR.[77]

More evidence of the contribution of MMPs to AHR in allergic asthma may be derived from the effects of MMPs on airway smooth muscle contraction in cell culture models. Several studies have demonstrated the importance of MMP-1, a collagenase, in airway smooth muscle cell contraction. Ohta et al demonstrated that IL-4 and IL-13 stimulate *MMP1* expression in bovine tracheal smooth muscle cells but have opposing effects on contraction of collagen gels containing smooth muscle cells, with IL-4 inhibiting contraction and IL-13 stimulating contraction. Both of these effects were mediated by MMP-1, and the authors provided evidence of changes in the collagen fiber structure surrounding the smooth muscle cells in response to either IL-4 or IL-13, suggesting that MMP-1 acts to either degrade or reorganize collagen/smooth muscle cell interactions to modulate contraction responses to each cytokine.[54] Similarly, Rogers et al found that ECM proteins, collagen type I and tenascin-C, stimulated significantly increased *MMP1* expression by airway smooth muscle cells in asthma as compared to cells from healthy patients. MMP-1 and tenascin-C co-localized to airway smooth muscle cells in biopsy tissue from asthma

patients, but not in tissue from healthy control patients. Furthermore, pretreatment of airway smooth muscle cells with a broad-spectrum MMP inhibitor, GM6001, or small interfering RNA specific for *MMP1* significantly reduced agonist-stimulated gel contraction, suggesting that matrix-induced MMP-1 mediates airway smooth muscle contraction in asthma.[81] In addition, Margulis et al demonstrated that human airway smooth muscle cells co-cultured with human mast cells in collagen gels undergo increased contraction in response to IgE cross-linking or IL-13 treatment and that this effect was dependent on MMPs. Activation of MMP-1 and MMP-2 by serine proteases was implicated in the mast cell-directed smooth muscle cell contraction.[82] Taken together, these studies highlight the actions of MMP-1 to stimulate airway smooth muscle contraction and implicate a role for this collagenase in AHR in the setting of type 2 cytokine-driven inflammation and allergen-induced asthma.

To this end, Bruce and Thomas published results of a small pilot clinical trial to investigate the effects of marimastat (British Biotech, Oxford, UK), a broad-spectrum MMP inhibitor, in nine steroid-naïve patients with mild atopic asthma. In this randomized, double-blind crossover trial, patients received a placebo twice daily for 3 weeks separated by a 6-week washout period, with allergen challenges at the beginning and end of each treatment phase. The authors found that house dust mite allergen-provoked airways hyperreactivity was significantly reduced in patients receiving marimastat as compared to placebo control, but no significant changes were observed with regard to effects of marimastat on airway inflammation, forced expiratory volume in 1 second (FEV_1), or asthma symptoms as compared to placebo control.[83] Although this trial was very small, the effect of MMP inhibition on AHR is somewhat promising and merits further investigation to determine if inhibition of specific MMPs, particularly MMP-1, may serve as therapies for airway inflammation or AHR in allergic asthma.

MMPs in airway remodeling in allergic asthma

Key cellular features of airway remodeling in allergic asthma include mucous cell metaplasia, airway smooth muscle cell hyperplasia and hypertrophy, airway fibroblast proliferation, invasion, and differentiation to a myofibroblast phenotype, and deposition of collagen and other ECM components in the submucosal region of the airway. IL-13 is a type 2 cytokine that directs most of these processes in allergic asthma. Elias et al, who developed mice with inducible lung-targeted overexpression of IL-13, elegantly

demonstrated the effects of IL-13 on airway remodeling and inflammation. They found that IL-13 overexpression alone was sufficient to induce most of the features of allergic asthma seen in human patients and other murine models of allergen challenge, but they also observed an emphysema-like phenotype. A characteristic of these IL-13 transgenic mice significantly increased *MMP9*, *MMP12*, *MMP2*, *MMP13*, *MMP14*, and *TIMP1* mRNA expression and MMP-9, MMP-12, and MMP-2 activity in the lung tissue as compared to nontransgenic animals. Furthermore, treatment of the IL-13 transgenic mice with GM6001 significantly reduced lung and alveolar size, reduced total numbers of cells in BAL fluid, and reduced numbers of eosinophils and lymphocytes in BAL fluid by >90%; however, GM6001 had no effect on IL-13-induced mucous cell metaplasia.[84] Taken together, this study demonstrated that MMP expression and activity mediated the IL-13-induced emphysema and effects on airway inflammation and remodeling.

Reports focusing on airway remodeling in human allergic asthma have shown that IL-13 stimulates significantly increased MMP-1 and MMP-2 protein and activity in airway fibroblasts derived from patients with mild allergic asthma as compared to healthy controls.[39,85] Treatment of airway fibroblasts derived from patients with asthma with GM6001 significantly reduced IL-13-induced airway fibroblast invasion, a process implicated in proteolytic penetration of the airway basement membrane and submucosa.[36] In addition to this direct role in degrading ECM, MMP-1 and MMP-2 were found to mediate IL-13 effects on ECM gene expression by airway fibroblasts in allergic asthma. Firszt et al showed that the action of MMP-2 was required for IL-13-induced *COL1A2* gene expression and collagen type I protein expression by airway fibroblasts in asthma. Furthermore, inhibition of MMP-2 blocked IL-13-induced activation of the profibrotic cytokine, transforming growth factor-β1 (TGFβ1), in airway fibroblasts in allergic asthma,[39] suggesting that MMP-2 is required for IL-13-induced processing of TGFβ1 from a latent form, bound to latency-associated protein, to the dissociated and active form of TGFβ1. Thus, MMP-2 serves to regulate *COL1A2* gene expression and collagen production by activating an upstream mediator. Similarly, Ingram et al found that both MMP-1 and MMP-2 mediate IL-13-induced suppression of elastin (*ELN*) gene expression by airway fibroblasts in allergic asthma, which is associated with loss of elastic fiber staining in patients with increased AHR. Treatment of airway fibroblasts with GM6001 or inhibitors specific for MMP-1 or MMP-2 in combination with IL-13 significantly

increased *ELN* gene expression as compared to IL-13 treatment alone.[85] Regulation of *ELN* gene expression may be related to proteolytic shedding of heparin-binding epidermal growth factor (HB-EGF), which has been shown to downregulate *ELN* mRNA expression in pulmonary fibroblasts.[86,87] Therefore, MMP-1 and MMP-2 play roles in mediating IL-13 effects on ECM degradation as well as modulation of gene expression to direct airway remodeling in allergic asthma.

A similar role for MMP-1 was reported by Rajah et al, in which they demonstrated that airway smooth muscle cell hyperplasia in allergic asthma was regulated by MMP-1-mediated proteolysis of insulin-like growth factor binding proteins (IGFBPs). These proteins suppress airway smooth muscle cell growth in healthy subjects, but in the setting of allergic asthma, increased secretion of MMP-1 by airway smooth muscle cells leads to increased cleavage of IGFBP-2 and IGFBP-3, rendering them inactive in the regulation of cell proliferation.[88] In addition, Al Heialy et al demonstrated that allergen-stimulated CD4+ T-cells adhere to airway smooth muscle cells and stimulate cell proliferation. The mechanism for this CD4+ T-cell-mediated mitogenic effect involves allergen-stimulated release of HB-EGF from the cell surface of the T-cells by MMP-9. The shed HB-EGF then activates epidermal growth factor receptor on the surface of airway smooth muscle cells to stimulate proliferation.[89] Thus, MMPs regulate both airway fibrosis and airway wall thickening in allergic asthma by directly modulating the action of growth factors and cell signaling.

Direct effects of both structural cell-derived and inflammatory cell-derived MMPs on airway remodeling through ECM proteolysis are well documented. Activity of MMP-2 and MMP-3, but not MMP-1, MMP-9, or TIMP-1, was significantly increased in bronchial fibroblasts from patients with asthma as compared to cells derived from healthy patients. These MMP-2 and MMP-3 activity levels were also found to be significantly and positively correlated with procollagen I and proteoglycan levels secreted from fibroblasts as well as negatively correlated with FEV_1 in asthma patients.[90] Araujo et al found that the fractional area of elastic fibers present in airway smooth muscle in large and small airway biopsy tissue from patients with fatal asthma was significantly increased as compared to cases of nonfatal asthma and nonasthma controls. The fractional areas of MMP-9-positive immunostaining within the airway smooth muscle were also increased in fatal asthma as compared to nonasthma controls, and MMP-12 staining significantly and positively correlated with elastic fiber staining and age of asthma onset in the fatal asthma patients.[91]

These data suggest that MMP-2, -3, and -12 participate in abnormal ECM turnover and degradation in asthma.

MMP-9 is also implicated in airway remodeling in allergic asthma. Tang et al reported significantly increased positive staining for MMP-9 in the lung tissue of ovalbumin-challenged guinea pigs as compared to saline-challenged controls. The positive staining was observed in both inflammatory cells and structural cells in the lung tissue.[92] Allergen-challenged *MMP9*-deficient mice exhibited significantly reduced peribronchiolar fibrosis and total lung collagen as compared to allergen-challenged wild-type mice.[93] Airway vascular wall remodeling in a model of allergen-induced guinea pig airways disease was also mediated by MMP-9. Percent collagen and elastic fibers were significantly increased in ovalbumin-challenged guinea pigs as compared to saline controls, but treatment with 1400W, a specific inhibitor of inducible nitric oxide synthase, significantly reduced this response as well as reduced immunostaining of MMP-9, TIMP-1, and TGFβ1 in airway vascular wall.[94] In human allergic asthma, MMP-9 activity and secretion in BAL fluid were significantly increased in asthma patients with mucus hypersecretion and in subjects with severe asthma as compared to mild/moderate asthma subjects and healthy controls.[95] Also, Pham et al demonstrated that MMP-9 protein and MMP-9/TIMP-1 ratios were significantly elevated in the BAL of subjects with nocturnal asthma as compared to asthmatic subjects without nocturnal symptoms. These effects were significantly correlated with percentage overnight fall in FEV_1 and with reduced airway elastin density.[96] Thus, MMP-9 secretion by inflammatory cells links airway inflammation and remodeling in allergic asthma.

MMPs as biomarkers of allergic asthma pathobiology

Investigators of the pathobiology of allergic asthma can utilize several relatively noninvasive techniques to search for potential measurable markers of disease diagnosis, classification, and severity that may serve to guide medical treatment. These noninvasive techniques include collection and analyses of cell types and protein content of induced sputum, blood serum and plasma, and exhaled breath condensate (EBC) in both children and adults with allergic asthma.[97] In order for a biomarker to be useful, it must be 1) reliably measured in biological fluid or tissue secretion; 2) consistently correlated with a biological process or disease state; and 3) measurable using noninvasive techniques that can be easily and inexpensively translated to routine clinic use. In studies of allergic asthma, several measurements

have emerged as accepted markers of airway inflammation and atopy that have allowed for classification of asthma into subphenotypes based on molecular, inflammatory, or cellular profiles. These markers include the measurement of fraction of exhaled nitric oxide in exhaled breath, inflammatory cell profiles of induced sputum, numbers of peripheral blood eosinophils, serum IgE levels, and molecular profiling of type 2 or "T2 high" gene signatures in blood.[3]

Recent studies have begun to investigate the association of MMPs in induced sputum, EBC, and serum with markers of airway inflammation and atopy in allergic asthma. The majority of these reports have measured MMP-9 protein or activity or MMP-9/TIMP-1 ratios and found associations with airway inflammation in allergic asthma. First, MMP-9 protein and activity levels in plasma were shown to be significantly associated with severity and inflammatory profile of disease, as patients hospitalized with acute asthma exacerbations had significantly increased MMP-9 in plasma as compared to atopic nonasthmatic patients and healthy controls. MMP-9 protein levels were positively and significantly associated with numbers of neutrophils in the plasma.[98]

Analyses of MMP-9 protein levels in induced sputum at baseline and following allergen challenge have provided evidence that MMP-9 has the potential to serve as a biomarker for the classification of allergic asthma patients into subphenotypes based on airway inflammation and remodeling indices. Zhang et al found that baseline MMP-9 protein levels in induced sputum collected from asthma patients with irreversible airway obstruction (as defined by prebronchodilator FEV_1 of <80% predicted and was improved by <15% predicted after albuterol administration) were significantly increased as compared to patients with reversible airway obstruction (as defined by prebronchodilator FEV_1 of <80% predicted and was improved by >15% predicted after albuterol administration), nonobstructed airways (defined by prebronchodilator FEV_1 of >80% predicted), and healthy controls.[99] In addition, baseline MMP-9 and TIMP-1 protein levels in induced sputum of atopic children with intermittent or moderate asthma were significantly increased compared to healthy controls, but no significant difference was reported between the two asthma groups. The MMP-9 measurements in the induced sputum of moderate asthmatic children were significantly correlated with the numbers of eosinophils.[100] Furthermore, Cataldo et al showed that MMP-9 protein and activity in induced sputum were significantly increased in allergic asthma patients following house dust mite allergen challenge relative to patients receiving the sham challenge. TIMP-1 levels were not significantly different between groups. Also, the change

in FEV_1 during the EAR was significantly and positively associated with sputum MMP-9 activity, while the percentage of sputum neutrophils was significantly and positively associated with MMP-9 protein levels.[101] In contrast, this group also reported that *MMP9* mRNA expression in sputum cell pellets was not changed in mild asthma as compared to normal controls; however, no information was given regarding atopy in the subjects and the patients included in the study exhibited a predominantly eosinophilic inflammatory profile in the sputum.[102] Similarly, Boulay et al collected induced sputum and plasma from mild allergic asthma patients before and after allergen provocation. MMP-9 protein and MMP9/TIMP1 ratio levels in sputum were significantly increased 6 hours and 24 hours after allergen provocation as compared to baseline and were significantly associated with numbers of neutrophils, but not eosinophils in sputum. This group also found that MMP-9 protein concentrations in induced sputum were significantly and positively correlated with the change in FEV_1 during the LAR.[103]

More information on the relationship of MMP-9 secretion and pathobiology of allergic asthma has been gathered through the analyses of EBC. MMP-9 protein concentrations in EBC from children with persistent allergic asthma were significantly greater than children with intermittent allergic asthma or healthy controls, and both groups with asthma were significantly higher than healthy controls. These measurements of MMP-9 protein were significantly and inversely correlated with both FEV_1 and peak expiratory flow measurements in the children, but positively correlated with levels of IL-4 and IL-10 cytokines in EBC.[104] In addition, Barbaro et al first classified subjects in their study based on eosinophil and neutrophil counts in induced sputum of mild and severe allergic asthma patients. Although no information is given regarding the levels of MMP-9 in the induced sputum, the group measured MMP-9 protein levels in EBCs from the patients. MMP-9 was significantly increased in severe allergic asthma subjects as compared to mild/moderate asthma subjects, and the greatest concentrations of MMP-9 were found in severe asthma patients that had predominantly neutrophils in their sputum as compared to eosinophilic severe or mild/moderate asthma patients. Significant and positive associations between MMP-9 in EBC and percentage of neutrophils in sputum and exhaled nitric oxide suggest the MMP-9 is reflective of airway inflammation.[105] Taken together, these studies suggest that MMP-9 protein concentrations and activity levels in induced sputum and EBC may be a useful tool to indicate airway inflammation and airway remodeling in allergic asthmatic patients and to facilitate

classification of patients based on inflammatory profiles, severity, or type 2 cytokine-driven inflammation.

In addition to MMP-9, two other MMPs have been evaluated in either induced sputum or serum from allergic asthma patients. *MMP1* and *TIMP1* mRNA were significantly increased in sputum pellets from patients with mild asthma compared to controls, and the fold change in *MMP1* mRNA expression was significantly and positively correlated with monocyte chemoattractant protein-1 (*MCP1*) mRNA in the sputum pellets of asthma patients, but negatively correlated with FEV_1 in asthma,[102] suggesting that *MMP1* gene expression in induced sputum of allergic asthmatics may serve as a marker of airway remodeling. Finally, a report by Blumenthal et al[106] found a significant association between concentrations of serum leukolysin (MMP-25), which is a membrane-type MMP expressed primarily on neutrophils and serum IgE levels in atopic asthma patients, suggesting that neutrophilic release of MMP-25 may be associated with atopy and allergen sensitization. Therefore, MMPs important for airway remodeling may also be measured noninvasively and could serve as biomarkers for the process in allergic asthma.

Polymorphisms of MMPs associated with allergic asthma

Several recent studies have identified polymorphisms in *MMP9*, *MMP1*, *MMP2*, and *MMP12* genes as potential risk factors for the development of allergic asthma in various populations. First, Nakashima et al identified 17 polymorphisms in *MMP9* in a Japanese population of 290 pediatric atopic asthma patients and 638 healthy controls. Two of the polymorphisms, 2127G>T (rs2274755) and 5546G>A (rs2274756), were found to be significantly associated with the risk for childhood atopic asthma. Also, two common haplotypes of these polymorphisms were found in significantly higher frequencies in atopic asthmatic children as compared to nonasthma children.[107] Likewise, the rs2274755 polymorphism of *MMP9* was identified, along with another polymorphism, rs17577, to be associated with risk for atopic asthma in a study of 403 Mexican pediatric patients as compared to healthy, nonatopic controls.[108]

In addition, two studies have investigated associations of *MMP1* and *MMP2* with asthma in Asian populations. A study of 131 Taiwanese adults with asthma as well as healthy controls identified significantly increased risk for persistent airflow obstruction (defined as FEV_1/forced vital capacity $<75\%$) in asthma patients with at least one 1G allele at position -1607 relative to the transcription start site
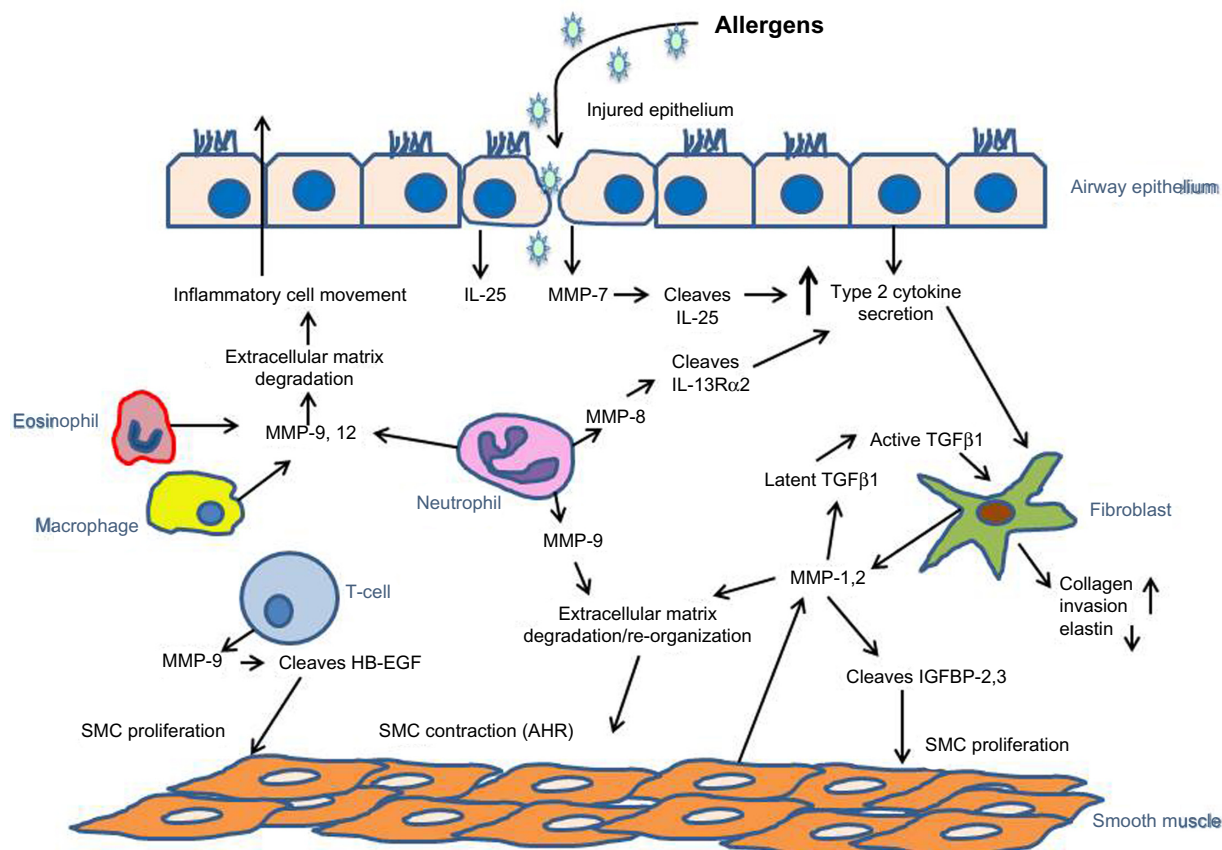
Figure 1 MMPs mediate allergen-induced airway inflammation, hyperresponsiveness, and remodeling.
Notes: Inhaled proteolytic allergens damage the airway epithelium and stimulate increased IL-25 and type 2 cytokine secretion. A cascade of airway cell responses results including increased production of MMPs by airway epithelium, smooth muscle, fibroblasts, T-cells, neutrophils, macrophages, and eosinophils. These MMPs participate in the allergic asthma response by further stimulating type 2 cytokine secretion, degrading, and re-organizing extracellular matrix and cleaving biomolecules. These actions serve to facilitate inflammatory cell egress from the tissue to the airway lumen, stimulate profibrotic responses, and promote smooth muscle cell contraction and proliferation.
Abbreviations: MMP, matrix metalloproteinase; IL, interleukin; IL-13Rα2, IL-13 receptor alpha 2; TGFβ1, transforming growth factor-β1; HB-EGF, heparin-binding epidermal growth factor; IGFBP, insulin-like growth factor binding protein; SMC, smooth muscle cell; AHR, airways hyperresponsiveness.

in the promoter of the *MMP1* gene as compared to patients homozygous for the 2G allele.[109] Also, Birbian et al reported a C/T single nucleotide polymorphism in the promoter of *MMP2* at position −1306 relative to the transcription start site that was associated with increased risk for allergic asthma in a Northern Indian population. The allele frequency of the C allele was significantly increased in allergic asthmatic patients as compared to the T allele, and overall, the homozygous CC genotype was significantly more frequent in patients with asthma than healthy control patients. The heterozygous CT and the homozygous TT genotypes were more prevalent in healthy controls, suggesting that the T allele offers some protective effect in allergic asthma.[110]

Finally, a polymorphism in *MMP12* was also implicated with increased risk for asthma. In a study of Scottish children and young adult asthma patients, presence of the serine variant of rs652548 in the *MMP12* gene conferred increased risk of asthma severity and exacerbations.[77] Taken together, all

of these studies point to the potential for polymorphisms of genes encoding MMPs to be associated with allergic asthma risk, but investigations of more diverse populations are required in order to draw definitive conclusion.

Conclusion

Allergic asthma is a complex, chronic disease that involves airway inflammation, hyperresponsiveness, and remodeling. The activity of MMPs and their endogenous inhibitors plays roles in all of these processes through degradation of ECM and/or modulation of cell signaling (Figure 1). It is clear that MMPs are secreted/released by inflammatory cells following allergen provocation and in response to type 2 cytokine signaling, and MMP-9, in particular, serves to facilitate inflammatory cell egress from the tissues to the airway lumen. Inflammatory cell- and structural cell-derived MMPs also contribute to AHR and remodeling by altering ECM turnover, which affects smooth muscle contraction, airway fibroblast

invasion, and submucosal accumulation of collagen. Furthermore, MMP-induced regulation of cell signaling through proteolytic shedding and activation of key growth factors, such as TGFβ1, stimulates airway cell proliferation and modulates matrix production, contributing to airway fibrosis. Because MMPs link these three processes, inhibition of their activity, either with pharmacologic inhibitors or overexpression of TIMPs, may have potential for providing benefit in certain patients with allergic asthma, and a limited number of studies suggest that MMP inhibition attenuates inflammation and AHR. But, it is important to understand which patients may be most responsive to those therapies, and to that end, the usefulness of MMPs as biomarkers is just beginning to be investigated, particularly with regard to MMP-9 as a marker of neutrophilic airway inflammation in allergic asthma. Similarly, studies of genetic polymorphisms in *MMP* genes suggest that, in certain populations, these polymorphisms confer increased risk for allergic asthma, but more information is needed to determine the impact of these polymorphisms in more broad populations. Therefore, MMPs are critical mediators of all three pathologic processes in allergic asthma, and more studies in the future will help us to gain a better understanding of how measurement and inhibition of these proteolytic enzymes in the airway may benefit patients with allergic asthma.

Disclosure

The authors report no conflicts of interest in this work.

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