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ADAM8: A new therapeutic target for asthma

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Abstract

Background—A proteinase with a disintegrin and a metalloproteinase domain-8 (ADAM8) has been linked to asthma.

Objective—To explore whether ADAM8 is a therapeutic target for asthma.

Methods—We reviewed literature on ADAM8's function and expression and activities in lungs of humans and mice with allergic airway inflammation (AAI). We used these data to generate hypotheses about the contributions of ADAM8 to asthma pathogenesis.

Conclusions—ADAM8 levels are increased in airway epithelium and airway inflammatory cells in mice with AAI and human asthma patients. Data from murine models of AAI indicate that ADAM8 dampens airway inflammation. It is not clear whether ADAM8 contributes directly to structural remodeling in asthmatic airways. Additional studies are required to validate ADAM8 as a therapeutic target for asthma.

Keywords

ADAM; airway hyper-responsiveness; allergy; asthma; disintegrin; eosinophil; epithelium; inflammation; leukocyte; macrophage; metalloproteinase; proteinase; remodeling; signaling

1. Introduction: asthma

1.1. Healthcare and economic burden

Asthma is a chronic inflammatory disease of the airways characterized by airway hyperresponsivness (AHR) and intermittent airflow obstruction. Asthma is associated with high morbidity and mortality and its incidence is increasing in most countries. Asthma affects approximately 300 million people worldwide and causes an estimated 255,000 deaths per year [1]. In 2006, 7.3% of the US population had asthma and asthma was responsible for 17 million physician consultations [2]. The total annual financial cost for all forms of asthma in the US is estimated to be ~$14 billion [3]. Thus, asthma represents a large economic and healthcare burden. Although patients with mild disease respond well to inhaled bronchodilators and/or corticosteroids, other patients need oral corticosteroids, which are associated with undesirable side effects, and some patients are refractory to these therapies. Thus, there is a pressing need to develop new and improved therapies for asthma.

1.2. Asthma pathogenesis

Asthma is thought to be caused by complex interactions between genetic and environmental factors, but its pathogenesis is still incompletely understood. Patients with asthma have

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increased numbers of $CD4^+$ T cells in the airways, which are predominantly of the T helper type 2 (T_H2) subtype. These T_H2 lymphocytes produce T_H2 cytokines including IL-4, −5, −9, and −13 which orchestrate the inflammatory response in asthmatic airways by recruiting and activating additional T_H2 cells and also mast cells and eosinophils [4]. IL-4 and −13 also promote isotype switching of B cells to IgE production. Inhalation of allergens in sensitized individuals increases B cell production of IgE, which binds to IgE receptors on mast cells inducing mast cell degranulation. Degranulating mast cells release cytokines, histamine, cysteinyl-leukotrienes, prostanoids and proteinases, which induce bronchoconstriction, edema, and additional recruitment of eosinophils and T_H2 lymphocytes to the airways thereby amplifying airway inflammation. However, this is a gross oversimplification of the complex inflammatory events occurring in asthma. Natural killer T cells [5], T_H17 lymphocytes [6], regulatory T-cells [7], dendritic cells (DCs [8]), macrophages [9], and polymorphonuclear neutrophils (PMNs [10]) also play important roles in asthma pathogenesis.

Structural changes also occur in asthmatic airways including mucus metaplasia and hyperplasia, activation of fibroblasts, interstitial collagen deposition and airway smooth muscle hyperplasia, which contribute fundamentally to asthma chronicity and severity [11-13]. Although these airway remodeling processes were initially thought to be secondary to chronic airway inflammation, extensive airway remodeling occurs early in this disease in children and often before the onset of symptoms [14,15]. It has been suggested that in asthma the airway epithelium is abnormally sensitive to the damaging effects of oxidants [16,17]. The injured epithelium communicates in a dysregulated and bi-directional fashion with the underlying mesenchyme to form the epithelial-mesenchymal trophic unit, which initiates and amplifies airway remodeling. Poorly controlled repair processes in the asthmatic epithelium lead to excessive release of growth factors that activate subepithelial fibroblasts to form myofibroblasts that deposit extracellular matrix (ECM) proteins along the airway basement membrane and secrete mitogens for airway smooth muscle cells (SMCs) and cytokines that amplify remodeling and inflammation [18].

1.3. Asthma and proteinases

Serine and matrix metalloproteinases (MMPs) have been shown to regulate mucus hyper-secretion [19], airway inflammation [20-22], subepithelial fibrosis [23], and airway SMC proliferation [24,25] in asthma. In 2002, the a disintegrin and a metalloproteinase (ADAM) subfamily of MPs was implicated in asthma pathogenesis when genetic polymorphisms in the *ADAM33* gene were found to be associated with asthma and AHR [26,27]. ADAM33, a product of mesenchymal cells, probably regulates airway remodeling rather than airway inflammation in asthma [17,28]. More recently, ADAM8 has been strongly associated with allergic airway inflammation (AAI) in humans and mice and additional studies of ADAM8 are beginning to shed light on its roles in asthma pathogenesis. Below we outline what is known about the biology of ADAM8 and its expression in AAI in humans and mice. We will also speculate about its potential contributions to pathologies occurring in the airways of asthmatic subjects and its potential as a new therapeutic target for asthma.

2. ADAM8

2.1 ADAM8 structure and chromosomal localization

ADAM proteinases are a subfamily of zinc-dependent MPs and are type I transmembrane proteins with a multi-domain structure [29]. ADAM8 is also known as membrane-spanning 2 (MS2) or cluster of differentiation antigen 156a (CD156a) and was originally cloned in 1990 from murine macrophages and macrophage cell lines [30]. The human ADAM8 gene maps to chromosome 10q26.3 and the mouse ADAM8 gene to region F3–F4 on chromosome 7 [31, 32]. There is 65.6% and 61.7% homology between human and murine ADAM8 at the

nucleotide and protein levels, respectively [31]. The functions of ADAM proteins are related to their multiple domain structure which includes a pro-domain, a metalloproteinase (MP) domain, a disintegrin domain, a cysteine-rich (CR) domain, an EGF-like domain, a transmembrane domain, and a cytoplasmic tail (Figure 1). ADAM8 has all of these domains and the human protein contains 808 amino acids, including 637 residues in the ectodomain, 25 residues in the transmembrane domain, and 146 amino acids in the cytoplasmic tail [31].

2.1.1 The pro-domain—Like other ADAMs, ADAM8 is initially synthesized as a latent pro-enzyme. The pro-domain maintains the MP domain in an inactive form through an interaction between a conserved cysteine residue in the pro-domain and the active site zinc atom. Although many proADAMs are activated by furin-mediated cleavage of the pro-domain in the trans-Golgi, proADAM8 is activated in the trans-Golgi by autocatalytic cleavage of the prodomain [33,34].

2.1.2 The MP domain—ADAM8 contains the catalytic site zinc-binding consensus sequence (HEXXHXXGXXHD) and is an active proteinase [33-35]. After proADAM8 is activated in the trans-Golgi it translocates to the cell surface. In some cells, the MP domain can further proteolytically cleave active ADAM8 with loss of the MP domain itself leaving a truncated form of the enzyme with the disintegrin domain at the NH_2 terminus [34,36]. The main function of the MP domain of ADAMs is thought to be in proteolytically cleaving and releasing (or shedding) signaling molecules and their receptors from cell surfaces. The best-known example of an ADAM ‘sheddase’ is ADAM17 which cleaves latent, membrane-bound, 26 kDa pro-TNF-α thereby releasing soluble, active 17 kDa TNF-α [37]. Recombinant active ADAM8 sheds adhesion molecules and surface receptors from cell surfaces (Table 1) and also cleaves short peptide substrates containing sequences in cytokines, cytokine receptors, and growth factors that are susceptible to cleavage by other proteinases (Table 1). Some of these potential substrates for ADAM8 have been implicated in asthma pathogenesis as outlined below. The role of ADAM8 in degrading ECM proteins has not been examined, but ADAM proteinases are generally weak ECM protein-degrading enzymes [29]. The MP domain of ADAM8 is not inhibited by any of the four members of tissue inhibitor of metalloproteinase family [33,34]. Although physiological inhibitor(s) of the ADAM8 MP domain are likely to exist, they have not yet been identified.

2.1.3 The disintegrin, cysteine-rich, and EGF-like domains—ADAMs have a disintegrin domain that is structurally similar to the disintegrin domain of hemorrhagic snake venom proteins, which contain RGD sequences that bind to platelet integrins to inhibit platelet activation and thrombosis. The disintegrin domain of ADAM8 contains an RX6DLPEF sequence rather than an RGD integrin-binding sequence. On osteoclast surfaces, ADAM8 disintegrin binds to α9β1 integrins on other osteoclasts in an RGD-independent manner to promote osteoclast fusion, activation, and differentiation [38,39]. However, it is not clear whether ADAM8 disintegrin domain binds to other integrins. ADAM8 overexpression in cell lines increases their capacity to adhere to ECM proteins and migrate [34,40], but the role of ADAM8 disintegrin in regulating the adhesion or migration of primary cells is not known. The disintegrin domain, along with the CR and EGF-like domains of ADAM8, is also required for optimal activation and processing of proADAM8 by its own MP domain [34,41]. However, it is not clear whether the CR and EGF-like domains of ADAM8 have other functions.

2.1.4. The cytoplasmic tail—This proline-rich domain contains a sequence similar to the consensus Src kinase homology 3 (SH3) binding sequence. SH3 domains are small, non-catalytic domains found in various intracellular signaling proteins including Src and Abl family protein tyrosine kinases. Although ADAM-9, −12, and −15 bind to Src family kinases

[42-44], there have been no studies examining whether ADAM8 binds to SH3 domains in intracellular proteins.

2.2. ADAM8 expression and regulation

In healthy adult mice, ADAM8 is expressed at low levels in the brain, bone marrow, ovary, and the respiratory tract epithelium ([45-47]; Table 2). However, in mice, ADAM8 is not required for embryogenesis, reproduction, or survival since ADAM8$^{-/-}$ mice have normal development, fertility, and lifespan [47]. In humans, ADAM8 is expressed by most leukocytes [36,45,48], lung epithelial cells [49], and osteoclasts ([38,39]; Table 2). In contrast, ADAM33, the other ADAM family member that has been linked to asthma, is expressed mainly by mesenchymal cells in the airways including SMCs and fibroblasts [50,51]. However, there is conflicting literature on whether lung epithelial cells express ADAM33 [52,53].

Pharmacological agonists, cytokines, bacterial products and hormones upregulate ADAM8 levels in various cells (Table 2). The murine ADAM8 promoter contains a nuclear factor-1 (NF-1) binding site which may regulate phorbol ester responsiveness [46], two purine rich (PU) boxes, two IL-6 response elements, three NF-IL-6 binding sites which may regulate bacterial lipopolysaccharide (LPS) responsiveness [32], and an interferon regulatory factor-1 (IRF-1) binding sequence which may regulate TNF-α responsiveness [45]. ADAM8 levels are increased in inflammatory disorders of the lung, bone and joints, and central nervous system (Table 3). ADAM8 is also expressed in several tumors where its expression correlates positively with tumor invasiveness, metastasis, and poor prognosis (Table 3).

2.3. Biology of ADAM8 in cells relevant to asthma pathogenesis

ADAM8 is expressed by all leukocytes expect T lymphocytes [36,45,48] and epithelial cells [49]. ADAM8 is expressed at low levels in the airway epithelia of healthy mice and humans where its expression increases during AAI ([47,49]; Table 2). However, ADAM8 is not known to be expressed by airway SMCs or fibroblasts. ADAM8 is expressed at low levels on the surface of unstimulated leukocytes but pro-inflammatory stimuli including T_H2 cytokines strikingly upregulate ADAM8 levels on leukocyte surfaces (Table 2). In most leukocytes, agonists increase ADAM8 at the steady state mRNA level. However, in PMNs, ADAM8 is stored as a preformed enzyme in their gelatinase and specific granules from where it translocates to their surface when PMNs are activated to degranulate [48]. The ectodomain of ADAM8 is also shed by unidentified MP(s) from the surface of activated PMNs [48] but the function of ADAM8 that is shed from PMN surfaces is not known.

ADAM8 may regulate the recruitment of eosinophils and PMNs into asthmatic airways. For example, ADAM8 may inhibit eosinophil recruitment into the airways during AAI by shedding vascular adhesion molecule-1 (VCAM-1). During AAI, activated eosinophils express α4 integrin, which binds to VCAM-1 that is induced on endothelial cells activated by T_H2 cytokines. The binding of α4 integrin to VCAM-1 promotes the initial rolling and tethering of eosinophils on endothelial cells prior to their trans-endothelial migration [54]. When eosinophils adhere to endothelial cells they form podosomes which are punctuate and highly dynamic adhesive structures [55]. ADAM8 localizes to the podosomes of adherent eosinophils [56] where it may contribute to shedding of VCAM-1 from subjacent endothelial cells which may reduce eosinophil extravasation during T_H2 airway inflammation. ADAM8 also sheds L-selectin from PMN surfaces *in vitro* [48]. Since L-selectin promotes PMN rolling on endothelial cells by binding to its ligands on endothelial cells during PMN transendothelial migration, ADAM8-mediated L-selectin shedding may also limit excessive or inappropriate efflux of PMNs and other leukocytes expressing both ADAM8 and L-selectin into sites of inflammation.

3. ADAM8 associations with asthma

3.1. ADAM8 levels are increased in the lung during AAI

ADAM8 was first linked to asthma in 2004 when King et al showed that ADAM8 (but not ADAM33) mRNA transcripts are increased in lungs of mice with Aspergillus- and ovalbumin (OVA)-induced AAI [57]. Initial robust increases in ADAM8 levels were linked to eosinophils, PMNs and macrophages infiltrating the airways. Less impressive increases in ADAM8 that persisted after inflammation had resolved were attributed to increases in epithelial-cell-derived ADAM8 [57]. Delivering or overexpressing T_H2 cytokines (IL-4 and 13) in murine lungs also increased ADAM8 mRNA levels in lung homogenates by an IL4Rα and signal transducer and activator of transcription-6 (STAT-6)-dependent pathway [57]. More recently, bronchial tissue from healthy human subjects was shown to have little or no ADAM8 staining in airway epithelia or submucosa. However, intense staining for ADAM8 protein was present in bronchial inflammatory cell infiltrates and airway epithelia from patients with moderate and severe asthma which was greater than the ADAM8 staining in bronchial biopsies from subjects with mild asthma [49]. ADAM8 mRNA levels were also increased in sputum cells from patients with mild asthma compared with healthy control subjects and modest positive correlations were observed between sputum ADAM8 mRNA levels and sputum PMN and eosinophil counts and sCD23 levels [58]. Although these authors reported a negative correlation between sputum ADAM8 levels and forced expiratory volume in 1 second (FEV1) measurements, the correlation was weak, only a small number of patients was studied, and most patients had mild disease (mean FEV1 measurement were ~90% of predicted values). Thus, this reported negative correlation between FEV1 and ADAM8 is not likely to be physiologically relevant.

Soluble ADAM8 levels were also increased in bronchoalveolar lavage (BAL) samples from patients with cigarette-smoke-induced acute eosinophilic pneumonia (AEP) and chronic idiopathic eosinophilic pneumonia (CEP [59]). However, for reasons that are not clear, ADAM8 was not increased in BAL samples from patients with drug-induced pulmonary eosinophilia [59]. This literature indicates that in most types of eosinophilic lung inflammation in humans and mice, ADAM8 levels are increased in the lung, and increases are mainly due to increased expression of ADAM8 in lung inflammatory cells and to a lesser extent in airway epithelial cells [60].

3.2. ADAM8 polymorphisms, asthma, and atopy

Interest in ADAMs in lung biology was sparked in 2002 when van Eerdewegh and coworkers used positional cloning to identify ADAM33 as a putative asthma susceptibility gene in an outbred Caucasian population [26]. Since then, strong associations between single-nucleotide polymorphisms (SNPs) in the ADAM33 gene and asthma have been reported in several other Caucasian populations [61-64]. However, for reasons that are not clear, other studies have shown only weak associations [65,66], or no association between ADAM33 SNPs and asthma [67]. In 2008, Tremblay et al [68] subjected a genome-wide association scan performed on 609 subjects in a Northeastern Quebec population with familial asthma and atopy to a ‘Genes to Disease’ computational analysis. This was done to identify candidate genes in genetic loci known to be associated with asthma (6q26) and atopy (10q26.3). This study identified a single SNP in the ADAM8 gene that was initially suggested to confer protection against allergic asthma and atopy but this polymorphism was not associated with non-allergic asthma. However, the associations between the SNP in the ADAM8 gene and asthma and atopy were modest in magnitude and not significant after multiple comparisons testing. No other studies assessing whether SNPs in the ADAM8 gene are associated with asthma or atopy in other populations have yet been published.

3.3. Studies of ADAM8 transgenic and ADAM8 deficient mice in murine models of AAI

In 2002, Higuchi at al generated a conditional C57BL/6 transgenic mouse line expressing a soluble form of the entire ADAM8 ectodomain (sADAM8) under the control of the α_1-antitrypsin promoter, which is mainly expressed by hepatocytes in the mice [69]. These transgenic mice had detectable levels of sADAM8 in their plasma and plasma sADAM8 levels increased when the mice were given LPS, which is a potent inducer of the acute phase response in hepatocytes [69]. When sensitized and challenged with OVA, sADAM8 transgenic mice had decreased inflammatory cell infiltrates in bronchial submucosal and perivascular areas in lung sections compared with non-transgenic control mice [70] but the leukocyte subsets that were increased were not identified. While the ADAM8 transgenic and non-transgenic control mice did not differ in BAL fluid (BALF) levels of IL-4 or −5, OVA-treated sADAM8 transgenic mice had reduced serum IL-5 levels, but the mechanism underlying this difference was not investigated. Serum from the sADAM8-transgenic mice had greater capacity than serum from non-transgenic control mice to cleave VCAM-1 from activated endothelial cells *in vitro* [70]. The authors concluded that sADAM8 limits AAI by shedding VCAM-1 from endothelial cells. However, this sADAM8-induced increased VCAM-1 shedding was very modest in magnitude and the authors did not measured levels of other potential ADAM8 substrates (see Table 1) in the transgenic versus non-transgenic mice. Thus, ADAM8 may limit the influx of leukocytes into the airways of the transgenic mice by shedding substrates other than, or in addition to VCAM-1, and/or by reducing levels of IL-5 which is critical for inducing eosinophil production and survival [4].

Recently, mice genetically deficient in ADAM8 ($ADAM8^{-/-}$ mice) versus wild type (WT) littermate control mice in a T_H1 skewed mixed SvEV129 × C57BL/6 strain were compared in an acute OVA-induced AAI model system. $ADAM8^{-/-}$ mice had increased BAL total leukocytes counts (due mainly to increased BAL macrophage counts), increased peribronchial infiltrates in lung sections, and increased AHR to methacholine challenge during OVA-induced AAI [71]. Although the levels of candidate substrates for the ADAM8 MP domain have not yet been measured in the mice, these data provide additional evidence that ADAM8 has potent anti-inflammatory activities during AAI in mice. In contrast, ADAM33, the other ADAM family member that has been linked to asthma, is not upregulated in the lungs of mice with AAI [57]. In addition, studies of $ADAM33^{-/-}$ mice in a murine model of acute allergen-induced AAI showed that ADAM33 plays no role in regulating AAI and AHR in mice. Since ADAM33 is predominantly a mesenchymal cell product and its catalytic domain promotes angiogenesis *in vitro* [72], it has been proposed that ADAM33 contributes mainly to airway remodeling in asthma [17,27]. However, this possibility has not yet been tested *in vivo*.

4. Potential activities of ADAM8 in asthma

Based upon experimental data from murine models systems and the known activities of ADAM8 MP domain *in vitro* (Table 1) we suggest that ADAM8 plays significant roles in limiting airway inflammation and mucus hyper-secretion but has more limited activities in structural airway remodeling in asthmatic airways. We predict that ADAM8 expressed by activated leukocytes and/or bronchial epithelial cells has critical activities in limiting airway inflammation and mucus hyper-secretion but has limited activities in other structural airway remodeling processes occurring in asthmatic airways as outlined below.

4.1. Potential activities for ADAM8 in reducing AAI

Experimental data from ADAM8 transgenic and $ADAM8^{-/-}$ mice indicate that ADAM8 reduces AAI and AHR in acute allergen challenge models [70,71] but there are some caveats when generating hypotheses from the results of these studies. First, it is not clear how relevant the activities of circulating soluble ADAM8 ectodomain present in the ADAM8 transgenic

mice are to physiological and pathological processes occurring in asthmatic airways given that ADAM8 is thought to function as a transmembrane proteinase and signaling through its cytoplasmic tail may contribute in critical ways to ADAM8's function in airway leukocytes and bronchial epithelial cells. While ADAM8 is shed from the surface of human PMNs [48] and sADAM8 has been detected in human plasma [40], the levels are low (267 pg/ml) and plasma levels of sADAM8 were not measured in the ADAM8-transgenic mice. Thus, it is not clear whether plasma sADAM8 levels in the transgenic mice are similar to those in human plasma especially since ADAM8 is mainly expressed by leukocytes and epithelial cells, which probably produce less sADAM8 than hepatocytes in the ADAM8 transgenic mice. Nevertheless, a recent study of ADAM8$^{-/-}$ mice provided additional evidence that ADAM8 limits OVA-induced AAI [71]. Second, the ADAM8 transgenic and ADAM8$^{-/-}$ mice studied thus far were both in T_H1-skewed strains that have reduced recruitment of eosinophils and T_H2 lymphocytes during AAI compared with T_H2-skewed strains of mice. In order to determine whether ADAM8 regulates eosinophil and T_H2 lymphocyte recruitment, studies of ADAM8$^{-/-}$ mice in a pure T_H2-skewed strain (such as the BALB/c stain) are needed.

Although studies of ADAM8 transgenic and ADAM8$^{-/-}$ mice have not yet dissected the mechanisms by which ADAM8 reduces AAI in mice, based upon ADAM8's known activities *in vitro*, we speculate that ADAM8's anti-inflammatory activities in AAI are due to ADAM8 MP domain shedding adhesion molecules, cytokines and/or receptors from the surface of leukocytes, bronchial epithelial cells, and/or endothelial cell as outlined below.

4.1.1. Shedding of adhesion molecules—Most probably, ADAM8 limits AAI by shedding adhesion molecules (Figure 2A) since ADAM8 expressed on activated leukocytes cleaves L-selectin and possibly P-selectin glycoprotein ligand-1 (PSGL-1) from leukocyte surfaces [48] and VCAM-1 from endothelial cells during leukocyte-endothelial cell interactions *in vitro* [70]. Since L-selectin promotes PMN rolling on endothelial cells by binding to its ligands on endothelial cells during PMN transendothelial migration, ADAM8-mediated L-selectin shedding may also limit excessive or inappropriate recruitment of PMNs and other leukocytes expressing both ADAM8 and L-selectin into asthmatic airways. L-selectin may be an important substrate for leukocyte-derived ADAM8 *in vivo* since L-selectin levels were elevated and correlated positively with ADAM8 levels in synovial fluid from patients with rheumatoid arthritis and in lung samples from patients with eosinophilic pneumonias and mild asthma [48,58,59]. In addition, when compared with non-transgenic mice with peritonitis, ADAM8 transgenic mice with peritonitis had reduced surface L-selectin levels on peripheral blood PMNs and peritoneal exudate PMNs, which were associated with reduced peritoneal PMN counts [69]. ADAM8 also cleaves a PSGL-1-like peptide *in vitro* [35] and PSGL-1 binds to CD34 on endothelial cells to promote leukocyte transendothelial migration. Thus, ADAM8 expressed by leukocytes may shed L-selectin and/or PSGL-1 from leukocyte surfaces to limit the influx of leukocyte subsets that express both ADAM8 and these adhesion molecules into the airways of subjects with asthma (Figure 2A).

ADAM8 that is expressed on the surface of activated eosinophils may inhibit eosinophil recruitment into the airways during AAI by shedding VCAM-1. ADAM8 cleaves VCAM-1-like peptides *in vitro* [35] and serum from sADAM8 transgenic mice has increased capacity to shed VCAM-1 from activated endothelial cells *in vitro* [70]. Although the latter activity was modest in magnitude, more efficient VCAM-1 shedding may occur when high levels of membrane-associated ADAM8 are concentrated in the sequestered microenvironments of the podosomes of activated eosinophils attached to lung capillaries [56]. Since binding of α4 integrin expressed by eosinophils to VCAM-1 expressed by endothelial cells is a critical event during eosinophil transendothelial migration [54,73], shedding of VCAM-1 by ADAM8 expressed on the surface of eosinophils bound to endothelial cells may limit airway T_H2 inflammation during AAI (Figure 2A).

4.1.2 Shedding of cytokines and their receptors—ADAM8 cleaves a number of peptides having sequences in cytokines and cytokine receptors that are cleaved by other proteinases (Table 1). ADAM8 cleaves a proTNF-α-like peptide *in vitro* [33,35] suggesting that ADAM8 expressed on activated macrophages or bronchial epithelial cells in asthmatic airways may shed and activate pro-TNF-α from the surfaces of these cells. However, ADAM17 is thought to be the main pro-TNF-α-activating enzyme *in vivo* [37]. If ADAM8 is confirmed to play important roles in the shedding of TNF receptor 1 (TNFR1 [35]), this might limit AAI by reducing the pro-inflammatory signaling of TNF-α in leukocytes and/or bronchial epithelial cells human subjects with asthma ([74] and Figure 2B). For example, if ADAM8 expressed by activated bronchial epithelial cells sheds TNFR1 from epithelial cells, this may reduce TNF-α-induced upregulation of epithelial ICAM-1 expression [75]. This, in turn, may reduce leukocyte adhesion to bronchial epithelial cells to limit leukocyte-mediated epithelial cell injury and loss of bronchial epithelial cells in asthmatic airways. IL-1β has pro-inflammatory activities during AAI in mice [76] and ADAM8 cleaves a peptide sequence occurring in one of the two IL1 receptors (IL-1 receptor II, IL-1 RII) *in vitro* [35]. IL-1 RII is a non-signaling decoy receptor which has a short cytoplasmic tail lacking the signaling Toll-IL-1R domain. IL-1RII is proteolytically shed from the surface of leukocytes and epithelial cells by other MPs and sIL-1RII acts as a decoy receptor that sequesters soluble IL-1β and reduces its binding to and signaling through the functional type I IL-1R [77]. If this receptor is confirmed to be an important substrate for ADAM8 *in vivo*, ADAM8 expressed by activated leukocytes and/or epithelial cells in asthmatic airways may shed IL-1RII from the surfaces of these cells to limit AAI and AHR (Figure 2B).

ADAM8 also cleaves a fractalkine-like peptide *in vitro* [35]. Fractalkine (CX3CL) is produced as a membrane-bound protein on endothelial, epithelial, and SMCs which promotes adhesion of leukocytes to these cells [78]. ADAM-10 and −17 shed CX3CL from these cells generating sCX3CL which binds to CX3CR1 on monocytes, T cells, and DCs to increase their migration [78,79]. Both forms of CX3CL are upregulated in asthmatic subjects [80]. Thus, if ADAM8 expressed on the apical surface of activated bronchial epithelial cells in asthmatic airways sheds CX3CL from the epithelial cell surface, this may increase the trafficking of leukocytes into asthmatic airways. Alternatively, ADAM8-mediated shedding of CX3CL from epithelial surfaces may reduce adhesion of leukocytes to bronchial epithelial cells and limit leukocyte-mediated epithelial cell injury and loss in asthmatic airways.

Stem cell factor (SCF, kit ligand) is produced by bronchial epithelial cells [81], eosinophils [82], alveolar macrophages [83], mast cells [83], and PMNs [84]. SCF is initially produced as a membrane-associated protein and its expression is increased in epithelial cells and macrophages in the airways of asthmatic subjects [83,85]. Mast cell chymase and unidentified MPs cleave membrane-associated SCF generating soluble SCF [86,87]. Both forms of SCF are active cytokines that bind to c-kit receptor on mast cells to promote mast cell chemotaxis, activation, degranulation and survival, and mast-cell-induced AHR [88,89]. Although ADAM8 cleaves a SCF-like peptide *in vitro* [35], it is not clear whether SCF is cleaved from the surface of ADAM8 expressed by activated leukocytes or bronchial epithelial cells in the airways of asthmatic subjects. It is also not clear whether ADAM8-mediated cleavage of SCF alters the biological activity of SCF.

4.1.3. Shedding of leukocyte immunoglobulin receptors—ADAM8 cleaves a CD16-like peptide *in vitro* [35] but it is not clear whether ADAM8 sheds CD16 from leukocyte surfaces. CD16 is a low-affinity receptor for IgG which is expressed by various leukocytes including granulocytes, mononuclear phagocytes, and mast cells [90]. IgG binding to CD16 promotes leukocyte degranulation and release of cytokines, enzymes, and other mediators. Neutrophil elastase [91] and unidentified ADAM proteinase(s) shed CD16 from leukocyte surfaces, and CD16 shedding is associated with increased PMN apoptosis [92]. While CD16

expression is increased on circulating and tissue leukocytes of asthmatic patients compared with healthy subjects [93,94], surface CD16 levels are reduced on alveolar macrophages after allergen challenge [95]. This suggests that significant CD16 shedding occurs from macrophage surfaces during AAI. If ADAM8 that is expressed on activated leukocytes in asthmatic airways induces significant shedding of CD16 from leukocyte surfaces, this may limit airway inflammation in subjects with asthma by inhibiting leukocyte degranulation and release of pro-inflammatory mediators and/or by reducing the survival of airway granulocytes (Figure 2B).

CD23 is a low affinity receptor for IgE which is expressed on B cells, monocytes, and macrophages. Soluble forms of CD23 (sCD23) are shed from cells by several ADAMs including ADAM8 *in vitro* [96]. Patients with asthma and atopy have increased leukocyte surface CD23 levels and increased sCD23 levels in plasma [97-99]. In addition, ADAM8 levels in lung samples correlate positively with lung sCD23 levels in patients with mild asthma and eosinophilic pneumonias [58,59]. However, it is not clear whether leukocyte-derived ADAM8 is a significant CD23 sheddase since CD23 shedding has not been measured in leukocytes isolated from WT versus ADAM8$^{-/-}$ mice. The effect of ADAM8-mediated shedding of CD23 during AAI is unclear. Genetic deletion or transgenic overexpression of CD23 increases or decreases serum IgE levels and AAI and AHR in mice, respectively [100,101]. However, at the cellular level, the function(s) of CD23 may depend upon the form that is expressed. For example, although membrane-bound CD23 on B cells increases B cell IgE-dependent antigen presentation to T cells [102], IgE binding to membrane-bound CD23 on B cells downregulates further IgE production. In addition, the binding of sCD23 to CD21 on B cells increases their IgE production [103,104] and sCD23 binding to CD11b/CD18, CD11c/CD18, and αv integrins on monocytes increase their production of pro-inflammatory cytokines [105]. Thus, shedding of leukocyte CD23 by ADAM8 expressed on the surface of activated leukocytes in asthmatic airways may increase rather than decrease airway inflammation.

4.2 Potential of ADAM8 to regulate airway remodeling in asthma

The activities of ADAM8 in airway remodeling are not clear since ADAM8 transgenic or deficient mice have not been studied in murine models of allergen-induced airway remodeling. Based upon ADAM8's expression profile (it is expressed by leukocytes and bronchial epithelial cells but not by mesenchymal cells) and its activities *in vitro*, we suggest that, unlike ADAM33, ADAM8 does not play a major role in directly regulating structural remodeling in asthmatic airways. However, by reducing airway inflammation ADAM8 may reduce mucus hyper-secretion indirectly in the airways of subjects with asthma (Figure 3).

4.2.1. Mucus hyper-secretion—Patients with asthma have goblet cell metaplasia and increased mucus secretion, and airway plugging by mucus contributes to asthma mortality [106]. T_H2 cytokines and other stimuli induce mucus hyper-secretion not only by increasing EGFR expression on airway epithelial cells but also by inducing shedding of membrane-associated ligands for this receptor, generating the mature active forms of these ligands which activate the EGFR [107]. Neutrophil elastase, MMP-9, ADAM10, and ADAM17 directly or indirectly shed membrane-associated ligands for the EGFR from lung epithelial cells to increase mucin gene transcription and promote goblet cell metaplasia [108-111]. Although the EGFR-ligand-shedding activities of epithelial-derived ADAM8 have not been studied, ADAM-10 and −17 are thought to be the main EGFR-ligand sheddases contributing to increased mucus production in airway epithelium [112]. Instead, ADAM8 may reduce airway mucus hyper-secretion by limiting AAI and reducing the airway burden of other leukocyte proteinases that increase mucus production (Figure 3).

4.2.2. Myofibroblast activation, subepithelial fibrosis, and airway SMC hyperplasia—In patients with chronic asthma, there is increased production of several

growth factors that drive differentiation of fibroblasts into myofibroblasts. Activated myofibroblasts secrete ECM proteins such as interstitial collagens and fibronectin and also release factors such as vascular endothelial growth factor and endothelin I which are mitogens for airway SMCs [113,114]. The MP domain of other ADAMs and metalloproteinases cleave and thereby activate latent growth factors [115,116], alter biological activity of cytokines that control fibroblast survival [117-119], or degrade ECM proteins produced by myofibroblasts [120-122]. Since ADAM8 has an active MP domain, epithelial-derived ADAM8 may also promote these processes as outlined below and as illustrated in Figure 3.

The activity of growth factors is regulated by proteinases. TGF-β1-3, which are important growth factors in asthmatic airways, are produced as large latent complexes in which a latency-associated peptide (LAP) is bound to a latent TGF-β-binding protein. These large latent complexes bind to ECM proteins. Neutrophil elastase and plasmin release complexes containing latent TGF-β from these ECM stores [123,124] and MMPs cleave LAP to activate TGF-β [115,116]. While the activities of ADAMs in activation of TGF-β have not been studied, ADAM proteinases activate IGFs, which also contribute to airway remodeling in asthma [125]. IGFs circulate in an inactive form bound to circulating IGF binding proteins (IGFPBs) which prevent IGFs from binding to their receptors on fibroblasts and airway SMCs [126]. ADAM9, ADAM12, ADAM28, and several MMPs degrade IGFBP thereby releasing active IGFs that can signal through their receptors on myofibroblasts to increase collagen deposition in tissues [117-119] and degraded IGFBP have been detected in asthmatic airways [125]. However, ADAM8's activities as an IGFBP-degrading enzyme have not been assessed. The MP domain of ADAM8 expressed by activated bronchial epithelial cells or leukocytes in asthmatic airways could also increase remodeling of asthmatic airways by shedding IL-1RII from the surface of epithelial cells or leukocytes [35] which may bind and sequester soluble IL-1β and thereby reduce IL-1β-mediated induction of fibroblast apoptosis [127]. Thus, if ADAM8 is confirmed to be a key IL1 RII sheddase, it could increase the survival of fibroblasts in asthmatic airways and promote collagen deposition in the basement membrane. Alternatively, ADAM8 expressed by activated leukocytes or epithelial cells may limit subepithelial fibrosis in asthmatic airways by the MP domain of the proteinase degrading ECM proteins deposited by activated (myo)fibroblasts (Figure 3). By altering the ECM composition of asthmatic airways, ADAM8 MP domain may also reduce the survival and differentiation of airway SMCs which require specific ECM components for these processes [128]. However, it is noteworthy that while the ECM-degrading activity of ADAM8 has not been assessed, most ADAMs studied thus far have limited capacity to degrade ECM components, especially interstitial collagens *in vitro* [29].

5. Summary and conclusions

Surprisingly little is known about the contributions of ADAM proteinases to pathologies occurring in the airways of asthmatic patients. ADAM33 was the first member of the ADAM family of MPs to be linked to asthma and AHR [26]. While ADAM33 plays no role in regulating allergen-induced AAI or AHR in mice [28], we and other have proposed that ADAM33 regulates airway remodeling processes in asthmatic airways [17,27,72]. More recently, ADAM8 has been linked to asthma since it is impressively upregulated by T_H2 cytokines in the lungs of mice and its expression is increased in the inflammatory cell infiltrates and airway epithelium of humans [49,129] and mice [57] with AAI and in BALF from patients with some eosinophilic pneumonias [59]. Although ADAM8 levels are increased during AAI in mice and humans, the role of ADAM8 in this disease is to dampen airway inflammation, since studies of OVA-sensitized and -challenged ADAM8 transgenic and $ADAM8^{-/-}$ mice show that this proteinase limits accumulation of leukocytes in the airways [70,71]. Although the mechanism (s) by which ADAM8 exerts its anti-inflammatory activities have not yet been dissected, we speculate that the MP domain of ADAM8 sheds adhesion molecules such as VCAM-1 and L-

selectin and/or leukocyte surface cytokine receptors (TNFR1 or IL-1RII) and/or CD16, all of which play important roles in leukocyte recruitment and activation in asthmatic airways. However, this needs to be confirmed by measuring the shedding of these molecules in transgenic mice over-expressing ADAM8 or in ADAM8$^{-/-}$ mice versus control mice with allergen-induced AAI.

There have been no studies of transgenic mice overexpressing ADAM8 or ADAM8$^{-/-}$ mice in murine models of allergen-induced airway remodeling. Thus, it is not clear whether ADAM8 plays a significant role in regulating airway remodeling processes in asthma. However, based upon its expression profile and the activities of ADAM8 MP domain *in vitro*, we predict that overall, ADAM8 would play a minimal role or no role in directly regulating subepithelial fibrosis or airway SMC hyperplasia. However, by dampening airway inflammation, ADAM8 may reduce mucus hyper-secretion indirectly by limiting the airway burden of other inflammatory cell proteinases known to promote mucus hyper-secretion. Additional studies of transgenic mice overexpressing ADAM8 or ADAM8$^{-/-}$ mice in models of acute and chronic allergen-induced airway inflammation and remodeling are needed to determine the activities of ADAM8 in these pathologies, to identify the critical cellular sources of ADAM8 (inflammatory cells versus bronchial epithelial cells), and to dissect the mechanism involved.

6. Expert opinion

There is an urgent need to develop new treatment strategies that more effectively reduce inflammation in asthmatic airways since there are many asthmatics with chronic symptoms that are refractory to current anti-inflammatory therapies and account for up to 50% of the health care costs of this disease [130]. In addition, current asthma treatments are ineffective at preventing and reversing the chronic remodeling processes in asthmatic airways, which are linked to AHR and a steeper decline in lung function in asthmatic patients.

Most previous studies of asthma pathogenesis have focused on pro-inflammatory molecules and pathways. There have been relatively few studies examining counter-regulatory anti-inflammatory molecules and pathways in asthma. Lipid mediators that inhibit inflammation or promote resolution of inflammation have recently been found to be reduced in patients with asthma and can limit AAI when administered to mice with AAI [131-134]. Thus, anti-inflammatory pathways are an emerging area for designing novel treatment strategies for asthma. Based upon experimental data in mice, ADAM8 is an important anti-inflammatory protein in AAI [70,71] and acute peritonitis [69] but the contributions of ADAM8 to airway remodeling processes in asthma have not been assessed.

Herein, we have speculated that ADAM8 limits AAI by its MP domain shedding adhesion molecules and/or cytokine receptors and IgG receptors to limit AAI. This hypothesis is based upon the results of studies examining the activities of ADAM8 MP domain *in vitro* and the results of correlative studies of ADAM8 levels and the levels of shed candidate substrates in lung samples from humans and mice with AAI. There are several important caveats when drawing conclusions from the results of these studies. First, other ADAMs and MMPs are increased in asthmatic airways, which can also shed these surface molecules [29,58]. Second, our conclusions about important substrates for the ADAM8 MP domain *in vivo* have mainly been based upon the activities of recombinant ADAM8 against peptide sequences containing sites known to be cleaved in intact surface proteins by other sheddases (see Table 1). Thus, it is not clear whether intact proteins that have secondary and/or tertiary structure are cleaved by the transmembrane form of ADAM8 expressed on cell surface and, if so, whether this increases or decreases the biological activity of the products generated. Third, as we have learned from studying MMPs, substrates cleaved efficiently by MMPs *in vitro* were often found not to be important substrates for MMPs *in vivo*, as assessed by subsequent studies of MMP-gene-

targeted mice in murine models of disease. MMPs were initially thought to function as ECM-degrading enzymes, but are now know to play more prominent roles in regulating inflammation and immunity *in vivo* [135]. Thus, it is not clear whether substrates for ADAM8 identified *in vitro* will later be confirmed to be important substrates for ADAM8 MP domain *in vivo*. Fourth, it is possible that ADAM8 domains other than the MP domain are involved in ADAM8's anti-inflammatory activities. The disintegrin domain could bind to leukocyte integrins to inhibit leukocyte-endothelial cell adhesion since ADAM8 disintegrin binds to α9β1 integrin on osteoclasts [38,39] and this integrin is also expressed on leukocytes and promotes leukocyte transendothelial migration [136]. Future studies should also investigate whether the SH3 domain binding sequences in the cytoplasmic tail of ADAM8 bind to and regulate the activity of SH3-domain-containing proteins such as Src family kinases, which play critical roles in regulating leukocyte function [137]. Before ADAM8 can be validated as a new target for asthma, we need more information about the mechanisms by which ADAM8 limits AAI, the critical cellular sources of ADAM8 in the lung, the target cells upon which ADAM8 acts, the ADAM8 domains(s) that contribute to its anti-inflammatory activities, and its activities (if any) in structural remodeling in asthmatic airways. This might be best achieved by additional studies of ADAM8$^{-/-}$ and transgenic mice overexpressing ADAM8 in lung epithelium or leukocytes in strains that develop robust T_H2 inflammation (such as the BALB/c strain) in acute and chronic allergen exposure models. We also need to address whether, as demonstrated for ADAM33 [26,138,139], there are SNPs in the ADAM8 gene that are associated with asthma. If so, it will also be critically important to assess whether SNPs in the ADAM8 gene limit its expression or reduce its functioning and thereby predispose individuals to develop asthma.

Although ADAM8 has not yet been tested as a new therapeutic target in mice with AAI, Schluesener used ADAM8 disintegrin domain as an adjuvant by fusing it to a polyvalent autoantigen vaccine and this modified vaccine reduced disease severity in rats with experimental autoimmune encephalitis [140]. These results suggest that ADAM8 disintegrin domain can inhibit immune reactions to antigens *in vivo*. If future studies confirm that ADAM8 is a promising new therapeutic target for asthma, approaches to increase its expression, translocation to cell surfaces, and/or retention in an active form on cell surfaces might be effective strategies in limiting airway inflammation in subjects with asthma. However, we need more information about the factors that control ADAM8 transcription, trafficking in leukocytes and epithelial cells, and its half life on cell surfaces. We also need more information on the physiological activities of ADAM8 in leukocytes, including whether ADAM8 plays roles in host defense against infection. We also need to determine whether ADAM8 has important functions in other organ systems, especially bone, since: (1) ADAM8 is expressed by osteoclasts and promotes osteoclast differentiation *in vitro* [38,39]; and (2) ADAM8 levels are increased in the joints of patients with rheumatoid arthritis and levels correlate positively with joint destruction [38,48]. ADAM8 is also expressed by several tumors and its expression correlates with invasiveness, metastasis, and poor prognosis (Table 3). Thus, the roles of ADAM8 in leukocyte, osteoclast, and tumor cell biology need to be clarified before strategies to increase the ADAM8 levels of activity *in vivo* can be validated as a new and safe target for asthma.

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Abbreviations

AAI, allergic airway inflammation

ADAM, a disintegrin and a metalloproteinase
AEP, acute eosinophilic pneumonia
AHR, airway hyper-responsiveness
BAL, bronchoalveolar lavage
CD, cluster of differentiation
CEP, chronic eosinophilic pneumonia
CR, cysteine rich
DCs, dendritic cells
ECM, extracellular matrix
EMTU, epithelial mesenchymal trophic unit
IGFBP, insulin-like growth factor binding protein
IRF, interferon-response element
LAP, latency associated peptide
LPS, lipopolysaccharide
MP, metalloproteinase
MMP, matrix metalloproteinase
NF-1, nuclear factor-1
OVA, ovalbumin
PMN, polymorphonuclear neutrophil
PSGL-1, P-selectin glycoprotein ligand-1
sADAM8, soluble ADAM8
sCD23, soluble CD23
SCF, stem cell factor
SH3, Src homology 3
SMC, smooth muscle cell
SNP, single-nucleotide polymorphisms
STAT-6, signal transducer and activator of transcription-6
TNFR1, TNF receptor-1
VCAM-1, vascular adhesion molecule-1
WT, wild-type

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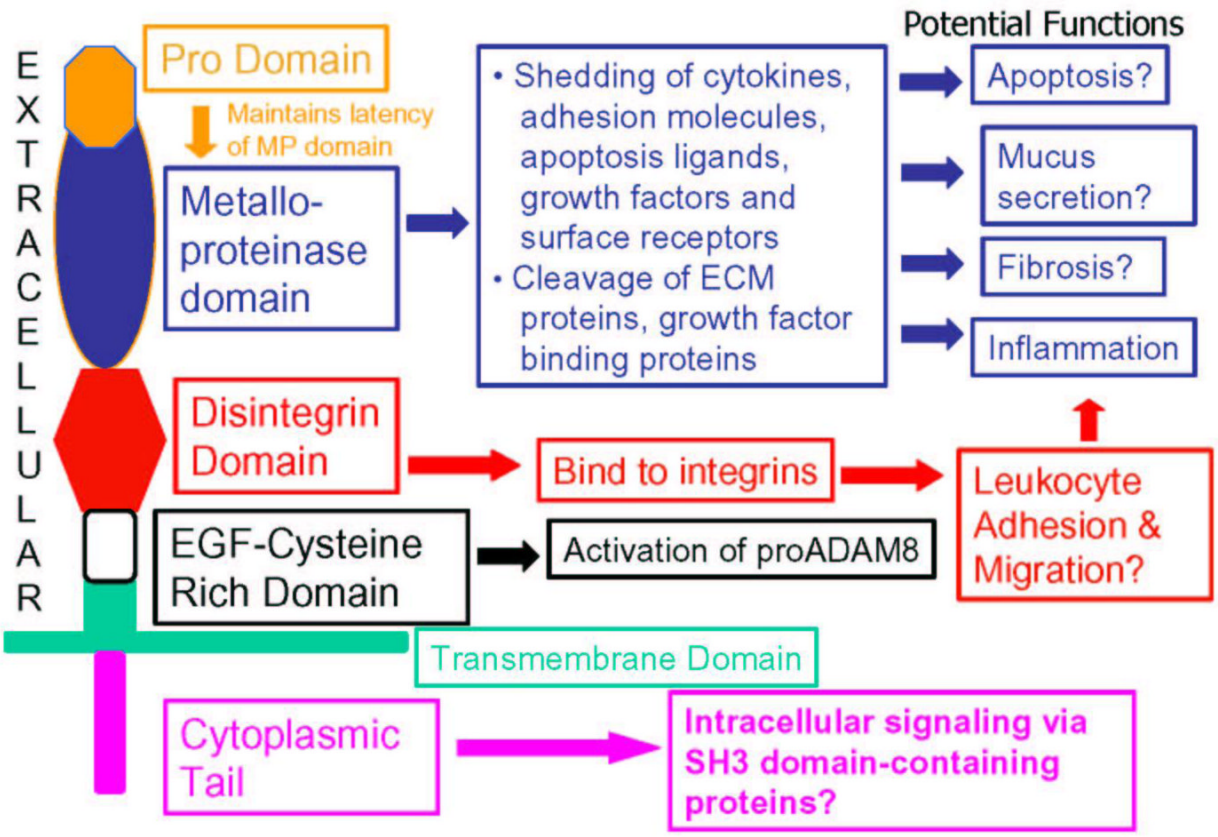

Figure 1. The domain structure of ADAM8 and known or potential functions of each domain

Structure & Potential Function of ADAM8

Most is known about the metalloproteinase (MP) and disintegrin domains of ADAM8. ADAM8 is an active MP and may cleave several cell proteins including adhesion molecules, cytokines, cytokine receptors, growth factors and leukocyte immunoglobulin receptors from cell surfaces. The disintegrin domain of ADAM8 binds to α9β1 integrin on osteoclasts but it is not clear whether it binds to other integrins expressed by leukocytes to regulate leukocyte adhesion or migration. The cytoplasmic tail of ADAM8 has SH3 binding domains but its role in binding to SH3-domain-containing intracellular proteins to regulate intracellular signaling has not been examined. ECM: extracellular matrix.

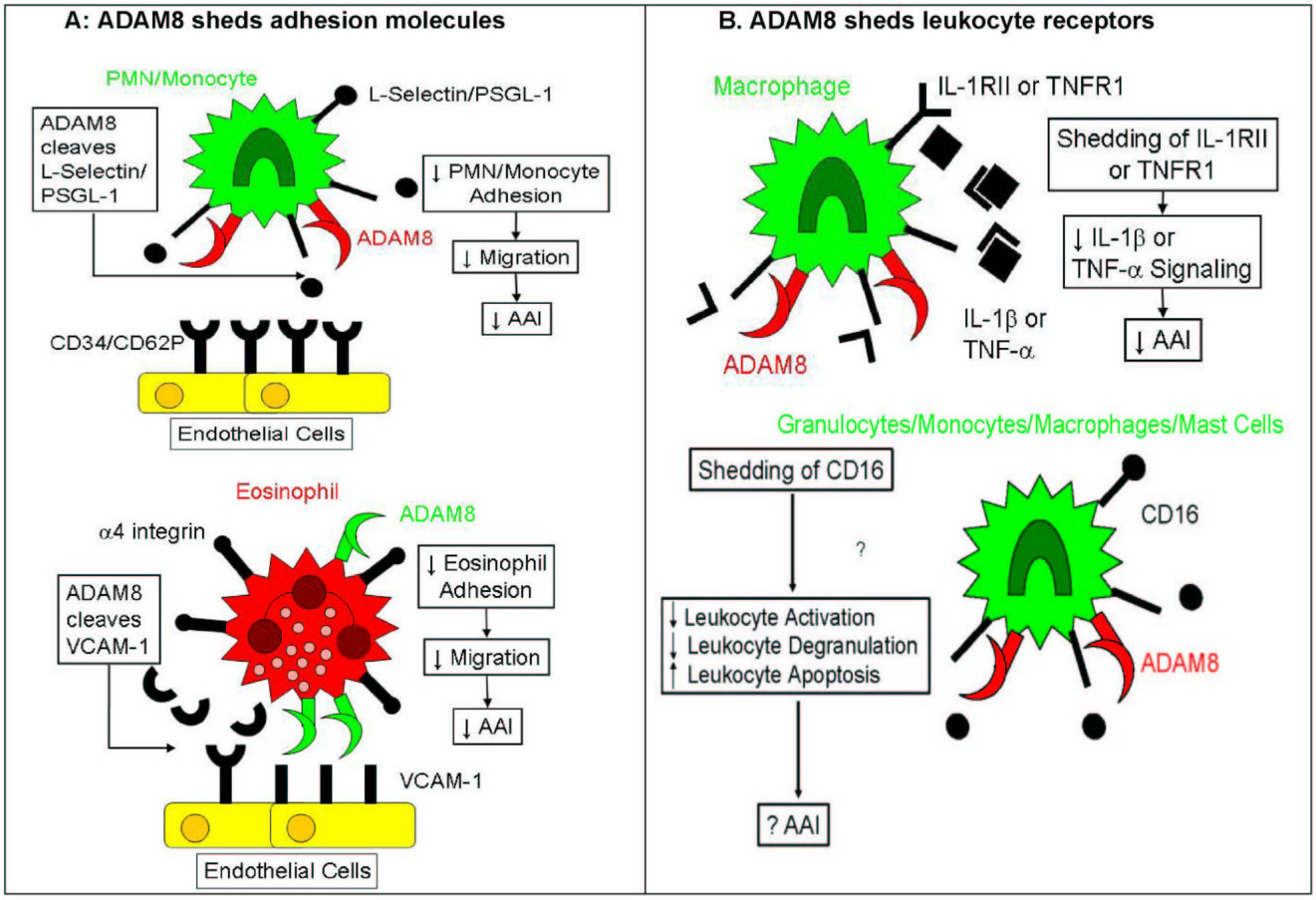

Figure 2. Schematic representation of the potential mechanism(s) by which ADAM8 may limit allergic airway inflammation (AAI)

Potential Mechanisms by which ADAM8 Reduces Allergic Airway Inflammation

In **A (top panel),** L-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) on polymorphonuclear neutrophil (PMNs) and monocytes bind to their ligands (CD62P and CD34, respectively) on endothelial cells to promote PMN and monocyte rolling on endothelial cells which is the initial step in the transendothelial migration of these leukocytes. ADAM8 expressed on the surface of activated leukocytes in the vasculature of asthmatic subjects may shed L-selectin and possibly PSGL-1 from leukocyte surfaces to reduce the egress of PMNs and monocytes from the vasculature into the airways. In **A** (bottom panel), activated eosinophils express α4 integrin which binds to vascular cell adhesion molecule-1 (VCAM-1) expressed on activated endothelial cells leading to the formation of podosome-like adhesive structures in which ADAM8 is localized on the surface of eosinophils. Eosinophil-derived ADAM8 may shed VCAM-1 from endothelial surfaces to reduce α4 integrin-VCAM-1-mediated eosinophil-endothelial-cell adhesion which may limit the migration of eosinophils into asthmatic airways. In **B** (top panel), ADAM8 expressed on activated leukocytes or bronchial epithelial cells in asthmatic subjects may cleave IL-1 RII and TNFR1 from leukocyte or epithelial surfaces which may decrease the pro-inflammatory signaling of IL-1β or TNF-α through their receptors in asthmatic airways. In **B** (bottom panel), ADAM8 may shed CD16 from the surfaces of various leukocytes. CD16 is a low-affinity IgG receptor and binding of IgG to this receptor promotes leukocyte activation, degranulation and survival. ADAM8 expressed on the surface of activated leukocytes in asthmatic airways may shed CD16 to reduce

leukocyte activation and decrease leukocyte survival, which may reduce inflammation in asthmatic airways.

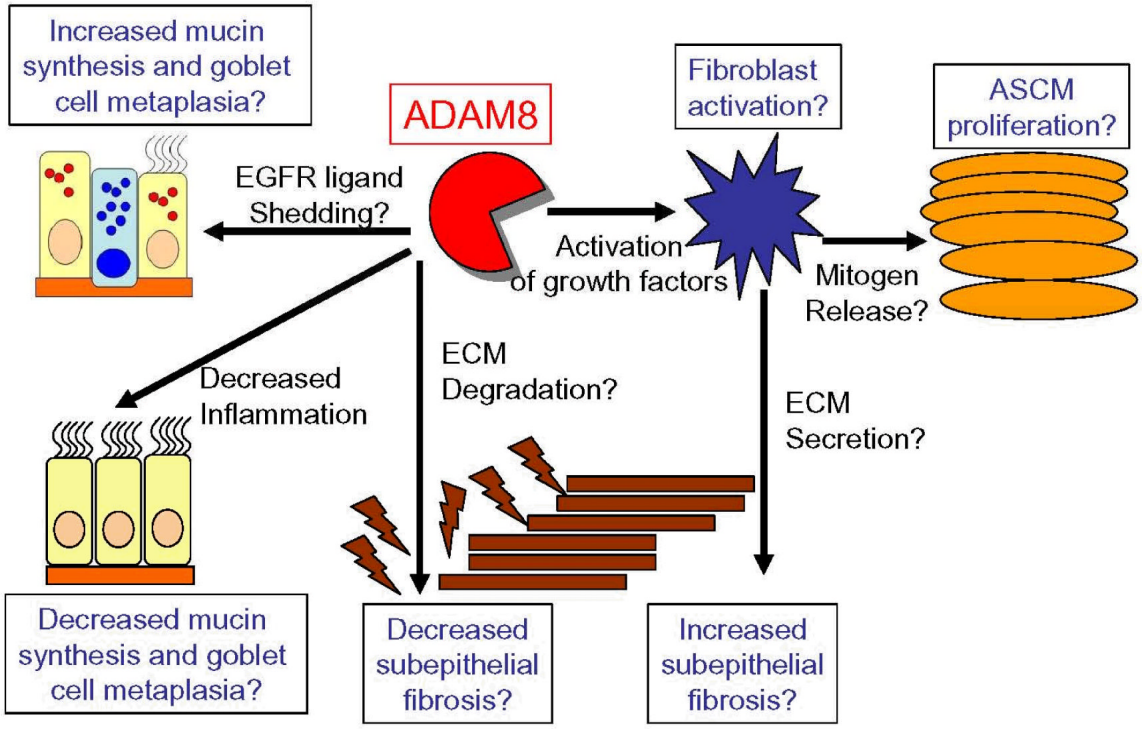

Figure 3. Schematic representation of the potential mechanisms by which ADAM8 may regulate airway remodeling in asthma

Potential Activities of ADAM8 in Remodeling of Asthmatic Airways

We currently have no data linking ADAM8 to airway remodeling in asthma. Based upon its anti-inflammatory activities in murine models of allergic airway inflammation (AAI), we speculate that ADAM8 may indirectly reduce mucin gene expression and goblet cell metaplasia by decreasing the airway burden of inflammatory cells and their mucin-inducing proteinases. The growth factor-activating activities of ADAM8 have not yet been tested. However, if the metalloproteinase domain of ADAM8 that is expressed on the surface of activated leukocytes or bronchial epithelial cells in asthmatic airways plays a significant role in activating TGF-β family members, IGFs, or ligands for the EGFR, this would promote fibroblast activation and deposition of extracellular matrix (ECM) proteins to increase subepithelial fibrosis and fibroblast secretion of mitogens that induce airway smooth muscle cells (ASMC) proliferation. If ADAM8 that is expressed by activated bronchial epithelial cells sheds EGFR ligands from epithelial cell surfaces, this may also promote mucin secretion and goblet cell metaplasia in the airway epithelium of subjects with asthma. Alternatively, ADAM8 that is expressed by activated airway leukocytes or activated bronchial epithelial cells in asthmatic airways may reduce subepithelial fibrosis by degrading ECM proteins deposited by airway myofibroblasts.

Table 1
Known and potential substrates for ADAM8 metalloproteinase (MP) domain

Substrate	Effect of ADAM8-mediated cleavage of the substrate	References
Cytokines:		
Pro-TNF-α*	Sheds active, soluble TNF-α Increase AAI	[33,35]
Fractalkine (CX3CL1)*	Decrease leukocyte accumulation in the airways	[35]
Stem cell factor (SCF)*	Regulate mast cell activation and survival	[33,35]
Receptors:		
TNF receptor 1*	Decrease AAI	[35]
IL-1 receptor II (decoy receptor)*	Decrease AAI	[33,35]
CD16*	Decreased leukocyte activation	[35]
CD23*	Increase IgE release by B cells Increase cytokine release by leukocytes	[35,96]
Adhesion molecules:		
L-selectin†	Limit accumulation of PMN and monocytes in the airways	[35,48]
VCAM-1†	Limit accumulation of eosinophils and B cells in the airways	[35,56,70]
P-selectin glycoprotein ligand-1*	Limit accumulation of PMN and monocytes in the airways	[35]
Neural cell adhesion molecule (NCAM or CHL1)†	Suppress neuronal cell death and regulate neuronal development	[141,142]
Growth factors:		
TGF-α*	EGF receptor activation to increase mucin production and airway remodeling	[35]
Other proteins:		
Amyloid precursor protein*	α-secretase activity generating anti-amyloidogenic peptides	[33,35]
Basic myelin protein*	*Promotes demyelination	[33-35]

AAI: allergic airway inflammation; CHL1: close homolog of L1; PMN: polymorphonuclear neutrophil; VCAM: vascular cell adhesion molecule

*Indicates that a short peptide containing sequences in the protein known to be cleaved by proteinases was tested as a substrate for ADAM8 MP domain.

†Indicates that the full length protein was tested as a substrate for ADAM8 MP domain.

Table 2
Expression and regulation of ADAM8 in humans and mice

Tissue/cells	Stimuli increasing ADAM8	References
Murine lung	OVA-induced AAI, IL-4, and −13	[57]
Mouse bronchial epithelial cells	Low level basal expression	[47]
	OVA-induced AAI, IL-4 and IL-13	[57]
Human bronchial epithelial cells	Asthma	[49]
Leukocytes:		
Eosinophils	OVA-induced AAI, IL-4, IL-13 Adhesion to endothelial cells	[31,56,57]
PMN	Phorbol ester and adhesion to endothelium	[48]
Monocytes	Macrophage colony stimulating factor	[71]
Murine monocytic cell lines	LPS and interferon-γ	[32]
Murine macrophages	OVA-induced AAI	[30,57]
Myeloid DC	LPS, TNF-α	
	IL-4, IL-4 and GM-CSF	[36]
B cells	Low level basal expression	[36]
	ADAM8 inducers not known	
T cells	Do not express ADAM8	[31,36]
Basophils	ADAM8 expression has not been assessed	
Mast cells	ADAM8 expression has not been assessed	
Murine central nervous system:		
Oligodendrocytes and neurons	Low-level basal expression	[47]
Neurons and glial cells	TNF-α	
	ADAM8 levels are increased in neurodegenerative diseases	[45]
Murine ovarian follicles	Phorbol ester, human chorionic gonadotrophin and forskolin	[46]
Osteoclasts, bone, cartilage	Low-level basal expression	[47]
	Macrophage colony stimulating factor	[38]
Murine salivary gland epithelium	Low-level basal expression	[47]
Murine kidney epithelium	Low-level basal expression	[47]

AAI: allergic airway inflammation; LPS: lipopolysaccharide OVA: ovalbumin;

Table 3
ADAM8 expression and activities in diseases other than asthma

Disease (species)	Expression and activities of ADAM8	References
Eosinophilic pneumonia (humans)	Soluble ADAM8 is increased in the BAL of patients with non-drug-induced eosinophilic pneumonias. Soluble ADAM8 levels correlate positively with soluble levels of CD23 and VCAM1 in BALF.	[59]
Osteoporosis (humans)	ADAM8 is upregulated in osteoclasts and promotes osteoclast fusion and differentiation.	[38,39]
Rheumatoid arthritis (humans)	Increased ADAM8 levels on PMNs and in joint pannus and synovial fluid. ADAM8 levels correlate positively with joint destruction.	[38,48]
Reperfusion injury in the kidney (mice)	ADAM8 expression is increased in hypoxia-induced renal injury.	[143]
Neurodegeneration (mice)	ADAM8 shed NCAM1 which promotes neurite outgrowth and branching.	[141,142]
Preterm delivery during pregnancy	Elevated ADAM8 levels in mid-trimester amniotic fluid are associated with an increased risk of preterm delivery.	[144]
Spinal cord injury (mice)	ADAM8 is expressed in the endothelial cells of mice with spinal cord injuries and its expression correlates with angiogenesis.	[145]
Tumors (all human):		
Brain	ADAM8 is highly expressed especially in glioblastoma multiforme. ADAM8 expression is associated with increased tumor invasiveness.	[146]
Lung	ADAM8 is increased in lung cancer tissue, especially in higher stage adenocarcinomas. sADAM8 is increased in serum of lung cancer patients.	[40]
Pancreatic	ADAM8 levels are increased in pancreatic ductal adenocarcinoma and associated with decreased patient survival. ADAM8 increases the invasiveness of pancreatic cancer cell lines.	[147]
Prostate	ADAM8 expression is increased and associated with unfavorable prognosis.	[148]

BAL: bronchoalveolar lavage; BALF: bronchoalveolar lavage fluid; PMN: polymorphonuclear neutrophil; NCAM: Neural cell adhesion molecule; VCAM: vascular cell adhesion molecule.