ADAM-family metalloproteinases in lung inflammation – potential therapeutic targets

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Running head: ADAMs in lung inflammation

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Abstract

Acute and chronic lung inflammation are driven and controlled by several endogenous mediators that undergo proteolytic conversion from surface expressed proteins to soluble variants by a disintegrin and metalloproteinase (ADAM) family members. TNF and epidermal growth factor receptor ligands are just some of the many substrates by which these proteases regulate inflammatory or regenerative processes in the lung. ADAM10 and ADAM17 are the most prominent members of this protease family. They are constitutively expressed in most lung cells and – as recent research has shown – are the pivotal shedding enzymes mediating acute lung inflammation in a cell-specific manner. ADAM17 promotes endothelial and epithelial permeability, transendothelial leukocyte migration, and inflammatory mediator production by smooth muscle and epithelial cells. ADAM10 is critical for leukocyte migration and alveolar leukocyte recruitment. ADAM10 also promotes allergic asthma by driving B-cell responses. Additionally, ADAM10 acts as a receptor for *Staphylococcus aureus* α-toxin and is crucial for bacterial virulence. ADAM8, ADAM9, ADAM15 and ADAM33 are upregulated during acute or chronic lung inflammation, and recent functional or genetic analyses have linked them to disease development. Pharmacological inhibitors that allow to locally or systemically target and differentiate ADAM-family members in the lung suppress acute and asthmatic inflammatory responses and *S. aureus* virulence. These promising results encourage further research to develop therapeutic strategies based on selected ADAMs. These studies need also to address the role of the ADAMs in repair and regeneration in the lung to identify further therapeutic opportunities and possible side effects.
Introduction

Acute or chronic inflammation in the lung can lead to a fatal loss of lung functions. Acute inflammation resulting from infection, aspiration of gastric juice or overventilation during intensive care is initially characterized by enhanced inflammatory mediator production, edema formation and recruitment of innate immune cells with the risk of lung damage. Asthma, chronic obstructive pulmonary disease (COPD) and lung fibrosis are fatal chronic diseases that are driven by persistent inflammation with a lack of resolution and impaired tissue regeneration. Targeting the immune response is the underlying principle of many treatment strategies against these diseases (reviewed in (7)).

The cytokines, growth factors, receptors and adhesion molecules, that drive the inflammatory process, are all under intensive scrutiny. Many of these molecules undergo functional modulation by limited proteolysis, bringing the participating proteases into the focus of attention. Among these proteases is a class of metalloproteinases that subdivides into the matrix metalloproteinases (MMP) and the families of a disintegrin and metalloproteinases (ADAM) and a disintegrin and metalloproteinase with trombospondin motif (ADAMTS). MMPs have already been intensively investigated with respect to their involvement in acute and chronic inflammation as well as tissue remodeling and repair (reviewed in (81; 138)). However, despite the many encouraging data obtained from MMP knockout mice, clinical trials with broad spectrum MMP inhibitors have been unrewarding (reviewed in (197)).

Recent data from human and animal studies do now suggest that also ADAMs are critically involved in inflammatory lung diseases, which has further raised interest in these proteases as potential drug targets. However, besides the growing number of promising reports for targeting of ADAM-family members in several disease models, there are also alerting findings hinting at potential side effects of such inhibition strategies in pulmonary inflammation. In the following, we will summarize and discuss the physiological and pathophysiological functions of selected ADAM-family members in the lung.

Overview on ADAM family proteases

The ADAM-family comprises approximately 34 members, 22 of which have been described in humans (reviewed in (4; 81); (37)). ADAMs are predominantly expressed as transmembrane proteins carrying an N-terminal prodomain, preceding a metalloproteinase domain which is followed by a disintegrin, a cysteine-rich, a transmembrane and finally a cytoplasmic domain (Figure 1A). ADAMs can function as adhesion molecules, as proteases
or they can combine both functions. The disintegrin domain has been associated with adhesive functions by mediating interaction with integrins or with extracellular matrix components. The metalloproteinase domain mediates proteolytic activity. A zinc atom bound in the proteases’ active site is vital for the proteolytic mechanism. In humans and mice ADAM8, 9, 10, 12, 15, 17, 28 and 33 display proteolytic activity, which was also predicted from structural comparison of the zinc binding site (4). Most ADAMs are synthesized as proenzymes, and display reactivity only upon proteolytic removal of the prodomain that blocks the zinc atom.

Substrates for proteolysis by ADAMs are generally other transmembrane surface proteins (Table 1). Proteolytic cleavage occurs in close proximity to the cell surface, results in the shedding of the substrates’ ectodomain into the intracellular space and generates a cell-associated fragment consisting of the transmembrane and cytoplasmic domain. Within the substrate molecule there seems to be no clearly defined amino acid motif defining its cleavage indicating that structural determinants distinct from the cleavage site contribute to substrate recognition by ADAMs. (10; 175). The protease itself generates some specificity by the substrate binding pockets in the proteases’ active site (18), and some by several of its more distant domains including the cysteine-rich domain (77), the juxtamembrane region and the transmembrane domain (36) that need to exist for recognition of selected substrates by certain ADAMs. Shedding significantly modulates the activity of growth factors, adhesion molecules, receptors and cytokines by the release of soluble ectodomains acting as either antagonists or agonists, by the loss of a functional cell surface molecule, or by intracellular signal transduction via the cell-associated cleavage fragment (Figure 1A). Through these mechanisms shedding is critical for embryonic and fetal development as well as for many physiologic and pathophysiologic processes in the adult. Among these multiple functions, it is especially the role of ADAMs in inflammation and cancer that suggests these shedding enzymes as potential drug targets (147; 158; 160).

Most of the known shedding events are mediated by the two closely related proteases ADAM10 and ADAM17 (Figure 1B, Table 1). The importance of these two proteases is underlined by the fact that their knockout in mice is lethal either during embryonic development or shortly after birth (11; 65). ADAM10 and ADAM17 are abundantly expressed not only during development, but also in adult tissue including the lung (30). Both proteases have been implicated in various inflammatory diseases, in wound healing, neurodegenerative diseases, and cancer development. Accumulating evidence from animal studies with either pharmacological inhibitors or gene targeted mice also points towards a crucial role in
inflammatory lung diseases. As detailed in this review, both ADAM10 and ADAM17 regulate pulmonary inflammation via several pathways in a cell-specific manner. In light of these cell-specific contributions this review will then summarize and discuss pharmaceutical approaches targeting ADAM10 or ADAM17 either systemically or locally.

Much less is known about the other proteolytically active proteases ADAM8, 9, 12, 15, 28 and 33. Their knockout phenotypes do not indicate a critical role in development and normal physiology. In contrast to the broad and high expression of ADAM10 and ADAM17 these other ADAMs are normally expressed at much lower levels, but may become upregulated under inflammatory conditions. Especially ADAM8 and ADAM33 have been associated with allergic airway disease, but their functional implication is still not well understood. Here, we will also review the evidence for this association and discuss potential mechanisms of action.
Table 1: List of substrates and binding proteins of ADAM-family protease substrates relevant in pulmonary inflammation

<table>
<thead>
<tr>
<th>ADAM</th>
<th>Growth factors, cytokines, chemokines</th>
<th>Adhesion molecules</th>
<th>Receptors, other molecules</th>
<th>Binding c</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>TNF peptide (44) SCF peptide (132) TGFα peptide (132) CX3CL1 peptide (132) d</td>
<td>L-selectin (57) PSGL-1 (31)</td>
<td>TNFR1 (8) CD23 (44)</td>
<td>α9β1 integrin (151)</td>
</tr>
<tr>
<td>9</td>
<td>HB-EGF (75) IGF-BP (122)</td>
<td></td>
<td>ADAM10 (185) ACE-1(38) extracellular matrix (159)</td>
<td>α1, α3, α6 and αe and β1 integrin (130)</td>
</tr>
<tr>
<td>10a</td>
<td>EGF (212) betacellulin (161) neuregulin 1(45) CX3CL1 (71) CXCL16 (1)</td>
<td>E-cadherin (109) VE-cadherin (166)</td>
<td>Notch (65) RAGE (152) CD23 (205) IL6R (118) FasL (165) Ephrin-A (77) CD44 (177)</td>
<td>S. aureus toxin receptor (73) tetraspanins (CD9, CD81, CD82) (5) tetraspanin 12 (210) tetraspanin 15 (145)</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>E-cadherin (179)</td>
<td>FGFR II (111) ADAM10(185) extracellular matrix (113)</td>
<td>α5β1, αvβ3, α9β3 integrins (40; 129)</td>
</tr>
<tr>
<td>17a</td>
<td>TNF (11) TGFα (161) amphieregulin (161) HB-EGF(161) epiregulin (161) neuregulin 1(69) CX3CL1(47)</td>
<td>L-selectin (142) JAM-A(86) VCAM-1(48) ICAM-1(189) ALCAM (54) α5β1 integrin (190)</td>
<td>TNFR1 and R2 (154) IL6R (3) IL15R (17) IL1RII (154) syndecan 1 and 4 (148) CD44(177) c-Kit (26) Mer (184)</td>
<td>α5β1 integrin (58) tetraspanin CD9 (63)</td>
</tr>
<tr>
<td>33</td>
<td>TRANCE peptide (225)</td>
<td>c-Kit peptide (225)</td>
<td></td>
<td>α9β1 integrin</td>
</tr>
</tbody>
</table>

a many more substrates are known and only those relevant for pulmonary inflammation are listed
b determined in either cellular cleavage assays or with synthetic peptides as indicated
c membrane-expressed interaction partners
d abbreviations are explained in the text
ADAM17

ADAM17 is not only required for development, but also triggers a number of distinct pathways involved in various pathologies (reviewed in (158)). Accumulating data point towards a critical function of this protease in acute lung inflammation.

ADAM17 was first discovered as the TNF cleaving enzyme, and initially this was thought to be the predominant function of ADAM17 directly linking it to inflammatory diseases (11; 125). The importance of TNF in rheumatoid arthritis and inflammatory bowel disease but also in acute respiratory distress syndrome (ARDS), asthma and severe COPD has long been recognized (7). The significance of ADAM17 in humans is underlined by the fact that loss of function mutations of ADAM17 are extremely rare; only one case has been found. The peripheral blood leukocytes from this patient showed impaired release of TNF and the patient suffered from repeated inflammatory skin and bowel diseases (12). Besides TNF, to date more than 77 substrates for ADAM17 are known (reviewed in (163)). Importantly, growth factors of the epidermal growth factor (EGF) family including transforming growth factor (TGF)$\alpha$, heparin-binding (HB)-EGF, amphiregulin, epiregulin and neuregulin (13; 161) are subject to cleavage by ADAM17. Once released the growth factors can bind to receptors of the ErbB-family including ErbB1 (also termed epidermal growth factor receptor, EGFR), ErbB3 and ErbB4 in a paracrine and autocrine fashion. This pathway is termed transactivation and provides critical transcriptional signals to regulate proliferation and differentiation. Transactivation via growth factor shedding is indispensable in developmental processes, but accumulating evidence also suggests essential roles in tumor development and inflammation. The mere fact that ErbB/EGFR signaling is involved in several inflammatory lung disorders including acute lung inflammation, asthma and fibrosis (193), together with the fact that ADAM17 is the shedding protease for ErbB-family ligands, already suggests that this protease plays a crucial role in pulmonary inflammation. As discussed below, beyond TNF and ErbB family ligands there exist many other substrates by which ADAM17 can regulate inflammatory events in the lungs: e.g. TNF receptors, IL6 receptor (IL6R), the tyrosine kinase Mer, L-selectin, the transmembrane chemokine CX3CL1, the vascular cell adhesion molecule VCAM-1, the intracellular cell adhesion molecule ICAM-1, the junctional adhesion molecule JAM-A, the proteoglycan CD44 and chemokine-presenting syndecans and c-Kit (Table 1, reviewed in (147; 163)).

It seems that all lung cells express ADAM17 albeit at different levels (30; 39). In addition, ADAM17 may become upregulated on the transcriptional level in the lung (39). However, it
has become clear that the protease is predominantly regulated by posttranscriptional mechanisms involving enzymatic modification, conformational changes, interaction with adapter molecules and/or cellular trafficking in a stimulus- and cell-specific manner (reviewed in (34)). The activity of ADAM17 can be upregulated by ligands for GPCR including PAF, thrombin, ATP and angiotensin, and bacterial toxins or products such as lipoteichoic acid. All of these are potent mediators of edema formation, blood pressure regulation, coagulation or pathogen recognition in the lung. Therefore, it appears likely that under inflammatory conditions in the lung increased levels of several shedding products are found in the alveolar fluid. And in fact, this has been described for, CX3CL1, JAM-A, CXCL16, TNF, TNFR, TGF\(\alpha\), IL6R and syndecans in human sputum samples (20; 89; 123; 153; 198) or murine alveolar lavage fluid (32; 33; 148).

Most data on the functional relevance of ADAM17 in the lung were obtained from the analysis of gene targeted mice. Targeted disruption of the zinc binding site within the metalloproteinase domain or general knockout of ADAM17 leads to various defects including an open eye phenotype with altered eyelid, hair, whisker development, aberrant heart valve development and defects in mammary morphogenesis (76; 142; 176). In their lungs these mice show decreased branching of the airways (222). Most developmental effects of ADAM17 deficiency including airway branching have been explained by the lack of growth factor shedding which is responsible for the transactivation of EGFR. The elucidation of the role of ADAM17 beyond developmental issues became possible by the generation of floxed mice for conditional knockout (67) that enabled several groups to assess the protease’s function in distinct cell types of the lung in various lung diseases (see Table 2).

**Endothelial ADAM17 - a regulator of vascular responses in acute lung injury**

Acute lung inflammation is characterized by an increased permeability of the pulmonary vascular endothelium. Several properties of the endothelium that regulate vascular permeability such as leukocyte adhesion, paracellular contact between endothelial cells and leukocyte transmigration are regulated by ADM17, as indicated by studies with endothelial-specific knockout mice.

Mice with preferential ADAM17 deficiency in endothelial cells (driven by Tie2-Cre) have been generated by several groups. In the first report no obvious vascular phenotype, but decreased pathological neovascularization was observed in a cornea pocket assay (206). Two other studies described decreased collateral vessel formation (103) and defective remodeling of the semilunar valves and cardiac dysfunction (208). It is thought that the endothelial effects of ADAM17
deficiency on collateral vessel formation are in large part due to the early deficiency of VEGF-induced Notch signaling in developing blood vessels (103), while the pathological neovascularization in the adult may be explained by a lack of receptor transactivation via growth factors such as TGFα and HB-EGF (206). Although the same promoter was being used in these three studies, it may be possible that the different driver lines with different insertion of the transgene led to knockout during different developmental stages thereby either already affecting the early Notch processing during development or rather regulating the later ErbB receptor transactivation. In models of lung inflammation, hitherto only those mouse lines with no obvious basal phenotype have been used.

Endothelial cells can attract leukocytes via the activity of chemokines that are either expressed as transmembrane chemokines such as CX3CL1 or presented on the endothelial surface via proteoglycans such as syndecans (reviewed in (61; 96)). Endothelial surface expressed CX3CL1 is special in that it can tightly bind to its receptor CX3CR1 on leukocytes in order to establish flow resistant cell-to-cell contacts (reviewed in (106)). At a later step integrins on leukocytes are contributing to establish firm adhesion by binding to cell adhesion molecules such as VCAM-1 and ICAM-1 on the endothelial cell surface. Subsequently the leukocytes migrate through the endothelial junctions, where they interact with junctional adhesion molecules including JAM-A. Notably, all of these endothelial surface molecules undergo cleavage by ADAM17 (47; 48; 86; 148; 189). On the one hand, the cleavage may result in reduced surface expression of these adhesion molecules and thereby downregulate endothelial adhesiveness for leukocytes. On the other hand, the cleavage could also be required to solve the endothelial attachment of leukocytes and that between neighboring endothelial cells as a prerequisite for leukocyte transit through the endothelial cell layer (72; 167). In fact, silencing or pharmacological inhibition of ADAM17 in pulmonary endothelial cells reduces endothelial permeability and transmigration of leukocytes (32).

Thus, ADAM17 affects the endothelium in multiple ways. The overall role of ADAM17 in acute inflammation (24h) was analyzed in a model of endotoxin-induced (intranasal challenge) acute lung inflammation that is characterized by cytokine release (TNFα and IL-6), leukocyte infiltration (neutrophils and monocytic cells) and edema formation 24h after intranasal LPS challenge. All these responses were considerably reduced in mice with cell-specific deficiency of ADAM17 in endothelial cells (32) underlining the importance of the protease in mediating endothelial dysfunction in the course of acute lung inflammation.
Smooth muscle ADAM17 - a relay in acute lung inflammation

Pulmonary smooth muscle cells have long been suspected to play a role in inflammatory lung diseases as these cells can produce relevant amounts pro-inflammatory mediators in vitro (209). The possibility of generating smooth muscle-specific knockout mice now allows to address this topic in a pathophysiological context.

Knockout of ADAM17 in smooth muscle cells (SMC) (driven by Tagln-Cre) does not display any obvious physiological phenotype (206). In the inflamed lung, smooth muscle cells may hold a specialized function by transmitting inflammatory signals from and to the endothelial cell layer. This concept is supported by in vivo and in vitro experiments on the role of ADAM17 for inflammatory mediator production by SMC. Mice lacking ADAM17 in SMC (driven by Tagln-Cre) showed considerably decreased lung damage, edema formation, neutrophil recruitment, and cytokine production in response to intranasal LPS challenge (33). Moreover, these mice were also protected in a model of acid-induced lung inflammation. In vitro experiments revealed that cultured lung airway SMC require ADAM17 for efficient induction of CXCL8 and IL6 by pro-inflammatory mediators such as LPS or by treatment with supernatants of acid-exposed epithelial cells. The LPS-induced response was explained by ADAM17-mediated shedding of TGFα and subsequent transactivation of SMC via EGFR leading to enhanced cytokine induction. The acid-induced response, however, was mediated by the release of neuregulins and ErbB4-transactivation (33). These findings do not only indicate an important role of lung interstitial cells in the transmission of inflammatory signals across the airways, but they also demonstrate that this process is governed by ADAM17. As a caveat, so far the Tagln-Cre-driven knockout mice do not allow to distinguish between airway smooth muscle cells, vascular smooth cells and pericytes. It may well be possible that all of these cell types relay inflammatory signals to the endothelium.

Epithelial ADAM17 - a regulator of epithelial barrier and defense function

Clearly, pulmonary epithelial cells are critically important in the pathogenesis of ARDS, but the great heterogeneity of epithelial cells from the trachea down through the bronchial tree until the alveoli, makes their study different. So far, a role for ADAM proteases in the pulmonary epithelium has been demonstrated for lung development and pulmonary inflammation, while fluid absorption – another important issue in ALI – has not yet been looked at.

Epithelial ADAM17 has been best characterized with respect to its role in lung development. Lung epithelial knockout (driven by SPC-rTA/TetO-Cre) results in developmental defects
including reduced saccular formation and decreased proliferation and differentiation of mid-distal
epithelium (211). In cultured fetal epithelial cells mechanical strain was found to induce
ADAM17 activity via its binding to \( \alpha_6\beta_1 \) integrin leading to enhanced HB-EGF shedding and
differentiation (199). *In vitro* findings on the transactivation of ErbB receptors via growth factor
shedding suggests a number of potentially pro-inflammatory properties of ADAM17 that
remain to be studied in more detail. The versatility of epithelial ADAM17 is indicated by a
number of *in vitro* studies with epithelial cells demonstrating that the protease is required for
epithelial activation by TLR ligands (88), for CXCL8/IL-8 expression (128), permeability
changes (41), for mucus hypersecretion induced by smoke, elastase or oxygen radicals (90;
150; 169), and for epithelial type II cell differentiation in response to mechanical-strain (200).
Another potentially interesting mechanism is bacterial induction of ADAM17 and shedding of
IL6R, because this can program epithelial cells to switch their chemokine production from
neutrophil attracting CXCL8 to monocyte attracting CCL2 (56). Yet, ADAM17 on epithelial
cells can also organize anti-inflammatory responses such as the shedding of TNF receptors
(218) that generates a soluble decoy receptor antagonizing TNF functions (89).

So far, the role of epithelial ADAM17 has not yet been analyzed in models of chronic lung
disease. In mice, epithelial overexpression of a dominant negative EGFR suppressed house
dust mite allergen-induced chronic asthma (92). The latter finding gives rise to the hypothesis
that transactivation of the epithelium by ADAM proteases may regulate airway
hyperresponsiveness and remodeling in chronic lung diseases. ADAM17 may also act on
goblet cells in the lung. Using tamoxifen-inducible general knockout ADAM17\(^{-/-}\) mice (driven
by R26CreER), it was found that ADAM17 deficiency reduced elastase-induced goblet cells
metaplasia and mucus production, suggesting that ADAM17 may accelerate chronic
inflammatory airway disease (136).

### Leukocytic ADAM17 – regulation of cytokine release and inflammatory cell recruitment

Leukocytes including neutrophils, macrophages, monocytes, and lymphocytes are clearly
critical for the pathogenesis of acute lung inflammation. Leukocyte recruitment is a hallmark
of acute inflammation and both resident and recruited leukocytes contribute to inflammatory
mediator production. On all of these cells ADAM17 turned out to be a critical protease
regulating cytokine and adhesion molecule function. The ADAM17 substrates and leukocyte
surface molecules TNF, L-selectin and IL6R open up numerous avenues into the regulation of
pulmonary inflammation by the protease. For instance, when exposed to LPS, mice with
hematopoietic cells that lack ADAM17 (driven by Vav-Cre), showed reduced levels of TNF and L-selectin and an altered pattern of neutrophil sequestration in the lungs (6) (see below). However, it is clearly not easy to reconcile to which extent each of the shedding pathways modulates the inflammatory response in the lung.

The general importance of ADAM17 as a shedding enzyme on leukocytes is evidenced by the finding that mice are protected against LPS-induced septic shock when their myeloid cells lack ADAM17 (driven by LysM-Cre oder Mx1-Cre) (67). Recent reports revealed that the rhomboid family member iRhom2 is required for the maturation and the surface expression of ADAM17 in leukocytes, but not in tissue cells and in fact, deficiency of iRhom2 protected mice from LPS-induced septic shock (120). In both instances the lack of TNF shedding has been made responsible for the observed protection. TNF is considered a cardinal pro-inflammatory cytokine that also contributes to inflammatory lung damage and edema formation (155; 216). However, in LPS-induced lung inflammation mice lacking ADAM17 in hematopoietic cells (driven by Vav-Cre) were not protected against lung inflammation as measured by edema formation (146). This may indicate that TNF shedding by leukocytes is more critical for the development of systemic sepsis and less relevant for lung inflammation. Yet, there exist other forms of acute lung alveolar damage that appear to be more dependent on TNF shedding. For example acute lung inflammation that develops after transfer of cytotoxic effector CD8+T-cells is clearly suppressed by ADAM17 deficiency in the transplanted cells (produced by liver chimeras of Adam17ΔZn/- and Rag1ΔZn/- mice) (28).

Besides release of soluble TNF, further shedding pathways must be considered relevant for inflammatory responses in the lung. This includes the shedding of adhesion molecules and the resulting effects on leukocyte sequestration. While ADAM17 deficiency seems not required for leukocyte migration in standard chemotaxis assays (146), this does not rule out the possibility that the protease interferes with the leukocyte-endothelial interactions. On the surface of leukocytes ADAM17 mediates L-selectin shedding, and loss of L-selectin from the cell surface causes reduced rolling and adhesion to the endothelium under flow conditions (174) leading to reduced neutrophil recruitment. Correspondingly, loss of L-selectin shedding by ADAM17 deficiency in leukocytes or disruption of the cleavage site within L-selectin has been correlated with more severe early peritoneal recruitment of neutrophils induced by E. coli or thioglycollate (100; 101; 183) (driven by Vav-Cre in 72 or by hematopoietic chimeras of wildtype mice with ADAM17ΔZnΔZm or ADAM17ΔEx5ΔEx5). On monocytes, ADAM17 does not limit cell migration by L-selectin shedding (183), but rather promotes monocyte transendothelial migration, which was associated with ADAM17 dependent surface regulation.
of MAC-1 (αMβ2-integrin) (190). Also in acute lung inflammation there may exist pathways by which ADAM17 can promote or suppress neutrophil or monocyte migration over the course of the inflammatory response. At an early stage (2h after LPS inhalation) ADAM17 deficiency was found to slightly enhance alveolar neutrophil recruitment (6) which may be explained by reduced L-selectin shedding leading to enhanced rolling. However, a reduction of neutrophil accumulation was observed at a later stage (8h), which correlated with diminished production of the neutrophil attracting chemokines CXCL1 and CXCL5 (6). At even later time-points (72h after intranasal LPS) no effect of ADAM17 deficiency (driven by Vav-Cre) on neutrophil or monocyte recruitment and CXCL1 production was observed (146). These observations illustrate that the contribution of ADAM17 towards leukocyte recruitment depends on the stage of the inflammation: ADAM17 controls both rapid events like adhesion molecule shedding as well as protracted events like cell activation and the induction of chemokine expression.

Also IL6R shedding might be relevant for pulmonary inflammation and organ cross talk as indicated by the finding that IL6-deficient mice develop less acute lung inflammation in a pancreatitis model (220). IL6 forms a soluble complex with the soluble IL6R, which is generated by proteolytic shedding of the transmembrane IL6R on neutrophils and monocytes (163). This IL6/IL6R complex can stimulate IL6R negative tissue cells and may represent an essential mediator of acute lung inflammation (220). It remains to be clarified to which extent IL6R shedding by leukocyte ADAM17 occurs in the lung. In LPS-induced lung inflammation ADAM17 deficiency had almost no effect on alveolar IL6R release (6; 146) which may be explained by the fact that IL6R can also be shed by ADAM10, especially in mice (46).

In yet another pathway, ADAM17 mediates shedding of the receptor tyrosine kinase Mer on phagocytic cells (184). Inhibition of Mer shedding enhances protective anti-inflammatory functions of Mer in acute lung inflammation and this can be subverted by neutralization of surface expressed Mer (23). These data suggest that ADAM17 can also abrogate protective anti-inflammatory pathways by removal of surface expressed Mer.

Tissue inhibitor of metalloproteinases - endogenous ADAM17 inhibitor

Physiological homeostasis is characterized by a system of checks and balances. This is also true for some ADAMs, although clearly this system has not been understood in full. The tissue inhibitor of metalloproteinases (TIMP3) is an endogenous inhibitor that blocks some MMPs and also ADAM17 (reviewed in (81)). In various mouse strains increased sensitivity
towards early bleomycin-induced fibrosis was correlated with lower TIMP3 expression and higher ADAM17 activity (143). TIMP3 deficiency has a negative impact on lung structure and function in response to sepsis (112). Further, TIMP3 deficiency prolongs bleomycin-induced lung inflammation with alveolar neutrophilia and prevents resolution of inflammation (53). In LPS-induced lung inflammation, lack of TIMP-3 was associated with increased macrophage polarization into the inflammatory M1 phenotype (52). Although TIMP3 seems to attenuate pro-inflammatory events in the lung, it is not clear to which extent this is mediated by the inhibition of ADAM17 or other metalloproteinases.

Table 2: Effect of general and conditional ADAM17 knockout on lung development and pathophysiology

<table>
<thead>
<tr>
<th>Transgene</th>
<th>Approach/Background</th>
<th>Model</th>
<th>Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>Adam17&lt;sup&gt;tm&lt;sub&gt;iz&lt;/sub&gt;&lt;/sup&gt;/tm&lt;sub&gt;iz&lt;/sub&gt; C57BL/6 x 129</td>
<td>Development</td>
<td>Die shortly after birth; reduced airway branching</td>
<td>(142) (222)</td>
</tr>
<tr>
<td>Endothelial</td>
<td>Tie2-Cre/Adam17&lt;sup&gt;flx&lt;sub&gt;lox&lt;/sub&gt;/lox&lt;/sup&gt; C57BL/6</td>
<td>LPS / acute lung inflammation</td>
<td>Reduced permeability, reduced neutrophil recruitment, reduced cytokine release</td>
<td>(32)</td>
</tr>
<tr>
<td>Smooth</td>
<td>Tagln-Cre/Adam17&lt;sup&gt;flx&lt;sub&gt;lox&lt;/sub&gt;/lox&lt;/sup&gt; C57BL/6</td>
<td>LPS or acid/ acute lung</td>
<td>Reduced transactivation via TGFα and neuregulins</td>
<td>(33)</td>
</tr>
<tr>
<td>Leukocyte</td>
<td>Vav-Cre/Adam17&lt;sup&gt;flx&lt;sub&gt;lox&lt;/sub&gt;/lox&lt;/sup&gt; C57BL/6</td>
<td>LPS / acute lung inflammation</td>
<td>Not relevant for neutrophil and monocyte recruitment; decreased neutrophil recruitment</td>
<td>(146) (6)</td>
</tr>
<tr>
<td>T-cells</td>
<td>transfer of influenza-specific CD8&lt;sup&gt;+&lt;/sup&gt; T cells C57BL/6</td>
<td>Lung injury</td>
<td>Decreased TNF production and survival</td>
<td>(28)</td>
</tr>
<tr>
<td>Epithelial</td>
<td>SPC-rTTA/TetO-Cre/Adam17&lt;sup&gt;flx&lt;sub&gt;lox&lt;/sub&gt;/lox&lt;/sup&gt; C57BL/6</td>
<td>Development</td>
<td>Reduced saccular formation in developing lung</td>
<td>(211)</td>
</tr>
<tr>
<td>General inducible</td>
<td>R26-CreER/Adam17&lt;sup&gt;flx&lt;sub&gt;lox&lt;/sub&gt;/lox&lt;/sup&gt; C57BL/6</td>
<td>Elastase exposure</td>
<td>Reduced goblet cell metaplasia and mucus production</td>
<td>(136)</td>
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</tbody>
</table>

ADAM10

Like ADAM17, ADAM10 contributes to developmental processes as well as lung inflammation. Yet, the two best studied functions of this protease are the cleavage of the amyloid precursor protein in Alzheimer’s disease and the cleavage of Notch (65). The latter cleavage event is induced by binding of Notch ligands to the Notch receptor thereby exposing the cleavage site to ADAM10. Notch cleavage can also occur via activated ADAM17 but this does not require binding of Notch ligands (14). Extracellular cleavage of Notch is then followed by further intramembrane processing via the γ-secretase complex leading to the
release of an intracellular domain of Notch that acts as a transcriptional regulator (62). This pathway is almost unique for Notch, and there exist only a few other surface molecules that may employ a similar mechanism for transcriptional activation. Notch appears to the most relevant substrate to explain the developmental effects of ADAM10 in the central nervous system, in somites, and in the cardiovascular system (65). Even in adults, Notch shedding by ADAM10 is crucial for regenerative processes (202) and myeloid development (203; 217). In inflammatory lung diseases, on the other hand, many other substrates of ADAM10 seem important, such as the low affinity IgE receptor CD23 (205), VE- and E-Cadherin (110; 166), EGF (212), the transmembrane chemokines CX3CL1 and CXCL16 (1; 71), and the receptor for advanced glycation endproducts (RAGE) (152). These pathways have been analyzed in vivo by the use of knockout mice with cell specific ADAM10 deficiency (Table 3) and by corresponding in vitro experiments with isolated lung cells.

**B-cell ADAM10 – critical roles in asthma**

The initiation of extrinsic asthma requires the differentiation of IgE-producing B cells. Overexpression of ADAM10 in early lymphoid and myeloid development resulted in abrogated B cell development and dysregulated myelopoiesis. In vitro experiments with hematopoietic cells revealed that overexpression of ADAM10 increases the proteolysis of Notch receptors and thereby shifts lympho- and myelopoiesis (51). B cell-specific ADAM10 deficiency in mice (driven by CD19-Cre) prevented marginal zone B cell development, that is dependent on Notch2 signaling (50). However, the interpretation of such experiments is confounded by the fact that ADAM10 deficiency in B cells is compensated in mice by increased ADAM17 activity and TNF release in the lymph node. The TNF release then disturbs follicular lymph node architecture, which was not found in TNF-deficient mice (43). On B cells, ADAM10 seems to possess several additional functions beyond its developmental effects. On these cells ADAM10 is the most relevant protease for cleavage of the low affinity IgE receptor CD23 (50; 205), which functions as a natural regulator of IgE synthesis (24). The results reported so far indicate a pro-asthmatic role of ADAM10 by driving eosinophilia and Th2 polarization (114). Further, when ADAM10 was conditionally deleted in plasma cells (driven by IgG1-Cre), only after IgG class switch the antibody response was impaired and this was correlated with the deregulation of several transcriptional regulators and repressors in plasma cells indicating that ADAM10 is required for terminal plasma cell differentiation and function (19).
Leukocytic ADAM10 – requirement for alveolar cell recruitment

ADAM proteases appear particularly relevant for leukocyte adhesion and migration. Pharmacological or genetic ablation of ADAM10 in leukocytes in vitro, revealed that ADAM10 is critical for the chemotactic migration of neutrophils and monocytes through endothelial cell layers (146). Further, signaling and adhesion events of leukocytes that are linked to cell migration such as p38 and Rho GTPase-family activation, actin polymerization, adhesion to fibronectin, and upregulation of α5 integrin were also dependent on ADAM10. The relevance of these findings was confirmed by in vivo analysis of mice with hematopoietic ADAM10 deficiency (driven by Vav-Cre), which impaired the LPS-induced neutrophil and monocyte recruitment into the alveoli (146). A similar effect was seen in mice with knockout of ADAM10 in only myeloid cells (driven by LysM-Cre), where the lack of pulmonary neutrophil sequestration correlated with the protection against edema formation (146). Thus, with regard to leukocyte recruitment ADAM10 has pro-inflammatory activities.

Endothelial ADAM10 – contributions to vascular development and inflammation

Endothelial cells are critically involved in lung inflammation by regulation of vascular permeability and extravasation of inflammatory cells. Recent data indicate that ADAM10 may not only contribute to early development of vascular structures but also to pathological processes including neovascularization and inflammatory responses of endothelial cells.

Two groups have produced mice that lack endothelial ADAM10 (driven by Tie2-Cre), unfortunately with conflicting results. In one study endothelial ADAM10 deficiency led to embryonic death at day 10.5 due to vascular defects (219), while the other study found no obvious physiological phenotype but reduced neovascularization (55). Again, different driver lines were
used in these studies which may have led to knockout of ADAM10 at different developmental stages, as already explained for cell specific knockout of ADAM17. Both, the developmental effects as well as the disturbed neovascularization are probably to a large part due to the lack of Notch shedding by ADAM10. ADAM10 deficiency thereby disrupts a critical step in the Notch signaling pathway as indicated by a decreased expression of critical downstream target genes (55; 219).

To date, only limited information exists on the functional role of endothelial ADAM10 in the adult lung during inflammatory diseases. In vitro data show that ADAM10 mediates VE-cadherin shedding and thereby facilitates enhanced endothelial permeability and leukocyte transmigration through endothelial cells (166). ADAM10 has also been identified as a sheddase for glycosylphosphatidylinositol (GPI)-linked ephrin-A (77). On the one hand shedding by ADAM10 may dissolve the complex of ephrin-A with its receptor ephA that has been implicated in cell to cell adhesion leading to cell detachment, and on the other hand soluble ephrin-A can provide signals via ephA receptors enhancing endothelial permeability (25). Further, shedding of the transmembrane chemokine CX3CL1 by ADAM10 promotes cell detachment during transendothelial migration of leukocytes (72). It can be speculated that endothelial ADAM10 knockout mice would display diminished acute inflammatory responses of the pulmonary vasculature. First experiments with such mice already indicate a pro-inflammatory role for ADAM10 in the early phase of LPS-induced lung inflammation by promoting edema formation and neutrophil recruitment (105).

Epithelial ADAM10 – multiple functions in lung infection

A completely different function of ADAM10 became evident by the discovery that ADAM10 is a receptor for S. aureus pore-forming H1a toxin (207). The toxin upregulates the protease on pulmonary epithelial cells leading to enhanced E-cadherin cleavage, which then causes barrier disruption and increased bacterial invasion leading to lethal infection of the mice (73). Moreover, as a further mechanism, S. aureus infection was found to upregulate PAF, which activates ADAM10 on lung epithelial cells resulting in ADAM10-mediated HB-EGF shedding and enhanced mucus production (95). Interestingly, S. aureus also induces the processing of the receptor for advanced glycation endproducts (RAGE) (196) that mediates the recognition of danger signals by epithelial cells. On epithelial cells ADAM10 contributes to the processing of RAGE (152). In humans, RAGE shedding is also associated with acute lung inflammation, lung fibrosis development, and COPD (127; 133). Yet, the functional role
of soluble RAGE generation in the lung remains unclear. *In vitro* studies suggest that soluble RAGE may act as an antagonist of transmembrane RAGE on epithelial cells (223) or act as an agonist by mediating leukocyte recruitment, survival and differentiation (201).

Respiratory infection of mice with an adenovirus encoding ADAM10 causes emphysema in mice (162). Given that the virus predominantly targets epithelial cells, this may indicate that the protease on epithelial cells could contribute to COPD. However, cell-specific knockout mice have not yet been investigated in animal models of this disease.

Table 3: Effect of general and conditional ADAM10 knockout on lung development and pathophysiology

<table>
<thead>
<tr>
<th>Transgene</th>
<th>Approach/Background</th>
<th>Model</th>
<th>Observation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>general</td>
<td>Adam10−/−; CD1 x 129SV; C57BL/6 x 129Sv; 129Sv</td>
<td>Development</td>
<td>Die at day 9.5</td>
<td>(65)</td>
</tr>
<tr>
<td>B cell</td>
<td>CD19Cre/Adam10flox/flox C57BL/6</td>
<td>OVA/ IgE-dependent asthma</td>
<td>Reduced eosinophilia, reduced TH2 response, reduced CD23 shedding</td>
<td>(114)</td>
</tr>
<tr>
<td>Plasma cell</td>
<td>IgG1-Cre / Adam10flox/flox unknown</td>
<td>OVA/ asthma</td>
<td>Impaired antibody response and deregulation of plasma cell transcription factors</td>
<td>(19)</td>
</tr>
<tr>
<td>Leukocyte</td>
<td>LysM-Cre/Adam10flox/flox Vav-Cre/Adam10flox/flox C57BL/6</td>
<td>LPS / acute lung inflammation</td>
<td>Reduced neutrophil recruitment</td>
<td>(146)</td>
</tr>
<tr>
<td>Epithelial</td>
<td>SPC-rtTA/TetO-Cre/Adami10flox/flox unknown</td>
<td>S. aureus infection</td>
<td>Resistance to lethal pneumonia</td>
<td>(73)</td>
</tr>
<tr>
<td>ADAM10 overexpression</td>
<td>Respiratory application of adenovirus encoding Adam10</td>
<td>Analysis of mice 8 weeks after infection</td>
<td>Emphysema</td>
<td>(162)</td>
</tr>
</tbody>
</table>

From all these findings ADAM10 and ADAM17 appear as a central hub for the organization of pulmonary inflammation by triggering various shedding pathways in many different cells. (Figure 2).
ADAM 8

The contribution of ADAM8 towards allergic airway inflammation is still under discussion that is founded on the adhesive and protease functions of ADAM8 related to cell migration. The disintegrin domain of ADAM8 binds to α9β1 integrin in an RGD-independent manner (151), the relevance of which – except for osteoclast cell-to-cell interactions – remains to be clarified. For ADAM8 only a few substrates have yet been reported. The protease can remove the prodomain from pro-ADAM8 leading to its own activation (164). Recombinant ADAM8 is capable of cleaving peptides corresponding to the membrane proximal domains of TNF, CX3CL1, stem cell factor (SCF), TNFR and TGFα (132). However, it is not clear whether ADAM8 is a sheddase for the full-length molecules on the cell surface, especially in vivo. ADAM8 was found to associate with P-selectin glycoprotein ligand-1 (PSGL-1) in neutrophilic HL60 cells through ezrin–radixin–moesin actin-binding proteins. Further, ADAM8 caused the proteolytic cleavage of this adhesion receptor and affected leukocyte rolling on activated endothelial cells (31). Moreover, upregulation of ADAM8 on the surface of neutrophils associates with shedding of L-selectin (57; 205). This may also affect leukocyte rolling, although L-selectin is predominantly shed by ADAM17 and it remains to be shown to which extent the shedding by ADAM8 is relevant for pathological processes. Likewise, while CD23 seems to be a substrate of ADAM8, it is predominantly shed by ADAM10 (205).

In healthy lungs of men and mice only very low levels of ADAM8 are found and ADAM8-deficient mice do not display any obvious defects (80). In vitro, pro-inflammatory stimuli can induce the expression of ADAM8 in various lung cells including epithelial cells, neutrophils and monocytic cells (reviewed in (22)). ADAM8 synthesis is upregulated in murine models of allergic airway disease (82) as well as in patients with asthma (42), allergic rhinitis (135), eosinophilic pneumonia (117), and ventilator induced pneumonia (182). In inflamed lungs, ADAM8 is expressed on inflammatory cells and the epithelium (30). Increased levels of soluble ADAM8 protein can be found in the sputum and bronchoalveolar lavage fluid of patients with asthma (141) and COPD (115; 134).

Pro-versus anti-inflammatory functions of ADAM8 in allergic asthma

Thus, ADAM8 upregulation is tightly associated with chronic inflammation. However, it is still controversial whether ADAM8 acts pro- or anti-inflammatory (22; 85; 140) (Table 4). In ADAM8 transgenic mice overexpressing a soluble form of the protease under a CMV promoter the asthmatic response was reduced (116). This has been explained by decreased
endothelial VCAM-1 expression due to its shedding by ADAM8, which then would lead to reduced leukocyte recruitment. However, this approach may not reflect the physiological situation in asthma in which ADAM8 is initially expressed as a transmembrane protease predominantly on the surface of leukocytes and epithelial cells. Studies with ADAM8-deficient mice in models of experimental asthma have returned conflicting results. Using a model of ovalbumin (OVA)-induced airway inflammation Naus et al. described a protection by ADAM8 deficiency, which was associated with decreased migration of T-cells, eosinophils and dendritic cells from the blood vessels into the lungs (131). By transfer experiments they demonstrated that ADAM8 in T cells alone was sufficient for this protection. Also, Paulissen et al. reported a protection against OVA-induced airway inflammation in ADAM8 knockout mice or mice treated with neutralizing antibodies against ADAM8 (139). This was linked to decreased DC recruitment and reduced production of the chemokines CCL22, which is produced by dendritic cells, and CCL11, an eosinophil chemoattractant. In contrast to these studies, Knolle et al. observed that ADAM8 knockout exacerbated inflammation and airway hyper-responsiveness in mice of a mixed SvEv129/C57Bl/6 and Balb/c background sensitized by OVA or house dust mite allergen (84). This coincided with higher leukocyte counts, intensified airway hyperplasia, more T\textsubscript{H}2 cytokines and increased airway hyperreactivity towards metacholine. These findings were explained by the increased survival of eosinophils and macrophages in the absence of ADAM8. These contradictory results were discussed with respect to different sensitization protocols, different mouse strains and different animal housing conditions used. Nonetheless, there could still be two independent ADAM8-mediated effects on the recruitment of immune cells on the one hand and the apoptosis of immune cells on the other hand. It seems possible that these two functions play different roles during the development and the chronification of the disease. Moreover, it remains to be clarified to which extent epithelial ADAM8 contributes to the disease development at different disease stages by regulating epithelial permeability, epithelial survival or repair (22).
Table 4: Effect of general ADAM8 knockout on lung development and asthma development

<table>
<thead>
<tr>
<th>Approach</th>
<th>Model/Background</th>
<th>Observation</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adam8&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Development 129/Ola x C57BL/6</td>
<td>Expressed from early to late development, but no obvious phenotype</td>
<td>unknown</td>
<td>(80)</td>
</tr>
<tr>
<td>Adam8&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>OVA/ asthma SvEv129 x C57BL/6</td>
<td>Acceleration of airway hyperresponsiveness and increased alveolar leukocyte counts</td>
<td>Decreased apoptosis of eosinophils and macrophages</td>
<td>(84)</td>
</tr>
<tr>
<td>Adam8&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>OVA/ asthma 129/Ola x C57BL/6</td>
<td>Protection</td>
<td>Decreased DC recruitment and chemokine production</td>
<td>(139)</td>
</tr>
<tr>
<td>Adam8&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>OVA and house dust mite/ asthma C57BL/6</td>
<td>Protection</td>
<td>Decreased T cell, eosinophil and DC migration</td>
<td>(131)</td>
</tr>
<tr>
<td>Adam&lt;sup&gt;8&lt;/sup&gt; transgenic mice</td>
<td>OVA/ asthma C57BL/6</td>
<td>Protection</td>
<td>Enhanced endothelial VCAM-1 shedding and less asthma</td>
<td>(116)</td>
</tr>
</tbody>
</table>

ADAM 33

ADAM33 has been linked to allergic airway inflammation, but its role in the pathophysiology of asthma remains to be demonstrated.

Recombinant soluble ADAM33 cleaves peptides representing membrane proximal domains of the stem cell factor c-kit, TNF-related activation-induced cytokine (TRANCE), insulin-β chain and amyloid precursor protein (224), but natural transmembrane substrates for transmembrane ADAM33 are yet unknown. Its disintegrin domain was found to support α9β1 integrin-dependent cell adhesion (15). For ADAM33 different splice variants have been reported (144).

ADAM33 is highly upregulated in patients with asthma (42), COPD (59) or sarcoidosis (168). Expression of ADAM33 is increased in the lungs of mice upon induction of allergic airway disease and in cultured human fibroblasts upon stimulation with the T<sub>H</sub>2 cytokines IL4 and IL33 (78). Besides cultured lung fibroblasts, the protease is found on cultured lung smooth muscle cells, but absent in cultured epithelial cells, endothelial cells or leukocytes (144; 192). Within human lung tissue, smooth muscle cells, leukocytes, the endothelium and the basal part of the epithelium were positive for ADAM33 (30). Soluble ADAM33 promotes angiogenesis in vitro (149), and a role in airway remodeling and repair processes has been proposed (66).
ADAM33 - a risk factor in allergic lung inflammation

By positional cloning a susceptibility locus for asthma and airway hyper-responsiveness has been identified within the ADAM33 gene (195). Additionally, ADAM33 was identified as a susceptibility gene for COPD (194), fibrosis (191), and impaired early life lung function (173). Initially, eight ADAM33 polymorphisms were described, and subsequent studies have identified up to 24 polymorphisms within different exons and introns of the ADAM33 gene, six of which are associated with asthma (70). When comparing several studies conducted in different populations it is still unclear whether there exist SNPs with association to asthma or COPD across all different ethnic groups (2; 99; 188; 221). Additionally, ADAM33 may be regulated by epigenetic mechanisms such as hypermethylation of the ADAM33 promoter, which was found to silence expression of the protease in bronchial epithelial cells (214). TGFβ stimulation was reported to downregulate ADAM33 expression in fibroblasts, which was associated with histone modifications, but not with altered methylation patterns within the promoter region (215). However, the methylation pattern in the promoter region was not changed in individuals with or without asthma (213) and therefore the contribution of this epigenetic modification of ADAM33 to the development of lung diseases remains unclear.

ADAM33 knockout mice (129SvxC57BL/6) showed no phenotype in experimental allergic asthma (21). However, such models in mice are clearly different from human disease and the role of ADAM33 may differ between mice and men. Thus, there is some evidence for a role of ADAM33 in human asthma, but a causal link between ADAM33 polymorphisms, asthma development and the function of the protease in this disease remains to be demonstrated.

Other ADAMs

Only limited knowledge exits on other ADAMs in inflammatory lung diseases. Recent studies have shown that ADAM9 and 15 are induced in the course of acute lung inflammation and may exacerbate the inflammatory process. Interestingly, both ADAM9 and ADAM15 are capable of cleaving ADAM10 (185) and may thus affect lung inflammation via the release of ADAM10 activity from the cell surface. However, as outlined below there may also exist more direct protease specific pathways.
**ADAM9 – a promoter of acute lung inflammation**

ADAM9 is expressed at a low level in the lung and is upregulated in experimentally induced acute lung inflammation, a finding that was explained by an increase in leukocyte derived ADAM9 (159). In vitro, the protease was found on monocytic cells, neutrophils, epithelial cells and vascular smooth muscle cells. In neutrophils, the protease is stored in granules and upregulated on the surface upon degranulation (159). Only a few substrates were described including the insulin B chain, insulin growth factor binding proteins (IGF-BP), APP, some ErbB ligands, angiotensin converting enzyme 1 (ACE-1), ADAM10, and extracellular matrix proteins (38; 122; 137; 157). Additionally, the disintegrin domain has been implicated in the binding of various integrins, including α1, α3, α6 and αv and β1 (108; 130). ADAM9 knockout mice (129SvxCD57BL/6) do not show an obvious phenotype other than reduced neovascularization in the cornea pocket assay (204). In the lung, ADAM9 deficiency (C57BL/6) conferred protection against LPS- or bleomycin-induced lung inflammation (159). This protection was explained by the ability of ADAM9 to degrade lung elastin fibers leading to reduced lung compliance and disruption of the alveolar-capillary barrier. Yet, an association with chronic inflammatory airway diseases has not yet been reported.

**ADAM15 – mediator of hyperpermeability in acute lung inflammation**

ADAM15 plays a role in endothelial barrier function, which suggests a possible role in lung inflammation. As a protease ADAM15 can remove fibroblast growth factor receptor FGFRII components from the extracellular matrix (ECM) and possibly also E-cadherin (111; 113; 126). ADAM15 also contains an RGD motif within the disintegrin domain, which is recognized by the integrins α5β1 and αvβ3 (129). The protease also binds to α6β3 integrins in an RGD-independent manner (40). Inhibition of airway SMC migration and adhesion by the disintegrin domain have been reported (102). In endothelial cells, ADAM15 can regulate permeability and neutrophil transmigration via Src/ERK1/2 signaling independent of junction molecule shedding (179). ADAM15 deficient mice show decreased susceptibility towards atherosclerosis development, which was explained by the protease’s role in endothelial barrier function (180). When ADAM15 deficient mice were investigated for LPS-induced acute lung inflammation, it was concluded that ADAM15 promotes endothelial hyperpermeability, but does not affect leukocyte migration (178).
Inhibition of ADAMs in acute lung inflammation

There is increasing evidence that treatment with exogenous synthetic metalloproteinase inhibitors can limit hallmarks of acute pulmonary inflammation (Table 5). Since these inhibitors were mostly broad spectrum inhibitors and were delivered systemically, their effects are most likely explained by the inhibition of distinct metalloproteinase activities on multiple cell types. As described above, ADAM10 and ADAM17 fulfill multiple roles in various cell types. For example, ADAM17 on endothelial cells and SMC acts pro-inflammatory in a murine model of acute pulmonary inflammation (32), whereas leukocytic ADAM17 seems to play a negligible role in acute lung inflammation (146) or could even limit early neutrophil migration by L-selectin shedding (101; 183).

Therefore, more protease-specific inhibitors are warranted and their application may need to be restricted in time and space. More selective inhibitors have been generated such as the inhibitory prodomain of ADAM17, which has been used in several in vitro studies (16; 98) and which blocks LPS-induced TNF shedding in the lung (97). This and the other inhibitors such as mouse and human cross-reactive inhibitory exosite antibodies against ADAM17 (91) need to be further tested in various lung inflammatory models.

It could also be worthwhile to specifically target ADAM10, but not ADAM17. In the lung, pro-inflammatory activities of ADAM10 on endothelial cells may synergize with its pro-migratory activities on leukocytes, its pro-asthmatic activities on B cells, and its function as the receptor for S. aureus toxin on epithelial cells (32; 73; 114; 166; 167). Treatment with selective ADAM10 inhibitors may represent a novel strategy to limit pulmonary inflammation. In fact, treatment with the preferential ADAM10 inhibitor GI254023X which blocks ADAM10 100-fold more potently than ADAM17 (71; 104) was found to reduce LPS-induced edema formation, cytokine production, and neutrophil recruitment (105) and prevent destructive effects of S. aureus toxin on lung epithelial cells in vivo (73). A different ADAM10 inhibitor reduced eosinophilia in a murine asthma model (114). GI254023X and most other tested compounds are peptidomimetic inhibitors with a reverse hydroxamate group, which chelates the zinc atom in the active site of the protease. Many hydroxamate-based compounds display hepatotoxicity when administered systemically and clinical trials had to be halted because of these problems. To circumvent these side effects inhibitors are developed that are not hydroxamate-based. The prodomain of ADAM10 that inhibits cellular shedding events (124) or inhibitory antibodies against ADAM10 may be useful approaches.
Still very little is known on the druggability of other ADAMs that may be relevant in the lung. Antibody-based neutralization of ADAM8 was found to protect mice against experimentally induced asthma (139). A patent describes a cyclic peptide ADAM8 inhibitor (UK Patent Appl. Nr. 0719997.9; WO2009047523) that blocks ADAM8 activity \textit{in vitro}. As this peptide comprises the integrin binding loop of ADAM8 it seems likely that it will also block the integrin binding activity of the protease. Recently this inhibitor was reported to prevent airway hypersensitivity and release of soluble CD23 in experimentally induced asthma in mice (29).

As a particular advantage, the lung opens the possibility of applying the inhibitors locally. In fact, intratracheal administration of a combined ADAM10 and ADAM17 inhibitor (GW280264X) in mice effectively prevented LPS-induced edema formation, cytokine production, and neutrophil recruitment in the lung and very similar results were made with GI254023X (105). Neutralizing antibodies against ADAMs, can also be applied locally and coupling of polyethylenglycol (PEG) to their F\textsubscript{ab}-fragments can be used to increase their local residence time following delivery in the respiratory tract. Also, local RNA interference may represent an option. Small inhibitory RNA or small hairpin RNA have been successfully used for highly selective targeting of ADAMs \textit{in vitro}, but not yet \textit{in vivo}. Application of siRNA via the airways will predominantly reach epithelial cells and alveolar immune cells. Moreover, siRNA can be coupled to nanoparticles or packed into liposomes and applied systemically which may be used to target pulmonary endothelial cells (27; 119). Thus, there exist several options for targeting ADAMs more specifically by either improving existing small molecule inhibitors, by developing humanized inhibitory antibodies or by siRNA techniques. Side effects may be circumvented by local drug delivery including microsprayers, nanoparticles, liposomes or PEG-coupled antibodies.
<table>
<thead>
<tr>
<th>Approach/Model</th>
<th>Inhibitor</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS/acute lung inflammation</td>
<td>TAPI-0</td>
<td>Reduced edema formation and leukocyte recruitment, inhibition of Mer shedding and restoration of Mer signaling; Protection, inhibition of Mer shedding, enhanced apoptotic cell clearance, reduced proinflammatory mediators, reduced leukocyte recruitment</td>
<td>(23)</td>
</tr>
<tr>
<td>Bleomycin/acute lung inflammation</td>
<td>TAPI-0</td>
<td>Reduced neutrophil recruitment</td>
<td>(94)</td>
</tr>
<tr>
<td>LPS/acute lung inflammation in rats</td>
<td></td>
<td>Reduced neutrophil recruitment</td>
<td>(172)</td>
</tr>
<tr>
<td>Bleomycin/acute lung inflammation</td>
<td>TAPI-2</td>
<td>Reduced alveolar leukocyte infiltration and reduced edema formation, reduced neuregulin shedding by epithelial cells</td>
<td>(41)</td>
</tr>
<tr>
<td>LPS/acute lung inflammation</td>
<td>GW280264X</td>
<td>Reduced neutrophil recruitment, edema formation, and cytokine release</td>
<td>(32; 105)</td>
</tr>
<tr>
<td>LPS/acute lung inflammation</td>
<td>GW280264X</td>
<td>Reduced neutrophil accumulation, albumin leakage, chemoattractant levels, and soluble E-cadherin</td>
<td>(186)</td>
</tr>
<tr>
<td>Post transplantation lung injury in rats</td>
<td>Y-41654</td>
<td>Reduced neutrophil accumulation, albumin leakage, chemoattractant levels, and soluble E-cadherin</td>
<td>(60)</td>
</tr>
<tr>
<td>LPS/acute lung inflammation</td>
<td>Recombinant ADAM17 prodomain</td>
<td>Reduced TNF release</td>
<td>(97)</td>
</tr>
<tr>
<td>OVA/asthma</td>
<td>ADAM8 neutralizing antibody ADAM8 inhibitor</td>
<td>Protective</td>
<td>(139) (29)</td>
</tr>
<tr>
<td>OVA/IgE-dependent asthma</td>
<td>INC008765</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung injury by H1a toxin</td>
<td>GI254023X</td>
<td>Reduced epithelial E-cadherin cleavage</td>
<td>(73)</td>
</tr>
</tbody>
</table>

Open questions - potential roles of ADAMs in chronic inflammation and infectious diseases of the lung

In addition to the overall pro-inflammatory role of ADAM10 and ADAM17 in the early phase of acute lung inflammation, both proteases may also critically regulate chronic inflammation. By constant generation of inflammatory mediators and proliferative stimuli the proteases may drive the chronification of the disease, but at the same time the protease can generate mediators that switch off inflammation or induce regeneration.

As mentioned, shedding of TNFR may serve to downregulate inflammation. Increased levels of soluble TNFR1 are found in the sputum of patients with COPD and here they may function to bind and antagonize soluble TNF. Moreover, shedding of adhesion molecules (e.g. L-
selectin, CX3CL1) and proteoglycans (CD44 and syndecans) at the endothelial surface may reduce endothelial adhesiveness for leukocytes and presentation of chemotactic factors and thereby downregulate the inflammatory response.

Growth factor shedding may be ambivalent in inflammatory diseases. On the one hand, transactivation via EGFs can positively contribute to the generation of inflammatory mediators, provide co-stimulatory signals for cell migration, induce mucus overproduction, hyperproliferation and collagen deposition, as explained above. In fact, development of ventilator-induced lung inflammation and lung fibrosis can be attenuated by EGFR inhibitors (9; 74; 121). Mice deficient in the EGFR ligand TGFα are resistant to bleomycin-induced lung fibrosis (107), and induced overexpression of TGFα in transgenic mice can induce fibrosis in an EGFR dependent manner (93). On the other hand, as a long term effect, transactivation of ErbB receptors could also stimulate healing responses by providing co-stimulatory signals for the regeneration of injured lung tissue. In fact, EGF mediates bronchial and alveolar epithelial wound healing in vitro (49; 187). This may explain why EGFR inhibition by continuous application of inhibitors (e.g. 14 days) had adverse effects on naphthalene- or bleomycin-induced acute lung inflammation by suppressing repair of the airway epithelium (64; 181). In addition to the cleavage of EGFR ligands, repair processes can also be regulated by ADAM10-mediated shedding of Notch and ephrins, both of which have been implicated in epithelial and endothelial regeneration after inflammatory lung injury (reviewed in (25; 156)).

Finally, ADAM10 and ADAM17 may also be involved in the recruitment or differentiation of regulatory anti-inflammatory immune cells including IL-10 producing monocyte and T cell subpopulations and thus be indirectly required for the resolution of chronic inflammation (35). On cytotoxic T cells, Fas ligand (FasL) undergoes ADAM10 mediated shedding, and this results in reduced surface expression of FasL and the reduced ability of cytotoxic T cells to induce apoptosis of target cells (165). Further, FasL-induced apoptosis was found to reduce eosinophilic inflammation in a mouse model of asthma (170); it also reduces the alveolar macrophage pool in the resolution phase of pulmonary inflammation (79). These proposed beneficial functions of ADAMs in chronic lung inflammation and the involved pathways remain to be examined in detail.

Also for ADAM9 and ADAM15 in the lung, the current knowledge is limited to acute inflammatory models, but both proteases could also be involved in mediating chronic lung inflammation. ECM degradation reported for ADAM9 may play a crucial role in tissue
remodeling during fibrosis. Hyperpermeability mediated by ADAM15 may accelerate chronification of lung inflammation. It is important that these proteases are investigated and compared in several models of acute and chronic lung inflammation.

From the current knowledge, it is less clear to decide whether ADAM8 and ADAM33 should be regarded as therapeutic targets in the lung and if so how they might be targeted. As mentioned, data on asthma development in ADAM8-deficient mice point towards both pro-inflammatory and protective functions of the protease in allergic airway disease. For ADAM33 there exists a genetic association of the molecule with asthma in humans, but pathologic functions of the protease are unknown.

Besides the many unknown functions of ADAMs in chronic lung inflammation, their role in host defense of the lung against microorganisms remains largely unexplored. Here, ADAMs may be directly involved by controlling the epithelial barrier against bacteria, by mediating epithelial regeneration via ErbB-ligand shedding (87), by shedding of scavenger molecules binding bacteria (CXCL16 (171), CD163 (83), CD36 (35)), or possibly also by shedding of pattern recognition receptors or by the activation of antibacterial leukocyte defense mechanisms. To date ADAM17 has been implicated in TNF production during LPS-induced sepsis (68) and in leukocyte recruitment during bacterial peritonitis (101), but except for *S. aureus* infection, in which ADAM10 functions as a receptor of the *S. aureus* toxin (73), the role of ADAMs in bacterial lung infections remains unknown. Therefore, to assess the side effects of local intervention strategies with ADAM inhibitors, models for viral, bacterial and fungal lung infection need to be tested in the future.
Acknowledgment

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Figure legends

Figure 1: A) schematic representation of an ADAM-family protease with metalloproteinase domain, disintegrin domain cysteine-rich domain, transmembrane domain and cytoplasmic domain. The adhesive function of the disintegrin domain and the proteolytic function of the metalloproteinase domain as well as the functional consequences of substrate shedding are indicated. B) Dendrogram based on the relative amino acid sequence homologies among most relevant catalytically active ADAMs and their isoforms.

Figure 2: Summary of ADAM10- and ADAM17-mediated shedding pathways and their cell-specific functions in lung inflammation: Cleavage events predominantly mediated by ADAM10 are marked in green, and those mediated by ADAM17 in red. Pro-inflammatory functions are given as white boxes, anti-inflammatory/protective functions as grey boxes. For abbreviations see text.
substrate recognition
proteolysis
Zn²⁺
soluble agonist (e.g. TNF)
or antagonist (e.g. TNFR)
removal of surface molecule (e.g. L-selectin)
degradation or signalling function (e.g. Notch)

A B

soluble ectodomain
adhesion molecule (e.g. integrins) or ECM interaction

substrate recognition
proteolysis

soluble agonist or antagonist
removal of surface molecule
degradation or signalling function

A

ADAM17-1/-2
ADAM10
ADAM12
ADAM28
ADAM33 (several isoforms)
ADAM19
ADAM15-1/-2/-3/-4/-5/-6
ADAM9/-1/-2