Role of Proteases in Lung Disease: A Brief Overview

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Abstract
Proteases play an important role in health and disease of the lung. In the normal lungs, proteases maintain their homeostatic functions that regulate processes like its regeneration and repair. Dysregulation of proteases–antiproteases balance is crucial in the manifestation of different types of lung diseases. Chronic inflammatory lung pathologies are associated with a marked increase in protease activities. Thus, in addition to protease activities, inhibition of anti-proteolytic control mechanisms are also important for effective microbial infection and inflammation in the lung. Herein, we briefly summarize the role of different proteases and to some extent antiproteases in regulating a variety of lung diseases.

Keywords
Protease · Antiprotease · Lung diseases · Infection · Inflammation

1 Introduction
The lung possesses a large number of anti-inflammatory components [1], which fight against microbial infections.

Serine, cysteine, aspartic, and metalloproteases are the principal classes of protease present in the human lung. A good number of evidence suggest that neutrophil serine proteases (NSPs) such as elastase, proteinase 3 (PR3), cathepsin G
(CatG), and matrix metalloproteases (MMPs) are major pathogenic determinants of chronic inflammatory lung disorders [1]. The lung proteases act in concert with the proteases of invading microbes, inactivate antiproteases, and antimicrobial compounds and thereby play a pivotal role in different types of lung diseases including chronic obstructive pulmonary disease (COPD), asthma, acute respiratory distress syndrome (ARDS), influenza, and cancer [1].

The lung proteases can either intracellularly or extracellularly regulate processes such as tissue remodeling, mucin production, neutrophil chemotaxis, and microbial destruction. Additionally, they regulate infection and inflammation in the lung, for example neutrophil elastase (NE), a serine protease, which plays critical role in the progression of a variety of lung diseases. It can regulate activities of CatB and MMP-2 in alveolar macrophages [2] and also activates proMMP-2, MMP-7, and MMP-9 [3–5], indicating that NE may act as a proinflammatory mediator. In some cases, NE regulates important signaling pathways that modulate innate immunity [6, 7]. NE’s multiple roles characterize it as a decisive factor controlling many aspects of infection and inflammation in the lung.

2 Pulmonary Hypertension

Pulmonary hypertension (PAH) occurs due to elevation of pulmonary artery pressure and if it is prolonged, then right ventricular failure may occur with subsequent fatality [8]. PAH often leads to secondary complications of many pulmonary disorders such as COPD, asthma and chronic bronchitis bronchopulmonary displasia, cystic fibrosis, chronic bronchitis, and emphysema [9, 10].

2.1 Serine Protease and Pulmonary Hypertension

The role of oxidants such as hydrogen peroxide, hydroperoxides, superoxide, and peroxynitrite in producing PAH is now well established [11–15]. Administration of the oxidant, tert-butylhydroperoxide (tert-buOOH) to the perfusate of isolated rabbit lungs causes pulmonary vasospasm [16]. Oxidant-induced pulmonary vasoconstriction can be blocked by the cyclooxygenase or thromboxane synthase inhibitor, indomethacin and is closely correlated with the thromboxane level in the effluent perfusate [13, 16–19], suggesting a critical role of thromboxane in pulmonary vasoconstriction. On the other hand, TMB-8, an intracellular Ca$^{2+}$ ([Ca$^{2+}]_i$) antagonists, has been shown to prevent oxidant-mediated pulmonary vasoconstriction [13]. Thus, oxidant-mediated PAH is triggered by an increase in [Ca$^{2+}]_i$. In many occasions PAH occur due to an increase in [Ca$^{2+}]_i$ caused by stimulants such as thromboxane A$_2$ and endothelin-1 that generates oxidants. Oxidant and Ca$^{2+}$ ionophore-mediated pulmonary hypertension has been observed to be inhibited by serine protease inhibitors, for example, aprotinin [19–21].
The mechanism by which oxidants stimulate production of the arachidonic acid (AA) metabolites has gained considerable interest. A report by Chakraborti et al. [22] indicated that oxidants, e.g., tert-buOOH stimulation of pulmonary artery endothelial and smooth muscle cells caused a marked increase in phospholipase A$_2$ (PLA$_2$) activity with subsequent generation of AA. Mepacrine, an inhibitor of PLA$_2$ inhibits tert-buOOH-induced increase in PLA$_2$ activity, thromboxane B$_2$ production, and PAH [19, 22]. Some investigators, considering the analogy of activation of pancreatic phospholipase A$_2$ [23], suggested that a serine protease might be involved in regulating PLA$_2$ activity [24]. Chakraborti et al. [25, 26] demonstrated that oxidant-mediated activation of PLA$_2$ activity in pulmonary endothelial and smooth muscle cells occur with the involvement of proteolytically activated protein kinase C$\alpha$ (PKC$\alpha$). They have also demonstrated that oxidants caused increase in $[\text{Ca}^{2+}]_i$ in pulmonary endothelial and smooth muscle cells can activate an aprotinin sensitive protease having mol mass of $\sim 43$ kDa [26]. The protease then proteolytically activates PKC$\alpha$ resulting in stimulation of cPLA$_2$ (Fig. 1), which generates thromboxane and that has been observed to be important in producing PAH [25, 26]. Oxidants elicit an increase in $[\text{Ca}^{2+}]_i$ due to proteolytic activation of

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**Fig. 1** Schematic representation of the underlying mechanism associated with oxidant (reactive oxygen species: ROS)-mediated cPLA$_2$ activation in pulmonary vascular endothelial and smooth muscle cells. A calcium channels; B membrane bound Ca$^{2+}$ stores; C anion channels; D diffusion; E inhibition of Na$^+$ dependent Ca$^{2+}$ uptake
PKC-δ by MMP-2 resulting in phosphorylation of a pertussis toxin sensitive protein (Gi) leading to inhibition of Na⁺ dependent Ca²⁺ uptake (Na⁺/Ca²⁺ exchanger) in the endoplasmic reticulum (ER) [27–31] (Fig. 2), whereas the role of cell membrane for an increase in [Ca²⁺]ᵢ has been observed to be due to phosphorylation of Gi via proteolytically activated PKCα by an aprotinin sensitive serine protease leading to inhibition of Na⁺ dependent Ca²⁺ efflux (Na⁺/Ca²⁺ exchanger) (Fig. 1) in pulmonary vascular cells [24–26].

In many systems, Ca²⁺-ATPase represents only about 1% of the total proteins [32]. In heart sarcolemmal vesicles, Ca²⁺ uptake via NCX produces maximum transport velocity and that has been demonstrated to be about 30-fold up than that elicited by the sarcoplasmic reticulum (S(ER)) Ca²⁺ pump system [33]. In addition
to the Ca$^{2+}$ pump, Na$^+$ dependent Ca$^{2+}$ uptake system is an important mechanism to sequester Ca$^{2+}$ in the ER of pulmonary vascular cells [29–33]. A decrease in Ca$^{2+}$ sequestration by proteolytic inhibition of Na$^+$ dependent Ca$^{2+}$ uptake has been observed to measure duration of free [Ca$^{2+}$], transient, which eventually produces vasoconstriction [34, 35]. In different systems, Na$^+$/Ca$^{2+}$ exchanger controls the contractility of smooth muscle cells [34, 35]. For example, contractile dysregulation in the myocardium could be related with activation of proteases [36]. Thus, the role of cell membrane associated aprotinin sensitive protease and ER MMP-2 on Na$^+$/Ca$^{2+}$ exchange in pulmonary artery endothelial and smooth muscle cells under oxidant triggered condition is an important mechanism for the pathological manifestation of pulmonary vasoconstriction [24–26, 29–31].

### 2.2 MMPs and Pulmonary Hypertension

PAH is characterized by persistent vasoconstriction and remodeling of pulmonary vasculature associated with activation of proteases, for instance, MMPs [34]. Remodeling of pulmonary artery is associated with an alteration of extracellular matrix (ECM) turnover with concomitant change in ECM proteins level. In PAH, dysregulation of ECM turnover has been suggested to play an important role in the pathological remodeling process [35, 36]. ECM degradation occurs by different proteases of which matrix metalloproteases (MMPs) has been shown to play the crucial role [37, 38]. Of the MMPs, MMP-2, and MMP-9 are able to cleave basement membrane associated type IV collagen, which increase remodeling of the pulmonary vasculature in PAH [39]. Given the potency of MMPs, its activity is tightly regulated at the transcriptional and post-translational level, where the tissue inhibitors of MMP (TIMPs) play a pivotal role [40, 41].

IL-1, a potent endogenously generated inducer of PAH, elicits its effect via an increase in the level of TGF and TNF in pulmonary smooth muscle cells. TGF causes an increase in the expression of the 92 kDa proMMP-9 and 72 kDa proMMP-2 mRNAs, while TNF triggers activation of proMMP-9 and proMMP-2 [42].

MMP-2 is produced upon activation of proMMP-2 by a variety of stimuli under different pathophysiological conditions. It has been observed that the activation of proMMP-2 occurs at the cell membrane. Interaction between MT1-MMP and TIMP-2 is an important phenomenon in the activation of proMMP-2. The MT1MMP-TIMP2 associates with proMMP-2 and forms a trimolecular complex, which triggers the activation of proMMP-2 and subsequently generates MMP-2 [41, 42]. The activation of proMMP-2 in pulmonary artery smooth muscle cells occur with the involvement of protein kinase C-α dependent and NF-κB-MT1MMP-mediated signaling mechanism. TNF-α augments mRNA and protein expression of MT1MMP, while the expression level of TIMP-2 diminishes. The increase in TNF-α leads to IKK activation, IB phosphorylation and degradation, and subsequently activation of NF-κB. Upon activation, NF-κB binds to the MT1-MMP promoter, thereby enhancing its expression and subsequently increases
proMMP-2 level in association with TIMP-2 that is modulated by protein kinase C-\( \alpha \) at the cell membrane [41, 42] (Fig. 3). This indicates therapeutic potentiality of PKC inhibitors in ameliorating the PAH, where activation of proMMP-2 is an important phenomenon.

ProMMP-9 activation by TNF-\( \alpha \) has been observed to occur with the involvement of an aprotinin sensitive serine protease [42]. TNF-\( \alpha \) was shown to inhibit aprotinin and TIMP-1 mRNA and protein expression, which trigger activation of proMMP-2 resulting in the stimulation of MMP-2 (Fig. 4). Under IL-1\( \beta \) stimulation, the aprotinin sensitive protease was not activated, although a discernible inhibition of TIMP-2 mRNA and protein expression were triggered by TNF-\( \alpha \) [42]. Thus, IL-1-induced stimulation of the two progelatinases occurs via different mechanisms.
Influenza viruses are highly infectious and trigger acute respiratory diseases with significant morbidity and mortality in humans and other animals [43–46].

Influenza viruses can be classified as A, B, or C. Influenza virus A, found in humans and other mammals and birds, played a nefarious role in causing the three twentieth century major influenza outbreak and also the influenza outbreak of swine origin that occurred in the recent past [47]. Many of the influenza A-related mortalities are attributable to secondary bacterial pneumonia [48, 49].

Haemagglutinin (HA) protein contributes critically to influenza virus-mediated pathogenicity. HA of influenza virus binds to sialic acid containing cell surface receptors. HA upon cleavage by a good number of host of protease(s) forms HA1 and HA2 subunits and that has been fused with host cell membrane, which subsequently initiates the infection process [49–52]. In most cases, the cleavage site of HA of avian and mammalian influenza viruses is a single arginine, albeit a single lysine amino acid has also been observed at the cleavage site in some cases. Cleavage can occur extracellularly by trypsin [53, 54] and proteases such as...
plasmin [55–57], tryptase of bronchiolar epithelial and mast cells [58], and also by bacterial proteases [59–61].

Several other proteases expressed in the lung are also able to facilitate influenza virus spread. Böttcher et al. [62] demonstrated that TMPRSS2 and TMPRSS11D, transmembrane serine proteases, (a.k.a. human airway trypsin-like protease: HAT) activate the influenza viruses H1N1 (A/Memphis/14/96), H2N9 (A/Mallard/Alberta/205/98), and H3N2 (A/Texas/6/96) upon cleavage of haemagglutinin (HA) and contribute to the high pathogenicity of these influenza viruses in the lung [63]. In addition to HAT, TMPRSS-2 and -4, the granzymes (Gzm) such as GzmA, GzmB, and GzmE are known to play a key role in the process of cleavage of 1918 H1N1 HA as a part of the progression of the influenza disease [62, 63].

The sites of virus replication in the microenvironments of respiratory tract represent complex extracellular proteases (such as trypsin and tryptase), which activate a family of receptors called protease activated receptors (PARs) [64, 65] and that play an important role in both virus replication and innate immune response [58, 66]. Four PARs (PAR1-4) are known to be activated by different proteases. After cleavage of the receptor(s) by proteases, the newly released amino terminal sequence binds and internally activates the receptor [67]. In the airways of IAV-infected mice, an increase in the level PAR2 upon IFNγ-mediated modulation has been shown to play a crucial role in influenza pathology [68, 69].

Multiple serine protease activities are implicated in mediating influenza virus infection. Inhibition of influenza A virus infection in cultured lung epithelial cells by serine protease inhibitors, for example, aprotinin markedly protects mice from infection [70]. Another serine protease inhibitor, camostat has also been shown to possess anti-influenza (Taiwan/1/86) virus pathology [71].

4 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is associated with the pathological manifestations of emphysema and chronic bronchitis. Emphysema is characterized by a marked destruction of the alveolar septa with concomitant decrease in lung plasticity and that results in gas trapping leading to a marked decrease in pulmonary oxygenation in the lung. Chronic bronchitis usually occurs with inflamed and thickened airways along with an increase in mucus production by the cells in the airways, which leads to a marked increase in cough and difficulty in breathing. Noxious particles present in cigarette smoke and automobile exhaustion have been observed to be an important causative agent of COPD [72, 73].

In the early 1960s, proteases have been shown to produce lung lesions in experimental animals similar to human emphysema. Initial studies in this scenario included metalloproteinases, papain and subsequently serine proteases, for example, porcine pancreatic elastase [74].
4.1 Serine Proteases and COPD

The identification of α1-PI (an endogenous serine protease inhibitor) and subsequent research confirmed that a strong association exists between the development of emphysema and inherited deficiency of the inhibitor [75]. A critical role of serine proteases have been established in the pathobiology of emphysema. Preliminary studies in this genetic condition of inherited α1-PI deficiency indicated that chronic bronchitis is associated with the early onset of the disease [76, 77].

The lung epithelium of normal individuals is protected from the detrimental effects of neutrophil serine proteases (NSPs) by a battery of antiproteases. Large quantities of NSPs (~20-fold w.r.t. normal subjects) released by neutrophils in acute and chronic inflammatory conditions overpowered antiprotease activities, leading to uncontrolled proteolysis and subsequently lung damage [74–76].

Alpha-1-PI deficiency increases activities of different enzymes secreted by activated neutrophils such as NE, cathepsin G (CatG), and proteinase 3 (PR3), all of which are capable of damaging different components of the ECM such as collagen, laminin, fibrillin, and elastin. However, several evidences suggest that it is the destruction of lung elastin that is important in causing emphysema, which generates COPD pathophysiology in animal model systems [75, 76].

Human neutrophil elastase (HNE), by destroying elastin, plays a critical role in the development of pulmonary emphysema [76, 77]. HNE has many other biological activities. For example, it stimulates mucin production [78], activates MMPs [5], inactivates TIMPs [79], and generates neutrophil chemotactic elastin-derived fragments [80, 81]. PR3 and CatG, two elastase homologues secreted in massive quantities from neutrophils at inflammatory sites, have also been shown to have proinflammatory functions acting through various mechanisms [80–82]. The most abundant is the α1-PI, which targets preferentially HNE. Secretory leukoprotease inhibitor (SLPI), an inhibitor of HNE and CatG, but not of PR3, has been shown to control excess proteolysis in the upper airways [83]. Elafin, derived from trappin-2 (pre-elafin) [84], is an NSP inhibitor that controls the activities of HNE and PR3 [85]. Other NSP inhibitors including 1-antichymotrypsin and monocyte/NE inhibitor (MNEI) were found to play relatively minor role as protease inhibitors [86, 87]. NSPs, therefore, could prove useful as therapeutic target for a number of inflammatory lung diseases.

There are differences in pathological manifestations among smoking and non-smoking α1-PI deficient COPD patients. The smokers with COPD frequently show pulmonary emphysema and bronchitis, while patients of the latter category often show emphysema without bronchitis. Although the chronic smokers usually suffer from the antiprotease inactivation in both the airways and respiratory units as a result of oxidant-mediated inactivation of α1-PI, the non-smoking α1-PI deficient individuals possess diminished antiprotease content primarily in respiring units, which are free of mucus glands and depends upon α1-PI for antiprotease defence [88].

Oxidative stress induced by cigarette smoke in COPD patients may promote the inflammatory state by recruiting additional neutrophils and upregulating the
inflammatory transcription factor, NF-κB and neutralizing TIMPs in addition to α1-PI and SLPI [89].

4.2 Urokinase Plasminogen Activator and COPD

In COPD patients, an increase in urokinase plasminogen activator (uPA) level in the airway epithelial and alveolar cells, and lung macrophages cause destruction of small airways and alveolus of the lung [90]. Urokinase plasminogen activator receptor (uPAR), in addition to functioning as a protease receptor, mediates intracellular signaling [91]. An increase in uPAR level in the macrophages has been observed in patients with COPD, which suggests the critical role of uPAR in inflammation and tissue remodeling including parenchymal destruction and fibrosis of small airways [92].

4.3 MMPs and COPD

MMPs are known to induce morphological changes in the lung that are prevalent in COPD. Several MMPs are known to play important roles in the pathogenesis of COPD [93]. Lung parenchyma and inflammatory cells such as neutrophils and macrophages are the major sources of MMPs in patients with COPD [94].

MMP-12 (a.k.a. macrophage elastase) is known to play an important role in COPD pathogenesis. A marked increase in MMP-12 expression in alveolar macrophages is associated with smoking associated emphysema [95]. In mouse, deletion of MMP-12 gene prevents cigarette smoke-induced inflammation, neutrophil influx, and emphysema in the lung [96, 97]. Genetic analysis of human COPD patients demonstrated that the common serine (codon 357) of the MMP-12 gene plays a crucial role in the pathological manifestations of matrix degradation, which has been observed to be related with the severity of the disease [98, 99].

Analysis of COPD lung tissue indicated an increase in the activity of MMP-1 and MMP-8, but not MMP-13 [100, 101]. An increase in MMP-1 activity was found in type II pneumocytes in patients with emphysema, but not in normal control subjects [101]. Neutrophil-derived MMP-8 levels were markedly increased in patients with COPD in comparison to the normal subjects [101]. Prominent increase in MMP-2 and MMP-9 expression has been observed in the lung of COPD patients [102]. During interleukin-10 (IL-10)-mediated airflow obstruction, an imbalance between MMP-9 and TIMP-1 results in an increase in MMP-9 activity was found in an animal model system [103].

Acrolein, a component of cigarette smoke, has been shown to initiate cleavage of proMMP-9, thereby producing active MMP-9. However, MMP-9 knockout mice do not completely inhibit cigarette smoke-induced emphysema, suggesting that other MMPs also play role in COPD pathogenesis [103]. Importantly, MMP-14, the membrane-type MMP (MT1-MMP), has been observed to be induced by acrolein which upon increase in mucin production leads to COPD [104] (Fig. 5).
Lung fibrosis (a.k.a. interstitial lung diseases) is a chronic disorder, exemplified by a marked increase in matrix degradation and intra-alveolar fibrosis leading to dyspnea, impaired oxygen transfer and alveolar collapse [105, 106]. Lung fibrosis occurs in the alveolar space and interstitium and is characterized by a widespread accumulation of differentiated fibroblasts (i.e., myofibroblasts) and ECM components.

Fibrotic disorders in the lung are associated with dysregulation of proteolytic activities. A considerable number of reports have suggested the involvement of cathepsins in this scenario. Enhanced proteolytic processing of CatB was observed in the lungs during an increase in TGF-β, suggesting that CatB may participate in fibrogenesis [107].

Microarray studies have revealed that in addition to NE, MMP-7 (a.k.a. matrilysin) is an important COPD marker. MMP-7 degrades decorin, the extracellular proteoglycan, which subsequently releases decorin-bound transforming growth
factor-β (TGF-β) [108] and that subsequently contributes to TGF-β activation, which is known as a critical marker of COPD [109].

6 Silicosis

An increase in the activity of MMP-2, MMP-9, and stromelysin has been demonstrated in alveolar macrophages from silica-treated rats, which contribute to extracellular matrix (ECM) and basement membrane (BM) degradation [110]. Administration of silica particles to mice causes upregulation of cathepsin K (CatK) expression and activity in silicotic lung homogenates compared to control lungs. Lung fibroblasts and macrophages were known as the main CatK-producing cells. Expression of CatK is inversely correlated to the level of TGF-β1, suggesting a protective role of CatK during silicotic process [111]. Mature active CatB, -H, -K, -L, and -S were identified in the broncho alveolar lavage fluids (BALFs) of patients suffering from silicosis. Among them, CatH has been observed to be the most abundant aminopeptidase, while CatB and CatL were mostly found thiol dependant endoproteases. Importantly, an increase in cathepsins/inhibitors ratio has been shown to favor uncontrolled proteolysis during silicosis [110, 111].

7 Cystic Fibrosis

In cystic fibrosis (CF), a marked increase in the activities of proteases could damage the airway architecture and that contributes to progressive bronchiectasis, a condition where the bronchial tubes of lungs are permanently damaged and enlarged due to infection in the bronchi [112, 113]. CF is an autosomal recessive genetic disorder caused by loss of expression or functional mutations to the cystic fibrosis transmembrane conductance regulator (CFTR) [114, 115]. CF affects multiple organs, albeit the pathology associated with CF appears to be due to its effect on the respiratory system. Non-functional CFTR channels in CF patients prevent the regulation of chloride and sodium ions across epithelial membranes leading to an increase in dehydrated mucus secretions in the lungs [112–116].

The key immune cell mediators seen in CF patients are polymorphonuclear neutrophils [115]. Upon recruitment, activated neutrophils release a wide variety of proteases, which induce inflammatory response and subsequently tissue damage [115, 116].

The impairment of mucociliary clearance mainly revolves around the interactions between NE and mucins. Mucins are a family of highly glycosylated proteins produced by epithelial cells and are the main components of the mucus found clogging of the airways in CF patients [117, 118]. NE has been shown to regulate the mucins via activation of TNF—converting enzyme, which upregulates their expression via epidermal growth factor receptor (EGFR) pathway [118–120].
CF patients cannot efficiently clear mucus due to damage by proteases to the cilia structures in the lungs and, therefore, are highly susceptible to chronic bacterial infections [121–123].

MMPs were found to play a crucial role in CF pathogenesis [124]. MMP levels are increased in the BAL of CF individuals [125]. MMPs produce proline-glycine-proline (PGP), a neutrophil chemoattractant derived from extracellular matrix, which regulates the immune response during CF [126].

A marked increase in NE has been shown in CF lung, which causes airway remodeling by degrading ECM proteins such as elastin and fibronectin [127]. The resulting alteration of airway epithelial cell membrane by NE induces neutrophil-mediated inflammation upon increase in the expression of proinflammatory cytokines, for example, IL-8, which results in neutrophil-mediated inflammation by upregulating the proinflammatory MMPs, CatG, and PR3 leading to tissue damage in the CF lung [6].

8 Asthma and Allergy

8.1 Asthma

Asthma is a chronic inflammatory disease of the airways and its occurrence and propagation is on the rise. The number of patients with asthma is estimated to attain a staggering figure of 100 million globally by 2025 [128]. Generally, asthma is triggered by the activation of adaptive immune response that upon inducing the lung triggers mucus production, increased IgE level, airway remodeling, and airway hyperactivity [129].

Manifestation of Asthma is characterized by acute inflammatory response and airway obstruction [130]. In both acute and chronic asthma, proinflammatory cells, including neutrophils, eosinophils, mast cells and macrophages enters into the lung tissue [131, 132]. These proinflammatory cells secrete a variety of extracellular proteases of which serine proteases and MMPs are important as these enzymes play prominent role in asthma pathogenesis [133–139].

Plasminogen can be converted to the active enzyme plasmin by tissue type plasminogen activator (PA) or urokinase-type PA (u-PA). Tissue PA and u-PA are associated with the dissolution of fibrin and also in the degradation of ECM components [140]. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of tissue type plasminogen activator and u-PA, and thereby contributes to matrix formation by preventing matrix degradation. Mast cells (MCs) in the airways of patients with asthma are crucial in initiating allergic inflammation [141]. MCs and bronchial epithelial cells (BECs) are the major source of plasminogen activator inhibitor (PAI-1). The interactions between the BECs and the MCs are important in maintaining persistent inflammation and structural changes in asthma [142].

IgE-mediated inflammation is well known for the pathogenesis of asthma. MCs-derived TGF-β upon cross-linking with IgE receptor enhances PAI-1
production in BECs. This increase in the production of PAI-1 has been suggested to play a critical role in the development of fibrosis that occurs adjacent to the epithelium [143]. Conceivably, drugs that inhibit activation of MCs, for example, by anti-IgE may prove useful in preventing airway remodeling in asthma.

### 8.2 Serine Protease and MMP in Asthma Pathophysiology

Proteolytic enzymes including NE and MMP-9 play important roles in tissue remodeling and repair in the airways [144]. The proteolytic enzymes levels are increased in asthma, which occurs due to an imbalance in the protease–antiprotease system.

In neutrophilic asthma, high levels of active NE and proMMP-9 were observed, whereas only a small amount of MMP-9 has been observed to be active. However, eosinophilic asthma was characterized with high level of active MMP-9 without free elastase. Thus, a differential profile of protease activity has been observed in asthma. A deficiency of antiproteases may explain the differential enzyme activity observed in eosinophilic and neutrophilic asthma. An increase in the level of MMP-9 bound to TIMP-1 in subjects with neutrophilic asthma in comparison to the eosinophilic asthma has been observed. This could explain about the presence of low level of active MMP-9 in neutrophilic asthma. This relative deficiency of TIMP-1 in subjects with eosinophilic asthma in comparison to neutrophilic asthma may explain about the high level of active MMP-9 that exists in the sputum of subjects in this group [144–146].

Alpha1-PI level has been observed to be increased in neutrophilic asthma, but its function was impaired leading to a marked increase in free elastase (NE) activity. Proteolytic inactivation of α1PI may lead to a form, which acts as an activator of neutrophils and that could result in superoxide (O$_2^-$) production [146]. However, role of proteolytic enzymes in specific inflammatory phenotypes of asthma is not clearly known. In contrast, IL-8 (a potent chemoattractant and an activator of neutrophils) plays an important role in eosinophilic asthma [6]. NE can induce production of IL-8 and its potency has been observed to be elevated upon proteolytic processing by MMP-9 [147].

Patients with persistent inflammatory asthma elicit more non-eosinophilic asthma progression than that of the eosinophilic asthma. These exacerbations are not prevented by corticosteroid treatment [148]. COPD and neutrophilic asthmatic patients generally show chronic airway inflammation associated with a marked airway neutrophilia, which are not discernibly responsive to inhaled corticosteroids [148, 149].

### 8.3 Cytokines and Asthma

Inflammation in asthma has been observed to be mediated by a specific subclass of T-lymphocytes referred primarily to Th2 lymphocytes, which causes inflammation and remodeling via secretion of specific cytokines [150].
Cytokines, for example, IL-11 play primary role in mediating asthma pathophysiology through its receptor (IL-11R). ADAM-10, a matrix metalloprotease, can release the IL-11R ectodomain upon cleavage of IL-11 receptor. Serine proteases such as NE and PR3 can also cleave the IL-11R. The resulting truncated soluble IL-11R (sIL-11R) activates the inflammatory cells. Thus, IL-11 signaling pathology proceeds upon proteolytic cleavage of its receptor [151].

An increase in the numbers of apoptotic airway epithelial cells in COPD has been observed to be associated with secondary necrosis [152, 153]. In severe asthma, conditions associated with increased airway neutrophilia, tissue damage and an increase in apoptosis of airway epithelial and smooth muscle cells have also been demonstrated [154]. Granzymes, a family of serine protease, have a repute to initiate immune-mediated cell death. Cytotoxic T cells and natural killer (NK) via granzyme-mediated pathway induces apoptosis of target cells, e.g., bronchial epithelial cells. Granzymes play critical roles in a variety of age-related chronic inflammatory diseases. There are five human granzymes identified so far in the lungs. These are granzyme A (GzmA-tryptase), granzyme B (GzmB-aspase), granzyme H (GzmH-chymase), granzyme K (GzmK-tryptase), and granzyme M (GzmM-metase). Granzymes, especially granzyme B and perforin, are stored in secretory granules of cytotoxic cells, and are released into the intercellular space following adhesion to the target cells. In presence of Ca²⁺, perforin pores in the cell membrane enable entry of granzyme B and subsequently induces caspase-dependent apoptosis [155], which may be an important mechanism of lung injury in asthma.

8.4 Allergy

Many aeroallergens like house dust mites and fungal allergens associated proteases play important role in asthma pathophysiology. Epidemiological studies suggested that sensitivity to fungal allergens could be an important cause of allergic asthma [156].

8.5 Alternaria Alternate and Asthma Severity

The fungus *Alternaria alternate* has been observed to cause asthma under certain circumstances [157, 158]. The allergen of the fungus possesses intrinsic proteolytic activities and that upon activating protease activated receptors (PARs) play a prominent role in mediating allergic airway diseases. Interleukin-33 (IL-33) has been observed to be associated with the development of allergic asthma [159]. IL-33 expression in the lung was found to be elevated in the asthmatic subjects and the asthma severity could be positively correlated with the IL-33 expression in the airways [160, 161].

Alternaria driven release of IL-33 occurs with the involvement of a serine protease specific to this aeroallergen. The Alternaria serine proteases cause marked
inflammation because of the capacity of the serine protease to drive IL-33 release, which in turn induces rapid onset of asthma exacerbations. Thus, targeting the protease—IL-33 signaling axis could prove useful as a therapeutic measure in this kind of asthma pathogenesis [162].

### 8.6 Aeroallergenicity of Acanthamoeba

The free living amoeba, *Acanthamoeba trophozoite*, is found in human airway cavities and possesses high protease activities, which can elicit allergic airway inflammation [163]. Intranasal inoculation of *A. trophozoite* or its excretory secretory (ES) proteins in mice have been shown to elicit allergic airway inflammation. ES proteins with strong protease activities stimulate dendritic cells and also able to enhance the differentiation of early T cells into mature IL-4 secreting T cells. Treatment of ES proteins in the protease activated receptor (PAR-2) knockout mouse showed inhibition of lung airway inflammation and Th2 immune responses with lower IgE level compared with the normal mouse. This suggests a role of PAR-2 in the aeroallergenicity of Acanthamoeba allergens [163].

### 8.7 Seasonal Rhinitis and Asthma

Allergic diseases like seasonal rhinitis and asthma are generally result from exposure to airborne pollens. Asthmatic patients allergic to pollen have been observed to develop a chronic inflammation of the airways leading to bronchial obstruction and hyper responsiveness. Upon inhalation, pollen grains release a wide variety of allergens with protease activities, which may act as inflammatory mediators and subsequently pathogenesis of respiratory allergies. The proteases were known to inactivate lung regulatory neuropeptides, for example, substance P and vasoactive intestinal peptide (VIP) [164] leading to dysregulation of the contraction-relaxation rhythm of the respiratory airways. The inhaled allergens were processed by dendritic cells (DCs) present at the subepithelial regions, and then present allergen peptides to native T-lymphocytes for stimulation of IgE production. Some pollen allergens exhibit proteases such as aminopeptidase and trypsin-like serine protease activities, which can cleave proteins from junctional complexes between epithelial cells [165]. A 98 kDa aminopeptidase of *Parietaria judaica*, for instance, has been shown to cause detachment of A549 human alveolar epithelial cells by degrading intercellular adhesion proteins from tight junctions and adherens junctions [165, 166]. Pollen proteases can induce degradation of cell junction proteins such as occluding, claudin-1 and E-cadherin, thereby help allergens to cross the epithelial barrier and contact with DCs for intensifying immune response [164–166].
8.8 Organic Dust Allergy

Organic dusts made for agriculture may lead to airway inflammation, which may cause sinusitis and chronic bronchitis to workers in agricultural industries [167–170]. Workers in livestock industries working in concentrated animal feeding operations (CAFOs) are susceptible to chronic airway diseases [171]. Extracts of dust collected from CAFOs are potent stimulators of lung inflammatory responses. Hog dust extract (HDE) contains active proteases, which play a critical role in lung inflammatory processes [172, 173].

Epidermal growth factor receptor (EGFR) signaling has been shown to play an important role in the proinflammatory response of bronchial epithelial cells (BECs) to HDE [172]. The proinflammatory effect of HDE has been suggested to be due to the proteolytic activation of PARs, especially PAR-2 [173]. In lung epithelial cells, actions of fungal, cockroach and dust mite allergen proteases are mediated by the cleavage and activation of protease activated receptor-2 (PAR-2) [174–176]. However, PAR-1 is unable to mediate the effects of these proteases. PARS play an important role in bronchial fibroblast proliferation; epithelial cell wound healing and hypersecretion of mucus [177, 178]. By inhibiting HDE proteases or abrogating activation of epithelial cells, PAR-2 could inhibit HDE-induced inflammatory indexes in bronchial epithelial cells (BECs) and subsequently inhibition of the allergic response [179]. Thus, targeting the protease activity of organic dusts made for agricultural usage and other air borne dusts may prove useful as a strategy for preventing airway inflammation in agricultural workers, who are generally exposed to dusty agricultural environments.

8.9 Cockroach Allergy

Bernton and Brown [180] first observed skin rashes upon exposure of cockroach over the skin of allergic patients. Subsequently, a considerable number of evidence have confirmed that exposure to cockroach can induce allergy. Proteases associated with cockroach can produce bleb formation to the skin, which could play a critical role in the development of allergic disease [181–183].

Cockroach allergens such as saliva, feces, cast skins, and dead bodies contain serine protease activities, however, feces (frass) was found to be the prominent source of allergens, which contains serine protease activity [184, 185]. Sensitization of wild type mice to German cockroach frass (GC frass) has been shown to increase allergic hyperactivity (AHR) due to a marked increase in serum IgE and also production of cytokines such as IL-13, IL-4, IL-5, and IL-17 [186]. Sensitization of PAR-2 deficient mice with GC frass, however, did not show a discernible increase in allergic airway inflammation indicating a role of PAR-2 in mediating allergic airway inflammation [185].
9 Lung Cancer

Lung cancer is one the most prevalent and lethal diseases worldwide. Despite recent advances in chemotherapy, the molecular basis of its progression to a metastatic disease remains unclear.

Exposure of bronchial epithelial cells of smokers produce oxidants and reactive oxygen species (ROS) with consequent cellular responses to activate NF-κB and other transcription factors that regulate inflammation-related genes and initiates several signaling pathways depending on both genetic and epigenetic factors and manifest COPD and lung cancer [187, 188].

9.1 MMPs and Lung Cancer

Lung cancers are of two major types: (i) small cell lung carcinoma (LC); and (ii) non-small cell lung carcinoma (NSCLC). ECM and basement membrane components are proteolytically cleaved by MMPs. These components play a pivotal role in cancer progression. Lung cancers express high levels of MMPs. MMP-7 and MMP-9 expressions were found to be markedly high in NSCLC in comparison to the normal tissue [189]. MMP-1 and MMP-3 promoter polymorphisms are associated in modifying susceptibility to NSCLC and also to an increase in the risk of lymphatic metastasis of these tumors [190]. MMP-2 and MMP-9 activities have been found to be associated with an increase in tumor spread [191].

Treatment of mice with CH1104I (dual inhibitor of MMP-2 and -9) has been shown to markedly inhibit metastasis of lung carcinoma, which suggests that inhibition of MMP-2 and -9 could significantly inhibit tumor invasion and metastasis [192]. MMP-2 and -9 expressions may have prognostic implications in patients with NSCLC [193]. Over expression of MMP-1 has been observed to induce the formation of lung metastases [194, 195].

9.2 High Temperature Requirement A (HtrA) Serine Protease and Lung Cancer

HtrA (a.k.a. DegP) is a heat shock-induced serine protease that is active in the periplasm of Escherichia coli. Homologues of HtrA were found in a wide range of bacteria and in eukaryotes. Till date, four human homologues of the bacterial serine protease HtrA have been described, which are named as HtrA-1, -2, -3, and -4 [196, 197]. They have a variety of functions including cancer [198].

HtrA1 has been observed to be downregulated in lung cancers [198]. In human cancer cells, HtrA1 over expression prevents cancer cell growth and proliferation suggesting HtrA1 as a tumor suppressor. A modest expression of HtrA1 has been observed in primary tumors and lymph node metastases. However, the exact functions of HtrA1 in cancer are mostly unknown. A previous report suggested that
HtrA1 elicits its function by inhibiting TGF-β pathway [199]. The role of TGF-β in cancer progression is well documented [200]. Accordingly, the TGF-β signaling pathway has been considered as both a tumor suppressor and promoter pathway of tumor progression and invasion. It, therefore, seems probable that activation of TGF-β signaling pathway occurs when HtrA1 is downregulated, thereby contributing to the cancer progression. Alternatively, over expression of HtrA1 has been shown to induce apoptosis [201]. Thus, loss of HtrA1 expression alters regulation of apoptosis and could lead to cancer progression [201].

HtrA1 degrades tubulin by disrupting microtubules (MTs), which suggest that HtrA1 could play an important role in regulating MT and tubulin stability and MT-associated cellular functions [245]. HtrA1 also regulates cell migration and offers a potential role in regulating MT organization associated with cell migration. However, the exact mechanism(s) by which HtrA1 regulates cell migration is currently unclear. Active HtrA1 upon removal of N-terminal Kazal-type trypsin inhibitory domains contributes to cell death through caspase dependent, as well caspase-independent mechanisms [202].

The role of HtrA2 protease in stress responses and apoptosis in lung cells has been established and a previous report suggested its involvement in cisplatin-induced death of renal cells [203]. Over expression of HtrA2 by cisplatin has been observed to follow the release of HtrA2 from mitochondria to the cytosol and it degrades anti-apoptotic proteins. This mechanism seems to be obligatory to trigger mitochondrial permeabilization for HtrA2 to participate in cell death [203, 204]. Therefore, HtrA2 plays a vital role in programmed cell death upon eliminating the caspase inhibitory activity of apoptosis [205, 206]. However, the detail mechanism(s) by which HtrA2 regulates lung cancer is currently unknown.

Smoking is a critical factor for lung cancers and the HtrA3 has been shown to be associated with smoking-induced lung cancer. HtrA3 expression has been found to be markedly downregulated in lung cancer cell lines and also primary lung tumors isolated from heavy smokers [207]. HtrA3, in contrast to the steady HtrA1 and HtrA2 expression, has been identified as a probable target for cigarette smoke-induced changes in normal human bronchial epithelial cells [207]. It has also been suggested that cigarette smoke-induced methylation of HtrA3 may play an important role in the etiology of smoking-linked lung cancer [207].

Studies on HtrA3 exon in lung cancer cell lines indicates that it possesses core xenobiotic response element (XRE) consensus sequence, 5-TNGCGTG-3 and is a target for methylation at CpG. XRE is located in the promoter region of the genes involved in metabolizing xenobiotic carcinogens, mainly aryl hydrocarbons from cigarette smoke and environmental pollutants, for instance, automobile exhaust [208, 209]. These compounds upregulate the gene products of XRE via aryl hydrocarbon receptors and multitude of other transcription factors [210, 211]. The degree of methylation of HtAr3 is similar when studied in A549 and H157 lung epithelial cells, when treated with NNK (Nicotine-derived nitrosamine ketone), a.k. a 4-(methylNitrosamino)-1-(3-pyridyl)-1-butanone, an important tobacco-specific nitrosamines, which play key role in carcinogenesis [208]. NNK suppressed the expression of HtAr3, whereas 5-azo-dc has been shown to induce it [208].
Differential expressivity of HtAr3 may be controlled by other cross-talking mechanisms such as histone deacetylation, micro RNA activation, loss of heterozygosity and genetic mutation. Along with that HTAr3, HtAr1, and HtAr2 are also believed to be upregulated by xenobiotic stress [208, 212]. However, more research is needed to ascertain them as therapeutic targets pertaining to lung cancer for epigenetic therapies as well as prognosis to forecast tumor response.

10 **Tuberculosis**

Upregulation of CatG, but not NE, has been shown to induce cell death of activated *Mycobacterium tuberculosis* infected macrophages. The substrate specificity of CatG and NE is distinct. CatG cleaves the C-terminus of aromatic or positively charged amino acid residues, while NE cleaves the C-terminus of small hydrophobic amino acid residues [213, 214]. Enhanced necrosis in infected macrophages by CatG may result from proteolysis of specific target sequences, which are currently unknown. It has been shown that serpinb3a inhibition of CatG is necessary to prevent necrosis induced by IFN-γ in *M. tuberculosis* infected macrophages [214].

Role of matrix metalloproteinases (MMPs) as an important mediator of tissue destructive response in TB has now been clearly known [215, 216]. MMPs can cleave ECM components [217]. In humans, MMP-1 cleaves fibrillar components (types I and III collagen) of ECM. The level of MMP-3, the activator of MMP-1 is high in respiratory secretions of TB patients than control subjects [216–218]. In rabbits, MMP-1, -3, -7, -12, and -13 expressions are elevated in granulomatous and cavitary pathologies in human respiratory secretions observed in vivo model systems of TB [218–220]. In a human monocyte infection model, a marked reduction in TIMP-3 has been observed to be correlated with TB pathogenesis [221]. In a mice model, TIMP-3 has been observed to be associated with cavity formation and subsequently ECM degradation, which are important for TB pathogenesis [221, 222].

11 **Acute Lung Injury**

Severe acute respiratory distress syndrome (SARS) was identified in 2003, which triggered death of thousands of people worldwide [223, 224]. A new type of coronavirus has been identified and found responsible for the SARS, which produces pneumonia and associated high fever and severe dyspnea and subsequently acute respiratory distress syndrome (ARDS) followed by death due to acute lung injury (ALI) [224, 225]. ARDS is characterized by accumulation of inflammatory cells and severe hypoxia that leads to pulmonary edema [226].
Renin-angiotensin system (RAS) has been observed to play a critical role in SARS. In animal studies, a prominent role of angiotensin converting enzyme (ACE) in the pathogenesis of ARDS has been suggested [227, 228]. ACE2, a homologue of ACE, was shown to be a key regulator for coronavirus infection that produces SARS. ACE2 has been shown to be expressed in the lungs of both healthy and diseased humans, and it protects against SARS-induced ALI [229–231]. Therefore, it seems conceivable that ACE2 might prove a novel therapeutic target for SARS-coronovirus-induced ARDS that develops in emerging lung infectious diseases including influenza [231].

12 Elafin and ALI

Elafin, a serine protease inhibitor having mol wt of 6 kDa, found in lung secretions. Elafin is formed by proteolytic cleavage of its precursor protein, trappin-2 [232]. The antiprotease activity of elafin is located in the C-terminal domain having specificity for NE and proteinase 3. The N-terminus transglutaminase substrate binding motif (GQDPVK) of elafin cross-links with extracellular matrix proteins [233, 234].

In ALI, the protease–antiprotease balance alters in favor of proteases leading to an increase in protease activity, and this protease burden can produce pulmonary edema [235]. A decrease in plasma elafin level has been observed to be correlated with altered elafin gene expression and seems a critical component for an increase in acute respiratory distress syndrome (ARDS) [236–238].

In ALI patients, the 20S proteasome was observed to be markedly higher compared with normal subjects [239], but elafin level was shown to be decreased with consequent proteolytic degradation of antiproteases by the 20S proteasome in lung patients with ALI. This decrease may contribute to an increase in NE activity in ALI regulation and expression; however, its biological role in the lung is currently unknown. The cleavage of elafin by 20S proteosome suggests that the increment of antiprotease levels in ALI patients could prove clinically beneficial in attenuating uncontrolled activity of NE. Elafin’s multifunctional properties could prove useful as the therapeutic target for ALI [239–241].

13 Elane and ALI

Elane has a potential catalytic activity to hydrolyze elastin. Under physiological conditions, lungs are protected from this enzyme by endogenous inhibitors such as α1-PI, α2-macroglobulin, and SLPI. However, in the course of ALI, the balance between elane and its endogenous inhibitors is deregulated in favor of the enzyme [242–244] leading to massive infiltration of neutrophils into the lungs and
subsequently tissue injury. Therefore, peptidic and non-peptidic elane inhibitors may prove useful for treating ALI associated with systemic inflammation [245, 246].

14 Age-Related Pulmonary Diseases

Granzymes, especially granzyme A and granzyme B, are the most abundant granzymes involving the membrane perforating molecule, perforin, which induce cell death [247]. Perforin facilitates granzymes entry into the target cell and that subsequently induces cell death [248]. Granzyme A has originally been thought to induce caspase-independent cell death; however, recent findings suggest that granzyme A may be involved in immune regulation of age-related lung disorders [249, 250]. In contrast, granzyme B induces apoptosis through caspase-dependent and -independent pathways [251]. An increase in granzyme A and B activities is known to promote generation of proinflammatory cytokines. ECM degradation and formation of autoantigens may exacerbate the inflammatory response [251]. Chronic inflammation is a hallmark of age-related cardiovascular and lung diseases. Thus, granzyme A and B may serve as important agent in promoting a positive feedback cycle that may be common to many persistent age-related disorders [251, 252]. However, role of other granzymes such as GzmH, GzmK, and GzmM in age-related ARDS are currently unknown.

15 Aspergillosis

Invasive pulmonary aspergillosis (IPA) elicited by the filamentous members of the genus Aspergillus can have devastating role in immune compromised individuals [253, 254]. Aspergillus fumigatus is responsible for the majority of IPA infections. Normal individuals are at little risk because of the effectiveness of their lung defences. However, inhalation of conidia becomes life threatening to subjects having weak immunity, which could allow the conidia to germinate into invasive hyphae in the lung [255].

The populations at greatest risk for IPA are patients with cancer, solid organ transplants, bone marrow transplants and those with advanced AIDS [256]. A. fumigatus secretes proteases like an alkaline serine protease (ALP), a metallo-protease (Mep), an aspartic protease (Pep) and a prolyl endopeptidase in the lungs [257–260]. A. fumigates secreted proteases are expressed in the lung during infection [261, 262].

A. fumigates binds to ECM proteins with the involvement of polysaccharides and glycoproteins of the conidial cell wall [263]. Several agents secreted from fungus such as proteases and toxins have been shown to influence its infection in host lung tissue [263].
16 Systemic Sclerosis

Systemic sclerosis (SSc) is an autoimmune disease, which could occur due to vascular injuries and fibrosis in skin and certain internal organs [264]. Some cytokines and growth factors like transforming growth factor-β (TGF-β) have been observed to stimulate fibroblast proliferation [264]. The disintegrin and metalloprotease-12 (ADAM-12) possess the extracellular cell binding functions. ADAM-12 is expressed in two alternative forms: (i) membrane-anchored form (ADAM12-L); and (ii) a short secreted form (ADAM12-S). An increase in the level of serum ADAM12-S level plays an important role in the pathological events of diffuse cutaneous systemic sclerosis (dcSSc) [264, 265].

ADAM-12 plays a critical role in fibrotic process. ADAM12-S has the ability to degrade physiological substrates such as the ECM substrates: fibronectin, type IV collagen [265] and also insulin-like growth factor binding protein (IGFBPs) [266, 267]. Degradation of IGFBPs augments the association between insulin-like growth factors, for example, IGF-I and its receptors. IGF-I down regulates collagenase activity with consequent increase in collagen production [268], which indicates that IGF-I could be an important mediator in the progression of fibrosis. Additionally, ADAM-12 has been observed to be upregulated in chronic wound suggesting that ADAM-12 could be related to the fibrotic process [269].

17 Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD) usually occurs in prematurely born infants. Due to deficiency of lung development, BPD patients require prolonged medical ventilation for oxygen. BPD causes an increase in morbidity and mortality in preterm infants. BPD is characterized by chronic inflammation, alveolar hypoplasia and respiratory infections [270, 271]. In an animal model of BPD, a significant high elastase activity along with excessive proteolytic degradation of the elastic fibers due to a marked decrease in the levels of endogenous protease inhibitors are usually observed in the lung secretion [272]. Additionally, a discernible increase in mRNA and protein expression and also activities of cathepsins-B, -H, -K, -L, and -S have been observed in new borne BPD tracheal aspirates [273]. BAL fluid of newborn preterm infants with BPD showed elevation of MMP-9 and a decrease in free TIMP-1 level [274, 275].

18 Lymphangioleiomyomatosis

Lymphangioleiomyomatosis, a rare and progressive lung disease, usually affects women of pre-menopausal age. This disease is characterized by the infiltration of smooth muscle cells that express contractile proteins, for example, desmin.
Immunohistochemical studies have demonstrated a strong expression of CatK restricted to lymphangioleiomyomatosis cells. CatK has been suggested as a marker for diagnosis of lymphangioleiomyomatosis [276].

19 Bronchiolitis Obliterans Syndrome

Bronchiolitis obliterans syndrome (BOS) is a complication, which usually occurs during chronic rejection of lung transplant patients. Elevated levels of MMP-8 and MMP-9 were observed in obliterative bronchiolitis patients after a few years of lung transplantation [277]. A marked elevation in gelatinase activity was found in BAL fluid from BOS patients that could be due to MMP-9 secretion by local neutrophils [278]. In lung transplant model, inhibition of matrix metalloproteases in the donor and recipient, respectively, before lung harvest and after lung transplantation have been shown to improve oxygenation and markedly decreased PMN leukocyte influx into the isograft [279]. Both MMP-8 and MMP-9 deficient mice were observed to be protected from BOS as evidenced by a marked decrease in neutrophil influx and collagen deposition [280, 281].

MMPs and TIMPs were suggested to play a crucial role important role during lung allograft rejection. While TIMP-1 and TIMP-2 over expression have not been observed to elicit consistent effect on the level of cytokines or rejection pathology, MMP inhibition via systemic administration of MMP inhibitors were shown to reduce lung allograft rejection [282].

20 Lung Surfactant Proteins and Proteases

Lung surfactants are a mixture of lipids and proteins complex, which form a thin film in the lung alveoli and that plays a vital role in respiratory function especially gas exchange [283]. Additionally, the surfactant also shows the first line of innate immune defence in the lung. Its mode of action appears to lie in the inhibition of microbial infectivity and attenuation of inflammatory responses [284]. SP-A, SP-B, SP-C, and SP-D are the major surfactant proteins, which elicit important roles to trigger immune response in the lung [285–289]. SP-A has been demonstrated to be an important surfactant component having relevant functional immune response during Staphylococcus aureus infection [290].

Surfactant protein D (SP-D) is an important target of numerous proteases present in the CF lung. Host defence appears to be impaired due to proteolysis of SP-D and may contribute to the supportive lung disease in CF. SP-D, a glycoprotein of the collecting family, is produced and secreted by alveolar type II cells and non-ciliated bronchial epithelial cells [291].
SP-D has been observed to be protective against a wide variety of pathogens such as *Pseudomonas aeruginosa*, Haemophilus influenza, and *A. fumigates* [291–293]. Upon binding with SP-D, these pathogens trigger their agglutination, enhanced killing and clearance [294, 295]. SP-D has been shown to cause a number of secretions, which present larger protection in the body. An important characteristic of CF is chronic neutrophil-mediated inflammation in the airways mainly with an increase in the levels of HLE and PR3 [296–298]. Proteolytic degradation of some proteins such as SP-A and SP-D was found in BALF of CF patients [299].

Proteases have been observed to modulate surfactant activity in addition to its action on mucus proteins. Secretory proteases of *P. aeruginosa* can degrade SP-A and SP-B from lipid–protein complexes [300]. Purified elastase or secretory protease IV of *P. aeruginosa* supernatants have also been shown to degrade SP-A and SP-D [301–303]. The protease IV-mediated degradation of SP-A and SP-D may cause a discernible loss of bacterial aggregation or increase bacterial phagocytosis by alveolar macrophages [304]. NSPs like NE, PR3 and CatG can cleave the surfactant proteins [305]. These proteases may also cleave SP-D within the conserved sub-region of the C-terminal lectin domain with the generation of a ~35-kDa fragment, which decreases bacterial aggregation and mannan binding of SP-D [306]. *P. aeruginosa* elastase digestion of SP-D has also been shown to produce the 35-kDa fragment that retain the N-terminal collagen tail, albeit devoid of functional C-terminal globular lectin domain, which consequently elicit loss of innate immune functions [300, 303, 304].

In CF, COPD and asthma, like chronic lung airway diseases, an increase in the epidermal growth factor receptor (EGFR) could be the mechanism for mucus production [307, 308]. EGFR phosphorylation has been observed to activate mitogen activated protein kinases (MAPKs)-dependent signaling pathways, which in turn stimulates MMPs, for example, ADAM-17 and also NSPs leading to the production of mucins [309–312].

Human airway trypsin-like protease (HAT) has been observed to enhance the synthesis of mucus glycoconjugates in airway epithelial cells [313]. HAT is a natural ligand for PAR-2 present in bronchial cells [314] and HAT-dependent upregulation of mucin genes have also been shown to occur via PAR-EGFR signaling pathway [313].

21 Particulate Matter

Particulate matter (PM) having diameter of about 10 nm (PM10) is a complex mixture of metals, polycyclic aromatic hydrocarbons, nitrates, sulfates and other chemicals [315], where traffic and industrial activities have an important impact on that composition. Adverse effects of PM10, especially on alveolar epithelia were related to inflammation triggered by phagocytic cells upon PM10 internalization [316]. An immediate response is to augment generation of cytokines and chemokines such as IL-1, IL-6, and IL-8, TNF-α [317, 318].
Airborne PM10 is a risk factor for the development of a variety of lung diseases including cancer [315, 316]. In vitro, treatment of PM10 induces an increase in MMP-2 and MMP-9 activities, which causes ECM degradation during acute lung injury [319]. PM10 was found to be responsible for lung diseases such as tuberculosis [320], emphysema [321] and COPD [322]. In A549 lung epithelial cells, PM10 causes a marked decrease in E-cadherin/β-catenin expression and subsequently induces potentially invasive characteristics and thereby could contribute to cancer development [323].

22 Conclusion and Future Perspective

In order to fight against infections, the lung is orchestrated with different antiproteases and anti-inflammatory components. In pulmonary diseases like COPD and asthma, the balance between host proteases and their secreted endogenous inhibitors shift toward the proteases. Proteases such as NSPs, MMPs, and cathepsins are known to act along with the bacterial proteases and play important role in the manifestation of a variety of pulmonary diseases. Thus, agents that restore lung protease–antiprotease balance by upregulating endogenous protease inhibitors and/or down regulating host protease activities, appear important to control excessive inflammatory responses in the lung.

Inflammatory cells, which are rich in oxidants and proteases cause proteolytic inactivation of protein inhibitors of proteases. SLPI and trappin-2/elafin are relatively stable; even though they may be cleaved and inactivated by proteases, for example, by cathepsins at their N-terminal end, albeit it does not affect their inhibitory potency [324]. In contrast, cleavage of elafin by P. aeruginosa proteases may inactivate its antiprotease activity [325]. To overcome the unwanted proteolysis of antiproteases, encapsulations of protease inhibitors within liposomes have been suggested [326]. Aerosol delivery of liposomes entrapped antiproteases may prove useful since it has several features such as sustained release and relatively high loading capacities.

NE has been chosen as a target for inhibition by synthetic compounds because it is a widely recognized serine protease, which has been shown to be associated with a variety of lung diseases including CF [327]. DX-890, a small protein inhibitor of NE, has been observed to be tolerable in rats and humans after phase 1 clinical trial [328]. This compound was shown to be involved in IL-8 release from CF neutrophils and to reduce neutrophil transmigration through the epithelial barrier. Outcome of phase 3 clinical trials will reveal the usefulness of DX-890 as a therapeutic measure for a variety of lung diseases.

In a guinea pig model system, the dual MMP9/MMP12 inhibitor, AZ11557272 was found to be protective toward cigarette smoke-induced emphysema [329]. This compound markedly reduces number of inflammatory cells in bronchoalveolar lavage (BAL) fluid and has also been shown to decrease smoke-induced air space enlargement, which suggests that MMP-2 and MMP-12 could be the potential
targets for therapeutic intervention in COPD. AS112108, another dual MMP-9/MMP-12 inhibitor, has been shown to inhibit the early inflammatory responses with a decrease in neutrophil numbers [330]. AS111793, a selective MMP-12 inhibitor, elicited dose-dependent inhibition in the levels of neutrophils and macrophages in bronchoalveolar lavage (BAL) fluid, and also on the concentration of several inflammation markers that express after cigarette smoke exposure [331]. However, it did not inhibit lung inflammation observed by lipopolysaccharide (LPS). Understanding the mechanism of these antiproteases will eventually lead us to gain an insight into the basic biochemical mechanisms that regulate COPD.

MMP-1 has been considered as one of the target proteases in lung cancer [332]. The generation of MMP-1 deficient mouse model suggested pro-tumorigenic role of the enzyme [333]. Further studies on MMP-1 and other MMPs in knockout mice will be required to evaluate the functional redundancy and relative relevance of MMP-1 and other MMPs in cell proliferation, regulation of inflammatory cells, and different stages of cancer progression.

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