Role of Proteases in Inflammatory Lung Diseases

Kaustav Chakraborty and Arindam Bhattacharyya

Abstract Proteases are enzymes that have the capacity to hydrolyze peptide bonds and degrade other proteins. Proteases can promote inflammation by regulating expression and activity of different pro-inflammatory cytokines, chemokines and other immune components in the lung compartment. They are categorized in three major subcategories: serine proteases, metalloproteases and cysteine proteases especially in case of lung diseases. Neutrophil-derived serine proteases (NSPs), metalloproteases and some mast cell-derived proteases are mainly focused here. Their modes of actions are different in different diseases for e.g. NE induces the release of IL-8 from lung epithelial cells through a MyD88/IRAK/TRAF-6-dependent pathway and also through EGFR MAPK pathway. NSPs contribute to immune regulation during inflammation through the cleavage and activation of specific cellular receptors. MMPs can also influence the progression of various inflammatory processes and there are many non-matrix substrates for MMPs, such as chemokines, growth factors and receptors. During lung inflammation interplay between NE and MMP is an important significant phenomenon. They have been evaluated as therapeutic targets in several inflammatory lung diseases. Here we review the role of proteases in various lung inflammatory diseases with emphasis on their mode of action and contribution to immune regulation during inflammation.

Keywords Serine Protease • Metalloprotease • Inflammation • Inflammatory lung diseases • Pro-inflammatory cytokines • Chemokines • Neutrophil

K. Chakraborty • A. Bhattacharyya (✉)
Department of Zoology, University of Calcutta, 35, Ballygunge Circular road,
Kolkata 700019, West Bengal, India
e-mail: arindam19@yahoo.com

1   Introduction

Numerous environmental pathogens, particulate matters, allergens and harmless antigens are present in the air we breathe. Although airways are the main port of entry for pathogens and allergens during inhalation of the inspired air (10,000 L per day in humans) [1], the lung is one of the most challenged organs of the body. For this reason, air-breathing animals have developed several defense mechanisms in this compartment [2, 3].

Inflammation, a host defense mechanism, is an immediate response of the body to tissue injury caused by harmful stimuli, such as pathogens, damaged cells or chemicals [4]. Lung inflammation is a broad term which covers various acute and chronic inflammatory diseases such as Acute Lung Injury (ALI), Acute Respiratory Distress Syndrome (ARDS), Emphysema, Airway hyper-reactivity (AHR) or Asthma, Allergic Asthma or Chronic eosinophilic inflammation, Chronic Obstructive Pulmonary Disease (COPD), Fibrotic lung disease, Pulmonary edema, Tuberculosis, Pneumococcal infection etc. This complex and dynamic process is characterized by an innate immune response, which involves a coordinated expression of inflammatory cytokines and implication of various cell types particularly immune cells aimed at clearing the pathogenic agent, damaged cells etc. [5]. Modulation of expression and activity of these inflammatory cytokines and other immune components are regulated by various proteases in the lung compartment. Here we present the information relative to some relevant proteases which have roles in different inflammatory lung diseases.

Proteases are enzymes that have the ability to hydrolyze peptide bonds and degrade other proteins. According to the active groups of their catalytic center they are categorized and in case of lung disease, basically three major protease group serine proteases, cysteine proteases, and the matrix metalloproteases (MMPs) are studied [6]. There is another group the ADAM (a disintegrin and metalloprotease) family of proteases, which has an emerging role in mucin production and cytokine processing [7]. A number of studies have elucidated the role of proteases in human diseases such as cancer, thrombotic and inflammatory disorders [8, 9]. Various inflammatory cells, such as neutrophils, mast cells, macrophages, and lymphocytes are the major source of proteases within the lung [10–12]. Other cells, including epithelial, endothelial, and fibroblasts, also synthesize proteases [13, 14]. Serine proteases, including Neutrophil Elastase (NE), Cathepsin G (CG), and Proteinase 3 (PR 3), are packaged in primary granules within neutrophils [15]. Some of the metalloproteases, MMP-8 and MMP-9, are also packaged into specific and gelatinase granules, respectively, in the neutrophil [16].

2   Proteases in Lung and Their Mode of Action

In the lung, proteases function either intracellularly or extracellularly after cellular activation. Proteases that may be present in the respiratory tract and activate PARs include the endogenous enzymes mast cell tryptase (activates PAR2), trypsin

(PAR1, PAR2 and PAR4), chymase (PAR1) and cathepsin G (PAR4), as well as exogenous enzymes such as Der p1 (PAR2) that are inhaled. However, these and other enzymes within the respiratory tract may also inactivate or disarm various PARs by cleaving them at other sites that remove the tethered ligand sequence [17]. Proteases have crucial role in chemotaxis of all of the various inflammatory cell types to the lung. MMP-9 and serine proteases increase eosinophil chemotaxis, and MMP-12 is responsible for eosinophil and macrophage accumulation [18, 19]. However, maximum work has focused on neutrophil chemoattraction to the lung by interleukin 8 (IL-8) and leukotriene B4 to study the effect of proteases on cell migration in inflammatory lung diseases.

3 Serine Proteases

3.1 Neutrophil-Derived Serine Proteases (NSPs)

Neutrophils are essential for host defense against invading pathogens. They are the first inflammatory cell lines to enter tissue during inflammation [20]. They engulf and degrade microorganisms using an array of weapons that include reactive oxygen species, antimicrobial peptides, and proteases such as Cathepsin G, Neutrophil Elastase and Proteinase 3. After release, these proteases also contribute to the extracellular killing of microorganisms, and regulate non-infectious inflammatory processes by activating specific receptors and modulating the levels of cytokines [21]. In addition to their involvement in pathogen destruction and the regulation of proinflammatory processes, NSPs are also involved in a variety of inflammatory human conditions, including chronic lung diseases (chronic obstructive pulmonary disease, cystic fibrosis, acute lung injury, and acute respiratory distress syndrome) [22–25]. In these disorders, accumulation and activation of neutrophils in the airways result in excessive secretion of active NSPs, thus causing lung matrix destruction and inflammation.

3.2 Neutrophil Elastase (NE)

NE is a serine-protease of the chymotrypsin family stored in primary (azurophilic) granules of PMNs along with proteinase-3 and cathepsin G, two other neutrophil serine-proteases. Intracellular stored NE comes in action when azurophilic granules are incorporated to the phagosome [26]. However, *in vitro* stimulation of PMNs with physiological relevant pro-inflammatory stimuli induces either transfer of NE to the plasma membrane (membrane-bound NE associated to by proteoglycans) [27, 28], or a secretion in the extracellular space specially in case of pulmonary chronic (CF and COPD) [29, 30] or acute lung injury [22], where high efflux of PMNs in the alveolar space increase the release of NE from necrotic PMNs. Functions of NE are not only concerned with degrading bacteria [31–33] and
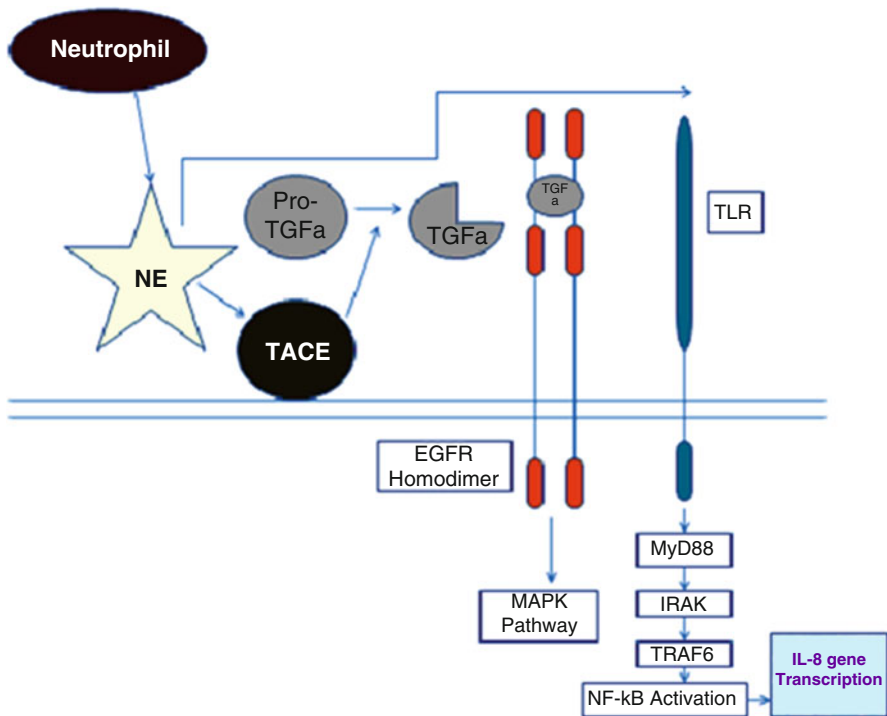
Fig. 1 Mechanism of neutrophil elastase (NE) induced-release of IL-8 from lung epithelial cells. Following its release from the azurophilic granules in response to pathogenic/pathologic insult, NE activates TNFa converting enzyme (TACE), which in turn cleaves proTGFa (pro-transforming growth factor a) to generate soluble TGFa as a ligand for the epidermal growth factor receptor (EGFR). EGFR co-localizes with toll-like receptor-4 (TLR4) and a signal transduction cascade is initiated via myeloid differentiation factor 88 (MyD88 or Mal), IL-1 receptor-associated kinases (IRAKs), tumor necrosis factor receptor-associated factor 6 (TRAF6), transforming growth factor-beta-activated kinase 1 (TAK1) and the IkB kinases (IKKs), leading to a degradation of inhibitor of NF-kB (IkB) proteins, activation of nuclear factor-kB (NF-kB) and increased IL-8 gene transcription

extracellular matrix molecules, they also operate on various bioactive molecules including chemokines, cytokines, growth factors and cell surface receptors [34–36], thus the "deleterious" concept of NE has changed towards a multifunctional molecule able to regulate inflammatory process and immune responses. Indeed, extracellular NE (free, chromatin-bound or membrane-bound) participates in: (1) direct killing of bacteria [31–33]; (2) processing and release of chemokines, cytokines and growth factors [34, 35], (3) modulation of immune cell activity through interaction with cell surface receptors [36, 37], (4) mucus secretion [38].

Proteases can also modulate cytokine activity and release from immune cells through mechanisms independent of cytokine receptors. For example, it has been shown that NE induces the release of IL-8 from lung epithelial cells [39–42] through a MyD88/IRAK/TRAF-6-dependent pathway [42] that also involves TLR4 [39] and also through EGFR MAPK pathway (Fig. 1) [43]. How NE activates TLR4 is

unknown but liberation of proteolytic fragments from host targets able to recognize PRR could be possible as described for TLR2 [44]. Serine proteases such as NE can induce IL-8 expression by bronchial epithelial cells (Fig. 1) and leukotriene B4 expression by macrophages [42, 45]. NE appears to be the most important regulatory factor present in the cystic fibrosis (CF) lung responsible for IL-8 expression because inhibition of NE activity in CF bronchoalveolar lavage fluid (BALF) almost completely blocks IL-8 message in bronchial epithelium [40]. It has been shown that NE generally act at least in part via an IL-1 receptor-associated kinase-1/ myeloid differentiation factor-88/nuclear factor-κB-dependent pathway in bronchial epithelial cells; this can be inhibited by a dominant negative variant of myeloid differentiation factor-88 [42]. This gives new therapeutic approaches targeted at inhibiting the NE-activated intracellular pathways rather than NE itself. It is quite clear that expression of IL-8 and leukotriene B4 are responsible for neutrophil migration to the lung, and given the high neutrophil and NE burden present in the CF lung this has lead to the "vicious cycle" hypothesis whereby NE is the main player behind IL-8 production and neutrophil influx into the CF lung. From further experiments it has been found that an initial inflammatory event can stimulate further inflammation i.e., epithelial cell injury in mice leads to secretion of the murine homolog of IL-8, which in turn binds to an adhesive component of the extracellular matrix, syndecan-1 [46]. MMP-7 cleaves this syndecan-1-murine IL-8 complex and this is crucial for attracting neutrophils to the damaged epithelial surface (Fig. 2).

3.3 Proteinase-3 (PR-3)

Proteinase-3 (PR-3) is a serine protease that cleaves TNF into membrane-associated TNF and soluble TNF form [47]. It has been hypothesized that PR-3-mediated TNF processing may be an important mechanism in inflammatory lung diseases [48]. PR-3 is able to degrade extracellular matrix, and its potential involvement in pulmonary inflammatory disease has been demonstrated by the induction of emphysema in hamsters following intratracheal instillation [49]. PR-3 is also enhanced in the sputum of cystic fibrosis patients, and correlates with disease severity [50]. The main sources of PR-3 are neutrophils and it has been hypothesized that there would be an increased contribution of PR-3 to TNF processing in diseases with abundant alveolar neutrophils such as usual interstitial pneumonia (UIP) [48].

NSPs contribute to immune regulation also through the cleavage and activation of specific cellular receptors. NE, PR3, and CG can process the N-terminal extracellular domains of protease-activated receptors (PARs), which are a subfamily of related G-protein-coupled receptors [51, 52]. These receptors are ubiquitously expressed in various tissues and cells and, more especially, in platelets and endothelial cells. Processing of PAR extracellular domains occurs through exposure of a tethered ligand that allows the auto-activation of the receptor and subsequent activation of an intracellular signaling cascade via phospholipase C [51, 52]. Four PARs have been identified so far; three of them, PAR-1, PAR-3, and PAR-4, can be activated by thrombin. Apart from thrombin, CG released from activated neutrophils
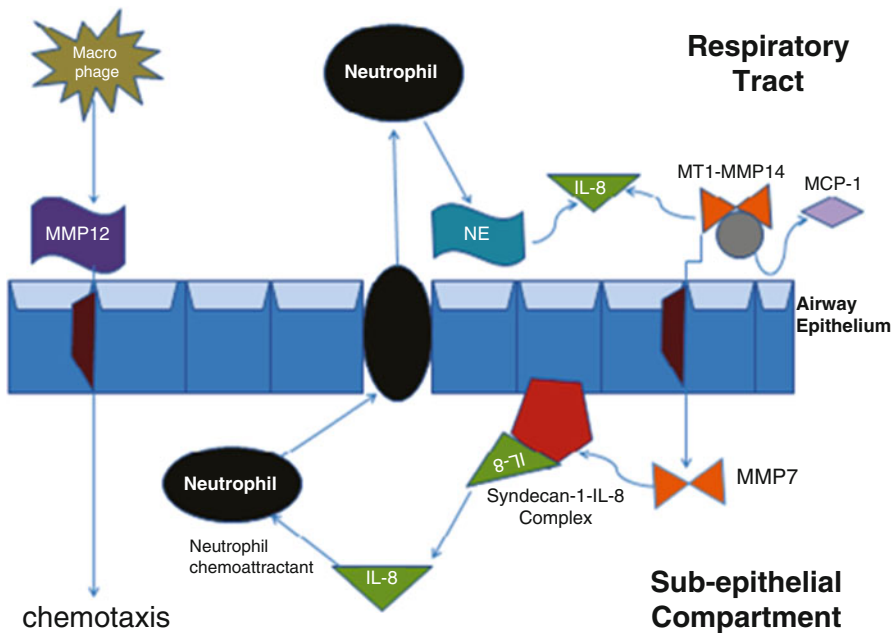
Fig. 2 Interaction of NE and MMPs during inflammatory responses in the respiratory tract. Neutrophil Elastase (NE) induces interleukin-8 (IL-8) synthesis, resulting in chemoattraction of neutrophils to the respiratory tract. MT1-matrix metalloprotease (MMP) 14 processes IL-8 and monocyte chemoattractant protein 1 (MCP-1), and MMP-7 cleaves the syndecan-1-IL-8 complex to generate active IL-8, which in turn acts as a neutrophil chemoattractant. MMP-12 released from macrophages also helps in chemoattraction of different inflammatory cell

can also activate PAR-4 at the surface of platelets and initiate their aggregation [53]. All three NSPs cleave PAR-1, which impairs their activation by thrombin [54]. Serine proteases cleave the amino acids at a specific site of the extracellular N-terminus of the molecule to expose a new N-terminal ligand domain that binds to another site on the same molecule, thereby activating the receptor. The amino acid sequence of each cleavage site is specific for the particular PAR, and mAb(monoclonal Antibody) assays for the protein and PCR assays for mRNA are available [55]. Activation of PAR2 results in the production and secretion of IL-8 and chemokine (C-Cmotif) ligand 2 [56, 57].

3.4 Human Airway Trypsin-Like Protease (HAT)

HAT belongs to the family of Type II Transmembrane Serine Proteases (TTSP) [58]. Members of this family present a short intracellular domain connected to a single-pass transmembrane domain followed by a large extracellular domain containing a highly variable stem region and a C-terminal serine-protease domain of the

chymotrypsin (S1) fold [58]. It is preferentially expressed in human bronchial and tracheal respiratory tract [59, 60], particularly in ciliated cells [61]. A soluble form after proteolytic cleavage is also found in the sputum of patients with chronic airways diseases [59]. Concerning the lung compartment, *in vitro* experiments have revealed various HAT activity such as: (1) fibrinogenolytic activity in lung airway anticoagulation processes [62]; (2) proteolytic inactivation of urokinase-type plasminogen activator receptor (uPAR) in lung epithelial cells [63]; (3) proteolytic activation of the hemagglutinin antigen of influenza virus leading to multicycle replication and propagation of influenza virus *in vitro* [64, 65]; (4) stimulation of lung fibroblast proliferation through a PAR-2-dependent MEK-MAPK mediated pathway [66]; (5) increase of intracellular Ca^{2+} concentration in bronchial cells through PAR-2-dependent mechanisms [67].
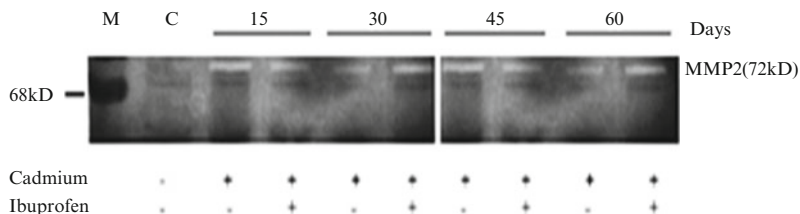
4 Metalloproteases

4.1 Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are zinc-dependent neutral endopeptidases that form a family of extracellular matrix proteolytic enzymes. They are primarily responsible for the degradation of extracellular matrix components during the remodeling processes essential for normal tissue growth and repair. In the lung, inappropriate expression and excessive activity of several MMPs, including MMP-12, have been implicated in the tissue-destructive processes associated with chronic lung diseases, including COPD and asthma [68–73]. It is well established that MMP-12 has definite role in the pathogenesis of COPD [74]. Patients suffering from COPD have increased secretion and activity of MMPs, especially MMP-2 and MMP-9, been identified in inflammatory cells and tissues isolated from those patients [75]. In our previous study, we found increased expression of MMP-2 in case of cadmium induced lung inflammation in mice model [76]. Kundu *et al.* (2009) performed SDS-PAGE and gelatine zymography to find out the expression of MMP-2 from lung cell extracts at different time points after the induction of cadmium chloride and found there was increased expression of MMP-2 even after use of ibuprofen, a non-steroidal anti-inflammatory drug (Fig. 3). Expression and activities of MMP-2 and MMP-9 are increased in case of Influenza virus infection both *in vivo* and *in vitro*. It has been demonstrated that H3N2 virus infection induces expression of MMP-2 and MMP-9 in murine lungs *in vivo* and alveolar epithelial cells *in vitro* [77]. Gelatinases (including MMP-2 and MMP-9) are zinc-dependent endopeptidases, degrade major components of the basement membrane such as gelatin and collagen IV, and exert deleterious effects on the epithelium and endothelium in the thin alveolar-capillary barrier [78].

A number of studies reveal that MMPs can also influence the progression of various inflammatory processes and there are many non-matrix substrates for MMPs, such as chemokines, growth factors and receptors [79]. MMP-14 is a protease who

Fig. 3 Increased expression of MMP-2 in cadmium induced lung inflammation by SDS-PAGE and Gelatin zymography. Detection of matrix metalloproteinase-2 (MMP-2) expression at different time points after the induction of Cadmium chloride (5 mg/kg body weight). Lung cell extracts were prepared at days of 15, 30, 45 and 60 from normal (N), Cadmium treated and Cadmium plus Ibuprofen treated (three individual animals per dose) mice. The zymography was developed and stained. The picture shows increased expression of MMP-2(72 kDa), which was not inhibited by Ibuprofen. Gel is representative of three comparable experiments indicate $p < 0.05$ with respect to the control

does the processing of IL-8 by removing a pentapeptide from the N-terminus of the protein, resulting in a more biologically active form. MMP-9 also process IL-8 to a form 20-fold more active as a chemoattractant [80, 81]. They process monocyte chemoattractant proteins (MCPs) to produce antagonists of chemokine receptors, indicating a role for MMP-14 in dampening inflammation [82]. Another potent pro-inflammatory cytokine IL-1β that requires proteolytic processing before activation not only by caspase-1 but also several MMPs, including MMP-2, MMP-3 and MMP-9. Interestingly, MMP-3 can degrade the mature IL-1beta cytokine, suggesting potentially dual roles for MMPs in either stimulating or inhibiting IL-1beta effects [83]. The mechanism by which MMPs control inflammation is the regulation of chemokine gradients and that includes both the immobilization of chemokines to the components of extracellular matrix and the generation of chemotactic concentration gradients which provide indications for leukocyte migration. Thus, MMPs can indirectly control influx of inflammatory cells by cleaving proteins in the pericellular environment that bind chemokines. One well established example of this mechanism is MMP7-dependent shedding of syndecan-1 in acute lung injury [46]. In response to lung injury, both CXCL1 (KC) and MMP7 are induced, and MMP7 sheds syndecan-1, a ubiquitous heparan sulfate proteoglycan, that releases the CXCL1-syndecan-1 complex to generate a chemokine gradient. MMP7-null mice that lack this shedding are unable to create a CXCL1 gradient, and thus, neutrophils fail to efflux into the alveolar space and instead remain in the perivascular space [84].

There is also interplay between NE and MMPs in inflammation. NE activates MMP-9 directly and indirectly by inactivating TIMP-1, the naturally occurring inhibitor of MMP-9 [85, 86]. Furthermore, NE may activate MMP-2 through a mechanism that requires MMP-14 expression [87]. Therefore, a variety of proteases liberated from neutrophils or actively expressed on epithelial cells may interact with each other, thereby perpetuating a cycle of inflammation. If we consider CF lung, there is high concentration of NE due to the increased neutrophil burden present in the CF lung and surplus the levels of MMP-14-processed monocyte chemoattractant

protein antagonists. This is supported by evidence showing significant IL-8 levels present in CF bronchoalveolar lavage fluid [40].

4.2 ADAM (A Disintegrin and Metalloprotease)

ADAMs are a family of type I transmembrane proteins belonging to the adamalysin subfamily of metalloproteinases [88]. Members of this family present a metalloprotease domain and a domain of interaction with integrin (disintegrin domain) [89], indicating that ADAMs are both proteases and adhesion molecules. At least 40 ADAMs has been described so far, 25 of which are expressed in humans [90, 91]. ADAMs family has been implicated in the control of membrane fusion, cytokine and growth factor shedding, and cell migration, as well as processes such as muscle development, fertilization, and cell fate determination.

Pathologies such as inflammation and cancer also involve ADAMs family members. ADAMs has been related to lung pathological processes such as cancer [90], asthma [92, 93] and idiopathic pulmonary fibrosis [94]. ADAMs can also modulate cell responses to various signals by acting as cell surface sheddase on membrane associated cytokines, apoptosis ligands, growth factors and receptors. In particular, sheddase activity has important physiological consequences. One of the most documented example is through the production of active tumor necrosis factor-α (TNF-α), a potent inducer of innate inflammatory responses. TNF-α is a homotrimeric transmembrane protein of 26 kDa able to induce inflammatory and cytotoxic effects after cell-to-cell contact [95]. ADAM-17 (also known as TNF-α convertase enzyme or TACE) cleaves transmembrane TNF-α to release the 17 kDa active soluble form of TNF-α [96]. Importantly, ADAMs are also implicated in the shedding of most of the EGFR ligands (EGF, transforming growth factor (TGF)-a, heparin binding (HB)-EGF, betacellulin, epiregulin and amphiregulin (AR)) [89]. It has been reported that Adam17 knock-out mice presented developmental defects resembling those in animals lacking TGF-a, HB-EGF, AR, or the EGFR [97–99]. In addition, sheddase activity on EGFR ligands has important physiological consequences for mucus production. Recently, it has been confirmed that endothelial ADAM10 and ADAM17 are both required for microvascular permeability [100]. To investigate this phenomenon Dreymueller *et al.* (2012) did LPS treatment on HMVEC-L (Human Microvascular Endothelial Cells) and found there is release of soluble JAM-A by LPS-challenged HMVEC-L. Release of soluble JAM-A was 1.3-fold enhanced by 4 h of LPS stimulation, further increasing to 2.3-fold by stimulation for 24 h and was completely inhibited by GW280264X-treatment, capable of blocking tumor necrosis factor-alpha-converting enzyme (TACE) and the closely related disintegrin-like metalloproteinase 10 (ADAM10). The knockdown of ADAM10 or ADAM17 by shRNA indicated the involvement of ADAM17 and to a lesser extent of ADAM10 in JAM-A release. They further examined whether ADAM10/17 activity might influence transendothelial migration of neutrophils. The inhibitor

GW280264X reduced transmigration in response to the neutrophil-attracting cytokine IL-8 by 70 %. Silencing or knock-out of either ADAM10 or ADAM17 alone was sufficient to abrogate transmigration in response to IL-8. Thus, endothelial ADAM10 and ADAM17 are both required for microvascular permeability and for IL-8-mediated transmigration of neutrophils *in vitro*.

4.3 TACE (TNF-α Converting Enzyme)

It has been reported that TACE mediates a critical step in the development of post-transplantation lung injury [101]. Goto *et al.* (2004) evaluated the role of TACE in acute inflammation using an inhibitor of the enzyme in a rat model of lung transplantation. Inhibition of this protease results decreased neutrophil accumulation in the alveolar space and other histological changes such as intercellular adhesion molecule-1 (ICAM-1) expression. In addition, significantly lower levels of monocyte chemotactic protein-1 (MCP-1), cytokine induced neutrophil chemoattractant-1 (CINC-1), high mobility group box-1 (HMGB1), and soluble epithelial cadherin and decreased neutrophil elastase activity were observed in bronchoalveolar lavage fluid from the rats treated with the inhibitor.

5   Mast Cell-Derived Proteases

Proteases are the most abundant class of proteins produced by mast cells. Many of these are stored in membrane-enclosed intracellular granules until liberated by degranulating stimuli, which include cross-linking of high affinity IgE receptor F(c)εRI by IgE bound to multivalent allergen [102]. It has been investigated that b-tryptase, a major protease released during mast cell activation, cleaves IgE and this tryptase-mediated IgE cleavage affects IgE binding to allergens [103]. From their study, IgE degradation products were detected in tryptase-containing tissue fluids collected from sites of allergic inflammation [103] and it has been confirmed that tryptase cleaves IgE and abolishes binding of IgE to allergens and F(c)εRI. It is a natural mechanism for controlling allergic reactions is supported by experiments performed with purified proteins, as well as by cellular *in vitro* and *in vivo* data [103].

The lung epithelial cells could be activated by airborne proteases from molds, mites, or pollens. Activation of other cells in the airway by various endogenous and exogenous proteases that increase production of IgE antibody and enhance infiltration of eosinophils, basophils, neutrophils, monocytes, and lymphocytes. Apart from that, smooth muscle contraction is enhanced, nerves are made more reactive, and airway responsiveness is increased [104]. Eosinophils and mast cells are degranulated and stimulated to produce inflammatory molecules, such as nitric oxide, major basic protein, leukotrienes, histamine, and mast cell tryptase itself. Mast cell tryptase is likely to be especially important in the late phase of the allergic response [105].

6 Proteases Involvement in Disease Progression

6.1 Chronic Obstructive Pulmonary Disease (COPD) and Pulmonary Emphysema

Chronic obstructive pulmonary disease (COPD) represents a group of diseases, including chronic bronchitis and emphysema, which are characterized by an airflow limitation that is not fully reversible [106]. The pathogenic roles of NSPs in COPD are attributed to their ability to break down connective tissue components and generate proinflammatory peptides from these components [107, 108], to induce mucus secretion by submucosal glandular cells and goblet cells, and to express proinflammatory cytokines from airway epithelial cells [109–111]. Pulmonary emphysema is a destructive lesion of the lung parenchyma. It has been demonstrated that the lesion of the matrix which results in emphysema is the end-result of crosstalk between macrophage metalloelastase and neutrophil elastase [112, 113]. However, the latter protease is responsible for the greater portion of the final proteolytic attack [112].

We have already mentioned before the role of MMP-12 in the pathogenesis of COPD. The most well studied MMPs in human COPD and emphysema are MMP-1, MMP-8, MMP-9, and MMP-12, which all have been implicated in tissue destruction in human COPD and emphysema [114]. The lymphocytes present in emphysematous lungs have a strong Th1 bias, expressing higher levels of CXCL10 (IP-10) and CXCL9 (MIG). This CXCL9 can upregulate the expression of MMP-12 in pulmonary macrophages [115], providing a mechanism for chronic and progressive destruction of lung parenchyma that is seen in emphysema.

6.2 Interstitial Lung Disease and Idiopathic Pulmonary Fibrosis (IPF)

Idiopathic pulmonary fibrosis (IPF), one of the most common forms of interstitial lung diseases, is a progressive fibrotic lung condition of unknown etiology [116]. The diagnosis of IPF is made by surgical lung biopsy, and the histopathological features include the presence of patchy inflammatory cells, foci of proliferating fibroblasts and myofibroblasts, and collagen deposition [117, 118]. From a study it has been observed that MMP-2 activity is increased in a dose-dependent manner in A549 cells treated for 48 h by TGF-β stimulation [119] because TGF-β plays a key role in stimulation of fibroblast proliferation and has been implicated in progression of IPF [120].

It has been postulated that NE may be involved in the early stages of lung inflammation during the development of pulmonary fibrosis [121]. Neutrophil elastase acts as a putative link between emphysema and fibrosis and this dual role of NE has been reported from a recent study that has highlighted NE as a common pathogenic mechanism linking pulmonary emphysema and fibrosis [122]. This study was done

in two animal models in which emphysema and fibrosis were induced either by bleomycin (BLM) or by chronic exposure to cigarette smoke. In order to study whether BLM-induced lesion is protease-dependent or not, a group of mice was treated with 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride (a serine proteinase inhibitor active against neutrophil elastase). In DBA/2 mice that develop both emphysema and fibrosis after chronic cigarette-smoke exposure, the presence of NE in alveolar structures is also associated with a positive immunohistochemical reaction of both TGF-β and TGF-α [123]. These results strongly suggest that neutrophil elastase may be a common pathogenic link between emphysema and fibrosis, acting as a regulatory factor in the generation of soluble cytokines with mitogenic activity for mesenchymal cells resulting either in emphysema or in fibrosis or both [122]. A recent study has provided evidence that different interstitial levels of NE burden in emphysema may be associated with different routes of collagen clearance (intracellular vs. extracellular) and different degrees of remodeling of the ECM in emphysema [124]. This point merits further investigation. The implication of NE in lung destruction and repair and its pathogenic role in emphysema and fibrosis could lead to a novel approach for therapeutic interventions.

6.3 Cystic Fibrosis (CF)

Cystic fibrosis (CF) is a common, inheritable genetic disorder that results in malfunctioning of the chloride channels of many exocrine epithelial linings including the airway epithelia [125]. The exact mechanism or pathophysiology of progressive lung inflammation in patients with CF is not clear, but patients with CF have increased levels of MMP-2, MMP-8, and MMP-9 in their BAL [126]. In a study of 23 children with CF, BAL fluid concentrations of MMP8 and MMP9 were higher in untreated children and lower in those who were treated with DNase [127]. In children with stable CF, there is a significant inverse relationship between MMP-9 and lung function, as measured by FEV1. Furthermore, levels of MMP-9 are higher in sputum of asymptomatic children with CF compared with controls, suggesting that MMP-9 and total neutrophil count may be useful markers of airway injury and airflow obstruction in persons with CF [126]. As discussed in Sect. 7, MMPs play a critical role in the host defense against pathogens in the lung.

Neutrophils express three closely related serine proteases, NE, CG and PR3, in a coordinated fashion. It has been reported that IL-6 is susceptible to cleavage by all three proteases [128] but now a days, degradation of SIL-6R in a time and dose dependent manner has also been proved in the context of cystic fibrosis [129]. It has been studied that on the basis of molar concentration, among these proteases, CG is the most potent protease with maximal degradation occurring within 60 min at a concentration of 250 nM. NE also shows potent activity at 250 nM but required up to 4 h for complete degradation of sIL-6R to occur. PR3 was the least active protease with minimal degradation of sIL-6R occurring in the presence of 1,000 nM protease after 1 h and complete degradation being observed after 4 h in the presence of 500 nM of protease. If we consider CF lung, unregulated proteolytic activity is

the main contributor to pathology of this disease and is also responsible for reduced expression and activity of a number of important components of the immune response [130]. Targeting of IL-6 by NSPs [128] has previously been suggested as an explanation for the surprisingly low pulmonary expression of IL-6 in CF patients [131] however, this is the first study to show specific proteolysis of this cytokine by serine proteases in CF BALF [129].

6.4 Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS)

These are inflammatory disorders of the lung most commonly caused by trauma, sepsis, and pneumonia, the latter two being responsible for approximately 60 % of cases [132]. Early in the initiation of ALI and ARDS, massive number of neutrophils accumulates in the vasculature of the lung. Neutrophils and their cytotoxic products, including oxidants and proteases, have main pathological importance in ALI and ARDS. It has been observed that there is increased elastolytic activity [133] in patients with ARDS [134]. Increased levels of HNE in plasma and in BAL have also been observed with at-risk patients who later developed ALI [135, 136]. Pathologic effects of HNE are associated with microvascular injury, causing endothelial damage, increased capillary permeability, and interstitial edema. HNE may also potentiate the inflammatory response by increasing the expression and release of cytokines [137] and by increasing mucin production [138]. In experimental animal models, intratracheal administration of exogenous HNE induces lung hemorrhage and ALI, whereas administration of pharmacological HNE inhibitors prevents lung injury, which further supports the role of NSPs in lung injury [22, 139, 140]. Owen *et al.* (2004) propose an interesting role of MMP-8 that it may be anti-inflammatory during acute lung injury, because MMP-8 null mice given intratracheal LPS have significantly greater accumulation of neutrophils in the alveolar space than wild-type mice [141].

In patients with acute respiratory distress syndrome, salbutamol (b2-agonist) increases MMP-9 activity in bronchoalveolar lavage fluid, and increases MMP-9 but decreases TIMP-1 and -2 expressions in distal lung epithelial cells [142]. Similar findings are reported for formoterol (b2-adrenoceptor agonist) in a rat model of pulmonary inflammation [143]. From another study of Zhang *et al.*, an interesting data found that formoterol and ipratropium bromide partially protect the lungs against the inflammation by reducing neutrophilic infiltration. This protective effect is associated with reduced MMP-9 activity known to play an important pro-inflammatory role in acute inflammatory process [143].

6.5 Asthma

Asthma is characterized by episodic dyspnea, lung inflammation, and in some patients, progressive irreversible airway dysfunction [144]. Expression of several MMPs has been associated with asthma; enhancement of MMP-1, MMP-2, MMP-3,

MMP-8, and MMP-9 all have been found in sputum and BAL from patients with asthma [69]. With severe asthmatic conditions, patients have increased MMP9 activity in BAL relative to that from patients with mild asthma or control patients. Wenzel *et al.* [145] found an increase in MMP-9 activity in the subepithelial basement membrane that is accompanied by higher TGF-β. These studies suggest that in patients with severe asthma, neutrophils play a key role in lung remodeling because they express both MMP-9 and TGF-β, which are involved in breakdown and repair of tissue, respectively. In asthma, MMP-9 is expressed in bronchial epithelium and submucosa, where its abundance correlates with tissue eosinophil number [146] because MMP-9 is also produced by eosinophils, macrophages, and neutrophils [147, 148]. In sputum, concentration of MMP-9 is positively correlated with neutrophil number [149] and also with the cumulative macrophage, neutrophil, and eosinophil count [150] during asthma. BAL taken from asthmatic patients after allergen challenge increases the mitogenic indices even more than a normal patient's BAL [151]. This phenomenon could contribute to the airway remodeling observed in patients with chronic asthma; however, the pathway underlying the proliferation remains unclear. EGFR, TGF-β, or platelet-derived growth factors are all candidates based on their presence in lung during disease and their relationship to various MMPs [152].

Asthmatic patients express an increased amount of PAR-2 on respiratory epithelial cells but not on smooth muscle or alveolar macrophages [153, 154]. Schmidlin and colleagues [155] have studied the effect of PAR-2 on ovalbumin challenge of immunized mice. Compared with wild-type animals, eosinophil infiltration was inhibited by 73 % in mice lacking PAR-2 and increased by 88 % in mice over expressing PAR-2. Similarly, compared with wild-type animals, airway hyperreactivity to inhaled methacholine was diminished by 38 % in mice lacking PAR-2 and increased by 52 % in mice over expressing PAR-2. PAR-2 deletion also reduced IgE levels to ovalbumin sensitization 4-fold compared with levels seen in wild-type animals. Mast cell chymase induces eosinophil infiltration, presumably by activating PAR-1 [156]. Secretary leukocyte protease inhibitor administered intratracheally before allergen challenge prevented bronchoconstriction, airway hyperresponsiveness, and leukocyte influx [157]. The actions of PARs and their activating proteinases in the airways have also been studied extensively to determine their role in various lung diseases [158].

6.6 *Pseudomonas aeruginosa Infection in Lung*

Pseudomonas aeruginosa (Pa) is an opportunistic pathogen that infects over 80 % of CF adult lungs [159] and is a major cause of ventilator-associated pneumonia (VAP) in hospitalized patients [160]. A broad spectrum of Pa virulence factors has been identified including proteases secreted by various mechanisms [161]. Proteases such as elastase (LasB), alkaline protease (AprA), staphylolysin (LasA) and Protease IV (a serine-endoprotease) have been identified in CF lung [162, 163]. There are other virulence factors (proteases) of Pa which are capable to degrade host

proteins, including matrix components and components of the immune system such as immunoglobulins and serum alpha proteins [164]. The most studied Pa protease is the elastolytic metalloproteinase LasB, a type II secretion system enzyme. There are several functions of Pa elastase has been reported such as it can alters epithelial barrier function [165], disables PAR-2 receptor in lung epithelial cells [166], cleaves uPAR leading to disruptions of uPAR-dependent cellular interaction [167], inactivates complement and immunoglobulins, and inhibits cell chemotaxis, phagocytosis and microbicidal activities in human leukocytes [168, 169].

The present findings reveal a protective role for extracellular NE against the pathogen. It has been demonstrated that NE, an endogenous effecter, could also participate in the orchestration of lung inflammatory response against *P. aeruginosa* infection by modulating the expression of cytokines (e.g., induction of the expression of the pro-inflammatory TNF-α, MIP-2, and IL-6) [5].

6.7   *Allergic bronchopulmonary Aspergillosis*

Allergic Bronchopulmonary Aspergillosis (ABPA) occurs in nonimmunocompromised patients and belongs to the hypersensitivity disorders induced by *Aspergillus* sp. Genetic factors and activation of bronchial epithelial cells in asthma or cystic fibrosis are responsible for the development of a CD4 + Th2 lymphocyte activation and IgE, IgG and IgA-AF antibodies production [170]. It appears that total serum IgE levels are extremely high, and not all of it is specific antibody to *Aspergillus* species antigens. The pathological changes such as pulmonary infiltrates of eosinophilic pneumonitis, granulomatous central bronchiectasis, and segmental pulmonary fibrosis are seen [171]. *Aspergillus* species produces proteases [172–176] which can desquamate epithelial cells and stimulate IL-6 and IL-8 production [172]. *Aspergillus* species proteases also initiate growth factor release from epithelial cells and are possibly responsible for the central bronchiectasis [177]. It still remains unclear what is abnormal about the response in the few patients in whom this disease develops and why it is especially common in individuals with cystic fibrosis.

7   Conclusions

As we have mentioned before that generally three major classes of proteases—serine proteases, MMPs, and cysteinyl proteases—have been identified in the lung. They are associated traditionally with various inflammatory lung diseases and airway extracellular matrix destruction. From ample evidences it has been proved that each protease family has a multitude of regulatory functions, which makes them of pivotal importance in inflammation, innate immunity, and infection. For example, if we consider role of NE in lung inflammation, recent observations suggest that it's role is more complex than the simple degradation of ECM components. Several

lines of evidence propose that NE aims specifically at a variety of regulatory functions in local inflammatory processes but their relevance under various pathophysiological conditions still remains poorly understood and need further investigation. NE, acting as a link between pulmonary emphysema and fibrosis that could lead to a novel strategy for therapeutic interventions.

Among various lung inflammatory diseases, in case of chronic infective lung diseases, the normal physiological processes become deregulated because of extracellular protease activity, which leads ultimately to upregulation of proinflammatory mediators, increased recruitment of inflammatory cells to the lung, impaired phagocytosis, enhanced mucin production, and inactivation of important innate and antimicrobial proteins. This results in sustained inflammation and predisposition to infection. One way to treat such protease-mediated events in chronic infective lung disease is with antiprotease therapy, which neutralizes excessive extracellular protease activity without compromising the normal physiologic role of proteases. Antiprotease trials have been performed using α_1-antitrypsin and secretary leukoprotease inhibitor, both of which have successfully inhibited NE activity *in vivo* in CF and α1-antitrypsin deficiency [178–180]. Other inhibitors of serine proteases and MMPs are being developed, including synthetic inhibitors to combat protease-induced lung destruction [181].

As these NSPs participate in a variety of pathophysiological processes, they appear as potential therapeutic targets for drugs that inhibit their active site or impair activation from their precursor. Overall, the available preclinical and clinical data suggest that inhibition of NSPs using therapeutic inhibitors would suppress or attenuate deleterious effects of inflammatory diseases, including lung diseases [182].

MMPs compromise a structurally and functionally related group of proteolytic enzymes, which play a key role in the tissue remodeling and repair associated with inflammation [183]. Among various MMPs, MMP-12 has massive importance for lung remodeling in patients suffering from COPD, and its importance was confirmed in MMP knockout mice that were protected against smoke-induced emphysema [184]. Presently it has been found that MMP-12 activity was higher in ex-smokers with COPD compared with that seen in smokers with COPD, and this suggests that the smoking effect keep increasing MMP-12 activity, is irreversible and more severe disease can be associated with deregulated MMP-12 function [74]. Other MMP such as MMP-2 would be the target for treatment of cadmium induced lung inflammation [76]. Animal models of human lung diseases and clinical studies have provided ample evidences for the involvement of MMPs in the development or progression of a number of common lung diseases such as COPD, emphysema, and asthma. However, merely detecting increases in MMPs expression in various lung inflammatory conditions may not provide sufficient information to understand the contribution of MMPs in human lung diseases [152].

Metalloproteinases of the ADAM family have been recognized as potential therapeutic targets in several diseases. However, due to their broad activity, systemic application of inhibitors may not represent an appropriate treatment strategy. ADAM17 mediates a number of shedding events that influence several components of acute lung inflammation including vascular leakage, leukocyte recruitment and

cytokine release. Particularly in endothelial cells, ADAM17 appears to act as a central regulator in pulmonary inflammation. So, in near future inhibition of ADAM17, possibly locally to reduce systemic side effects, may be a promising approach for the treatment of Acute Lung Injury [100].

Given that they are involved in the pathogenesis of various diseases, they can be good therapeutic targets along with their specific protease inhibitors [185]. There could be a further option of combining protease modulators with monoclonal antibodies against cytokines to treat such inflammatory lung diseases. From clinical point of view whether anti-protease treatment or use of these proteases along with their specific inhibitors for curing these inflammatory lung diseases is beneficial, needs further investigation and research.

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