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Diverse Activities for Proteinases in the Pathogenesis of Chronic Obstructive Pulmonary Disease

Emer Kelly and Caroline A. Owen*
Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA USA

1. Introduction

COPD remains a major public health problem. It is the fourth leading cause of chronic morbidity and mortality in the United States, and is projected to rank fifth in 2020 in burden of disease caused worldwide, according to a study published by the World Bank/World Health Organization. COPD is a preventable and treatable disease, with some significant extra-pulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. Smoking remains the major risk factor for this disease, but inhalation of other pollutants and genetic factors also play a role.

Inhalation of cigarette smoke and other pollutants leads to a chronic inflammatory process in the small airways and the lung parenchyma, which includes an influx of macrophages, polymorphonuclear neutrophils (PMN), T lymphocytes (with CD8+ T cells exceeding the numbers of CD4+ T cells), and B lymphocytes (1-4). This inflammatory process over a prolonged period, leads to destruction of the alveolar walls leading to airspace enlargement, loss of lung elasticity, closure of small airways, and irreversible airflow obstruction. Pathological changes also include mucous metaplasia and mucus hyper-secretion. The small airways narrow due to the combined effect of mucus plugging, inflammation in the airways walls and lumen, and subepithelial fibrosis and can become obstructed (1). COPD is a complex disorder with many processes at play but there is strong evidence that proteinases make critical contributions to all the pathologic processes detected in the lungs of COPD patients.

2. Classification of proteinases

Proteinases are named for their action, i.e. to cleave the internal peptide bonds of polypeptides. In human biology they are classified into 4 groups based on the chemical

* Corresponding Author

nature of their active site: serine, metallo-, cysteine, and aspartic proteinases. Serine proteinases and MMPs are the major players in extracellular proteolysis and are optimally active at neutral pH. Cysteine and aspartic proteinases work mainly in the cell in the breakdown of proteins in lysosomes. These are optimally active at acidic pH. These acid proteinases can potentially breakdown extracellular proteins if they can keep catalytic activity at neutral pH or are released into an environment having an acidic pH, such as the pericellular environment of activated macrophages (5,6). Proteinase inhibitors are generally specific to individual classes of proteinases. Proteinases of the serine, metallo- and cysteine proteinase classes have been shown to have activities that contribute to COPD pathogenesis.

2.1 Serine proteinases

Members of this group that are implicated in COPD include PMN-derived serine proteinases, urokinase-type plasminogen activator, granzymes, and plasmin.

PMN-derived serine proteinases

Neutrophil elastase (NE), proteinase 3 (PR3), and cathepsin G (CG) make up this group. The proteinases are stored in an inactive form within granules in PMN (Figure 1) and pro-inflammatory monocytes {8}. When the cells are stimulated by pro-inflammatory mediators they degranulate releasing the enzymes (7,8). These serine proteinases have a broad action against extracellular matrix (ECM) proteins (especially elastin) and non-ECM proteins (7). Figure 2 illustrates how the catalytic triad at the active site of NE (His^{41}-Asp^{99}-Ser^{173}) cleaves the internal peptide bonds of proteins.

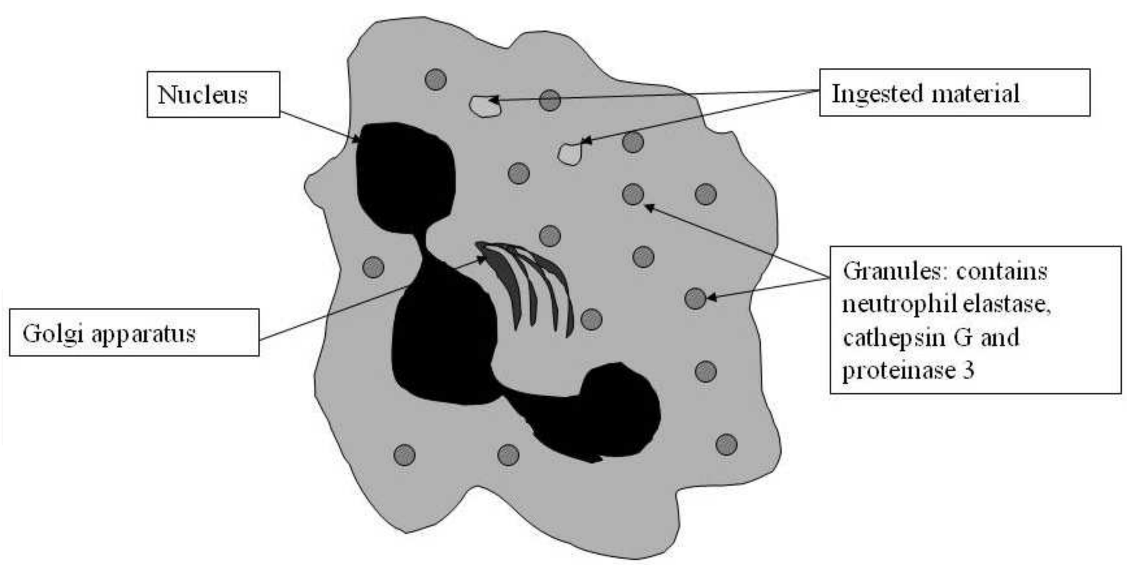

Fig. 1. Structure of neutrophil: Proteinases are stored in an activated form in the azurophilic granules within the neutrophil

Urokinase type plasminogen activator (uPA)

This enzyme is expressed by PMN, monocytes, and macrophages. This enzyme is also stored in and released from the specific granules of PMN. The expression of uPA is regulated at the transcriptional level in mononuclear phagocytes by pro-inflammatory

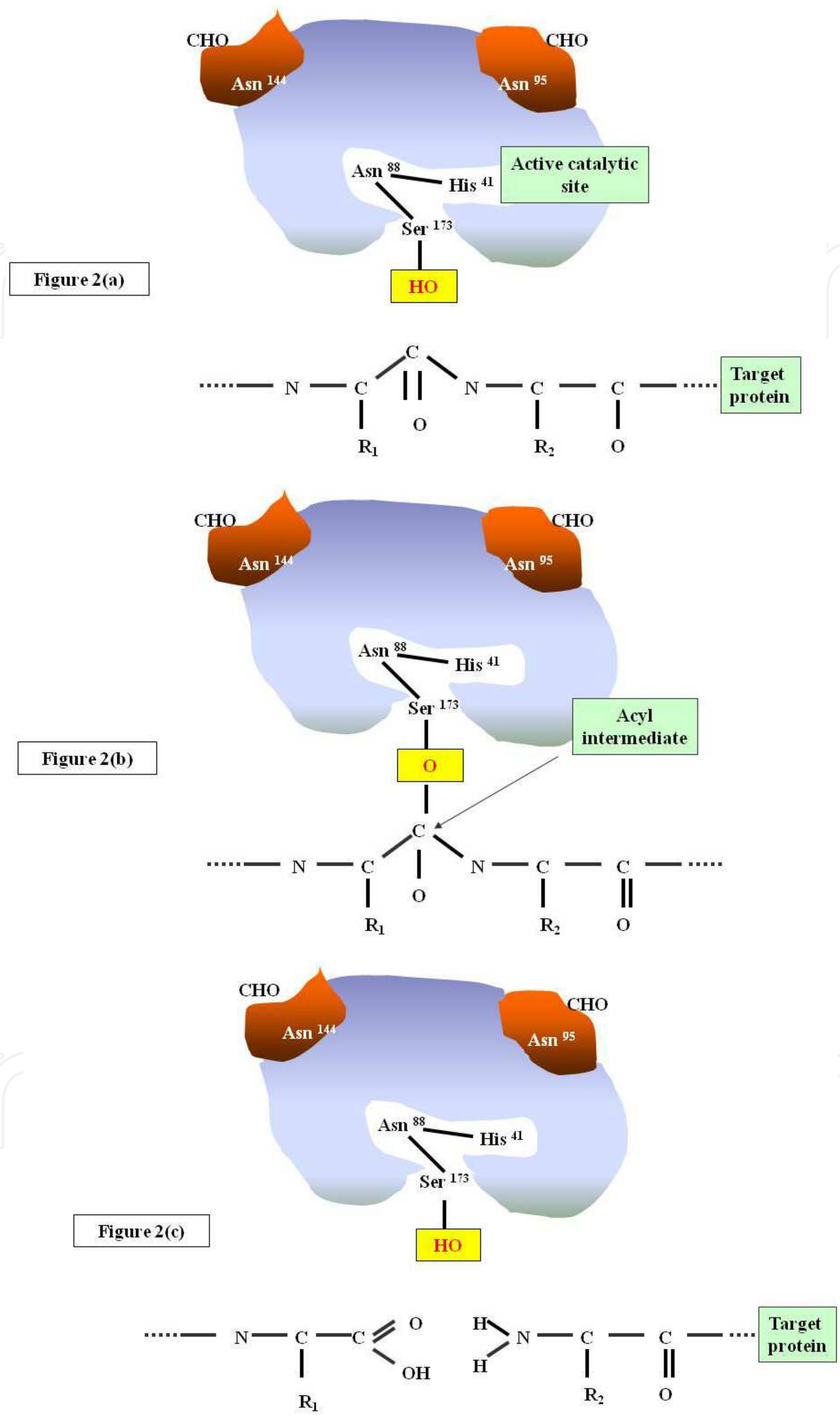

Fig. 2. (a) Mechanism by which NE cleaves a target protein. The NE molecule has two complex carbohydrate side chains attached to Asn[95] and Asn[144]. The catalytic site of the NE

molecule is an indentation of the molecule and is composed of the molecule and the triad His41-Asp88-Ser173, in which the γ-oxygen of serine becomes a powerful nucleophile able to attack a suitably located carbonyl group on the target substrate. The bond to be cleaved must fit into the active site pocket of the NE held there by charge interactions mediated by the residues forming the pocket. The peptide bond under attack is between two amino acid residues recognized by their side chains R_1 and R_2. (b) An acyl-enzyme intermediate molecule is formed between serine and the carbonyl group on the target protein. (c) The acyl-enzyme complex is hydrolysed with subsequent regeneration of active NE and cleavage of the protein.

mediators (9,10). On release from cells, uPA binds to a specific receptor (uPA receptor) on phagocyte surfaces, where it functions as a cell-associated proteinase. The main action of uPA is to activate the serine proteinase, plasmin from its inactive form, plasminogen. Plasmin lyses blood clots by breaking down fibrin, but also cleaves and activates latent growth factors, latent pro-metalloproteinases (MMP), and protease-activated receptor-1 (PAR-1) on macrophages, which drives macrophage MMP-12 production (11-14). Through this action, it plays an important role in ECM degradation and fibrotic processes in the lung.

Granzymes (GRZ)

These **gran**ule-associated en**zymes** are predominantly expressed by CD8+ T lymphocytes and are stored in the lytic granules (15). The main GRZ family members in human CD8+ T cells are GRZ A and B. Once activated by antigen, the CD8+ T cells commence rapid exocytosis of GRZ and perforin-containing granules. Release of perforin alters the properties of the cell membrane of the target cells, heralding the entry of GRZ into the target cell, and GRZ A and GRZ B then initiate caspase-independent and caspase-dependent apoptosis, respectively.

For this group of proteinases there exists naturally occurring inhibitors. Serine proteinase inhibitors (Serpins) in plasma and interstitial fluids include α_1-anti-trypsin (AAT), α_1-antichymotrypsin, plasminogen activator inhibitors, α_2-plasmin inhibitor, and the universal inhibitor, α_2-macroglobulin (α_2-M), which inhibits all four classes of enzymes (16). Secretory leukocyte proteinase inhibitor (SLPI) and elafin are inhibitors synthesized locally in the respiratory tract by epithelial cells.

2.2 Metalloproteinases

Included in this group of proteinases are the MMPs and the members of the ADAMs family.

MMPs

These proteinases have an NH_2 terminal pro domain, an active site zinc atom, and a COOH terminal hemopexin domain that regulates the binding of the enzymes to their substrates. They are stored in a latent form as the inactive proenzymes or proMMPS, a state maintained by an interaction between the active site zinc atom and the cystein residue in the pro domain. Disruption of this interaction is required for activation of the proMMPs. This is facilitated by the actions of other proteinases and oxidants in the extracellular space (17,18)]. The intracellular serine proteinase, furin, is responsible for activation of some MMPs (19,20).

MMPs can be synthesized de novo by cells activated by pro-inflammatory mediators or growth factors. PMN, however, store preformed MMP-8, MMP-9, and MT6-MMP (MMP-25) in their cytoplasmic granules, and release the enzymes when they degranulate (7). Macrophages express MMPs-1, -3, -7, -9, -12, and -14 (21,22), and lung epithelial cells and fibroblasts produce MMPs-2, -9 and -14.

MMPs are classified into 6 groups based upon a similar domain organization and substrate specificity: 1) the interstitial collagenases (MMPs-1, -8, and -13); 2) the gelatinases (MMPs-2 and -9); 3) the stromelysins (MMPs-3, -10, and -11); 4) matrilysin (MMP-7); 5) metalloelastase (MMP-12); and 6) membrane-type MMPs (MT-MMPs), integral membrane proteinases with either a transmembrane domain or a glycosylphosphatidyl-inositol anchor to the cell membrane (23,24). The interstitial collagenases degrade interstitial collagens. The other subgroups have a broader range of substrates including denatured collagens (gelatins), basement membrane proteins, and pro-inflammatory mediators. MMPs-7, -9 and -12 also degrade elastin (7).

ADAM

This is a family of type I transmembrane proteinases, named ADAMs because they contain **a** **d**isintegrin and **a** **m**etalloproteinase domain (25). The metalloproteinase domain of ADAMs plays a role in regulation of inflammation, apoptosis and possibly fibrotic processes by shedding membrane-anchored cytokines such as pro–tumor necrosis factor (TNF-α), other cytokines, growth factors, apoptosis ligands and receptors for these molecules from cell surfaces (25-27). The disintegrin domain is involved in cell adhesion and migration which it accomplishes by binding to integrins (25).

The inhibitors of the MMPs include the universal inhibitor, α_2-M, and the four members of the tissue inhibitors of metalloproteinases family (TIMPs1-4), which are synthesized by connective tissue cells and leukocytes and form non-covalent complexes with MMPs (28,29). Although the inhibitors of ADAMs have not been fully elucidated, it is known that ADAM-17 is inhibited by TIMP-3 but not TIMP-1 or -2 (30,31).

2.3 Cysteine proteinases

This group includes the cathepsins B, H, L, and S, which have been implicated in COPD. Cathepsin S and L are potent elastases *in vitro* (5,6) and contribute to macrophage-mediated ECM degradation. Inhibitors for this group, again include the universal inhibitor, α_2-M, but also the cystatin superfamily and the kininogens (32).

3. Evidence for activities for proteinases in COPD

The proteinase/anti-proteinase hypothesis for the pathogenesis of COPD is not a new concept. It dates back to experimental work done over 50 years ago. The basis of the concept was 2 key observations. The first came from the keen observations by Laurell and Eriksson who noted that deficiency of AAT was associated with early onset, severe panlobular emphysema (33). AAT has since been shown to be the major inhibitor of NE in the lower respiratory tract. The second observation was made when instillation of papain (an enzyme with elastase activity) into rat lungs was shown to cause progressive airspace enlargement (34). Over the years, other elastolytic proteinases have been shown to cause airspace enlargement when instilled into the lungs of animal models. The concept proposed that the imbalance between proteinases (especially elastases) and their inhibitors lead to pulmonary emphysema. Emphysema, however, does not account for all COPD patients and whereas AAT deficiency is a cause of COPD, AAT deficiency only accounts for approximately 2% of COPD. Other factors have now been implicated in airspace enlargement in COPD, including other classes of proteinases (MMPs and cysteine proteinases), oxidative stress, and apoptosis of lung structural cells. COPD is a clinically and pathologically heterogeneous disease and includes chronic inflammation in the alveolar space, airways, and lung interstitium; mucus hypersecretion; and subepithelial fibrosis in the small airways. Although the proteinase/ antiproteinase concept does not account for all of the complex pathologies that make up COPD it certainly has far-reaching effects, many of which have been investigated in *in vitro* studies, and studies of human samples from COPD patients and animal models of COPD

4. Proteinase biology in cells relevant to COPD pathogenesis

The role of proteinases in COPD has been studied at a cellular level with *in vitro* studies.

Lung inflammation and airspace enlargement

The serine proteinases, NE, CG, PR3, and GRZ, can promote lung inflammation in COPD patients, through their direct action stimulating the release of pro-inflammatory mediators from airway epithelial cells and macrophages in vitro (35,36) and many proteinases also have an indirect action proteolytically cleaving mediators to alter their biologic activities. The metalloproteinases, MMPs-8 and -9, cleave and activate various chemokines *in vitro* (37,38). ADAM-17 and several MMPs shed and activate membrane-associated, latent pro–TNF-α from macrophage surfaces (25,27,39). NE, MMP-12, and MMP-9 cleave elastin, and MMPs cleave AAT, generating fragments of these two molecules that are chemotactic for inflammatory cells (40,41). Serine, metallo-, and cysteine proteinases acting together can degrade elastin, interstitial collagens, and basement membrane proteins *in vitro* (7). The degradation of these ECM proteins leads to the enlargement of lung airspaces.

Airway pathologies

The proteinases play a role in the characteristic airway pathologies of COPD, including increased mucus production, poor clearance of this mucus and resulting bacterial infections and further inflammation. NE, MMP-9, and ADAMs-10 and -17 increase epithelial cell expression of MUC5AC, a major mucin protein, by activating epithelial growth factor receptor (EGFR) through shedding of membrane–bound pro–transforming growth factor (TGF)-α. The released soluble, active TGF-α, activates the EGFR (42-44). The 3 major serine proteinases, NE, CG, and PR3 potently stimulate goblet cell degranulation (45). Tissue kallikrein is a serine proteinase expressed by inflammatory cells and submucosal glands, which also stimulates mucin synthesis in airway epithelium *in vitro* by shedding and activating pro-EGF, another EGFR ligand (46). NE damages epithelial cells (47) and inhibits ciliary beat frequency of lung epithelial cells (48).

Plasmin, MMP-9, NE, and ADAMs may also induce sub-epithelial fibrosis in COPD airways, because they activate latent growth factors such as TGF-β (11,49,50) and insulin-like growth factors *in vitro* (51,52). These growth factors are known to induce fibroblasts to produce and secrete interstitial collagens. It remains unclear whether these proteinases induce sub-epithelial fibrosis in the small airways of human COPD patients.

5. Mechanisms by which proteinases contribute to individual lung pathologies in COPD patients

Most of the evidence for the mechanisms by which the proteinases act in the disease process of COPD comes from studies of clinical samples from human COPD patients and animal models of COPD.

5.1 Human COPD samples

Following on from the initial discovery that lack of inhibition of NE in patients with AAT deficiency was associated with emphysema, studies from Damiano et al further supported crucial activities for NE in pulmonary emphysema (53). They showed that the amount of NE bound to lung elastin is strongly correlated with the degree of emphysematous change and additional studies demonstrated stable binding of active forms of NE to elastin *in vitro* (54). Since then, additional studies have confirmed increased levels of NE in lung samples from COPD patients and demonstrated elevated levels of CG, PR3, uPA, and MMPs -1, -2, -8, -9, and -14 in various lung samples from smokers and COPD patients when compared to healthy subjects (53,55-65).

Inflammatory cells are the main source of these proteinases in COPD but production of proteinases by lung structural cells and immune cells has also been demonstrated. For example, cigarette smoke increases MMP production by lung epithelial cells (64), and fibroblasts (66). T lymphocytes from blood and BAL samples from COPD patients have increased levels of GRZ and perforin compared to samples from asymptomatic smokers and nonsmokers (67). Elevated levels of GRZ B in BAL samples from COPD patients show a correlation with bronchial epithelial cell apoptosis, suggesting that GRZ B promotes epithelial cell death in the lung and contributes to airspace enlargement in COPD patients.

5.2 Animal models of COPD

Animal models of COPD provide the strongest evidence for the roles of proteinases in COPD.

Acute cigarette smoke exposure models

Exposing mice to smoke for up to 30 days leads to an influx of PMN and macrophages to the lung (68). This is due to direct effects of inhaled smoke on lung capillaries, leading to leakage of thrombin and plasmin into the alveolar space (69,70). These proteinases cleave and activate PAR-1 on macrophages, leading to an increased synthesis of MMP-12 by macrophages (13,14). MMP-12 is responsible for shedding pro-TNF-α from activated macrophages, likely leading to an increase in E-selectin expression on endothelial cells (39). This facilitates transendothelial migration of PMNs. The presence of these increased PMNs and macrophages, releasing serine proteinases, increases lung collagen and elastin breakdown. Delivering human AAT to mice acutely exposed to cigarette smoke prevents PMN influx and ECM destruction. This is probably due to AAT inhibiting both PMN serine proteinase-mediated ECM destruction and thrombin- or plasmin-induced increases in macrophage MMP-12 production (14,71). Further evidence for the role of MMP-12 comes from a study showing that the minor allele of a single-nucleotide polymorphism (SNP) in MMP-12, is associated with a positive effect on lung function in adults who smoke and also a reduced risk of COPD in adult smokers (72).

Chronic smoke exposure models

When wild type (WT) mice are exposed to cigarette smoke for 3-6 months they develop pulmonary changes of airspace enlargement, inflammation and small airway subepithelial fibrosis, making this a good model to investigate the role of proteinases in COPD (73,74).

Work with proteinase deficient mice has confirmed the role of MMP-12 and NE in chronic inflammation and airspace enlargement and MMP-9 and possibly MMP-12 in sub-epithelial fibrosis. MMP-12 deficient mice (MMP-12$^{-/-}$mice) when chronically exposed to cigarette smoke show no increase in macrophages and no airspace enlargement, and so are completely protected from the changes seen in the wild type model (73). In the absence of MMP-12 mediated elastin degradation, the remaining elastin fragments attract monocytes (75). T lymphocytes also play a role in these processes with CD8+ T-cell-deficient (CD8$^{-/-}$) showing a blunted response to smoke exposure and protection from emphysema (76). This is mediated by a CD8+ T cell product, interferon gamma (IFN-γ) inducible protein 10 (IP-10), which induces production of MMP-12 and degradation of the lung ECM. Carrying this through to the human disease, there has been demonstration of increased Th1 cells associated with increased levels of IP-10 and MMP-12 in lung tissue from human COPD patients (77).

NE$^{-/-}$ mice are 60% protected from airspace enlargement and have decreased influx of PMN and monocytes into the lung compared to smoke-exposed WT mice [(78); Fig. 3]. NE likely contributes to airspace enlargement directly by degrading elastin and other ECM protein components of the alveolar walls (78).

There is also a direct action of cigarette smoke on the pulmonary airways. When rodent airways are exposed acutely to cigarette smoke, increases in growth factor and collagen production are detectable within 2 hours, and before inflammation occurs in the airway walls (79). This suggests that smoke directly promotes small airway subepithelial fibrosis and that smoke-induced inflammation and proteinase production are unnecessary for this process. However, in guinea pigs chronically exposed to cigarette smoke for up to 6 months, inflammatory cell MMPs amplify this process, since delivering a synthetic dual inhibitor of MMPs-9 and -12 to these animals significantly reduces small airway fibrosis (80). The use of MMP inhibitors in human COPD patients remains to be explored.

Transgenic murine models

These models are used to investigate over-expression of various proteinases, in contrast to the study of a deficiency of a protein in the knock-out murine models. Transgenic mice over-expressing MMP-1 in the lung develop enlarged airspaces (81), which may either reflect abnormal alveolar development or destruction of mature interstitial collagens by MMP-1. Adult transgenic mice over-expressing a Th1 cytokine (IFN-γ), a Th2 cytokine (IL-13), or a cytokine with Th1 and Th2 activities (IL-18) in airway epithelial cells spontaneously develop obvious lung inflammation, increased lung levels of MMPs and cysteine proteinases, and airspace enlargement (82-84). In mice over-expressing IL-13, the metalloproteinases MMPs -9 and -12 play critical roles in promoting airspace enlargement, with MMP-12 also promoting inflammation and driving the increased expression of other MMPs in the lung (85). In transgenic mice over-expressing IFN-γ, cathepsin S stimulates lung epithelial apoptosis, lung inflammation, and airspace enlargement (86).

Alveolar septal cell apoptosis models of airspace enlargement

In patients with COPD there is apoptosis of alveolar septal cells (87,88) and leukocytes (89,90), and apoptosis of the endothelial and epithelial cells that make up the alveolar walls. This leads to the development of emphysema. Septal cell apoptosis and airspace enlargement in the absence of overt lung inflammation can be induced rapidly in experimental animals by: 1) pharmacologic blockade of vascular endothelial growth factor receptors in rodents (91); and 2) transfection of murine alveolar epithelial cells with caspase-3, a pro-apoptotic cysteine proteinase (88). However, increased elastase activity due to acidic proteinases is detected in BAL samples after transfection of alveolar epithelial cells with caspase-3 (88). Thus, proteinases released from dying structural cells may degrade the lung ECM, thereby acting together with septal cell apoptosis to cause loss of alveolar units and airspace enlargement.

6. Regulation of proteinases in the lung

Proteinases are a significant factor in the pathogenesis of COPD, but do not act in isolation. They interact with other mediators and other pathways and are also regulated by inhibitors. Studies of the $NE^{-/-}$ and MMP-$12^{-/-}$ mice chronically exposed to cigarette smoke demonstrated interactions between these two classes of proteinases, with MMP-12 cleaving and inactivating AAT to increase NE-mediated lung injury, and NE cleaving and inactivating TIMP-1 to amplify MMP-12-mediated lung destruction (78). Proteinases also interact with reactive oxygen species (ROS), and ROS production is increased in the lungs of COPD patients. ROS are present in inhaled cigarette smoke itself, or are released by phagocytes activated by inhaled smoke. ROS are known to activate proMMPs *in vitro* and are thought to exacerbate lung inflammation and injury in COPD patients (92). Transgenic mice over-expressing the antioxidant enzyme Cu-Zn superoxide dismutase in the lung are protected from developing chronic lung inflammation, increased lung MMP levels, and emphysema in response to intratracheal instillation of porcine pancreatic elastase, or chronic exposure to cigarette smoke (93). However, mice deficient in a phagocyte-specific component of the NADPH oxidase, which generates superoxide anions (O_2^-), develop greater airspace enlargement in response to cigarette smoke than WT mice (94). This is due to ROS-mediated inactivation of MMPs via oxidative inactivation of residues in the catalytic domain of MMPs (95). Thus, phagocyte-derived O_2^- (and ROS derived from O_2^-) in COPD lungs may constrain rather than promote phagocyte MMP-mediated lung injury (94,96). It is noteworthy that clinical trials have failed to demonstrate protective effects of antioxidant supplementation in COPD patients, and this could be linked, in part, to antioxidants inducing reductions in ROS-mediated inactivation of MMPs (97).

6.1 Inhibitors of proteinases

Proteinase inhibitors are present in the extracellular matrix. To maintain their action, proteinases need to circumvent these inhibitors through inactivation of the proteinase inhibitor, evading them and / or overwhelming them.

6.2 Inactivation of proteinase inhibitors

Serpins can be cleaved and inactivated by MMPs (98-102), NE (103,104), cathepsin B (105), and bacterial proteinases (106). Serine proteinases cleave and inactivate TIMPs (107).

Proteolytic inactivation of AAT and TIMP-1 by MMP-12 and NE occurs in the cigarette smoke exposure model of emphysema in mice (78). ROS present in cigarette smoke or released by leukocytes activated by smoke, inactivate α_2-M, and AAT, and SLPI in vitro by converting the methionine at the active sites of these inhibitors to methionine sulfoxide. This reduces their capacity to inhibit serine proteinases (108-111). It is not clear if oxidative inactivation of proteinase inhibitors occurs in COPD patients. Some studies have detected oxidized AAT in lung samples from COPD patients but others have not (112-114). Also, ROS can inactivate proteinases as outlined above. It is difficult to know if previous work analyzing the oxidation state of proteinase inhibitors in lung samples from COPD patients actually includes events in cellular microenvironments. Adding to the complexity of studying this process is the fact that ROS are short-lived molecules and are active only at short distances from the cells generating them before they are muted by antioxidants.

6.2.1 Evasion of inhibitors

In another effort to preserve their function, proteinases can evade inhibitors by binding tightly to substrates, being released into sequestered microenvironments, or binding to cell surfaces.

Tight binding of proteinases to substrates

NE binds very stably to elastin in an active form, and AAT and SLPI have reduced activity against elastin-bound NE compared to soluble NE (54,115,116). In the lungs of humans with emphysema, NE is bound to interstitial elastin (53) and this lung elastin-bound NE likely retains catalytic activity and takes a major role in the destruction of elastin fibers in pulmonary emphysema (Fig. 4). MMPs-1, -2, and -9 bind to various ECM proteins, which may increase the retention, stability, and bioactivity of proteinases in the lung and aid their roles in extracellular proteolysis (117,118).

Sequestered microenvironments

Inflammatory cells can, via integrin-mediated adhesion to matrix or to cells, form small pockets of microenvironment. Large inhibitors such as AAT (119) and α_2-M (120) cannot enter these sealed pockets (Fig. 4).

Membrane binding of proteinases

MT-MMP and ADAMs are integral membrane proteinases, and some members of these families are resistant to inhibition by physiologic inhibitors. ADAM-17, for example, is resistant to inhibition by TIMPs-1 and -2 but not TIMP-3 (31), and MT1-MMP is resistant to inhibition by TIMP-1 but not TIMP-2 (121). NE, CG, PR3, MMPs-8 and -9 (which lack transmembrane domains or glycosylphosphatidyl-inositol anchors) are also expressed on the surface of activated PMN (122-127). These surface-bound proteinases degrade lung ECM proteins and proteinase inhibitors and induce goblet cell degranulation (122,126-128). The membrane-bound element of these proteinases confers a resistance to their inhibitors when compared to the soluble variety (122-124,126,127).

6.2.2 Overwhelming of inhibitors

A more obvious way to overcome the inhibitors is for the proteinases to overwhelm them with sheer numbers. This can happen with release of massive quantities of enzymes from large numbers of inflammatory cells, or when high concentrations are released from individual cells (quantum proteolysis).

Brisk recruitment of inflammatory cells in the lung

COPD exacerbations are characterized by an influx of inflammatory cells into the airways. These cells release active forms of NE, MMP-8, and MMP-9 (58,62,129,130). Macrophage clearance of the PMN recruited into the lung under normal circumstances would occur but in the case of the COPD lung this is hampered by a number of mechanisms. First, cigarette smoke impairs expression of recognition molecules for apoptotic PMN on the macrophage surface (131). Second, NE cleaves recognition molecules for apoptotic PMN from the macrophage surface (132). Third, when PMN ingest *Hemophilus influenzae,* which frequently colonizes the respiratory tract of COPD patients, PMN necrosis is rapidly induced (133).

Quantum proteolysis and PiZZ AAT deficiency

NE is present at millimolar concentrations in each azurophil granule of PMN, which is more than 100-fold higher than the concentration of AAT, its inhibitor, in plasma (134). The release of an azurophil granule into the extracellular space is thus accompanied by a transient burst of proteolytic activity as it greatly outnumbers the proteinase inhibitors. This activity fades as the granule contents diffuse, and the proteinase-inhibitor ratio falls below 1:1 (134). In patients with an inherited deficiency of AAT, the proteinase activity lasts longer, leading to more destruction of the lung. Quantum bursts of NE-mediated proteolytic activity associated with PMN migrating on ECM proteins are 10-fold larger in area and 4-fold longer in duration when PMN are bathed in serum from PiZZ patients compared to serum from healthy PiMM subjects (135), due to defective confinement of PMN-derived NE-mediated ECM degradation. The PiZ AAT mutant proteins polymers formed in this disease are also chemotactic for PMN (136,137).

7. Potential strengths and limitations of proteinase inhibitors and anti-inflammatory drugs as new therapeutic strategies to limit proteinase-mediated lung pathologies in COPD

7.1 Proteinase inhibition

Perhaps the most obvious role for intervention in this setting is to replace AAT in patients with COPD who have known severe, inherited AAT deficiency (AATD). Although we do not have conclusive randomized controlled trials, human clinical research has shown that AAT augmentation reduced exacerbation frequency and slows the rate of lung function decline in these patients (138). More recent work has attempted augmentation of AAT through gene therapy. This involves administration of recombinant adeno-associated virus (rAAV) vectors expressing human AAT (rAAV1-CB-hAAT) to patients with AATD (139). These studies are currently in phase 2 clinical trials and have shown increased expression of normal (PiM) AAT in serum occurs safely in patients for up to 90 days. Further optimization of the vector is likely to be required to generate sustained therapeutic AAT plasma levels. The concept of augmentation of AAT in COPD, outside the setting of AATD, is less clear.

Secretory leukocyte peptidase inhibitor (SLPI) and elafin are naturally occurring antiproteinases with anti-NE activity whose roles in COPD are not fully eludicated but may have potential as future treatment options (140). A number of synthetic low molecular weight inhibitors have been developed and are potential therapeutic agents for COPD. These include irreversible inhibitors such as the peptide chloromethyl ketones (141) and reversible inhibitors such as peptide boronic acids, peptide aldehydes (142), substituted tripeptide ketones (143), or β-lactams (144). One of the problems with the low-molecular-weight reversible inhibitors is that they can release NE, allowing it to destroy tissue. Although the irreversible inhibitors such as chloromethyl ketone have been shown to function effectively *in vivo* in hamsters to reduce many of the effects of intratracheally administered NE, the toxicity of chloromethyl ketones prevents clinical use.

Some support for potential use of these inhibitors comes from *in vitro* studies showing that low-molecular-weight, synthetic inhibitors of serine proteinases and MMPs effectively inhibit both soluble and membrane-bound proteinases (122,123,126,127), and studies of animal models of COPD showing that proteinase inhibitors effectively block both airspace enlargement and lung inflammation. In animals acutely exposed to cigarette smoke, delivery of synthetic or natural inhibitors of serine proteinases and synthetic inhibitors of MMPs blocks PMN influx into the lung and ECM destruction (68,145,146). In other animal work, a therapeutic effect demonstrated with daily oral delivery of synthetic MMP inhibitors to mice. This prevented airspace enlargement and macrophage accumulation in the lungs of mice exposed to cigarette smoke for 6 months (74). In additional experiments in which MMP inhibitor therapy was initiated after mice were exposed to cigarette smoke for 3 months to initiate airspace enlargement, therapy prevented progression of airspace enlargement as smoking continued (74). These results suggested a role for proteinase inhibition in potentially preventing disease progression in human COPD patients. However, it remains unclear which proteinases should be targeted. The counter argument to these theories is that proteinases have been shown to have beneficial as well as deleterious roles in the lung (roles in innate host defense, dampening inflammation, and inhibiting tumor growth and metastasis), which may prove to limit the usefulness of their inhibition.

7.2 Anti-inflammatory strategies

Strategies to reduce the burden of lung inflammatory cells in COPD would thereby reduce the amount of proteinase that they are responsible for releasing. Inhibitors of phosphodiesterase E4, the major isoenzyme in inflammatory cells, decrease inflammatory cell migration, activation, and release of proteinases. Clinical trials of phosphodiesterase E4 inhibitors in COPD have resulted in one selective PDE4 inhibitor, roflumilast (Daxas ®), being approved for use in humans and available in Canada and the European Union in 2011 for the treatment of a specific population of patients with severe COPD (147). Other anti-inflammatory approaches, such as inhibiting NF-κB activation to reduce pro-inflammatory gene expression, could also potentially inhibit proteinase- and oxidant-mediated lung injury in COPD patients.

8. Conclusions

Proteinases have diverse activities in the pathogenesis of COPD. With over 40 years having elapsed, since the initial breakthroughs showed a role for these enzymes in this disease, much work has elucidated many further elements of the roles they play. It is clear that the proteinase-antiproteinase balance is not the sole cause of all the pathology seen, but it continues to be a major contributor and a potential target for future therapies.

9. Abbreviations

ADAM. Proteinase **a d**isintegrin and **a m**etalloproteinase domain; cathepsin G (CG), chronic obstructive pulmonary disease (COPD), epithelial growth factor receptor (EGFR), extracellular matrix (ECM), g*ranzymes (GRZ), i*nducible protein 10 (IP-10), interferon gamma (IFN-γ), membrane-type MMPs (MT-MMPs), metalloproteinase (MMP), neutrophil elastase (NE), polymorphonuclear neutrophils (PMN), protease-activated receptor-1 (PAR-1), proteinase 3 (PR3), reactive oxygen species (ROS), secretory leukocyte proteinase inhibitor (SLPI), serine proteinase inhibitors (Serpins), transforming growth factor (TGF)-α, tumor necrosis factor (TNF-α), urokinase-type plasminogen activator (uPA), wild type (WT), α_1-anti-trypsin (AAT), α_2-macroglobulin (α_2-M)

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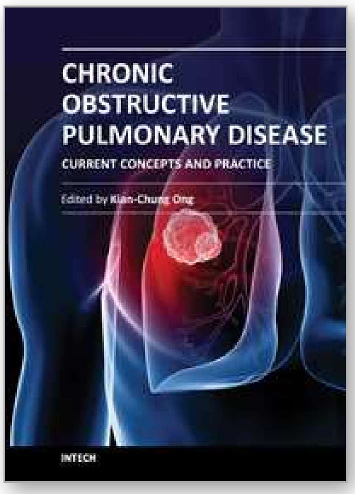

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A decade or so ago, many clinicians were described as having an unnecessarily 'nihilistic' view of COPD. This has certainly changed over the years... This open access book on COPD provides a platform for scientists and clinicians from around the world to present their knowledge of the disease and up-to-date scientific findings, and avails the reader to a multitude of topics: from recent discoveries in the basic sciences to state-of-the-art interventions on COPD. Management of patients with COPD challenges the whole gamut of Respiratory Medicine - necessarily pushing frontiers in pulmonary function (and exercise) testing, radiologic imaging, pharmaceuticals, chest physiotherapy, intensive care with respiratory therapy, bronchology and thoracic surgery. In addition, multi-disciplinary inputs from other specialty fields such as cardiology, neuro-psychiatry, geriatric medicine and palliative care are often necessary for the comprehensive management of COPD. The recent progress and a multi-disciplinary approach in dealing with COPD certainly bode well for the future. Nonetheless, the final goal and ultimate outcome is in improving the health status and survival of patients with COPD.