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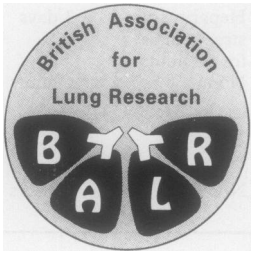
# *New perspectives on basic mechanisms in lung disease · 6*




# Proteinase imbalance: its role in lung disease

T D Tetley

The extracellular component of lung tissue was once regarded as relatively inert scaffolding that, once assembled, remained unaltered. We now know that the extracellular matrix is not a simple frame accommodated by cells but a dynamic material that provides a precise template on which the cells assemble in a controlled, defined manner to form the complex architecture of lung tissue.[1] During the host defence response, inflammatory cells are attracted to the lung to combat microorganisms and other irritants deposited in the lungs.[2] A significant component of their defence strategy is production of potent proteolytic enzymes to facilitate clearance of foreign and noxious agents but which, in excess, can also destroy the extracellular framework, disrupt resident cells, and stimulate further inflammation.[2,3] The activity of these enzymes is usually tightly controlled and repair is rapid so that little if any permanent damage occurs—for example, following pneumococcal pneumonia.[4] Inadequate inhibition of proteinase activity, however, can lead to the generation of more inflammatory mediators, continued inflammation, and impeded repair of tissue damage.

## Candidate cells

Neutrophils are conspicuous by their presence in many pulmonary inflammatory diseases. They contain potent proteinases, including serine and metalloproteinases, which are stored in the granules and are released during phagocytosis, during cell activation in response to various stimuli, or following lysis of the cell.[5] These cells form only a small percentage of the phagocytic migratory cells in the healthy lung.

By far the most prominent phagocytic cell in normal lung is the alveolar macrophage, a resident cell responsible for day to day removal of foreign agents deposited in the peripheral lung. The macrophage may exert control on unwanted neutrophil enzyme activity during resolution of inflammation via apoptosis.[4,6] Neutrophils usually have a short half life (1–2 days in tissue) and need to be removed by controlled mechanisms—that is, apoptosis—which prevent dispersion of harmful cellular components, including proteinases, into the surrounding tissue.[4] Where apoptosis is inadequate or inhibited, uncontrolled neutrophil enzyme release might occur as a result of necrosis. Macrophages themselves contain lysosomal proteinases with optimum reactivity at acid pH to degrade internalised unwanted material within the lysosomes.[7,8] Release of these enzymes as a result of leakage or cell death would be significant if the pH of the environment was favourable. Macrophages also synthesise and release matrix metalloproteinases which react at neutral pH and could be more significant to extracellular tissue turnover.[9] Degradation of extracellular matrix by macrophages involves interactions between cells and matrix[10] that could bypass inactivation by tissue proteinase inhibitors and pH.

Other inflammatory cells—for example, lymphocytes and eosinophils—contain proteolytic enzymes, but it is unclear what contribution they make to major changes in extracellular matrix turnover and, for simplicity, will not be covered here. Fibroblasts and other mesenchymal cells synthesise matrix metalloproteinases which are critical during pulmonary morphogenesis and remodelling and repair of damaged, regenerating lung tissue.[9]

## Emphysema

### NEUTROPHIL PROTEINASES

Neutrophil elastase (NE), a serine protease, was the first enzyme suggested to play a major part in lung disease. Despite the number of neutrophil proteinases, NE has arguably received the lion's share of attention. This is because of studies some 30 years ago which showed that an inherited deficiency of serum $\alpha_1$-antitrypsin predisposes the individual to early onset emphysema.[11] Subsequent studies established that $\alpha_1$-antitrypsin, now termed $\alpha_1$-proteinase inhibitor (PI), was primarily an inhibitor of a neutrophil derived enzyme that could degrade elastin, namely NE.[12] Importantly, NE can cause an emphysema-like condition in experimental animals when given intratracheally.[13] It was therefore proposed that, in those with PI deficiency, there was an elastase-antielastase imbalance in favour of NE which caused emphysema.

Although only 10–20% of tobacco smokers develop emphysema, they have normal serum levels of PI and account for more than 95% of patients with the disease. The increased protease load in these individuals was thought to result from an increase in neutrophil num-

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bers, up to ten fold. In addition it evolved that PI could be inactivated by oxidation of the methionine residue at the reactive site of the molecule.[12] The other major pulmonary inhibitor of NE is secretory leukoproteinase inhibitor, SLPI (also called antileukoprotease or bronchial mucus proteinase inhibitor), produced by non-ciliated bronchiolar cells, which can be inactivated in the same way.[14] The neutrophil, which generates oxygen free radicals when activated, can therefore, at the same time as releasing granule proteinases, "switch off" PI and SLPI in the pericellular region[14] to allow unrestricted NE action. In addition it is possible that oxidants in cigarette smoke inactivate these inhibitors of NE. Increased neutrophil numbers, combined with oxidative inactivation of inhibitors of NE, is therefore believed to occur in smokers who develop emphysema, tipping the balance between NE and its inhibitors in favour of elastase and elastolysis. PI deficient subjects who are smokers develop emphysema much earlier (about 20 years earlier), which probably reflects the severely limited antiprotease backup in the lungs of these subjects (less than 20% of normal).

The evidence that NE plays a central role in emphysema is, however, mostly circumstantial[15]; studies to show increased tissue levels of NE related to tissue pathology are controversial,[16–18] as are studies of NE levels and activity in bronchoalveolar lavage (BAL) fluids from normal subjects and in healthy and sick smokers.[19–21] One recent study shows a relationship between elevated NE and reduced antielastase levels in BAL fluid and emphysema.[22] There is also a correlation between high levels of plasma elastase specific fibrinopeptides and the degree of PI deficiency.[23] PI deficient subjects who smoke have even greater levels of plasma elastase specific fibrinopeptides, consistent with the observed increased susceptibility of these subjects to develop emphysema.[23]

The damage in emphysema affects more than elastic tissue; the affected area, the terminal respiratory unit, is often completely demolished and is replaced by a poor imitation that is functionally inadequate, incapable of gaseous exchange due to disrupted, disorganised alveolar walls and loss of capillaries, and incapable of elastic recoil due, at least in part, to deranged connective tissue. This suggests that destruction of the respiratory units involves a spectrum of proteolytic (and possibly other) enzymes. It has been shown, however, that if neutrophils adhere to type II epithelial cells (rodent) they can cause type II cell death.[24] Furthermore, cultured rodent type II cells and bronchiolar Clara cells are susceptible to detachment by human NE and are only partially protected by PI or SLPI.[25] These studies support the role of the neutrophil and NE in disruption of the terminal respiratory units.

That NE has broad substrate specificity also supports the proposal that NE is a key enzyme in the aetiology of emphysema. It is capable of degrading a wide range of extracellular matrix proteins including collagen, elastin, proteoglycan, fibronectin, and laminin,[26] as well as fibrinolytic and coagulation factors, complement, immunoglobulins G and M[26] and surfactant apoprotein A.[27] Thus, NE could account for much of the tissue damage observed in emphysema.

In addition, neutrophils also contain cathepsin G (CG)[26] and proteinase-3,[28] serine proteinases which are stored in the azurophilic granules and which are probably released alongside NE. Like NE these enzymes have broad substrate specificity[26 28] and co-release with NE could favour cooperative action. Degradation of extracellular matrix by NE and CG, for example, has been shown to be synergistic,[29] while proteinase-3 delivered intratracheally will degrade elastin and induce a bullous emphysema in experimental animals.[28] Other analogies with NE are the nature of proteinase inhibition in that proteinase-3 is inhibited by PI,[28] suggesting that PI deficiency or inactivation could favour increased proteinase-3 activity, while a missense mutation in $\alpha_1$-antichymotrypsin, the major inhibitor of CG, is associated with chronic lung disease.[30]

Neutrophils also store and release collagenase (from secondary granules), gelatinase (from secondary and tertiary granules), and metalloproteinases (dependent on metal ions such as $Zn^{2+}$ for activity).[9 26] These proteinases are stored in a proenzyme form, however, and need to be activated.[9] Although NE and CG have been shown proteolytically to activate neutrophil collagenase,[26] continued NE and CG action may eventually destroy neutrophil collagenase.[5] Activation of collagenase can be accomplished by plasmin,[9] a serine proteinase (see below), and both collagenase and gelatinase can be activated by oxidants generated by stimulated neutrophils.[5] In combination, activated neutrophil collagenase and gelatinase degrade a wide range of collagens as well as fibronectin and elastin.[9] Metalloproteinases are inhibited by serum $\alpha_2$-macroglobulin, which is found in only small quantities in lung tissue, and tissue inhibitors of metalloproteinases (TIMP).[9] Although TIMP is present within the lung, it is susceptible to degradation by NE[31] which could, therefore, potentiate metalloproteinase activity. Similarly, PI can be inactivated proteolytically by a mouse macrophage metalloelastase[32] and, thus, NE activity might be enhanced if an analogous protein exists in man, which now seems likely.

Since the neutrophil proteinases described above will be stored within the neutrophil granules, an increase in lung neutrophils means that there will be a parallel increase in all these proteinases, not just in NE. It therefore seems possible that neutrophil proteinases cooperate in the proteolytic events that lead to emphysema, particularly if the cells undergo necrosis and spill granule contents rather than undergoing programmed cell death.[4]

## MACROPHAGES

Interestingly, most of the increased phagocyte population in the lungs of smokers results

from a 5–10 fold increase in macrophage numbers (macrophages form almost 90% of total phagocytes). Despite the evidence favouring a dominant role for neutrophil proteinases in emphysema, it is possible that macrophages, in addition to producing mediators of inflammation, are involved in proteolytic mechanisms of lung injury since they synthesise and secrete enzymes which, when combined, degrade a similar spectrum of extracellular matrix components to the neutrophil proteinases.[9 26] Macrophage proteinases are thought to contribute to other chronic inflammatory conditions such as rheumatoid diseases; it could be argued that this may be true for emphysema, which also develops chronically. Together, macrophage metalloproteinases—collagenase, stromelysins 1 and 2, and gelatinase—will degrade matrix collagens (I-V, VII, IX-XI), proteoglycans, elastin, fibronectin, laminin, and gelatin at neutral pH.[9 26] In contrast to neutrophil metalloproteinases, these proteins are not stored and production is regulated mostly at the level of gene transcription,[33] followed by activation of the proenzyme[9] and inactivation by extracellular antiproteinases.[9] Like neutrophil metalloproteinases, macrophage metalloproteinases need to be activated extracellularly by other proteinases. Phagocytes have receptors for urokinase plasminogen activator (uPA)[34] and hence can store and utilise uPA when needed. uPA will activate tissue or cell plasminogen to form plasmin, a serine proteinase that will, in turn, proteolytically activate procollagenase, prostromelysin, and progelatinase B (but not progelatinase A).[9] Furthermore, degradation of elastin by macrophages depends on contact between cell and matrix[10]; cell receptor bound uPA is resistant to inactivation by $\alpha_2$-antiplasmin and can therefore degrade plasminogen to produce plasmin which, apart from activating metalloproteinases, degrades fibronectin and laminin to "uncover" the elastin, making it available for proteolysis.[35] This mechanism might also facilitate degradation of elastic tissue by macrophage lysosomal proteinases such as cathepsins by creating a discrete compartment to maintain acidic conditions.[35]

Many of the proteolytic processes described above may be relevant to a greater or lesser extent in other lung diseases, but with a different outcome depending on the aetiology, the inflammatory cell profile, and on the presence of other mediators.

## Proteinases and airways disease

Pulmonary emphysema frequently coincides with chronic obstructive airways disease suggesting that at least some of the pathology is based on the same mechanisms. The fact that instillation of elastase into rodent lungs results in changes in the airways which resemble those in man[36] supports this suggestion. Sputum from subjects with chronic bronchitis contains many inflammatory cells, a large proportion of which are neutrophils.[37] Enzyme analysis of sputum from patients with chronic bronchitis indicates elastase activity due to NE at times of active disease,[38] implying that the antiproteinase screen has been overwhelmed (see mechanisms above). Sputum from patients with cystic fibrosis and bronchiectasis similarly contains inflammatory cells and NE activity at times of active disease.[39 40] NE will cause persistent airway epithelial secretory cell metaplasia in animal models[36] and is a potent secretagogue.[41] Examination of human biopsy samples has shown that the beat frequency of respiratory epithelium cilia was reduced after addition of high levels of NE in vitro and was accompanied by epithelial disruption[42]; at lower, more physiological levels of NE there was still cell–cell and cell–matrix disruption, although cilia beat frequency was not inhibited.[42] In other studies, monolayers of cultured human airway epithelium became detached after addition of NE, but NE was not found to be cytotoxic.[43] Excessive NE activity within the airways could therefore account for the accumulation of mucus seen in patients with chronic bronchitis because of mucous gland hypertrophy, resulting in increased secretion of mucus coupled with reduced mucociliary clearance. The loss of epithelium, mucous gland hyperplasia, elevated production of mucus, and reduced clearance seen in patients with bronchiectasis and cystic fibrosis could also be a result of the multiple actions of NE. Whether or not excessive NE contributes to destruction of the muscular tissue is unclear, but loss of elastic tissue and development of fibrosis could well be partly due to NE, but might also result from proteolytic activity of infecting organisms.

During cystic fibrosis and bronchiectasis, infection of the airways by *Pseudomonas aeruginosa* and release of elastolytic enzymes by this organism may be significant. *Ps aeruginosa* elastase had similar effects to NE on cell disruption and detachment when applied to monolayers and biopsy specimens of human respiratory tract epithelial cells,[42 43] but had little effect on cilia beat frequency.[42] Significantly, *Ps aeruginosa* elastase is a metalloenzyme that can inactivate PI (SLPI is less susceptible)[44 45] and, apart from having direct effects on parenchymal and other components of the respiratory tract, could exacerbate NE activity. As mentioned earlier, an influx of neutrophils does not simply mean increased NE, and many other neutrophil enzymes such as proteinase-3 or the metalloproteinases may have relevance to airways disease. For example, CG is also a secretagogue for airway secretory epithelial cells.[41] The presence of airway macrophages could be significant because of the putative cooperative activity between macrophage and neutrophil proteinases that has already been addressed. Denudation of airway epithelium during chronic bronchitis, cystic fibrosis, and asthma may partly be a result of the action of NE at the basal surface of epithelial cells since monolayers of bovine airway epithelial cells in vitro were found to be more susceptible to NE applied to the basal surface than to the apical surface of the cells,[46] especially once these cells had formed tight junctions.

## Adult respiratory distress syndrome (ARDS)

In direct contrast to emphysema, ARDS is an acute condition caused by a massive influx of neutrophils that far outweighs the numbers of macrophages in the lungs. Considering the increase in lung neutrophils and the abundance of NE within these cells, high levels of pulmonary NE could be important because of its wide spectrum of substrate specificity. With this in mind, several groups have determined levels and activity of this enzyme in BAL fluid from patients with ARDS. In most studies of this type, high levels of immunologically reactive NE have been detected[47 48]; much of this is complexed to PI, which is itself elevated in BAL fluid from these subjects as a result of oedema and leakage of serum proteins into the lung tissue. Some of the PI in BAL aspirate is uncomplexed but is inactive because of oxidation.[49] Nevertheless, elevated, active elastase with reactivity analogous to NE has been shown by some investigators,[50 51] but not others,[47 48] to be present in BAL fluid from patients with ARDS. Discrepancies between studies might reflect the diversity of subjects who are diagnosed with ARDS and the stage of sampling. The presence of inactivated NE at the epithelial surface does not mean that it has not reacted with interstitial lung tissue components before inactivation. Indeed, high levels of oxidatively inactivated PI suggest that PI has been switched off and at least part of this process is likely to be due to activated, degranulating neutrophils.

In addition to elevated NE, neutrophil derived collagenase has also been detected in BAL aspirate from patients with ARDS.[52] It is unclear how much the fibrotic response that sometimes develops in these patients specifically relates to neutrophil enzyme activity. Oedematous lung injury has been induced with oxidant injury in association with NE in an isolated lung preparation.[53] Oxidative inactivation of PI was suggested as one mechanism contributing to NE toxicity in the preparation, where NE and oxidants were shown to act synergistically in promoting oedema. Although these studies imply that there is increased elastolytic potential in the lungs of subjects with ARDS, they do not directly implicate NE, as exemplified by the various other sources of elastolytic potential described above. Neither do they favour elastolysis as having a central role in ARDS. They do, however, support degradation of extracellular matrix components as a part of the aetiology of the ARDS syndrome. Although there is little evidence that macrophage derived proteinases contribute to the fibrosis observed in patients with ARDS, a macrophage derived peptide has been identified in BAL aspirate from patients with ARDS that is chemoattractive for neutropils[54] and, thus, may indirectly raise the lung protease burden. The fibrotic process observed in ARDS could involve mechanisms similar to those that are suggested to occur during other fibrotic diseases.

## Other fibrotic lung diseases

Interstitial pulmonary fibrosis is known to be caused by several agents and may also occur for unknown reasons. Whatever the aetiology, the damage stimulates a repair mechanism akin to the scarring that occurs in other tissues. The repair process is not always sufficiently restricted, however, and affects large regions, if not all, of the lung.[55 56] It is difficult to know the common factors between fibrosis dominated by different types of inflammatory cells—for example, predominantly macrophages, or lymphocytes, or eosinophils.

In animal models of fibrosis a consistent early feature is an increase in numbers of inflammatory neutrophils which declines over the first few days[57] although sometimes they continue to be elevated for months.[58] Elevated neutrophil numbers have also been detected in BAL aspirate from some subjects with idiopathic pulmonary fibrosis and other fibrotic lung conditions.[55 56] Some workers therefore favour the concept that neutrophils are the most damaging of the inflammatory cells in the development of pulmonary fibrosis, and certainly the evidence for their central role in ARDS would support this. Furthermore, immunological levels, though not activities, of NE and myeloperoxidase are higher in patients with interstitial fibrosis.[59] Neutropenic subjects, however, can develop mild ARDS[60] and, in animal models, depletion of neutrophils in advance of lung injury[61] leads to more lung collagen. This suggests that neutrophils restrict the extent of the fibrosis, possibly by proteolytic mechanisms, but possibly by oxidation of lung mediators or components, or both. There is only a small amount of evidence to support a major causative role for neutrophil proteases in fibrosis.

BAL aspirate from some individuals with fibrosis of varying aetiology contains high levels of collagenase,[62–64] thought to be neutrophil derived, and it has been suggested that this is related to the development of fibrosis in these subjects. On the other hand, analysis of lung tissue samples from subjects with hypersensitivity pneumonitis before and after treatment with prednisone[65] indicated that, when lung tissue collagenase levels were low, the subjects developed fibrosis; in those who improved or recovered, interstitial collagenase levels were 2–3 times higher than in those with fibrosis. Low levels of interstitial collagenase leading to reduced collagen turnover may therefore account for the build up of collagen in fibrosis. It has been suggested that this is the result of repressed collagenase production by fibroblasts during fibrosis,[65] although increased production of TIMP and reduced activation of latent enzyme could also account for low collagenase activity; this illustrates the need for concordant action of proteinases and inhibitors in metabolism of the extracellular matrix.

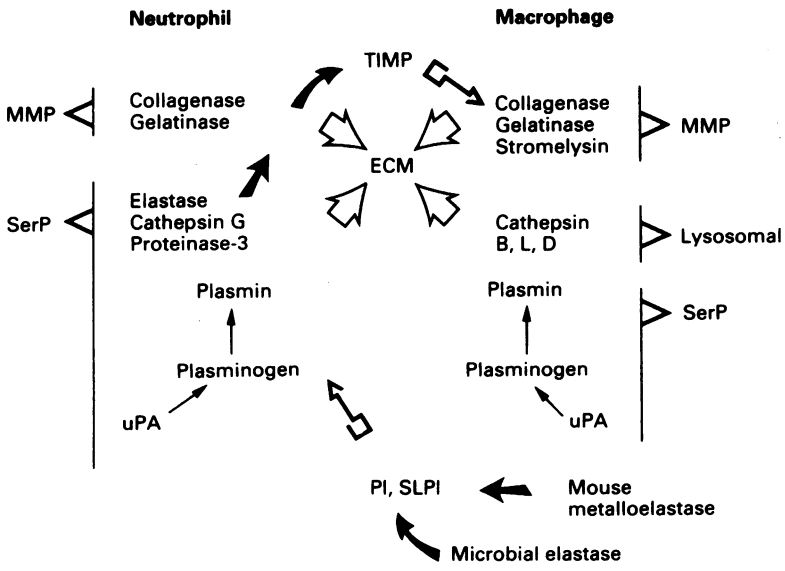
An alternative inflammatory cell associated with numerous fibrotic lung disorders is the mast cell.[66] These cells contain copious amounts of tryptase which seems more likely

to stimulate fibrosis via its mitogenic activity on fibroblasts than by having a direct effect on levels of extracellular connective tissue.[67] The relationship between the pathology of pulmonary fibrosis and altered proteinase activity is therefore unclear and could well be related to reduced interstitial proteinase—that is, collagenase—activity and lower connective tissue degradation accompanied by fibroblast proliferation and enhanced collagen synthesis,[56] rather than by obvious enzymatic destruction of lung tissue.

## Summary

The hypothesis, some 30 years ago, that NE was the sole proteolytic agent responsible for the development of emphysema seems naive in retrospect. The availability of technology to measure NE facilitated the early research into the relationship between NE and lung disease. Despite an abundance of information on the activity of NE in the lung, it will probably require prospective studies in man with specific NE inhibitors or control at the gene level to establish a causal relationship between NE and lung disease. Parallel research has resulted in the isolation and characterisation of NE inhibitors other than PI and, indeed, alternative proteolytic enzymes that might contribute to lung disease. It is perhaps impossible now to think that a single proteinase, however omnipotent it may be, causes lung diseases as diverse as emphysema and fibrosis.

An important aspect that is emerging is the interrelationship between proteolytic enzymes produced by different, or sometimes the same, cells that could potentiate tissue proteolysis. The evidence suggests that there is likely to be coordinated action between neutrophils, macrophages, and possibly mesenchymal proteinases which can activate or inactivate each other. In addition, one class of proteinases often appears able to proteolytically inactivate inhibitors of the opposite class, which presumably could amplify proteolysis if it occurred in vivo. Although the work on this aspect of proteinase activity is in its infancy, one suspects that part of the normal regulation of proteinase activity might include compartmentalisation. For example, the neutrophil stores proteinases before appropriate release and can inactivate PI to enable proteolytic action pericellularly, whereas degradation of extracellular matrix by macrophages requires interaction between the cell and matrix which is facilitated by cell receptor bound uPA. Disintegration of these "compartments" due to oedema, proteolysis, or for mechanical reasons could, firstly, expose further extracellular matrix substrates to inflammatory and damaged cell proteinases but, secondly, might enhance proteinase potential by the cooperative action of these enzymes. It seems increasingly likely that, where proteinases play a part, there is a cocktail of proteinases that is characteristic of the injury that develops (fig). What remains unclear is why only a proportion of those susceptible, such as smokers or those with acute lung injury, develop irreversible lung disease. This suggests that there are other factors acquired or inherited that need to be considered.




*Putative interaction of neutrophil, macrophage, and other proteinases with extracellular matrix (ECM) and proteinase inhibitors. ⇨—Proteolysis of ECM. ➡—Proteolytic inactivation of tissue inhibitor of metalloproteinases (TIMP), alpha 1-proteinase inhibitor (PI) and (possibly) secretory leukoproteinase inhibitor (SLPI). ⊏→—Augmentation of proteinase activity due to inactivated inhibitor. MMP—matrix metalloproteinases; SerP—serine proteinases; uPA—urokinase plasminogen inhibitor.*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