

Invited Review

Proteinase-antiproteinase imbalance in the pathogenesis of Emphysema: The role of metalloproteinases in lung damage

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Summary. Pulmonary emphysema refers to a lung disorder characterized by a diffuse destruction of the alveolar walls resulting in enlargement of the distal airspaces. The disease is usually a chronic, progressive, and disabling disorder. The concept of proteinase/antiproteinase imbalance evolved from the identification of patients with α 1-antitrypsin deficiency, and from the development of experimental emphysematous lesions using different enzymes. For a long time, this concept was seen as an elastase/antielastase imbalance, with the consequent degradation of elastin. Recent evidence, however, suggests that an intricate process of pulmonary remodeling occurs during the development of emphysema, where a complex network of serine proteases and metalloproteinases capable of degrading different extracellular matrix molecules, primarily, but not exclusively fibrillar collagens and elastin, are implicated in the pathogenesis of this disease.

Key words: Pulmonary emphysema, Metalloproteinases, Elastase, Collagenase

Introduction

Chronic obstructive pulmonary disease (COPD) is a prominent cause of morbidity and mortality among the adult population (Celli et al., 1995). The disease is characterized by fixed and irreversible air-flow limitation in the lungs, and by emphysema, which ultimately provokes the loss of alveolar surface area for gas interchange, thus being responsible for most of the features of chronic and irreversible respiratory failure.

Tobacco smoke is the most frequent identifiable risk factor for COPD. Cigarette smoke consists of an extremely complex mixture of compounds released into the respiratory tract, including highly reactive oxygen species. Actually, cigarette smoke imposes a huge

oxidant burden on the lungs, producing 10^{17} oxidant molecules per puff (Church and Pryor, 1985).

Emphysema is usually a progressive, and often disabling disease characterized by the abnormal and permanent enlargement of respiratory regions of the lung distal to the terminal bronchioles, accompanied by the destruction of the alveolar walls (Snider et al., 1985). According to their locations in the secondary pulmonary lobule, two major forms of emphysema have been described: panlobular and centrilobular. The most common form is represented by the centrilobular emphysema which is strongly associated with cigarette smoking, and results from destruction of alveoli around the proximal respiratory bronchiole. This form of emphysema shows predilection for the upper part of the individual lobes. In advanced stages of the disease, the emphysematous spaces may coalesce into large bullae which may reach several centimeters in diameter. On the other hand, panlobular emphysema exhibits a usual lower-lobe distribution and it is generally observed in patients with severe α 1-antiprotease deficiency; this form of the emphysema is characterized by the air space enlargement throughout the acinus.

Pathogenic mechanisms of pulmonary emphysema

The proteinase/antiproteinase imbalance hypothesis

This hypothesis, as a key concept in the pathogenesis of emphysema, arose from two independent reports in the early 1960s. Firstly, Laurell and Eriksson (1963) identified a number of subjects with deficiency of α 1-antitrypsin, several of whom had also severe emphysema of early onset. α 1-antitrypsin - more appropriately α 1-antiprotease - is a major serine proteinase inhibitor, that is the principal inhibitor of neutrophil elastase. Although the liver appears to be the primary source of α 1-antiprotease inhibitor, recent evidence suggests that lung-derived epithelial cells, both bronchial and alveolar, are able to synthesize a fully active form of this inhibitor (Cichy et al., 1997).

Forthwith, after Laurell and Eriksson's disclosure,

Proteinase/antiproteinase imbalance in emphysema

Gross et al. (1964) demonstrated that the use of a proteolytic enzyme (papain) was capable of inducing experimental lung lesions histologically similar to the human emphysema. These two pieces of evidence, suggested that emphysema could result as a direct consequence of an excessive proteinase burden in the lung milieu.

Although α 1-antiprotease (α 1-AP) deficiency is the major studied form of genetic susceptibility associated to the development of emphysema, other genetic polymorphism may also play a role. Recently, for example, an association between polymorphism in the gene for microsomal epoxide hydrolase (*mEPHX*) - an enzyme involved in the metabolism of highly reactive epoxide intermediates - and susceptibility to human emphysema was reported (Smith and Harrison, 1997). In that study, the proportion of individuals with innate slow *mEPHX* activity (homozygotes) was significantly higher in patients with COPD and emphysema, suggesting that genetic polymorphism involving xenobiotic enzymes may have a role in the susceptibility to oxidant-related lung disease. This is an important issue because an imbalance between oxidants and antioxidants has also been considered relevant in the pathogenesis of smoking-induced COPD and emphysema. The injurious effects of the increased oxidant burden in smokers include lipid peroxidation and oxidative protein damage, which, in turn, may alter the biological activity of receptors or antiproteinases enhancing the pathological response (Rahman and MacNee, 1996).

Neutrophil elastase, the first piece of the proteinase/antiproteinase puzzle

A central role for neutrophil elastase was supported by several lines of evidence. In the 1970s it was shown that neutrophil elastase was also capable of provoking lung emphysema in experimental animals (Janoff et al., 1977). Likewise, the enzyme was demonstrated in close association with interstitial elastic fibers in both emphysematous human lungs and in strains of mice spontaneously developing pulmonary emphysema (Damiano, 1986; de Santi 1989). Therefore, the prevailing theory for almost 20 years was that diffuse emphysematous lesions developed as a result of an abnormal balance between neutrophil elastase and α 1-antiprotease in the lower respiratory tract (Janoff, 1985; Niewoehner, 1988). This imbalance would be responsible for an excessive degradation of elastin, resulting in loss of the elastic properties of the lung. Elastin is an important lung extracellular matrix molecule, composed of highly cross-linked, hydrophobic tropoelastin monomers, which provides resilience to elastic fibers, allowing the characteristic lung mechanical behavior.

According to this "elastase/anti-elastase imbalance" hypothesis, cigarette compounds provoke the recruitment of neutrophils to the smoker's lungs, with the subsequent release of elastase into the lung

connective tissue in concentrations that awfully surpass the ability of the proteinase inhibitor systems to inactivate them, thus resulting in destruction of elastic fibers, and in the development of emphysema. Besides, since tobacco smoke carries an enormous amount of free radicals, it may induce the inactivation of the α 1-proteinase inhibitor, through the oxidation of a methionine located in the active site of the inhibitor. Actually, *in vivo* studies supported the notion that the α 1-AP present in lung lavage from cigarette smokers exhibits reduced inhibitory capacity, which appeared to be related to the oxidation of the methionine residue (Gadek et al., 1979; Carp et al., 1982). Furthermore, alveolar inflammatory cells from smokers with emphysema also have the ability to inactivate α 1-AP spontaneously whereas cells from smokers without detectable emphysema do not (Wallaert et al., 1993).

Neutrophils versus macrophages in the pathogenesis of emphysema. Which is the culprit cell?

Additional support for a deleterious role of neutrophils, is the evidence suggesting that cigarette smoking provokes an increased lung neutrophil traffic in human and experimental animals (MacNee et al., 1989; Bosken et al., 1991). Cigarette smoke may increase neutrophil chemotaxis and sequestration in the lung microcirculation, through an upregulation of interleukin-8 and neutrophil adhesion molecules (di Stefano et al., 1994; McCrea et al., 1994; Kanazawa et al., 1996). Interestingly, in the human disease, a weak but significant relationship between neutrophil sequestration and microscopic emphysema (alveolar wall per unit lung volume) has been found (Selby et al., 1994). Sequestered neutrophils may release a number of injurious products, including elastase and reactive oxygen species.

However, most of the experimental evidence supporting a role for neutrophils grew largely from circumstantial evidence and is actually inconclusive. Thus, for example, the search for neutrophil elastase in the human disease has given controversial results, (Damiano et al., 1986; Fox et al., 1988), and some studies do not support the existence of high levels of elastase in association with elastin. Furthermore, a more severe elastase-induced emphysema was revealed in animals made neutropenic than in control animals with normal levels of neutrophils (Kuhn et al., 1980). Additionally, macrophages and not neutrophils, are the most abundant inflammatory cells found in bronchio-alveolar lavage (BAL) of cigarette smokers, as well as in respiratory bronchioles where emphysematous changes are first apparent (Niewoehner et al., 1974; Merchant et al., 1992). In this context, it has been recently shown that the extent of alveolar septa destruction in human emphysematous lungs, is associated to the number of alveolar macrophages and T-lymphocytes present in the areas of lung destruction (Finkelstein et al., 1995). Interestingly enough, a negative correlation was found between the number of neutrophils and the emphy-

sematous lesions. Taken together, morphometric and BAL cell profile strongly suggest that macrophages are the most abundant inflammatory cells in the areas where lung is being destroyed, and that they are in close relationship to the alveolar disruption. Of course, nobody can negate a role for neutrophils, mainly immediately after smoking, and during lung infections; a frequent complication in COPD patients which worsens the natural history of the disease.

Both alveolar macrophages and neutrophils not only produce a number of enzymes, but are also a potential source of oxidants in the lower respiratory tract of cigarette smokers. As mentioned before, these oxidants can in turn inactivate α 1-AP, rendering it ineffective as an inhibitor of neutrophil elastase (Hubbard et al., 1987).

On the other hand, the contribution of other cells in the development of emphysema warrants further studies, and particularly, the participation of fibroblasts is a long way from being elucidated. There is some evidence suggesting that cigarette smoke extract inhibits fibroblast proliferation and migration, at least *in vitro* (Nakamura et al., 1995). If tobacco induces dysfunction of pulmonary fibroblasts it may affect quantitatively and qualitatively the production of extracellular matrix proteins altering the dynamics of tissue repair after the destructive events. In other words, emphysema could also be seen as a deficiency in tissue repair after alveolar disruption. Since fibroblasts are the main local cells responsible for the synthesis of the structural components of the alveolar walls including collagens, elastin, fibronectin, and proteoglycans, putative fibroblast alterations provoked by tobacco smoke might also participate in the pathological response. Additionally, fibroblasts are an important source of a variety of matrix metalloproteinases (MMP), tissue inhibitors of metalloproteinases (TIMP), and α 2 macroglobulin (Raghu and Kavanagh, 1991; Pardo et al., 1992); all of them playing a central role in matrix remodeling.

On the other hand, fibroblasts participate in the pathological changes of the COPD airways. It is well known, for example, that fibrosis as well as peribronchial inflammation are key lesions in chronic bronchitis and in the permanent narrowing of the small airways, the partners of emphysema in COPD (Chanez et al., 1997).

Metalloproteinases, the missing pieces of the proteinase/antiproteinase puzzle

The metalloproteinases are a family of secreted or transmembrane proteins that are capable of degrading virtually all extracellular matrix and basement membrane components. The MMP family consists of at least four different subclasses of zinc- and calcium-dependent endopeptidases that are synthesized as proenzymes and become activated through proteolytic cleavage. Based on their substrate affinity and structural domains, MMPs have been classified into collagenases,

gelatinases, stromelysins, and membrane-type metalloproteinases (Matrisian, 1992; Woessner, 1994). The collagenase subfamily includes interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase 3 (MMP-13), which have substrate specificity for fibrillar collagens (Goldberg et al., 1986; Hasty et al., 1990; Freije et al., 1994). The gelatinase subfamily is composed of two members, Gelatinase A (MMP-2) and Gelatinase B (MMP-9). Their substrate specificity involves the degradation of type IV collagen, the major structural component of basement membranes, denatured collagens (gelatin), as well as insoluble elastin (Collier et al., 1988; Wilhem et al., 1989; Senior et al., 1991). The stromelysin subgroup comprises, stromelysins 1 and 2, (MMP-3 and MMP-10) two highly homologous enzymes, and stromelysin 3 (MMP-11) which share substrate specificity with matrilysin (MMP-7) for proteoglycans, fibronectin, laminin, and the globular domain of type IV collagen (Basset et al., 1990; Birkedal-Hansen et al., 1993). Metalloelastase (MMP-12) with elastin degrading ability has also been classified in this subgroup (Shapiro et al., 1993). New members of the MMP family have been recently cloned (MMP-18 and MMP-19) although their substrate affinity has not yet been determined (Cossins et al., 1996; Pendas et al., 1997). Finally, the membrane-type metalloproteinases (MT-MMP) include four different enzymes (Sato et al., 1994; Takino et al., 1995; Will et al., 1995; Puente et al., 1996). Their physiological roles are not precisely defined yet, but they have been involved in cell surface activation of progelatinase A. Interestingly, MT1-MMP has been shown to digest interstitial collagens (Ohuchi et al., 1997).

Macrophages are a major source of metalloproteinases capable of lung destruction, including interstitial collagenase-1 and collagenase-3 (MMP-1, MMP-13) (Pardo et al., 1996; Selman et al., 1996), and at least four different enzymes which are also able to degrade insoluble elastin: metalloelastase (MMP-12), matrilysin (MMP-7), and gelatinases A and B (MMP-2, MMP-9) (Shapiro, 1994; Busiek et al., 1995; Shipley et al., 1996).

Evidence supporting a role for metalloelastase was recently found in mice made genetically deficient in MMP-12 by gene targeting (Hautamaki et al., 1997). Whereas wild-type mice revealed an increased inflammatory cell recruitment and emphysematous lesions when they were chronically exposed to cigarette smoke, macrophage elastase-deficient mice did not present increased numbers of lung macrophages and did not develop emphysema.

Likewise, an upregulation of gelatinase B, has been described in human and experimentally-induced emphysema (Rosenbluth et al., 1995; Segura et al., 1995; Horiba et al., 1997). Cells expressing gelatinase B are located along alveolar walls and in the alveolar spaces and interstitium, and most likely represent activated alveolar and interstitial macrophages, neutrophils and probably some epithelial cells. Therefore, at least two

metalloproteinases with elastin substrate specificity seem to be involved in emphysematous lung destruction.

A putative role for interstitial collagenase

The interstitial compartment of the alveolar walls is the space between the alveolar and capillary basal laminae. Fibrillar collagens and elastin are the major extracellular matrix components of this compartment and play a vital physiological role in ventilation, enabling the tissue to stretch during inflation, preventing over-inflation and facilitating subsequent recoil. Actually, collagens are the most abundant proteins in the lung parenchyma and to date, four molecular types of collagen (I, III, V, and VI) have been found in the interstitium, whereas type IV collagen is an important component of the basement membranes (Turino, 1985; Specks et al., 1995). In addition, a number of other macromolecules, such as proteoglycans and fibronectin are also part of this dynamic and complex structure.

During the development of emphysema, the alveolar walls are completely destroyed and in more advanced stages, the emphysematous spaces may coalesce into larger bullae where gas interchange is non-existent. In this context, breakdown of interstitial fibrillar collagens appears to be a fundamental step in the micro-environmental disruption of alveolar walls. Moreover, the disappearance of huge numbers of delicate alveolar-capillary structures should involve the multiple action of different extracellular-matrix proteolytic enzymes released into the local milieu.

The first important contribution supporting a putative role for interstitial collagenase in the pathogenesis of pulmonary emphysema was reported by D'Armiento et al. (1992) who demonstrated that transgenic mice overexpressing MMP-1 in their lungs developed morphological changes consisting in disruption of alveolar walls and coalescence of the alveolar spaces remarkably similar to human emphysema. Interestingly, no evidence of inflammation or fibrosis was observed, and furthermore a decrease of collagen fibers was noticed whereas elastin appeared to be normal. The association of collagenase transgene and emphysematous lesions were further confirmed since there was a positive relationship between the levels of collagenase expression and the severity of the emphysematous lesions.

Subsequently, similar findings were found in our laboratory using an experimental model induced by tobacco smoke in guinea pigs (Selman et al., 1996). During cigarette smoke exposure lungs developed first a multifocal inflammation in the lower respiratory tract, and, after several weeks, a varied degree of emphysematous lesions. These pathological changes coincided with a marked increase in interstitial collagenase expression and activity. The increase of collagenase activity was progressive and occurred in the presence of a higher degree of inflammation and alveolar walls rupture, suggesting that active collagen breakdown is

taking place during the development of emphysema.

Some recent reports have suggested that an up-regulation of interstitial collagenase also occurs in human emphysematous lung tissues. Finlay et al. (1997a), showed that alveolar macrophages obtained from patients with pulmonary emphysema have elevated mRNA transcripts for interstitial collagenase and gelatinase B when compared with normal volunteers. Moreover, unlike the other MMPs examined (gelatinases A and B, and metalloelastase) where expression was detectable in all study samples, mRNA transcripts for MMP-1 were distinguishable only in 20% of control samples compared with 100% of emphysematous patients. Intriguingly, transcript levels of metalloelastase were as in control samples, placing in some stress previous findings in transgenic mice (Hautamaki et al., 1997). Collagenase activity has been also found elevated in BAL fluid from patients with emphysema and seems to be a better indicator for the presence of the disease than elastase (Finlay et al., 1997b).

Nevertheless, a complex and probably compartmentalized inflammatory/repair, proteinase/anti-proteinase process seems to take place in the lungs since, although measurements of collagen have given contradictory results, two recent studies have suggested that areas of alveolar wall fibrosis with net increase in interstitial collagen are present in human pulmonary emphysema (Cardoso et al., 1993; Lang et al., 1994). Similarly, a pattern of thickened fibrils and disorganized deposition of collagen was observed in emphysematous human lungs, and in elastase-induced experimental emphysema (Finlay et al., 1996). In long-term smoke-induced emphysema in guinea pigs, morphometric and ultrastructural evidence of collagen breakdown and resynthesis have also been found (Wright and Churg, 1995). In general terms, these findings might be interpreted as representing a disturbance in both pathways of collagen homeostasis with an excessive collagen degradation participating in alveolar wall destruction, whereas an increased collagen synthesis may produce discrete foci of interstitial fibrosis. Intriguingly enough, although pulmonary emphysema and diffuse lung fibrosis have been classically considered to represent two separate disorders, some studies have revealed an occasional association between both disorders (Hiwatari et al., 1993).

A vicious circle between enzymes and inhibitors may perpetuate alveolar disruption

Excessive activity of interstitial collagenases may more extensively affect the lung connective tissue metabolism because both, macrophage/fibroblast collagenase and neutrophil collagenase, are able to hydrolyze and inactivate $\alpha 1$ -proteinase inhibitor (Michaelis et al., 1990; Desrochers et al., 1991). The obvious consequence is that collagenases may participate in this pathological process not only through the abnormal remodeling of the lung fibrillar collagens,

but also by enhancing an imbalance in the serine-proteinase/ α 1-proteinase inhibitor thus contributing to increased elastolytic activity. Similarly, other metalloproteinases, such as stromelysins 1 and 3 are also able to inactivate α 1-antiproteinase and consequently, they also may intensify the activity of neutrophil elastase (Winyard et al., 1991; Anderson et al., 1995).

On the other face of these interrelated enzymatic activities, some serine proteases, such as neutrophil elastase, are capable of degrading tissue inhibitor of metalloproteinases, thus increasing collagenolytic and other metalloproteinases activities (Okada et al., 1988).

In summary, a large and growing body of evidence strongly suggests that the emphysematous lesion involves more than neutrophil elastase and elastic tissue disruption, and that alternative proteolytic enzymes, i.e. interstitial collagenase, and gelatinase B, contribute to the lung disease. This complex network is further exemplified by findings showing the presence of structural changes or altered processing of heparan sulfate in patients with emphysema which may alter the stability of the alveolar extracellular matrix (Van de Lest et al., 1996). Actually, experimental inhibition of proteoglycan synthesis results in considerable emphysematous lung destruction (Van Kuppevelt et al., 1997).

Therefore, it is now impossible to conceive that a single proteinase, regardless of how all-powerful it may be, could be responsible for the destruction of alveolar walls.

Treatment of COPD has primarily been supportive and has included among others, the use of exercise and rehabilitation, supplementary oxygen, bronchodilators, antibiotics etc., and it has also been directed at changes in lifestyle; particularly smoking cessation. Additionally, lung reduction surgery and lung transplantation are currently under research. In general, however, management of COPD has been largely unsuccessful. In this context, new directions for effective therapy may arise from a better understanding of the complex mechanisms involved in the pathogenesis of this disorder. Since the lung is a readily accessible target organ for gene therapy, continuing improvements in DNA recombinant technologies will contribute to the development of new therapeutic strategies.

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