Review

The role of collagenase in emphysema

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Received: 23 March 2001
Revisions requested: 16 May 2001
Revisions received: 19 June 2001
Accepted: 7 August 2001
Published: 19 September 2001

Respir Res 2001, **2**:348-352

(Print ISSN 1465-9921; Online ISSN 1465-993X)

Abstract

The extracellular matrix is essential for the integrity of the lung and when disrupted can lead to the architectural changes seen in emphysema. The etiology of emphysema is believed to be due to an imbalance in the proteases and antiproteases within the lung. Studies have focused on elastolytic enzymes as the primary agents in disease pathogenesis, however, recent data suggests that collagenases may also be involved in the destruction of lung tissue in emphysema. It is hoped that this expanded understanding of the pathophysiology of emphysema will lead to improved therapy in the treatment of the disease.

Keywords: collagen, collagenase, elastase, emphysema, matrix metalloproteinases

Introduction

Emphysema is a common, debilitating pulmonary condition, the impact of which is felt worldwide, placing an enormous economic strain on international health care infrastructures. In the UK, data from 1996 indicate that the medical cost of chronic obstructive pulmonary disease (COPD) was approximately £846 million or about £1154 per person per year [1]. In the USA, over 106,000 people die from COPD yearly making it the fourth leading cause of death nationwide [2]. Moreover, while death rates for heart disease and stroke have declined by roughly 50% over the past 30 years, the death rate for COPD has risen by 71% [2]. In 1993, it was estimated that 14.7 billion dollars were spent in the USA on direct COPD-related health care expenditures, costing roughly the equivalent of £764 per person per year [1]. As the life expectancy of the populations of industrialized nations continues to increase, it is certain that the costs of emphysema in terms of lives lost and strains on limited health care resources will continue to grow.

Elastase/anti-elastase hypothesis

In light of these statistics on the global impact of this disease, it is surprising to realize that even the basic pathophysiology of emphysema is still being debated. While it is clearly established that cigarette smoke is the principal cause of emphysema, the mechanism by which cigarette smoke exposure leads to the destruction of lung architecture seen in emphysema is controversial. Elastin is an important component of the extracellular matrix (ECM) that is believed to confer upon the lung the resilience needed to undergo repetitive physiologic stress. For over 30 years, the leading hypothesis has been that emphysema resulted from an elastase/anti-elastase imbalance. This theory was formulated after it was noted that smokers with a congenital deficiency of alpha-1-antitrypsin (α1-AT) had an excessive incidence of emphysema [3]. Given the increased neutrophil content seen in the lungs of smokers and the fact that α1-AT inactivates neutrophil elastase, it was believed that elastin degradation via neutrophil elastase was the pivotal factor responsible for the develop-

α1-AT = alpha-1-antitrypsin; COPD = chronic obstructive pulmonary disease; ECM = extracellular matrix; MMP = matrix metalloproteinase; MMP-1 = collagenase; MMP-12 = metalloelastase; MT-MMP = membrane type matrix metalloproteinase.

ment of emphysema. The finding that the intratracheal administration of papain, a powerful elastase, could lead to the formation of emphysematous alveoli in the lungs of experimental animals further supported this elastin-based model of disease. These two findings have led to the widespread and persistent acceptance of the elastase/anti-elastase imbalance theory.

Recently, investigators have questioned the elastin-based model of disease for several reasons. Not all patients with congenital deficiency of α1-AT who smoke will develop emphysema, and patients who have a partial deficiency do not have an increased risk of emphysema [4]. While nearly all smokers have accumulation of neutrophils in their lungs, only a small minority develop emphysematous changes, and neutrophil number does not correlate with parenchymal destruction in the disease [5]. Studies to date have also failed to demonstrate elastase excess or inhibitor deficiency in smoking-induced emphysema [6,7]. Despite these reservations, the contribution of neutrophil elastase to emphysema pathogenesis cannot be ignored. Importantly, the survival time of the neutrophil in the lung is very brief, lasting only a few hours. Thus, studies examining a single time-point in the course of a prolonged disease may easily underestimate the true impact of this cell type in pathogenesis. Furthermore, a lack of elastase excess does not preclude that significant elastin degradation is occurring. Several investigators have shown that the concentration of elastase at the site of release from the azurophilic granule is supraphysiological and, thus, cannot be inhibited by even normal concentrations of inhibitors [8,9]. Campbell and colleagues have demonstrated that elastase enzyme-inhibitor kinetics do not follow a linear pattern. Rather, after inhibitor levels fall below a certain threshold, proteolytic bursts can occur in an exponential fashion [8]. This may explain why patients with a partial deficiency of α1-AT do not develop emphysema. Although elastase does not represent the mechanism for all emphysema, there is still a role for this enzyme in the disease process. It is important to realize that multiple factors are involved in the pathogenesis of emphysema [10] and no single agent will be totally responsible for the pathology seen in this disease.

Studies performed on a metalloelastase (matrix metalloproteinase [MMP]-12)-knockout mouse recently provided support for the involvement of elastolytic enzymes in emphysema [11]. These mice do not express the MMP-12 gene and have a normal lung phenotype. When the animals are exposed to cigarette smoke they do not develop emphysematous changes in the lung as wild-type animals do [11]. This study demonstrates that the macrophage is crucial to the development of emphysema in the mouse smoking model since, in the absence of these cells, emphysema does not develop. There is no direct evidence, however, which demonstrates that excess activity of MMP-12 is involved in the human disease. In fact, experiments examining human alveolar macrophages and lung tissue from patients with emphysema and from normal volunteers did not demonstrate increased levels of MMP-12 in the patients with emphysema in either site [12,13]. The lack of MMP-12 involvement in the human disease, therefore, may be accounted for by the fact that rodent and human macrophages differ significantly in their repertoire of MMPs [14].

Collagen degradation

Fibrillar collagen, another component of the ECM, is vital for maintaining the normal lung architecture. Type I collagen, in fact, is the major structural element of the lung, comprising 50–60% of the ECM [15]. Histological studies have demonstrated that fibrils from type I and III collagen are widely distributed in the lung. They are present in the adventitia of pulmonary arteries, the interstitium of the bronchial tree, the interlobular septa, the bronchial lamina propria and the alveolar interstitium, where the pathological changes of emphysema are known to occur. There are a number of correlative studies which suggest that collagenase, a classical member of the MMP family which degrades the fibrillar collagens, may be involved in several lung diseases [16,17]. In addition, studies suggest that collagen is degraded or damaged in pulmonary emphysema, for example, antibodies to collagen have been found in the serum of patients with emphysema [18]. Emphysematous changes have also been reported in patients with the inherited disorder, type VI Ehlers-Danlos syndrome, which causes a decreased structural integrity of collagen fibrils [19]. It has also been shown that collagen is affected in the various emphysema animal models. In papain-induced emphysema, dissolution of collagen fibrils is seen shortly after instillation of the enzyme [20]. Similarly, collagen is rapidly degraded in elastase-induced emphysema [21] and studies have shown that short exposure to toxic amounts of oxygen in rats will lead to emphysematous changes and collagen degradation with no changes in elastin [22].

The importance of collagen in this disease was raised when it was shown that a transgenic mouse line that expressed human MMP-1 (collagenase) in the lung developed emphysema with no effect on lung elastin content [23]. This was the first time that it was shown that emphysema could occur via an elastin-independent mechanism and it has led to a shift from thinking of emphysema as solely a disease of elastin degradation to one that incorporates collagen into the disease paradigm. The presence of collagen adds tensile strength to the lung tissue and allows the lung to maintain the structure needed to carry out the physiologic function of gas exchange. Collagen fibrils support this function by maintaining alveolar interdependence in the lungs, thereby helping to preserve the stability of small pulmonary airspaces. It is not surprising,

therefore, that damage to collagen fibrils may upset the balance of forces in the lung and lead to the development of overextended, emphysematous-appearing alveoli.

Studies examining collagen content in emphysematous lungs have yielded conflicting results. While we have demonstrated in the transgenic mouse model that the development of emphysema was associated with a loss of collagen without elastin degradation [23], other investigators have reported that the amount of collagen was actually increased in the septal regions of emphysematous patients [15,24]. This may reflect the fact that matrix remodeling is a dynamic process, with collagen degradation followed by repair ultimately increasing collagen deposition in the lung. It may seem counterintuitive that collagen content would be increased in a disease that is characterized by the destruction and dilation of small airspaces in the lung. By means of alveolar interdependence, however, increased collagen deposition in certain alveoli could expose surrounding alveoli to potentially deleterious forces of distension. If an alveolus in the earlier stage of emphysema is surrounded by alveoli that are in the post-repair phase, the effects may be even more exaggerated.

Increased collagenase in emphysema

Over the past several years, additional studies have emerged to implicate collagenase and collagen breakdown in this disease process. Wright and Churg exposed guinea pigs to cigarette smoke and demonstrated that the development of emphysema was associated with morphometric evidence of collagen breakdown and repair [25]. Following these findings, Selman and colleagues [26] demonstrated that smoke exposure in guinea pigs caused increased expression of collagenase and enhanced collagenolytic activity within their lungs. *In situ* hybridization showed that the collagenase expression was localized to alveolar macrophages, epithelial cells and fibroblasts. This increased collagenase activity was associated with a significant decrease in the lung collagen concentration in the smoke-exposed guinea pigs. These studies suggest that collagenase is involved in the pathogenesis of emphysema in animal models.

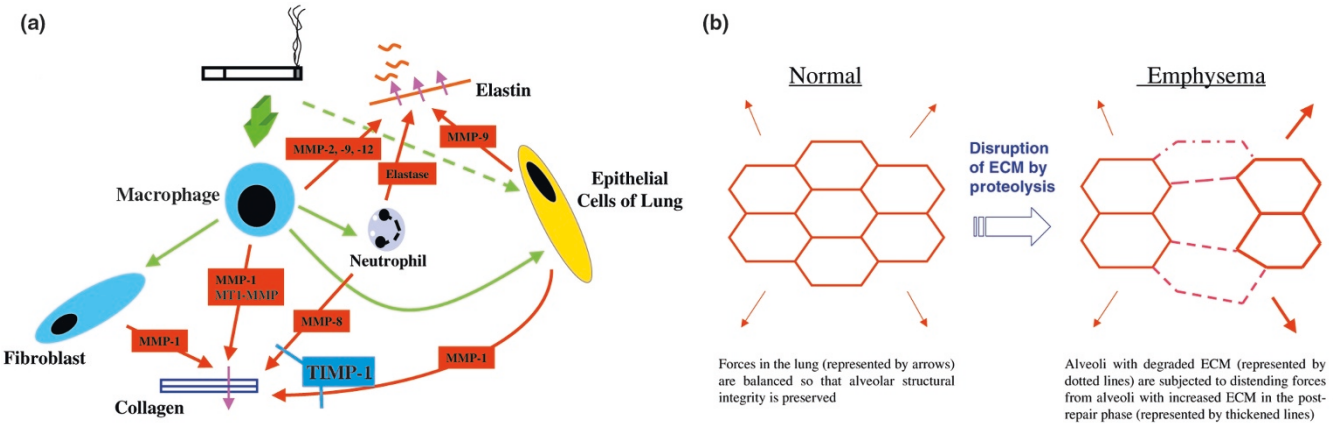
Recently, studies in humans have also demonstrated a role for collagenase in emphysema patients. Finlay and colleagues [12] showed that alveolar macrophages from the bronchoalveolar lavage of emphysema patients had augmented production of collagenase compared to matched controls. Ohnishi *et al.* reported a significant increase in collagenolytic activity within the lung parenchyma of emphysema patients [13]. More recently, our laboratory demonstrated the presence of MMP-1 mRNA, protein and collagenase activity within the lung parenchyma of patients with emphysema when compared to normal controls [27]. In this study, lung samples were examined from 23 patients with emphysema undergoing lung reduction surgery or lung transplantation. All but two of the patients had stopped smoking. When immunolocalization studies were performed on these samples the type II pneumocyte was identified as the cell type producing collagenase [27].

Other investigators have provided affirmative evidence for the presence of MMP-1 in the lung parenchyma of emphysema patients. Segura-Valdez *et al.* examined lung tissue from 10 COPD patients and found increased expression of collagenases 1, 2 and 3 [28]. Immunohistochemical analysis also revealed that MMP-1 was found to be present in alveolar and interstitial macrophages, endothelial cells and epithelial cells from COPD patients. The emerging data, while not conclusive, appears to point to a significant presence of collagenase in the lung parenchyma of patients with emphysema. These studies introduce the concept that cells other then inflammatory cells may participate in the destruction of the lung in emphysema.

Conclusion

Our scope of understanding of the basic pathophysiology of emphysema has advanced significantly in the past decade. It is becoming increasingly evident that elastin degradation alone cannot explain the morphological changes that occur in smoking-induced emphysema. The macrophage, which is the predominant cell in the airways and alveolar spaces of emphysema patients, contains an array of enzymes capable of digesting both fibrillar collagen (MMP-1, MT1-MMP, MMP-13) and elastin (MMP-9, MMP-12 and cathepsins). Of these, MMP-1 and MMP-9 have been most consistently demonstrated in the lungs of COPD patients. It is likely that cigarette smoke mediates its destructive changes through both alterations in collagen and elastin. The disease model set forth is outlined in Fig. 1. Cigarette smoke leads to the recruitment of macrophages into the lung. These macrophages secrete cytokines which further augment the inflammatory response, leading to the induction and release of proteolytic enzymes by macrophages and neutrophils. This proteolytic cascade leads to both collagen and elastin degradation. The macrophages and cigarette smoke then stimulate the parenchymal lung cells in patients susceptible to emphysema to produce proteolytic enzymes. This results in a significant imbalance of collagenase leading to altered fibril arrangement. The initial damage is followed subsequently by a repair process that may result in increased collagen deposition in the lung. These structural changes alter the balance of opposing forces in the lung and lead to the pathology of emphysema. The specific molecular changes, which occur in the parenchymal lung cells in response to cigarette smoke and inflammatory cytokines, will be the focus of future studies investigating the pathogenesis of emphysema.

Figure 1



Proposed proteolytic cascade in emphysema. **(a)** The exposure to cigarette smoke leads to recruitment of macrophages into the lung. Macrophages secrete cytokines which further augment the inflammatory response leading to the induction and release of proteolytic enzymes by macrophages and neutrophils, including matrix metalloproteinases (MMPs) (MMP-1, -2, -8, -9, -12 and membrane type matrix metalloproteinase-1 [MT1-MMP]), inhibitors (tissue inhibitor of matrix metalloproteinase [TIMP-1]) and neutrophil elastase, that lead to the remodeling of the lung architecture. This proteolytic cascade leads to both collagen and elastin degradation. The cigarette smoke exposure and inflammatory cells also stimulate the lung parenchymal epithelial cells to secrete proteolytic enzymes which contribute to the destructive process in emphysema. The macrophages and cigarette smoke then stimulate the parenchymal lung cells in patients susceptible to emphysema to produce proteolytic enzymes which results in a significant imbalance of collagenase leading to altered fibril arrangement. The initial damage is followed subsequently by a repair process in which fibroblasts participate inremodeling the collagen matrix of the lung. These structural changes alter the balance of opposing forces in the lung and lead to the pathology of emphysema. **(b)** Disruption of the extracellular matrix (ECM) content by proteolysis upsets the balanced forces within the lung and leads to alterations in alveolar structure.

Much work lies ahead to define the role of collagenase in the development of emphysema more clearly. Future experiments will need to identify the factors that regulate protease production within the pneumocyte and may explain why only 10–20% of smokers develop emphysema. As newer and more efficacious MMP inhibitors are developed, it is conceivable that these agents may be utilized in the future to halt the destructive changes associated with this disease. Given the tremendous economic, physical and emotional toll that this disease inflicts, it is imperative that research into understanding and treating emphysema is supported. It is only through such efforts that specific therapies for emphysema will be developed.

Acknowledgements

We thank K Chada, A Marks and R Cole for critical reading of the manuscript and N Kolesnikova for assistance in the preparation of Figure 1. This work is supported by a National Institutes for Health RO1 grant AG16994-0 (J D'Armiento). J D'Armiento is a recipient of a Burroughs Wellcome Fund Career Award in the Biomedical Sciences.

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