Abstract

Chronic obstructive pulmonary disease (COPD) is a major health problem worldwide, and we have little specific therapy to offer these patients. One potential strategy to limit loss of lung function in COPD would be to inhibit matrix-degrading proteinases. Several serine proteinases and matrix metalloproteinases are expressed in association with COPD in humans. Application of gene-knowledge regarding the mechanisms of COPD, elastase to a mouse model of cigarette-smoke-induced emphysema has uncovered roles for these proteinases in airspace enlargement, and has identified many interactions between these proteolytic systems.

Introduction

Chronic obstructive pulmonary disease (COPD) and other diseases related to cigarette smoking are epidemic worldwide, and despite increasing knowledge regarding the mechanisms of COPD, there has been limited translation into effective pharmacotherapy. Strategies to inhibit lung destruction could halt the progression of airspace enlargement and perhaps airflow obstruction in COPD.

COPD is defined as “a disease state characterized by the presence of airflow obstruction due to chronic bronchitis or emphysema” [1]. Exposure to cigarette smoke leads to a variety of changes in both airways and lung parenchyma that result in the syndrome termed COPD. A variety of proteinases participate in COPD, with their major effect being destruction of the extracellular matrix (ECM), particularly elastin. However, proteinases also play a role in regulating inflammation through the generation of chemokines and cytokines, and by blazing trails for cells through tissue barriers. Neutrophil elastase (NE) is also a potent secretagogue [2]. Thus proteinases participate in multiple activities in several anatomical sites in the lung during the development of COPD.

Potential role in COPD of proteinases at different anatomical sites

Exposure to cigarette smoke causes excess mucus production, non-specific hyperresponsiveness, and cough. In susceptible individuals this is followed by small-airway narrowing, and ulti-
mately slow loss of ECM and alveolar units leading to the insidious onset and progression of breathlessness. Throughout, the course is complicated by infections of the upper respiratory tract that might lead to stepwise loss of lung function. Thus COPD involves changes at multiple anatomical levels in the lung, resulting in airflow obstruction and dyspnoea.

**Large airways**

Chronic bronchitis is defined by cough and sputum production that results from cigarette smoke-induced mucous gland enlargement and goblet cell hyperplasia in the large airways. Whether these abnormalities cause airflow limitation directly is controversial. The serine proteinase NE causes goblet cell degranulation and mucin production [3]. Epithelial cells undergo squamous metaplasia with loss of cilia. This not only predisposes the tissue to carcinogenesis, but the impaired mucociliary clearance increases the risk of airway infections. Both bacterial and inflammatory host proteinases may promote airway remodelling, further decreasing bacterial clearance.

**Small airways**

The small airways (≤ 2 mm in diameter) are a major site of airflow obstruction in COPD. Changes that predispose to airway narrowing include goblet cell metaplasia, smooth muscle hypertrophy, excess mucus, oedema and inflammatory cellular infiltration. Reduced surfactant production may increase surface tension at the air–tissue interface, predisposing to airway narrowing or collapse. Airway remodelling with subepithelial fibrosis might be a critical factor in small-airway narrowing [4]. Little is known regarding the mechanisms of airway fibrosis in COPD, but there are several potential mechanisms whereby a proteinase can predispose to fibrosis. This might seem counterintuitive, since proteinases degrade matrix; however, the net effect on collagen depends on whether a particular proteinase can degrade collagen as well as other non-matrix substrates. For example, matrix metalloproteinases (MMPs) degrade insulin-like growth factor binding proteins, releasing profibrotic insulin-like growth factor [5]. Plasmin and other proteinases could release and activate transforming growth factor-β. MMP-2 has been shown to cause fibroblast proliferation [6]. Thus proteinases have multiple effects on cells and matrix that could result in the net accumulation of collagen.

Respiratory bronchiolitis might be of particular importance. Mononuclear inflammatory cells collecting in distal airway tissues may cause the proteolytic destruction of elastic fibres in the respiratory bronchioles and alveolar ducts, where the fibres are concentrated as rings around alveolar entrances. The resulting distortion and narrowing of these structures could be involved in early airflow obstruction in cigarette smoking-related COPD. In addition, small-airway patency is maintained by the surrounding lung parenchyma, which provides radial traction on bronchioles at points where alveolar septa attach. Loss of bronchiolar attachments as a result of ECM destruction may cause airway distortion and narrowing in COPD [7]. The concept of decreased alveolar attachments leading to small-airway obstruction is appealing, because it underscores the mechanistic relationship between loss of elastic recoil and increased resistance to airflow in small airways.

**Alveoli**

Emphysema, a major component of COPD, is defined as enlargement of peripheral airspaces of the lung, including respiratory bronchioles, alveolar ducts and alveoli, accompanied by destruction of the walls of these structures [1]. Emphysema results when cigarette smoke causes inflammatory cell recruitment. These inflammatory cells, and perhaps activated structural cells of the lung, have the capacity to release elastolytic proteinases that locally overwhelm or evade inhibitors, causing destruction of lung elastin and other ECM proteins. Lung destruction coupled with failure to repair abnormal structures leads to airspace enlargement characteristic of emphysema. Much research has focused on identifying specific proteinases involved in this process.

**Specific proteolytic enzymes and their association with COPD**

Inflammatory cell proteinases are believed to be largely responsible for destruction of the alveolar matrix in emphysema. A synthesis of data on the inflammatory cell response following exposure to cigarette smoke suggests the following sequence of events. Macrophages patrol the lower airspace under normal conditions. Acutely, following cigarette smoke exposure, macrophages may be activated and neutrophils arrive quickly. Subacutely, macrophages accumulate in respiratory bronchioles. Chronically, macrophages, neutrophils and CD8+ > CD4+ T cells accumulate in the airspace. Thus proteinases associated with
these inflammatory and immune cells might participate in emphysema. In addition, non-inflammatory mechanisms of emphysema are required to explain airspace enlargement seen experimentally, associated with vascular endothelial growth factor receptor-2 inhibition [8] and starvation-induced emphysema [9]. Epithelial cell proteinases are probably induced in these instances, with resultant lung destruction.

Serine proteinases
Serine proteinases associated with COPD belong largely to the SA clan, S1 (trypsin/chymotrypsin) family; they include NE, proteinase 3 and cathepsin G. S1 serine proteinases are characterized by conserved His, Asp and Ser residues that form a charge-relay system that functions by transfer of electrons from the carboxyl group of Asp to the oxygen of Ser, which then becomes a powerful nucleophile that is able to attack the carbonyl carbon atom of the peptide bond of the substrate. These enzymes are synthesized as preproenzymes in the endoplasmic reticulum and are processed by cleavage of the signal peptide (pre-) and removal of a dipeptide (pro-) by cathepsin C, and are then stored in granules as active packaged proteins. Distinct subsets of serine proteinases are expressed in a lineage- and developmentally restricted manner in immune and inflammatory cells. For example, NE, proteinase 3 and cathepsin G are major components of primary or azurophil granules that are formed during a very specific stage during the development of myeloid cells. These proteinases are also present in a subset of peripheral blood monocytes, but not in differentiated tissue macrophages. Other immune cells, such as mast cells, also contain significant stores of serine proteinases.

As discussed above, NE is a potent secretagogue, and the resultant mucus might aggravate airflow obstruction in COPD. However, the main role of NE relates to its capacity to cause alveolar destruction. NE has been prominent in our understanding of the pathogenesis of emphysema since the inception of the elastase/anti-elastase hypothesis 40 years ago. This hypothesis was built upon two seminal observations. First, instillation of elastases, and only elastases, results in emphysema [10]. Secondly, patients with a deficiency of α1-antitrypsin, the endogenous inhibitor of NE, are at increased risk of emphysema [11]. Yet, despite these compelling observations, and the fact that neutrophils are recruited to the lung in response to cigarette smoke, it has been difficult to convincingly establish a role for NE in emphysema. The association of NE with human emphysema has been inconsistent, the extracellular release of NE has been questioned, and other proteinases have been shown to play a role in experimental models of emphysema. For example, as discussed below, wild-type mice exposed to cigarette smoke develop emphysema, while mice deficient in the MMP macrophage elastase (MMP-12−/−) do not. NE-deficient (NE−−) mice have now also been generated by gene targeting [12] and exposed to cigarette smoke. Smoke-exposed NE−− mice developed only 40% as much airspace enlargement as wild-type mice (N. Goldstein and S. D. Shapiro, unpublished work). It appears that MMPs and NE interact in many ways to enhance lung destruction; for example, NE activates MMPs, inhibits tissue inhibitors of metalloproteinases (TIMPs) and promotes monocyte migration into the lung. In turn, MMPs degrade α1-antitrypsin.

MMPs
The MMPs comprise a family of 24 matrix-degrading enzymes believed to be essential for development and tissue remodelling and repair. Abnormal expression of MMPs has been associated with many destructive processes, including tumour cell progression, arthritis, atherosclerosis, arterial aneurysms and pulmonary emphysema. MMP family members share 40–50% identity at the amino acid level and possess common structural domains. MMP catalytic activity is dependent on co-ordination of a zinc ion at the active site, and is inhibited by TIMPs-1–4. MMPs are secreted as inactive proenzymes and are activated at the cell membrane surface or within the extracellular space by proteolytic cleavage of the N-terminal domain. An exception to this is the storage of neutrophil MMPs in specific granules within the cell.

MMPs are characterized by their capacity to degrade the ECM, although they also cleave a variety of non-ECM proteins. For example, MMPs cleave and activate latent tumour necrosis factor α, thereby regulating inflammation; they cleave plasminogen, generating the angiogenic fragment angiostatin [13]; and, as mentioned, MMPs [14], particularly MMP-12 [15], degrade and inactivate α1-antitrypsin, thus indirectly enhancing the activity of NE. Thus MMPs play both direct and indirect roles in matrix destruction associated with emphysema, and may indirectly influence cytokine release and angio-
genesis that could in turn influence the development and progression of COPD.

Proteases expressed by neutrophils and macrophages that have the capacity to degrade elastin are perhaps the best candidates for causing lung destruction in COPD. These include MMP-9, a product of both cell types, and MMP-12, a macrophage elastase. However, other ECM components such as collagen may also be targets of both inflammatory cell and structural cell proteinases. Correlative studies in human emphysematous lung tissue have demonstrated the presence of MMP-1 (collagenase), MMP-2 (gelatinase A), MT1-MMP (membrane type-1 MMP) [16], MMP-9 (gelatinase B) (D. Rosenbluth, S. D. Shapiro and R. M. Senior, unpublished work) and MMP-12 [17]. Reverse transcription-PCR of cultured macrophages found a correlation between MMP-1 and MMP-9 in smokers with emphysema as opposed to smokers without emphysema [18]. This study suggests that expression of certain MMPs might predict those smokers that are susceptible to emphysema.

The application of gene-deficient mice to disease models represents a means of performing highly controlled experiments in mammals. To determine directly the contribution of individual elastases to the development of emphysema, mice have been generated by gene targeting that are deficient in specific proteinases. These mice have been applied to a model of cigarette smoke-induced emphysema. Long-term exposure of mice to a regime of 2 cigarettes/day for 6 days/week was well tolerated, and resulted in inflammatory cell recruitment and airspace enlargement similar to human emphysema [19]. Unlike wild-type mice, MMP-12-deficient mice were protected from the development of emphysema, despite heavy long-term smoke exposure. Surprisingly, MMP-12−/− mice also failed to recruit monocytes into their lungs in response to cigarette smoke. Because MMP-12 and most other MMPs are not expressed by monocytes, it appeared unlikely that MMP-12 is involved in the transvascular migration of monocytes. The current working model is that cigarette smoke induces constitutive macrophages to produce MMP-12, which cleaves elastic fibres and generates fragments that are chemotactic for monocytes. This positive-feedback loop perpetuates macrophage accumulation and lung destruction. The concept that proteolytically generated elastin fragments mediate monocyte chemotaxis is not original. Independent studies by Senior et al. [20] as well as Hunninghake et al. [21] from the early 1980s demonstrated that elastase-generated elastin fragments are chemotactic for monocytes and fibroblasts. Gene targeting studies reinforce the suggestion that this process is a major mechanism of macrophage accumulation in a chronic inflammatory condition in vivo.

Cysteine proteinases

Cysteine proteinases utilize the thiol group of a cysteine residue as a nucleophile. Similar to serine proteinases, a proton donor from histidine forms a catalytic dyad (and in some cases triad) required for endopeptidase activity. Cysteine proteinases represent a large, diverse group of plant and animal enzymes with amino acid identity at the active site only [22]. Cysteine proteinases are inhibited by cystatins, which are also ubiquitously expressed. Human alveolar macrophages produce the lysosomal thiol proteinases cathepsins B, H, L and S, which have been implicated in COPD. CD4+ and natural killer cells also express cathepsin S, which functions in antigen processing [23]. These enzymes have an acidic pH optimum, but cathepsin S retains ~ 25 % of its elastolytic capacity at neutral pH (making it similar to NE). Cathepsin K is a potent elastase that is expressed predominantly in osteoclasts, but also in macrophages in the vasculature and perhaps other tissues.

Cathepsins are likely to be induced in COPD, due to their expression by macrophages, T cells and perhaps cell types. These enzymes clearly have the capacity to cause lung destruction if targeted to the cell surface or extracellular space, particularly in acidic microenvironments. There have been few data regarding the direct role of cysteine proteinases in COPD. Recently, inducible overexpression of interleukin-13 in transgenic mice resulted in inflammation and the release of metallo- and cysteine proteinases, resulting in emphysema [24]. This lesion was partially abrogated by cysteine proteinase inhibitors [24].

Summary

Although the proteinase/antiproteinase hypothesis has remained intact for nearly 40 years, many fundamental questions related to this hypothesis are still unanswered. With respect to inflammation, we need to understand cigarette-dependent and -independent mechanisms of inflammation that initiate and perpetuate inflammation in COPD. Inflammatory cells are the presumed source of injurious proteinases; however, we are considering the role of resident cell pro-
teinases and asking if proteinases serve additional functions in the disease process. We have long held elastin degradation to be essential for the development of emphysema, but definitive evidence is lacking and the involvement of other ECM proteins is intriguing. However, while collagen is lost from the alveolar space, it appears to accumulate in the small airways. Understanding the complexities of matrix turnover is essential. Finally, when the damage has been done, how do we go about repairing emphysematous lung tissue? Understanding the mechanisms of normal lung development is a reasonable place to start.

References


Role of newly synthesized fibronectin in vascular smooth muscle cell migration on matrix-metalloproteinase-degraded collagen

E. Stringa*, D. White†, R. S. Tuana*, V. Knauper‡ and J. Gavrilovic§*†

*School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K., †Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA 19107, U.S.A., ‡Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis and Musculoskeletal and Skin Disease, National Institutes of Health, Bethesda, MD 20892, U.S.A., and §Department of Biology, University of York, York YO10 5YW, U.K.

Abstract

The migration of vascular smooth muscle cells (VSMC) is known to be a key process in the development of a number of vascular lesions, although the precise mechanisms involved have still to be elucidated. In the present study, the production of endogenous fibronectins by VSMC migrating across intact and matrix-metalloproteinase-degraded collagen type I has been explored. Cellular fibronectin seems to play a role in the enhanced migration seen when VSMC are exposed to degraded collagen and platelet-derived growth factor-BB. VSMC were found to synthesize both exon IIIA-containing fibronectin (which predominated) and exon IIIIB-containing fibronectin. When these cells were exposed to substrates consisting of recombinant exon IIIA- or exon IIIIB-containing fibronectin, rates of mi-

© 2002 Biochemical Society 102