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# Role of Connective Tissue Proteases in the Pathogenesis of Chronic Inflammatory Lung Disease

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**The normal structure and function of the human lung is dependent on the maintenance of the connective tissue matrix. These structural macromolecules provide the template for normal parenchymal cell architecture on which efficient gas exchange depends. In addition, the organization and amount of this extracellular matrix accounts for much of the mechanical behavior of the lung parenchyma during the respiratory cycle. The preservation of this intricate connective tissue scaffold depends on the lung's capacity to prevent enzymatic disruption of the component matrix proteins. Specifically, the integrity of the normal connective tissue skeleton of the lung is determined by the maintenance of a balance between proteases capable of cleaving these structural elements and the specific protease inhibitors. The normal extracellular matrix is preserved when the local concentrations of protease inhibitors prohibits expression of active connective tissue proteases within the lung parenchyma. Conversely, the disruption of lung structure during the course of acute and chronic inflammatory diseases of the lung is often associated with an imbalance of protease-antiprotease activity. The consequence is the expression of unimpeded proteolytic attack on the connective tissue matrix of the lung. In this context, the nature of the pulmonary lesion and its physiologic consequences, reflect the specificity of the expressed proteases for the individual connective tissue components. Experimental evidence suggests that the differential expression of collagenase and elastase, prototypes of connective tissue proteases, may determine whether the pathologic outcome is fibrosis (e.g., idiopathic pulmonary fibrosis) or destruction (e.g., emphysema) of the alveolar structures.**

The structure and function of the normal lung are intimately dependent on the integrity of the connective tissue elements comprising the alveolar structures (*1*). These macromolecules provide the scaffolding that permits the normal cellular architecture necessary for effective gas exchange and account for the normal mechanical behavior of the lung during the respiratory cycle.

One of the most devastating consequences of inflammation in the lung results from the action of cellular enzymes that cleave the polypeptide backbone of the connective tissue proteins within the alveolar structures (*2,3*). When the local concentration of these connective tissue proteases exceeds the capacity of the lung to check their activity, the resultant disruption of the connective tissue matrix precludes its critical participation in the complex process of gas exchange.

The nature and physiologic consequences of the pulmonary lesion resulting from proteolytic injury reflect the specificity of certain proteases for the structural macromolecules comprising the extracellular matrix of the alveolar structures. In fact, both collagenase and elastase, classes of proteases whose substrates include the principal structural elements of lung connective tissue, have been implicated in several diffuse, chronic lung diseases (*4–6*). In this context, we will consider: (1) the components of normal lung connective tissue; (2) the proteases relevant to their disruption in diffuse lung disease; (3) the modulation of protease activity within the alveolar structures; and (4) connective tissue proteases and antiproteases in human lung disease.

## Connective Tissue Matrix of the Human Alveolar Structures

The major components of the lung's connective tissue include collagen, elastic fibers, proteoglycans and other glycoproteins (*1,7*). Of these matrix proteins, collagen is the most prevalent, comprising 6 to 20% of lung dry weight (*8*).

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## Collagen

Collagen is found in the alveolar structures in at least four different molecular forms. Among the pulmonary connective tissue constituents, the molecular structure of collagen is the best characterized. The collagen molecule is a rodlike unit formed by three polypeptide chains (α-chains) arranged in the form of a triple helix (*9*). These triple helical molecules polymerize to form collagen fibrils, the structures that ultimately account for the tensile strength of collagenous connective tissue.

An unusually repetitive primary structure of the collagen α-chain plays a critical role in conferring a unique helical structure to the collagen molecule. Along the collagen α-chain, glycine, proline and hydroxyproline residues appear as triplet of gly-pro-X (where X is commonly hydroxyproline), a structure that is repeated frequently along the length of the polypeptide chain (*9,10*). Although the precise nature of the interchain interactions that produce the unique triple helix of the collagen molecule have not been completely delineated, it is known that the relative position and frequency of each of these amino acids, particularly glycine and hydroxyproline, play an important role in the formation of the helix. In addition to this common triplet repeat, the α-chains also contain lysine and hydroxylysine, residues that are involved in formation of covalent crosslinks that permit the assembly of collagen molecules into fibrils (*9,10*).

The principal differences among the collagen types are the amino acid sequence within the polypeptide chains and the types of polypeptide chains comprising the triple helical molecule. Differences in the level of hydroxylation of proline and lysine residues, glycosylation of the lysine residues, the presence of disulfide crosslinks and frequency of covalent crosslinks account for the distinct macromolecular arrangement of each of these collegen types and result in a variable capacity of the different collagens to form fibrils.

Type I collagen, composed of two $\alpha_1$ (I)-chains and one $\alpha_2$ (I)-chain, is the most abundant collagen type present in the alveolar structures (*1,8,10*). Type I collagen is distributed diffusely within the alveolar interstitium but is not present in the endothelial or epithelial basement membranes.

Type III collagen comprises three identical $\alpha_1$ (III) polypeptide chains. Like Type I, it is an interstitial collagen and partially corresponds to "reticulin" demonstrable by light microscopy.

Type IV collagen is the major collagen type comprising epithelial and endothelial basement membranes in the lung; it is not found in the interstitial matrix (*10*). Type IV collagen is composed of at least two distinct polypeptides [$\alpha_1$(IV), $\alpha_2$ (IV)], but it is not clear whether these chains combine to form the Type IV molecule, or whether the different chains segregate into separate Type IV collagens composed of homogeneous α-chains (*9*).

Type V collagen is also present in the alveolar structures but its exact location is controversial. While some investigators assign it solely to the basement membrane, others find it throughout the interstitium (*9–11*). In general, it is considered to be a "pericellular" collagen that forms a surface matrix around cells such as smooth muscle cells. Type V collagen is composed of three chains [$\alpha_1$ (V), $\alpha_2$ (V), $\alpha_3$ (V)], but like Type IV collagen, the exact chain distribution within each Type V molecule is controversial (*9,10*).

In the human alveolar structures, the ratio of Type I and Type III collagen is approximately 3:1 (*8*). Together, Type I plus Type III comprise 90 to 95% of the total collagen, with Type IV and Type V making up the remainder. The contribution of each collagen type to the structure and function of the alveolar interstitium is incompletely understood. By virtue of its tensile strength, abundance, distribution, and ability to form fibers up to 200 nm in diameter, Type I likely plays a dominant role, determining alveolar shape and distensibility (*1,10*). In addition, Type I collagen contributes to the cellular organization of the interstitium by serving as a site of attachment for fibroblasts through another matrix glycoprotein, fibronectin (see below) (*11*). The role of Type III is less clear, but it also serves as an anchor for fibronectin-mediated cell attachment. The mechanical properties of Type III are not known, but there is evidence that it may modulate Type I fiber formation (*10*). Type IV collagen likely modulates basement membrane structure and mechanical properties (*11*). Although not yet studied with lung cells, it is known that Type IV can serve as an attachment site for epithelial cells (*12*) through laminin, another basement membrane consituent (see below). The role of Type V collagen in lung structure and function is unknown. There is some recent evidence that it may play a role in fixing basement membranes to the underlying stroma (*9–11*).

## Elastic Fibers

Elastic fibers are composed of at least two components, elastin and microfibrils. Elastin is composed of large, extremely insoluble polymers of crosslinked, identical protein subunits called tropoelastin (*1,10*). The tropoelastin polypeptide has a molecular weight of approximately 70,000 daltons and contains large proportions of nonpolar amino acids such as leucine, proline and valine. Like collagen, elastin also contains hydroxyproline residues; however, these are infrequent and represent only 1% of tropoelastin's amino acids (compared to 10% for collagen) (*10*). Unlike other connective tissue components present in the alveolar structures, elastin contains no carbohydrate side chains (*13*). The tropoelastin units of elastin are linked together by covalent bonds through the lysine residues. While two of these crosslink units, desmosine and isodesmosine, are unique to elastin, a third, the lysinonorleucine crosslink, is also present in collagen (*10*). Current information suggests that the tropoelastin monomer is

similar throughout the lung; there is little evidence of elastin polymorphism (*14*). While there is a relative paucity of information regarding the microfibrillar component of elastic fibers, it is distinct from elastin by virtue of the prevalence of polar, acidic amino acids, and the presence of cysteine and carbohydrate side chains (*13*).

Light microscopic evaluation suggests that elastic fibers make up a branching fiber network throughout the interstitium of the alveolar structures (*15*). At the ultrastructural level, elastic fibers appear either cylindrical or as fenestrated sheets. The elastin component appears as an amorphous material that forms the core of the elastic fiber while the microfibillar component forms cylinders or cables 10 to 12 nm in diameter (*16*). In the mature lung, elastic fibers are found in close proximity to collagen fibers and proteoglycans (*17*).

Elastic fibers have rubberlike properties, an attribute that undoubtedly plays a major role in modulating elastic recoil during the respiratory cycle. In this context, destruction of elastic fibers is central to the pathogenesis of lung disorders associated with loss of elastic recoil (*2,3*).

## Proteoglycans

The proteoglycans are the principal constituents of the so-called "ground substance" of the alveolar structures. These macromolecules are organized into a central protein core from which extend a number of carbohydrate side chains. Of the proteoglycan molecule, 10 to 30% is polypeptide in nature and the remainder carbohydrate (*10*). The carbohydrate side chains are repeating disaccharide subunits called glycosaminoglycans; in the alveolar interstitium they include hyaluronic acid, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, heparin, and heparin sulfate (*10,17*). The proteoglycans are distributed throughout the interstitium; by analogy to other tissues it is likely that proteoglycans are also present in basement membranes. The contribution of proteoglycans to the mechanical properties of the lung is unknown. However, by virtue of their ability to retain water, proteoglycans may significantly influence function. They may also contribute to lung structure by maintaining the proper spatial relationships between other extracellular components and cells.

## Other Glycoproteins

Fibronectin, a major cell surface glycoprotein, is present in the stroma and basement membranes of the alveolar structures. Fibronectin mediates cell–matrix (and possibly cell–cell) interactions by virtue of its ability to bind to some collagens (particularly Types I and III), and some glycosaminoglycans (*9,10,18*).

Laminin is a large glycoprotein present in basement membranes. It polymerizes into large aggregates and interacts with other connective tissue components, notably Type IV collagen (*18*). As such, laminin is thought to anchor epithelial and endothelial cells to Type IV collagen.

# Connective Tissue Proteases of Potential Relevance to Chronic Inflammatory Diseases of the Alveolar Structures

It is logical to expect that the proteases with potential relevance to the pathogenesis of lung disease are those whose substrates include the major structual connective tissue proteins (Table 1).

## Collagenase

Given the critical contribution of collagen to the structure of the normal lung, the presence of enzyme

**Table 1. Connective tissue proteases of potential relevance to chronic inflammatory diseases of the alveolar structures.**

| | | | | Collagen type | | | | Elastic fibers | | Other glycoproteins | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protease | Cellular source | Presence in normal lung | Form when released | I | III | IV | V | Elastin | Microfibrillar component | Proteoglycans | Fibronectin | Laminin |
| Collagenase | Alveolar macrophage | Yes | Latent | Yes[a] | Yes[a] | | Yes[b] | No | ? | No | No | No |
| | Fibroblast | Yes | Latent | Yes | Yes[a] | | | No | ? | No | No | No |
| | Neutrophil | No | Active | Yes[a] | No | | | No | ? | No | No | No |
| | Eosinophil | No | ? | Yes[a] | Yes[a] | | | No | ? | No | No | No |
| Elastase | Alveolar macrophage | Yes | Active | ? | ? | | ? | Yes | ? | ? | ? | ? |
| | Neutrophil | No | Active | No[c] | Yes[d] | | No | Yes | ? | Yes | Yes | ? |
| Cathepsin G | Neutrophil | No | Active | No[c] | No | No | ? | Yes[e] | ? | ? | ? | ? |

[a] Site of attack at or near classic collagenase sensitive site ¾ of the length of the molecule from the N-terminus of Type I and Type III collagen (*20*).
[b] Cultured macrophages produce a metallo proteinase that degrades Type V collagen (*35*).
[c] Attacks N- and C-terminal nonhelical ends (telopeptides) of the collagen molecule, but does not degrade Type I helex (*37*).
[d] Site of cleavage is in a region of the triple helix of Type III that is less tightly coiled (*32,41*).
[e] Cleavage of native elastin occurs at a rate 10-fold less rapid than neutrophil elastase (*45*).

within the alveolar structures capable of cleaving the collagen molecule would be expected to have a profound effect on structure and function of the lung. Mammalian collagenases are unique among proteases in their ability to cleave helical Type I collagen at neutral pH (*19*). The classical human collagenases are metalloproteases; i.e., they require the presence of divalent cations in order to express their activity. Furthermore, collagenases are commonly secreted in a latent form, necessitating partial proteolysis for the generation of the active enzyme (*20*).

The action of a classical collagenase is distinguished by a remarkable degree of substrate specificity. Among all the structural proteins of the lung, the action of human collagenases is restricted to native, triple-helical collagen (*19,20*). Cleavage of Type I collagen by this class of proteases typically occurs at the leu–gly peptide bond approximately three-quarters of the length of each component α-chain from its amino terminal end. This proteolysis cleaves the Type I molecule into two pieces, an *N*-terminal piece that represents three-quarters of the molecule and a *C*-terminal piece that represents the remaining one-quarter. Because neither piece can maintain a stable triple helical conformation at body temperature, they denature, leaving the cleaved component α-chains which are then vulnerable to attack by nonspecific proteases (*21,22*).

The alveolar macrophage (*23*), lung fibroblast (*24*), neutrophil (*25*) and eosinophil (*26*) are potential cellular sources of collagenase within the lower respiratory tract of man. With the notable exception of the neutrophil, collagenase release from these cells appears to depend upon *de novo* protein synthesis of the enzyme. In contrast, the bulk of the neutrophil's collagenase activity exists in a preformed state that is stored after synthesis (*27*). Recent studies have demonstrated that the neutrophil collagenase is contained in the "specific" (secondary) lysosomal granules of the neutrophil and is readily released into the extracellular milieu in response to a variety of phagocytic and inflammatory stimuli (*28*).

For the alveolar macrophage (*23,24*), the fibroblast (*25*) and possibly the eosinophil (*26*), collagenase is released in an inactive form. The mechanisms that control activation *in vivo* are not clear; in most cases, however, it is likely modulated by other neutral proteases (*29*). At least for the rabbit, the alveolar macrophage releases a latent collagenase as well as a nonspecific neutral protease capable of activating the collagenase (*29*).

The mechanisms regulating the release of collagenase by the fibroblast and eosinophil are unclear. For the neutrophil, most inflammatory stimuli (e.g., phagocytosis, immune complexes) result in rapid collagenase release. The same is true for alveolar macrophages from experimental animals (*23*). For human alveolar macrophages, however, collagenase release appears to be constitutive i.e., unaffected by stimulation (*24*).

Not only does collagenase demonstrate specificity for the general class of macromolecules termed "collagens," but certain collagenases are specific for certain collagen types. Alveolar macrophage, fibroblast and eosinophil collagenases are examples of classic collagenases: they degrade collagen Types I and III. In contrast, neutrophil collagenase readily attacks Type I but not Type III (*27,30*).

In the normal alveolar structures, the only known sources of collagenase are the alveolar macrophage and the fibroblast (*31*). Presumably, these collagenases are responsible for the turnover of interstitial collagen that continually occurs in the normal lower respiratory tract. In a number of chronic inflammatory disorders, however, neutrophils and/or eosinophils are also present, significantly adding to the collagenase burden (*32*). Furthermore, many of these disorders are also characterized by increased numbers of alveolar macrophages and/or fibroblasts in the alveolar structures, further increasing the potential sources of collagenases.

## Elastase

In contrast to the collagenases, the elastases are neutral proteases that attack all of the connective tissue structural proteins of the alveolar structures (*33–36*) (Table 1). Their designation as "elastases" derives from their ability to cleave the peptide bonds of the native, insoluble elastin molecule (*37*). However, at least one prototype of this class, neutrophil elastase, is capable of degrading collagen (*32,34,35*) elastic fibers (*37*), the protein portion of proteoglycans (*36*) and fibronectin (*33*).

Studies of alveolar macrophage and neutrophil elastases have demonstrated that while both are capable of cleaving elastin, they are different enzymes (*23,24*). For example, while the elastase of the human neutrophil has a serine-dependent active site and does not require free divalent cations for its elastolytic activity (like human and porcine pancreatic elastase) (*38*), the human alveolar macrophage releases an elastolytic enzyme that is inhibited by metal chelating agents (*24*). This suggests that, like the murine peritoneal macrophage, the human alveolar macrophage elastase is a metalloprotease rather than a serine protease (*23,24*). Furthermore, as in the case of collagenases, there are potentially important differences in the packaging and amounts of elastaselike enzymes produced by the human alveolar macrophage and the neutrophil. The alveolar macrophage releases only small quantities of its elastase under conditions of cell culture and requires *de novo* protein synthesis to do so (*24*). Like the regulation of collagenase release, the human alveolar macrophage releases elastase in a constitutive fashion (*24*). In contrast, the neutrophil carries large amounts of its serine elastase in a preformed state and rapidly releases this elastase in response to inflammatory stimuli (*2*). Neutrophil elastase is consigned to the primary (i.e., azurophilic) lysosomal granules (*27*).

Since the alveolar macrophage is the only known source of elastase in the normal lower respiratory tract,

it is presumed that its elastase accounts for turnover of elastin that normally takes place in the alveolar structures. In some chronic inflammatory disorders, however, there are two potential sources: the increased number of alveolar macrophages and neutrophils (*31*). Interestingly, recent studies by Campbell et al. have demonstrated that human alveolar macrophages have a surface receptor that recognizes neutrophil elastase (*39*). This is likely mediated by mannose receptors, a general class of receptors on the macrophage surface that recognizes carbohydrate residues on neutrophil elastase (*40*).

### Other Proteases

In addition to collagenase and elastase, there are undoubtedly other proteases capable of destroying at least some of the proteins that make up the connective tissue framework of the alveolar structures. For example, pepsin can attack the telopeptides of collagens Type I and III, trypsin attacks Type III collagen near the "collagenase" site (*41*), cathepsin B attacks the protein portion of proteoglycans (*42,43*), trypsin destroys fibronectin (*18,44*) and thrombin cleaves laminin (*45*). The relevance of such proteases to chronic inflammatory disease of the lower respiratory tract is unknown. However, the alveolar macrophage does release at least one nonspecific neutral protease (*29*) and the neutrophil carries cathepsin G (a nonspecific neutral protease that can also attack elastin, albeit at a very slow rate) in its primary granules (*46*). Such enzymes likely play a role in disorders of the alveolar structures, but their relative importance and substrate targets in these situations are not completely understood (*2*).

## Modulation of Protease Activity within the Alveolar Structures

In view of the susceptibility of the connective tissue framework of the lung to the action of collagenase and elastase, it is apparent that the normal structure-function characteristics of the lung must be dependent on homeostatic mechanisms that serve to minimize the access of these proteases to the protein skeleton of the lung. This function is subserved by: (1) regulation of the influx of protease producing cells to the lung parenchyma and (2) antiproteases, a class of proteins that modulate protease activity by forming intermolecular complexes with proteases.

### Regulation of the Influx of Protease Producing Cells

The extent of protease activity within the alveolar structures depends, to a large extent, on the burden of protease producing cells present. Beyond that present in the normal lung, the presence of protease producing cells in the lower respiratory tract depends on the status of the mechanisms capable of modulating the directed migration (i.e., chemotaxis) (*47*) of inflammatory cells from the circulating blood pool into the lung parenchyma. The chemotaxis of inflammatory cells from blood to lung depends upon chemotactic factors within the alveolar structures and chemotactic factor inhibitors that modulate the action of the chemotactic factors (*21*).

The chemotactic factors most relevant to the collagenase and elastase burden of the lung are those that attract neutrophils. In chronic lung disorders, most neutrophils are attracted by a chemotactic factor released by alveolar macrophages (*48*). Following a variety of stimuli, the macrophage releases at least two chemotactic factors that attract neutrophils, a low molecular weight lipid and a protein (*48,49*). In acute inflammatory disorders where there is significant transudation of plasma into the alveolar structures, C5a, a fragment of the C5 component of complement undoubtedly also plays a role in attracting neutrophils (*50*).

Modulation of chemotactic factor activity is provided by circulating inhibitors of chemotactic factors (so called "chemotactic factor inhibitor") as well as inhibitors of chemotaxis directed at the inflammatory cell itself (cell-directed inhibitors) (*47*). Through this system of regulatory proteins, the influx of protease-bearing inflammatory cells (primarily, the circulating neutrophil) can be checked, preventing excessive infiltration of the alveolar structures with neutrophils and their preformed proteases (*2*).

The collagenase and elastase burden of the lower respiratory tract may also be increased by expanding the numbers of alveolar macrophages in the alveolar structures. While more selective for neutrophils, both the macrophage derived chemotactic factor and C5a also attract monocytes (*51*), the cellular precursor of alveolar macrophages (*52*). In addition, fragments of elastin attract monocytes (*22,53*), providing a mechanism that expands macrophage numbers in those cases where elastase is allowed to work unimpeded on lung elastin, resulting in elastin fragments.

In normal lung, neutrophils are rarely found (*31*), suggesting that either few chemotactic factors are present and/or chemotactic factor inhibitors are preventing those chemotactic factors present from operating. Consistent with this concept are the findings that: (1) alveolar macrophages from normal individuals are not spontaneously releasing the neutrophil chemotactic factor (*48*); and (2) no active elastase or collagenase can be detected in epithelial fluid obtained from the lower respiratory tract of normal individuals (*31*).

### Antiproteases of the Alveolar Structures

In those instances when chemotactic activity overrides the chemotactic inhibitory mechanisms, and inflammatory cells such as polymophonuclear leukocytes are permitted to enter the alveolar structures, a second line of defense for the connective tissue elements

is provided by the antiproteases present within the extracellular milieu of the lung (Table 2). Given the critical contribution of elastin and collagen to the structural and functional characteristics of the normal lung, the antiproteases most relevant to the integrity of lung structure are those that inhibit the action of collagenase and elastase (*2*).

There are two elastases of potential importance to the alveolar structures: neutrophil elastase and alveolar macrophage elastase.

There are three physiologic inhibitors of neutrophil elastase that have been considered as possible antielastases of the lower respiratory tract, including: $\alpha_1$-antitrypsin, $\alpha_2$-macroglobulin, and the so-called "bronchial mucus inhibitor" (*22*). However, direct assessment of the antineutrophil elastase protection of the human alveolar structures has shown that, by far, $\alpha_1$-antitrypsin is the major antineutrophil elastase (*22*). Although it is a serum protein produced by hepatocytes, $\alpha_1$-antitrypsin has a molecular weight (52,000 daltons) that permits ready diffusion through the lower respiratory tract. In fact, $\alpha_1$-antitrypsin is found in the same concentration in lung (relative to albumin) as in serum, and $\alpha_1$-antitrypsin provides greater than 95% of the functional antineutrophil elastase protection to the alveolar structures (*22*). In contrast, $\alpha_2$-macroglobulin, another serum antiprotease, is mostly excluded from the human alveolar structures as a result of its large molecular weight (725,000 daltons) (*22,54*). While resident alveolar macrophages can be shown to produce $\alpha_2$-macroglobulin in cell culture (*55*), locally produced $\alpha_2$-macroglobulin likely makes little contribution to the total antineutrophil elastase activity of the lower respiratory tract. In fact, the ratio of $\alpha_1$-antitrypsin to $\alpha_2$-macroglobulin recoverable from the alveolar structures is approximately 50:1 on a weight basis and greater than 200:1 on a molar basis (*2,22*). Importantly, although it is the principal antineutrophil elastase of the upper respiratory tract, the bronchial mucus inhibitor does not appear to be produced or distributed at the alveolar level (*22*).

The concept that $\alpha_1$-antitrypsin is the major antineutrophil elastase of the lower respiratory tract has been confirmed by studies performed in patients with serum $\alpha_1$-antitrypsin deficiency. The lower respiratory tract of these individuals is virtually devoid of functional antineutrophil elastase activity, strongly arguing against a significant role for $\alpha_2$-macroglobulin and the bronchial mucus inhibitor in contributing significantly to the antineutrophil elastase screen of the lower respiratory tract (*2,22*).

The physiologic inhibitory profile of the human alveolar structures for human alveolar macrophage elastase is not well defined. Unlike neutrophil elastase, the alveolar macrophage elastase is not inhibited by $\alpha_1$-antitrypsin, the principal antineutrophil elastase of the human lower respiratory tract. In addition, there is disagreement regarding resistance of macrophage elastase to $\alpha_2$-macroglobulin, the antiprotease with the broadest inhibitory profile (*7*). However, in view of the paucity of elastaselike activity produced by the human alveolar macrophage and the fact that human macrophage release of elastase does not appear to be augmented by inflammatory stimuli, the relevance of this macrophage protease and its inhibitory profile to human lung disease remains to be clarified.

While the human alveolar structures appear to have ample antielastase protection, current evidence suggests that it is relatively devoid of anticollagenases. Evaluation of normal individuals by bronchoalveolar lavage has demonstrated that there is significantly less functional anticollagenase activity within the human lower respiratory tract than antineutrophil elastase activity (*56*). This finding implies that when both

**Table 2. Antiproteases of potential relevance of protease-antiprotease balance within the human alveolar structures.**

| | | Protease inhibition profile | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Alveolar macrophage | | | | | | | |
| Antiprotease | Source | Collagenase | Elastase | Collagenase | Neutrophil elastase | Cathepsin G | Molecular weight | Present in lower respiratory tract | Present in upper respiratory tract |
| $\alpha_1$-Antitrypsin | Serum (liver) | 0 | 0 | 0 | Yes | — | 52,000 | (51 ± 11 μg)[a] | Yes[b] |
| $\alpha_2$-Macroglobulin | Serum (liver), lung fibroblasts | Yes[c] | Yes[c] | Yes | +[d] | Yes | 725,000 | (2 ± 0.4 μg)[a] | Yes[b] |
| Bronchial mucus inhibitory | Bronchial epithelium | 0 | ? | 0 | Yes | Yes | 11,000 | (< 1)[a] | Yes[b] |
| $\beta_1$-Anti-collagenase[e] | Serum | ? | 0 | + | 0 | 0 | 50,000 | Probably | ? |
| $\alpha_1$-Anti-chymotrypsin | Serum | ? | ? | 0 | 0 | Yes | 42,000 | ? | ? |

[a] Quantification of antiproteases in the normal lower respiratory tract is expressed as micrograms of antiprotease/mg albumin in order to correct for the variable dilution during bronchoalveolar lavage (*22*).
[b] Data of Gadek et al. (*22*).
[c] Data of Gadek et al. (*24*).
[d] In the presence of equivalent amounts of $\alpha_1$-antitrypsin and $\alpha_2$-macroglobulin, neutrophil elastase binds to $\alpha_1$-antitrypsin exclusively until the protease binding sites of $\alpha_1$-antitrypsin are saturated, i.e., $\alpha_2$-macroglobulin is a "second-line" inhibitor of neutrophil elastase 21.
[e] Data of Wooley et al. (*58*).

collagenase and elastase are released within the alveolar structures, there is a greater opportunity for the expression of uninhibited collagenase activity, i.e., the specificity of proteolytic assault on the connective tissue of lung may be regulated by the quantity of specific antiprotease available at the inflammatory focus within the lung parenchyma.

The precise nature of the anticollagenase activity of the lower respiratory tract is unknown. Although early studies suggested $\alpha_1$-antitrypsin could inhibit neutrophil collagenase, it is now recognized that this is not the case (*56–58*). As yet, no anticollagenases have been identified that are selective for the collagenase of any one cell type relevant to lung inflammation; i.e., alveolar macrophage, neutrophil, or eosinophil collagenases. The principal physiologic anticollagenases relevant to preservation of lung collagen are likely those found in normal human serum, i.e., $\alpha_2$-macroglobulin and $\beta_1$-anticollagenase (*57,58*). As noted earlier, $\alpha_2$-macroglobulin in excluded from the lower respiratory tract as a result of its prohibitively large molecular size (*22*). In contrast, based on its molecular weight of 50,000 daltons, $\beta_1$-anticollagenase should have access to the alveolar structures. However, the serum concentration of $\beta_1$-anticollagenase is only 30 to 50 mg/dL (*57*), approximately one-fifth that of $\alpha_1$-antitrypsin. Although it has not been measured directly, a similar ratio of $\beta_1$-anticollagenase and $\alpha_1$-antitrypsin within the lower respiratory tract would account for the observed differences between anticollagenase and antielastase activity at this site.

The chymotrypsinlike protease of the neutrophil, cathepsin G, is a protease whose role in lung disease remains to be defined. This protease has not been identified within the lungs of patients with any chronic lung disease. In contrast to the other two neutral proteases of the neutrophil, collagenase and elastase, cathepsin G has no specific connective tissue substrate to attack in the lung (Table 1). While it has been shown that cathepsin G is capable of degrading lung elastin, it does so at a rate that is 10-fold slower than equimolar amounts of neutrophil elastase (*45*). However, it is of interest that oxygen radical generation by the neutrophil is dependent on the presence of a chymotrypsinlike protease associated with the cell membrane (*59*). The identity of this chymotrypsinlike neutrophil protease and the question of its relationship to cathepsin G remains to be clarified. Nevertheless, this evidence provides a mechanism through which a neutrophil protease may participate in oxidant mediated lung cell injury as well as proteolytic injury of the extracellular matrix.

Since cathepsin G is presently an uncertain candidate for participation in lung disease, it is understandable that the source and amount of anticathepsin G activity in the normal human lower respiratory tract has not been the subject of extensive study to date. Although human serum contains a potent inhibitor of cathepsin G in the form of $\alpha_1$-antichymotrypsin (*42*), direct measurement of this serum antiprotease in human lower respiratory tract fluid has not been done.

## Connective Tissue Proteases and Antiproteases in Human Lung Disease

Current concepts of the role of proteolysis in human lung disease derive from the theory that maintenance of normal alveolar structure is dependent on the existence of a homeostatic balance between the connective tissue-specific proteases, i.e., elastase and collagenase, and the antiproteases that inhibit these proteases. In this scheme, disease results from an imbalance between the connective tissue proteases and the antiproteases such that expression of connective tissue proteolysis is permitted (*2*). Theoretically, this can occur in two ways: either from an excess of protease or from a relative deficiency of antiprotease. A strong case can be constructed for the relevance of this "protease-antiprotease" theory to the pathogenetic mechanisms operative in two major classes of chronic lung disease: destructive lung disease (i.e., emphysema) and the interstitial (fibrotic) lung diseases.

## Destructive Lung Disease

The earliest evidence suggesting a role for antiproteases in the pathogenesis of destructive lung disease was derived from the studies of Laurell and Eriksson (*60*). While studying serum protein electrophoretic patterns in large numbers of individuals, these investigators noted that, in rare individuals, the $\alpha_1$-globulin band was missing. Remarkably, they found that all of these individuals also had early onset destructive lung disease (*60*). It is now recognized that the missing protein was $\alpha_1$-antitrypsin, the major antielastase of the alveolar structures.

The earliest direct evidence suggesting a role for proteases in destructive lung disease came from the studies of Gross et al. (*61*). By the instillation of papain (a protease with a broad spectrum of activity) into the lungs of experimental animals, these workers were able to produce a lesion which had remarkable similarity to that of human emphysema, i.e., it caused diffuse destruction of alveolar septal walls (*61*). Taking the lead from these studies, other investigators subsequently demonstrated that the severity of the destructive lesion correlated with the relative elastolytic activity of the instilled protease i.e., the relative ability of the enzyme to destroy elastin (*62*). Furthermore, the production of emphysema by the instillation of human neutrophil elastase into experimental animals provides compelling evidence of the potential role of this enzyme in human destructive lung disease (*5,6*). As will be discussed below, there is now clear evidence that active elastase and collagenase can be found in the lungs of at least some individuals with destructive lung disease (*63*).

Theoretically, the maximum opportunity for the expression of free elastase activity within the emphysematous lung would occur in a clinical setting in which both the elastase burden is increased and the antielastase screen in reduced. Indeed, recent studies have shown that individuals with $\alpha_1$-antitrypsin deficiency have both mechanisms contributing to the pathogenesis of their destructive lung disease (*63*).

The $\alpha_1$-antitrypsin-deficient patient has less than 10% of the normal functional antielastase activity within the lower respiratory tract (*22*). Formerly it was presumed that these individuals developed destructive lung disease as a result of the "normal" quantity of neutrophil elastase present within the lung. However, it now appears that there is an influx of neutrophils into the alveolar structures of the $\alpha_1$-antitrypsin-deficient patient who develops destructive lung disease. The mechanism by which neutrophils are attracted to the lungs of these patients is unclear, but it is known that their alveolar macrophages are spontaneously producing a chemotactic factor for neutrophils and that they have a deficiency of a serum chemotactic factor inhibitor (*64*). Regardless of the mechanism, the neutrophil influx in the $\alpha_1$-antitrypsin-deficient lung represents increased elastase activity in concert with deficient antielastase protection, i.e., the optimal situation for demonstration of active elastase within the emphysematous lung. In fact, analysis of fluid recovered from the lower respiratory tract of the majority of $\alpha_1$-antitrypsin-deficient patients demonstrates the presence of active neutrophil elastase (*63*). Furthermore, these individuals also have active neutrophil collagenase within the alveolar structures (*63*). Thus, it is probable that in the case of this form of destructive lung disease, all the connective tissue components of the connective tissue network of the alveolar structures are degraded (*2*).

Recently, several studies have served to demonstrate the potentially important parallels that exist between the inherited form of destructive lung disease (i.e., $\alpha_1$-antitrypsin-deficiency) and the acquired form of emphysema (i.e., cigarette smoking). Current evidence now suggests that exposure to cigarette smoke adversely affects both sides of the protease–antiprotease balance normally protecting the human lung i.e., the antiprotease activity is reduced and protease activity increased in the lung as a result of cigarette smoking. It is known that the oxidative products of cigarette combustion reduce the capacity of $\alpha_1$-antitrypsin to inhibit elastase *in vitro* and in the lungs of experimental animals (*65,66*). In addition, evaluation of the $\alpha_1$-antitrypsin recovered from the lungs of individuals who smoke cigarettes has shown that, although present in normal amounts, the lung $\alpha_1$-antitrypsin of smokers retains only approximately 60% of its elastase inhibitory activity (*67*). In addition, Hunninghake et al. have shown that the lungs of these individuals contain a significant number of neutrophils (*68*). This two-pronged assault on the elastase–antielastase balance of the alveolar structures provides a means by which cigarette smoking results in proteolytic injury to the human lung similar to that which occurs in the genetic form of destructive lung disease, $\alpha_1$-antitrypsin-deficiency (*2*).

## Interstitial Lung Disease

The principles of protease–antiprotease balance operative in destructive lung disease appear to have application to certain aspects of the pathogenesis of the chronic interstitial diseases. In idiopathic pulmonary fibrosis (IPF), a prototype of the interstitial lung diseases, the presence of active collagenase within the lower respiratory tract of affected individuals is associated with marked abnormalities of lung structure and function (*4*). Typically, the alveolar structures of these patients are distorted by derangement of normal collagen structure; there is fragmentation and fraying of the collagen fibers as well as deposition of collagen in a disorderly array (*69*). It is likely that disruption of the collagen fibers apparent at the electron microscopic level reflects specific proteolytic attack by an active collagenase. In this context, bronchoalveolar lavage analyses of these patients have demonstrated that they have an active collagenase in their lower respiratory tract (*4*).

There are several cell types that may represent the source of the active collagenase present in the lower respiratory tract of patients with idiopathic pulmonary fibrosis. In addition to the alveolar macrophage, it is known that the lung fibroblast produces collagenase as does the neutrophil and eosinophil (inflammatory cells that are both present in the alveolar structures of these individuals). However, recent studies of the collagen substrate profiles of the collagenase produced by each of these cell types, when matched with the profile of the enzyme recovered from the IPF lung, strongly suggest that the neutrophil is the source of this collagenolytic activity (*70*).

Evaluation of the antielastase screen of the IPF lung demonstrates that it is close to normal i.e., adequate antielastase protection is provided by $\alpha_1$-antitrypsin, but there is very little anticollagenase protection (*70*). In this context, it becomes apparent why IPF, cigarette smoking and $\alpha_1$-antitrypsin-deficiency are all associated with neutrophils in the alveolar structures, yet IPF is a fibrotic (i.e., collagen derangement) disease, while cigarette smoking and $\alpha_1$-antitrypsin-deficiency are destructive disorders. The most tenable hypothesis to explain the net expression of active neutrophil proteases in the fibrotic and destructive diseases is that the specific form of the disease reflects the modulating effect of specific lung antiproteases. In support of this premise, it has been shown that the IPF lung is devoid of functional anticollagenase (*70*), yet contains nearly 75% of the normal antielastase activity (*67*). In contrast, the $\alpha_1$-antitrypsin-deficient lung is devoid of both anticollagenase and antielastase, and the cigarette smoker's lung has little anticollagenase and 40 to 50% normal antielastase (*2*). Therefore, the net effect of neutrophil accumulation and protease release in the

IPF lung is the persistant action of neutrophil collagenase and the absence of active neutrophil elastase (i.e., the elastase is bound irreversibly by $\alpha_1$-antitrypsin). Conversely, the accumulation of neutrophils in the $\alpha_1$-antitrypsin deficient lung and in the cigarette smokers' lung results in the continuous expression of both collagenase and elastase since these individuals possess insufficient amounts of both protease inhibitors. Thus, it is probable that the specificity of the pulmonary lesion in these two prototypes of chronic diffuse lung disease is a reflection of the local balance between neutrophil connective tissue proteases and their physiologic inhibitors.

Continued study of the quantity and specificity of the connective tissue proteases and antiproteases of the alveolar structures and the factors that serve to maintain the balance necessary to preserve normal lung structure should further the understanding of the pathogenetic mechanisms operative in chronic lung diseases. Importantly, an understanding of these biologic mechanisms offers the opportunity for the development of specific therapy in these chronic disorders.

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