Irreversible abnormalities of lung architecture are hallmarks of many acute and chronic diseases. Lung cavities in gram-negative bacterial pneumonias and pulmonary tuberculosis, the loss of normal lung parenchyma characteristic of pulmonary emphysema, and the parenchymal alterations in idiopathic pulmonary fibrosis are examples of common changes of lung structure due to nonneoplastic lung disease. How is lung structure affected in these and other pulmonary diseases? This question is the focus of much current investigation. An important part of the answer appears to involve the destructive effects of inflammatory cells on the connective tissue ("extracellular matrix") of the lung.

In this concise review, we present some information about the relationship between inflammation and damage to extracellular matrix (ECM). We emphasize the role of proteolytic enzymes from inflammatory cells, but it should be noted that: (1) inflammatory cells may alter ECM by nonenzymatic means, such as through the action of oxidants;3 (2) even at inflammatory foci, structural cells such as fibroblasts and endothelial cells may release enzymes that degrade ECM;4 (3) honeycombing in interstitial lung diseases may result from collapse and aggregation of alveoli with enlargement of adjacent airspaces, rather than from alveolar septal destruction;5 and (4) preservation of normal tissue architecture is the rule, rather than the exception, in most acute inflammatory processes in the lungs. Finally, new information about the composition and turnover of pulmonary ECM keeps emerging, so that concepts of ECM injury must also evolve. For example, recent data from rodent lung explants show a high rate of synthesis of type IV procollagen, suggesting that the normal lung has a rapid turnover of its basement membrane in vivo.7

**The Inflammatory Response**

Along with increased permeability of the microcirculation, an integral feature of inflammation is the recruitment of polymorphonuclear leukocytes, monocytes, eosinophils, and lymphocytes from the circulation to specific sites within tissues. At such sites these recruited cells ingest and kill microorganisms, remove foreign and cellular debris, and assist in tissue repair by modulating the activity of surrounding mesenchymal and endothelial cells.

Inflammatory cells accomplish some of their critical functions by releasing degradative enzymes into the pericellular space. Among these enzymes are proteinases, whose potential substrates are not only foreign materials but also host proteins (including ECM components). Indeed, the capacity of inflammatory cells to degrade components of ECM (collagens, elastin, fibronectin, laminin, and proteoglycans) is probably essential to enable inflammatory cells to exit the circulation and reach sites where they are needed to mount inflammatory responses.8 In contrast to their beneficial role, it should not be surprising that inflammatory reactions may at times be excessive, inappropriate, or prolonged. Damage to normal ECM may thus result from inflammation under some circumstances. Evidence for proteolytic injury to pulmonary ECM by inflammatory cells is particularly compelling in pulmonary emphysema and cavitory infections such as tuberculosis.

**Inflammatory Cell Proteinases**

Since the ECM is primarily a mixture of proteins and proteoglycans, proteinases from inflammatory cells are
capable of causing extensive ECM injury. Proteinases, which are enzymes that cleave peptide bonds within proteins,
are classified both according to their mechanism of activity (eg, serine proteinases, metalloproteinases), and by the biologic substrate(s) on which they act (eg, elastases, collagenases). The latter classification is useful when considering the potential roles of proteinases in tissue injury and remodeling, so long as one realizes that enzymes with similar substrate specificities (eg, elastases) may have quite dissimilar mechanisms of catalytic action and that individual enzymes may degrade more than one component of the ECM.

The optimal pH for catalytic activity of inflammatory cell proteinases may be neutral or acid. The “neutral” proteinases have attracted the most attention in considerations of matrix damage, because they have active catalytic function at the pH found in the extracellular space. By contrast, “acid” proteinases have until recently been largely dismissed as contributing to matrix damage and have been thought to be of greatest importance in protein degradation occurring within acidic intracellular compartments. Some recent data, however, suggest the possibility that acid proteinases can be released and express activity in pericellular spaces. Three groups of inflammatory cell proteinases having particular relevance to ECM degradation are elastases, collagenases, and plasminogen activators.

Elastases

Elastases are enzymes that degrade elastin. Elastin is an intensely hydrophobic macromolecule that has a critical structural and mechanical role in the lungs and is quite resistant to degradation by most proteinases. The importance of elastin to the integrity of lung structure has been most directly shown by experiments in which emphysema is produced by instilling elastases intratracheally into experimental animals. Proteolytic enzymes lacking the capacity to digest elastin do not cause emphysema to develop in the animals.

There are several elastases associated with inflammatory cells. Neutrophils contain a large quantity of a well-characterized serine elastase. It is contained within azurophil granules that can be released in response to a variety of stimuli including phagocytosis. Monocytes also have an elastase which appears to be quite similar, if not identical, to that of neutrophils. The quantity of monocyte elastase, its location within the cell, and the capacity of monocytes to degrade extracellular matrix are still under active investigation. Human alveolar macrophages display elastase activity which may be due to several enzymes: (1) a metalloproteinase elastase similar to one which has been well described in murine macrophages; (2) “neutrophil elastase” which the cells have synthesized or internalized from the extracellular space by receptor-mediated endocytosis; and (3) cysteine proteinases, such as cathepsin L, that have an acidic optimal pH. Recent work has indicated that cathepsin L, which is found in many cells, is strongly elastolytic. Extracellular activity of an enzyme such as cathepsin L might be permitted by maintenance of an acid pH in the immediate pericellular environment.

Collagenases

The collagen molecule is composed of three polypeptide chains arranged in a rope-like triple helical conformation. This triple helical structure renders collagen resistant to proteolytic attack by all enzymes other than a specific biochemical class of proteinases called collagenases. Collagenases are enzymes that catalyze the initial and also the rate-limiting step in collagen degradation by cleaving the collagen helix under physiologic conditions. The most thoroughly studied collagenases are the so-called interstitial collagenases, typified by the enzyme secreted by human fibroblasts, which cleave collagen types, III, and III by a common mechanism, catalyzing the scission of all three constituent collagen polypeptide chains at a single locus three fourths the distance from the amino-terminal end of the collagen molecule, thereby producing three fourths and one fourth length fragments that spontaneously denature at body temperature into randomly coiled gelatinous peptides. Unlike native collagen molecules, which are highly resistant to proteolytic degradation, the denatured gelatinous fragments produced by collagenase activity are susceptible to rapid cleavage by a variety of proteolytic enzymes.

As shown in Figure 1, numerous collagenolytic enzymes are associated with inflammatory cells and fibroblasts. It has recently been determined that human alveolar macrophages secrete an interstitial collagenase that is identical to the well-characterized collagenase released by fibroblasts. Neutrophils contain a recently purified interstitial collagenase that is structurally and catalytically distinct from the enzyme elaborated by fibroblasts/macrophages. Neutrophil collagenase is contained within pre-formed specific granules of the cells, from which it can be rapidly (within minutes) released in response to a variety of stimuli. In marked contrast, since the fibroblast/macroage collagenase is secreted as it is synthesized and no intracellular storage pools have been identified, macrophages and fibroblasts are necessarily much slower than neutrophils in their response to regulatory signals in the pericellular environment.

Both the fibroblast/macroage and the neutrophil collagenases are secreted as inactive zymogen forms, or “proenzymes.” These proenzymes neither bind to collagen nor degrade it. The mechanisms which
permit such proenzymes to attain catalytic activity in the extracellular space are under active investigation. It has been reported that neutrophil-derived oxidants are capable of activating latent neutrophil collagenase and that plasmin can activate the fibroblast/macrophage proenzyme, but the exact physiologic mechanism of activation of either collagenase zymogen in vivo remains to be defined.

Other collagenases are involved in the proteolysis of basement membrane collagen (type IV) and pericellular (type V) collagen. Recent evidence indicates that human monocytes produce a metalloproteinase that can specifically attack type IV collagen. This enzyme disappears when the cells age in vitro in culture, and is not secreted by alveolar macrophages. These observations raise the possibility that the function of this type IV collagenase is to facilitate movement of monocytes out of the microcirculation. Human alveolar macrophages have been reported to release a proteinase with degradative activity against type V collagen.

Neutrophil elastase is capable of cleaving both collagens type III and IV, but it is noteworthy that the rate of catalytic cleavage of type III collagen by neutrophil elastase is less than 2 percent that of the rate exhibited by fibroblast/macrophage collagenase.

**Plasminogen Activators**

Plasminogen activators convert plasminogen to plasmin, a proteinase that can degrade fibrin and other proteins. There are two principal plasminogen activators, urokinase and tissue plasminogen activator. Although typically viewed as enzymes involved in thrombolysis, plasminogen activators have also been implicated in many cellular responses involving tissue degradation and cellular movement including implantation of the fertilized ovum, uterine involution postpartum, and neuronal growth and migration as well as inflammation. Plasminogen activators are widely distributed among many cell types. Neutrophils synthesize a plasminogen activator. Alveolar macrophages have membrane-bound plasminogen activator and can secrete the activator into their microenvironment. The plasmin activator of human alveolar macrophages is similar or identical to urokinase. In vitro, macrophages demonstrate greater elastolytic activity against elastin in ECM if the matrix is also exposed to plasmin produced by plasminogen activator activity, suggesting that plasmin activity: (1) exposes elastin to elastase activity by degrading surrounding ECM molecules; (2) acts synergistically with elastase on elastin; or (3) activates an elastase proenzyme. An important point that has emerged from studies with plasminogen activators and elastases is the complexity of ECM degradation. A single enzyme is not likely to be either sufficient or efficient in fully degrading ECM.

**Proteinase Inhibitors**

Both plasma and the interstitial spaces are replete...
with proteinase inhibitors. These inhibitors may be classified by the type of catalytic activity of the enzymes which they inhibit. For example, α1-proteinase inhibitor inhibits serine proteinases and exhibits the most rapid reaction time with neutrophil elastase. In contrast, the tissue inhibitor of metalloproteinases (TIMP; also called human fibroblast collagenase inhibitor) is a specific inhibitor of a variety of metal-dependent enzymes. The tissue localization of these and other proteinase inhibitors is not known with precision, but it is probable that at inflammatory foci in which there is increased vascular permeability, even very large molecules such as α1-macroglobulin (750,000 daltons), a broad-spectrum inhibitor of all known endopeptidases, enter the extracellular space and may be present in sufficiently high concentrations that they can add to the inhibition of proteinase activity.

Especially interesting have been recent observations that inflammatory cells not only release proteinases into the pericellular space, but can also concomitantly secrete inhibitor(s) of those proteinases. Thus, there are enzyme-inhibitor pairs: (1) neutrophil elastase/α1-proteinase inhibitor (and α2-macroglobulin) released by monocytes; and (2) procollagenase/TIMP secreted by macrophages; and plasminogen activator and a plasminogen activator inhibitor elaborated by endotoxin-stimulated macrophages. The implications for the regulation of extracellular proteolysis of concomitant release of an enzyme and its inhibitor are just beginning to be explored. Presumably, proteolysis of matrix molecules is determined by the relative quantities of proteinases and inhibitors secreted, as well as the timing and site(s) of their release.

**Mechanisms of Extracellular Matrix Damage During Inflammation**

Since both proteolytic enzymes and proteinase inhibitors coexist in the vicinity of the extracellular matrix, how is it possible for the enzymes to function? Although the answer to this question is not known with certainty, some relevant concepts are depicted in Figure 2 and discussed below.

**Compartmentalization** of proteinases and their inhibitors can exist in the interstitial space as a result of: (1) close apposition of inflammatory cells to matrix proteins, thereby forming a microenvironment with high concentrations of released enzymes relative to inhibitors at the interface; (2) exclusion of inhibitors from penetrating into zones of contact between cells and a substrate, as has been observed for other molecules such as immunoglobulins; and/or (3) accumulation of large concentrations of inflammatory cells so that locally high concentrations of proteolytic enzymes overwhelm local inhibitory defenses. In each

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**Figure 2.** Schematized representation of an inflammatory cell's interaction with extracellular matrix, showing mechanisms which might permit matrix degradation despite the presence of proteinase inhibitors. E = proteolytic enzyme(s); I = proteinase inhibitor(s); E-I = inhibited enzyme; I* = inactivated inhibitor. The depicted intracellular organization of the proteinase(s) and inhibitor(s) is not intended to represent specific cellular compartments. Some proteinases of inflammatory cells are secreted as proenzymes which must be activated to attain catalytic activity (not shown). Note that enzyme released freely into the extracellular space is subject to inhibition (E-I), but that E released into zones of close contact between the cell and the matrix is more likely to remain active. Also, E tightly bound to matrix protein and membrane-bound enzyme(s) may be resistant to inhibitors. Some inhibitor in the extracellular space may be inactivated. Following initial proteolytic attack, matrix fragments may be internalized for final degradation. For cells which secrete both a proteinase and an inhibitor of the proteinase, it is not known whether both are released at the same site(s) along the cell membrane.

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**Extracellular Matrix Injury (Campbell, Senior; Welgus)**

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of these scenarios, the ratio of inhibitor to proteinase for the whole tissue might incorrectly suggest that proteinase inhibition should be effective.

**Tight binding of enzymes to substrate** may make them less susceptible to inhibition. This has been demonstrated in the case of elastase binding to elastin. Studies in which neutrophil elastase has been found in association with interstitial elastin in human emphysema/tous lungs suggest the possibility that this elastase remains persistently active. It should be noted that the "substrate concentration" for an enzyme bound to insoluble matrix is nonuniform, because the substrate is not in solution and may also not be homogeneous. At the surface or within an ECM macromolecule, however, the effective substrate concentration for a proteinase should be considered to be quite high. The effectiveness of competitive proteinase inhibitors in the surrounding interstitial fluid may thereby be reduced once an enzyme has bound to an ECM substrate.

**Membrane-bound enzymes** may possess catalytic activity that is resistant to at least some proteinase inhibitors as has been shown for membrane-bound plasminogen activators of macrophages.

**Inactivation of inhibitors** in the pericellular microenvironment by inflammatory cells may promote local proteolysis. Oxidants derived from inflammatory cells have been shown to inactivate α-proteinase inhibitor; furthermore, metalloproteinase elastase (which is not inhibited by α-proteinase inhibitor) can proteolytically inactivate this inhibitor. These concepts have recently been reviewed. Neutrophils are able to release oxidants locally at the site of their contact with a surface. This could provide a microenvironment at the site of contact between a neutrophil and insoluble matrix in which proteolysis is facilitated by oxidative inactivation of α-proteinase inhibitor. Although controversy exists about the functional status of α-proteinase inhibitor in lungs of otherwise healthy human smokers and nonsmokers, this inhibitor may be substantially oxidized during lung inflammation in the adult respiratory distress syndrome and in rheumatoid synovial fluid.

**Intracellular proteolysis**: Studies in which macrophages have been cultured on matrix suggest that matrix degradation is accomplished by a two-stage process. In the first stage, secreted proteinases cleave matrix macromolecules such as collagen and elastin into fragments. In the second stage, the fragments are engulfed by inflammatory cells, exposed to acid proteinases in lysosomes, and degraded to constituent amino acids. In this complex process, the physical state of the extracellular space and the means by which the enzymes are presented to the extracellular matrix may be critical to the resulting proteolysis. Intracellular proteolysis would be unaffected by inhibitors of neutral proteinases which are present in the extracellular space.

The rate of reaction between a proteinase and an inhibitor may be another important determinant of the effectiveness of the inhibitor in preventing a released proteinase from degrading nearby matrix macromolecules. It has been hypothesized that a half-time of association of 100 msec is the maximum allowable for effective inhibition. Accordingly, many inhibitors have extremely rapid association with proteinases. This concept has been reviewed recently.

In summary, although all biologic fluids are rich in inhibitors against inflammatory cell-derived neutral proteinases, these defenses may be evaded or rendered ineffective by several mechanisms, one or more of which may be operative in a particular tissue environment. Further studies using models such as those in which viable inflammatory cells are placed in contact with either surface-bound radiolabeled proteins or ECM in the presence of inhibitors may help to elucidate mechanisms which permit released proteinases to retain enzymatic activity.

**Biologic Activity of Peptides Released from Extracellular Matrix**

One of the recent insights about ECM is that the constituent molecules may be more than inert structural building blocks. They may exert effects on cells involved in the synthesis of matrix and also on inflammatory and neoplastic cells. Expression of some of these activities may occur with intact ECM molecules as well as with proteolytically generated fragments and synthetic peptides that mimic sequences in the intact molecules. In some instances, most notably with fibronectin, the biologic activities have been precisely localized to particular domains of the ECM molecule. Peptides generated from degradation of collagen, elastin, and fibronectin possess chemotactic activity for a number of cell types including inflammatory cells and fibroblasts. In some studies, peptides derived from ECM components have been found to elicit pulmonary inflammation when instilled intratracheally. Thus, one can envision the possibility that ECM degradation may be a factor in perpetuating inflammatory responses which, in turn, lead to further matrix damage.

**Conclusions**

Proteinases from inflammatory cells appear to have an important role in causing damage to the lung's ECM and ultimately in producing the alterations of lung structure and function characteristic of various non-neoplastic lung diseases. Much progress has been made in the biochemical characterization of the lung extracellular matrix, inflammatory cell proteinases, and proteinase inhibitors, but detailed understanding
of how inflammatory cells inflict damage and the ways in which proteinase inhibitors protect matrix is still not at hand. A major challenge for continuing research activity will be to develop a comprehensive scheme of the mechanisms of matrix proteolysis by inflammatory cells in vivo.

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