LUNG INJURY

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Ronald G. Crystal

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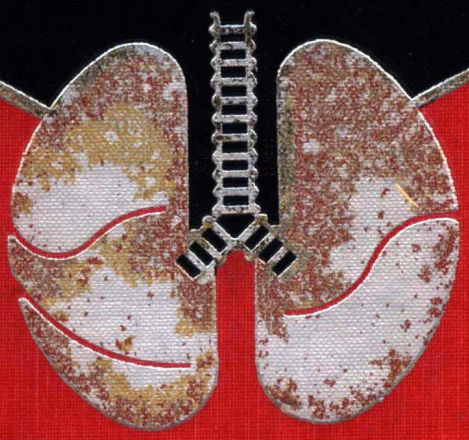

Raven Press

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RAVEN PRESS NEW YORK

Raven Press, Ltd., 1185 Avenue of the Americas, New York, New York 10036

Made in the United States of America

Library of Congress Cataloging-in-Publication Data

Lung injury / editors, Ronald G. Crystal, John B. West.
p. cm.
Includes bibliographical references and index.
ISBN 0-88167-848-1
1. Lungs—Pathophysiology. 2. Lungs—Wounds and injuries.
I. Crystal, Ronald G. II. West, John B. (John Burnard)
[DNLM: 1. Lung—injuries. WF 600 196337]
RC756.L838 1991
617.5'42044—dc20
DNLM/DLC
for Library of Congress 91-32568
CIP

The material contained herein appeared in *The Lung: Scientific Foundations*, edited by Crystal, West, et al. Raven Press, Ltd., New York © 1991.

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To my family
Janet and Zachary

RGC

To my wife Penelope

JBW

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Preface

This book brings together, in one convenient volume, the most clinically relevant material on lung injury from our two volume book, *The Lung: Scientific Foundations*. The 33 chapters selected provide a comprehensive and current review of lung injury, defense and repair, and therapeutic interventions.

The book focuses on the issues of interest to the clinician such as infections and host defenses, immune injury, airborne contaminants, acute lung injury, injury from drugs, fibrotic processes, genetic modulation of susceptibility to lung disease, the lung in the intensive care setting, and the lung following transplantation. The intent is to provide a concise, but complete review that will help the clinician deal with the large number of patients presenting with an increasing variety of lung injuries or suspected lung injuries.

Ronald G. Crystal
John B. West

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SECTION I

Lung Injury, Defense, and Repair

Section I covers the fundamental mechanisms involved in lung injury, the various aspects of lung defense, and repair mechanisms. Individual chapters reflect different approaches ranging from physiology to molecular biology but, taken together, provide an integrated picture of the complex processes involved in lung injury and repair, which are relevant to normal lung function and to many lung diseases.

The first chapter is devoted to proteases and antiproteases. There have been enormous advances in our understanding of lung proteases and how their activity is controlled by antiproteases. The balance between protease and antiprotease activity is critical and fundamental to our understanding of emphysema. Advances in molecular biology have provided important new insights into regulation of antiproteases and have led to new therapeutic options using genetically engineered antiproteases; in the future, these advances will lead to gene therapy for α_1-antitrypsin deficiency.

The second chapter deals with oxidants and antioxidants. The different types of reactive oxygen species, their source, and the mechanism by which they induce cellular injury are discussed in detail. Reactive oxygen species are increasingly recognized to be important in a number of inflammatory lung diseases, and, as with proteases, the balance between oxidants and antioxidants is of critical importance. Intracellular antioxidant defenses are now much better understood, and the development of antioxidants as therapeutic agents is also discussed. The prospect of developing far more effective antioxidants will undoubtedly lead to a greater interest in the role of reactive oxygen species in lung diseases beyond emphysema.

The respiratory epithelium is exposed to many inhaled gases and particles and has involved sophisticated defense systems. The third, fourth, and fifth chapters deal with particulates and infectious agents. The principles of particle deposition in the respiratory tract are discussed in some detail, since they are fundamental to our understanding of the site of deposition of inhaled particles in the respiratory tract and are relevant to understanding the principles of aerosol therapy. Mucociliary clearance is an important defense mechanism in the airways from the larynx to terminal bronchioles, and we now have a much greater understanding of the factors which alter clearance. Cough is an important defense mechanism in larger airways, and the different factors that are involved in cough control are discussed. Macrophages are the predominant defense mechanism in the alveoli, and the multiple responses of these phagocytic cells are becoming increasingly complex. It is clear that macrophages may show very different patterns of response with different stimuli, and this may be an important determinant in the way in which diseases may develop. Many minerals are inhaled and may lead to different patterns of lung injury. The cellular basis of these responses is discussed. The different mechanisms of the lung also include various immune mechanisms that are particularly important in defending against infections. Immunodeficiency predisposes to chronic lung infection and highlights the critical role of lung immune defense mechanisms. Immune mechanisms may also lead to lung injury such as granuloma formation and immune-complex-related lung injury, which are discussed in detail.

The sixth chapter deals with environmental contaminants. This is a highly topical area of research, and we are exposed to an increasing number of contaminants in the atmosphere as a result of industrialization. The multiple environmental contaminants are discussed, and cigarette smoking is given special consideration. The ways in which cigarette smoking leads to lung injury are still debated, and multiple interactive factors seem likely.

The seventh chapter considers other mechanisms of lung injury and discusses injury from inflammatory cells (particularly neutrophils and eosinophils), which

play an important pathogenetic role in many airway and parenchymal diseases. An increasing number of drugs are implicated in lung injury, and many different patterns of pathologic responses to drugs have now been recognized, although the particular mechanisms involved are often far from understood. Adult respiratory distress syndrome represents a type of gross lung injury with many different causes, but common underlying mechanisms, such as neutrophil-dependent endothelial cell injury and the role of cytokines, are now providing clues toward a greater understanding of the syndrome.

The eighth chapter discusses lung fibrosis, which is essentially a reaction to lung injury and is part of the normal repair process in the lung. The role of mesenchymal cells and their regulation is discussed, giving insights into the mechanisms of fibrosis in various chronic fibrosing lung conditions. Animal models have thrown light on the mechanisms involved in lung fibrosis, and molecular approaches are increasingly being used to understand the regulation of matrix protein production.

In the ninth chapter, the role of genetic factors in susceptibility to lung injury is discussed. It is clear that many lung diseases in which injury is important have a genetic basis, while most emphasis has been placed on α_1-antitrypsin deficiency, it is becoming increasingly clear that genetic factors also apply to many other lung diseases. This may be due to defects in epithelial cells, disordered mucosal immunity, or defects in lung function.

Section I thus covers a wide range of topics relating to lung injury and defense which are relevant to virtually all lung diseases and are fundamental to the function of the normal lung.

CHAPTER 1.1

Proteases

Richard C. Hubbard, Mark L. Brantly, and Ronald G. Crystal

"Proteases" are proteins that function as enzymes and that have the capacity to degrade proteins by hydrolyzing peptide bonds (1–3). Other terms used to describe this class of enzymes include "proteinases" and "peptidases," to separate enzymes that cleave proteins (the "proteinases") from those that act to cleave oligopeptide substrates (the "peptidases") (see ref. 1 for a review of this nomenclature). In modern usage, the proteases are divided into "exopeptidases" (proteases whose function is restricted to N- or C-terminal peptide linkages) and "endopeptidases" (proteases not so restricted). The proteases are further subdivided on the basis of critical components of the catalytic mechanisms they use to cleave the peptide bonds, including: serine, cysteine, aspartic, metallo, and unclassified (1–3). Beyond this mechanistic categorization, proteases vary in their substrate specificity, defined by primary, secondary, and tertiary structures of their targets. Some proteases are highly specific and will attack only one type or class of proteins, whereas others have a broad range of potential targets.

In the lung, some proteases function within cells while others are released by cells into the local milieu. In the latter category, a variety of proteases are capable of modifying the extracellular matrix components and/or the cells of the lung parenchyma. Extracellular proteases also play a central role in the lung by modifying proteins that participate in the complement system, in coagulation, and in other protein cascade systems. In this chapter we will focus on extracellular proteases relevant to the structure of the normal lung, as well as on those relevant to the derangements of the lung parenchyma in a broad variety of acute and chronic lung disorders. In general, these proteases are endopeptidases; they function in the pH and ionic conditions of the extracellular milieu of the lung, and their catalytic function depends on serine or metallo-type processes. For a general description of how such proteases evolved and how they function, several reviews are available (1–3). We will limit the discussion to human "proteases." With regard to proteases relevant to the lung of experimental animals, the overall concepts for the human proteases are generally applicable, but there are sufficient differences such that the reader should consult the literature specific to each species.

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ANTIPROTEASES VERSUS NATURAL SUBSTRATES

By necessity, proteases capable of attacking extracellular components of the lung parenchyma must be controlled. In the lung, as elsewhere, this is provided by antiproteases, proteins that inhibit proteases, usually by interacting with the catalytic site of the protease. The known extracellular lung antiproteases relevant to the proteases discussed in this chapter are α_1-antitrypsin, secretory leukoprotease inhibitor (SLPI), α_1-antichymotrypsin, α_2-macroglobulin, and tissue inhibitor of metalloprotease (TIMP). For a full discussion of these antiproteases, see Chapter 1.2.

In general, antiproteases function as such because the concentration–time kinetics of their interaction with the protease is more favorable than that of the protease with tissue components. If the protease concentration in the local milieu overwhelms the local antiprotease screen, the result is degradation of one or more protein components of the lung parenchyma. Furthermore, while interaction with an antiprotease generally provides permanent inhibition of the protease (depending on the protease and the antiprotease, as well as on the concentration of each), once a pro-

tease interacts with and cleaves a natural substrate, the protease is usually free to continue to destroy other tissue components.

The evidence that a variety of proteases are capable of functioning to cleave normal lung parenchymal components comes from several sources, including instillation of the proteases into the airways of experimental animals, *in vitro* addition of protease to explants of lung tissue or cultures of lung parenchymal cells, and *in vitro* studies with purified lung components, particularly components of the extracellular matrix.

SOURCES OF EXTRACELLULAR PROTEASES RELEVANT TO LUNG PARENCHYMAL COMPONENTS

Because of the abundance of antiproteases in plasma (4), proteases released outside of the lung likely never reach the lung; therefore, the only sources of proteases relevant to destruction of lung parenchymal components are derived from inflammatory cells within the lung (or possibly originating in pulmonary capillaries) and from lung parenchymal cells. Without question, the major sources of proteases potentially injurious to the lung are inflammatory cells, particularly the neutrophil (Table 1). Other inflammatory cells capable of releasing proteases within the lung are alveolar macrophages, T-lymphocytes, eosinophils, basophils, and mast cells. There is also evidence that cells which comprise the lung parenchyma, such as fibroblasts, can release proteases. In general, parenchymal cell-derived extracellular proteases are thought to function in normal growth and development and in normal extracellular protein turnover, whereas inflammatory cell-derived extracellular proteases are a major source of lung parenchymal derangement in lung disease.

NEUTROPHIL PROTEASES

Because of the prominent role of the neutrophil in releasing proteases into the extracellular environment in inflammatory reactions, neutrophils have been referred to as the "secretory organs of inflammation" (5). At least six proteases that function extracellularly have been identified in human neutrophils, including neutrophil elastase, cathepsin G, collagenase, gelatinase, proteinase-3, and plasminogen activator (1,6–9). Except for plasminogen activator, all are stored within cytoplasmic granules in the intact neutrophil and are disgorged extracellularly, or intracellularly into phagolysosomes as part of the neutrophil's response to various stimuli (6–11). The proteases are stored within neutrophils in one of three types of granules: azurophilic (primary), specific (secondary), and tertiary (C-particle). Azurophilic granules—so named because they contain myeloperoxidase, which imparts a bluish color to the granules when the cell is stained with the common Wright–Giemsa stain—contain neutrophil

TABLE 1. *Characteristics of proteases relevant to the lung*[a]

Protease	Category[b]	Molecular mass[c] (kDa)	Cell source	Substrate[d]
Neutrophil elastase	Serine	29	Neutrophil, basophil, mast cell	Elastin, type I–IV collagen, fibronectin, laminin, proteoglycans
Cathepsin G	Serine	30	Neutrophil	Fibronectin, proteoglycans, elastin, type IV collagen
Collagenase	Metalloprotease	75, 57	Neutrophil, fibroblast, macrophage	Type I, III–V, and VII collagen; gelatin; fibronectin
Gelatinase	Metalloprotease	225, 94, 30	Neutrophil, fibroblast, macrophage	Laminin, elastin, fibronectin, gelatin, Type V and VII collagen
Proteinase-3	Serine	27	Neutrophil	Type I and III collagen
Plasminogen activator	Serine	54	Neutrophil, macrophage	Elastin
Cathepsin D	Aspartic	42	Monocyte, neutrophil, fibroblast, macrophage	Proteoglycans, type IV collagen, fibronectin
Cathepsin L	Cystiene	25	Macrophage	Type IV collagen, fibronectin
Cathepsin B	Cystiene	29	Macrophage	Elastin
Granzymes 1–6[e]	Serine	60–70	T cell	Cellular matrix

[a] Only proteases listed are those known to be from humans.
[b] Classification of proteases by active-site catalytic mechanisms.
[c] Molecular mass observed in nonreducing conditions.
[d] Substrates listed are restricted to extracellular matrix components.
[e] Several human granzymes have been reported, but the biological function of only granzyme 1 has been evaluated.

elastase, cathepsins B, D and G, and proteinase-3. Collagenase is contained within specific granules, whereas gelatinase is contained within the tertiary and specific granules. All of these proteases are stored in an inactive form. It is likely that the granule form protects the neutrophil from self-destruction by its own proteases, thus enabling the neutrophil to serve as a transport "mule" capable of delivering and discharging its potent burden of proteases directly to an inflammatory locus.

The stored neutrophil proteases are released from their respective granules in different ways depending on the manner in which the cell responds to various stimuli (Fig. 1). For example, intracellular degranulation occurs following phagocytosis by opsonized bacteria, in a process that includes fusion of storage granules with portions of plasmalemma that form the phagocytic vacuoles (8–10). This process is not perfect, however, and invariably there is exocytosis of granule proteases into the extracellular milieu. With some stimuli, such as phorbol esters, lipopolysaccharides, and *N*-formyl-methionyl-leucyl-phenylalanine (FMLP), there is direct exocytosis of protease granules (8–11). Furthermore, the direct extracellular release of the contents of azurophilic and specific granules appears to be under separate control (10). For example, the proteases contained within the azurophilic granules are those which are used principally for extracellular release as part of inflammatory responses, a process that also includes the respiratory burst and myeloperoxidase-mediated oxidant production (8). In contrast, migration of neutrophils, through filters *in vitro* and through tissues *in vivo* in response to chemotactic stimuli, results in preferential discharge of specific granules (10,11). Relevant to this process, some neutrophil plasma-membrane receptors, including the FMLP receptor and the complement receptor CR3 (which binds the C3b fragment C3bi), exist in a preformed pool on the specific granule membrane. In this reaction, C3b-directed neutrophil chemotaxis through tissues is probably aided by C3b, inducing specific release of collagenase and thus likely helping neutrophil movement through tissues.

In addition to "leak" associated with phagocytosis and direct exocytosis, granule proteases can reach the extracellular milieu following neutrophil damage and death. The neutrophil is a short-lived cell with a lifespan of 1–2 days within the circulation and 1–2 days after leaving the circulation (12). It is likely that many neutrophils die within the lung, as a result of either (a) normal senescence or (b) inflammation within pulmonary capillaries or in the parenchyma. In these circumstances, disruption of the cell results in the indiscriminate release of all cellular enzymes, potentially leading to uncontrolled proteolysis. Another mechanism that can result in nonselective liberation of gran-

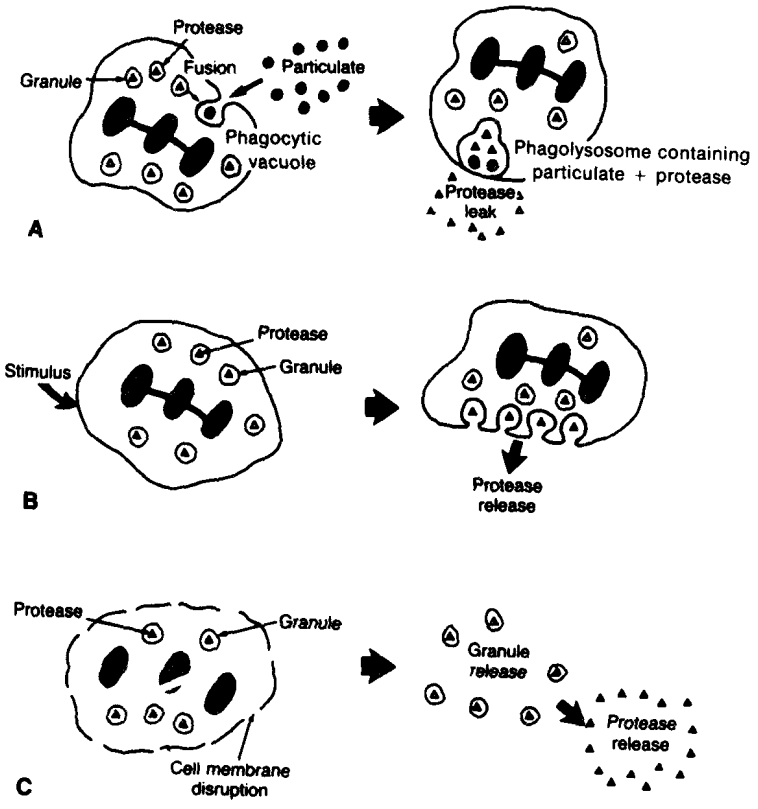

FIG. 1. Mechanisms by which neutrophils release proteases into the extracellular milieu. **A:** "Leaky phagocytosis." Proteases are released from neutrophils during the process of phagocytosis of particulates (such as bacteria). As the cell engulfs the particulate into a phagocytic vacuole, there is fusion of the vacuole with cytoplasmic granules containing proteases. The process of degranulation into the forming phagolysosome is associated with some "leak" of proteases outside of the cell. **B:** Inflammation. Proteases are released extracellularly at the surface of the neutrophil in response to an inflammatory stimulus. **C:** Cell death. Neutrophil autolysis leads to release of cytoplasmic granules containing proteases into the extracellular space.

ule enzymes is the toxic accumulation of soluble agents within lysosomes (e.g., material leached from silica) following endocytosis, resulting in rupture of lysosomes from within, leading to cytolysis.

Neutrophil Elastase

Neutrophil elastase, NE (EC 3.4.21.37), is the major protease contained within the azurophilic granules of neutrophils (6–9,13). NE is also the most potent of the neutrophil proteases with respect to its broad substrate specificity and kinetics of proteolysis. In this regard, NE plays a major role in the pathogenesis of a variety of human lung disorders (see below and Chapters 2.1, 2.4, 5.2, 7.1, 7.3.1, 9.1, 9.2, and 10). Because of the central importance of this protease in the lung, NE will be used as a prototype protease to demonstrate the general structure and expression of a protease gene and the structure and function of the protease itself.

NE is coded for by a single-copy, 4-kb gene on chromosome 11 at q14, including five exons and four introns (14). The exon structure predicts a primary translation product of 267 amino acid residues, including a 29-residue N-terminal precursor containing (a) a 27-residue "pre" signal peptide followed by a "pro_n" dipeptide and (b) a 20-residue C-terminal peptide. Following synthesis, the inactive precursor molecular form is trimmed, glycoslyated with complex carbohydrates, transported to the Golgi, and ultimately carried to the azurophilic granules. Although NE is carried by neutrophils within their granules, mature neutrophils do not synthesize neutrophil elastase (15). *In situ* hybridization studies have demonstrated that the NE gene is first detectable within blast cells and maximally expressed during the promyelocyte stage (15,16). Thereafter, neutrophil elastase mRNA levels decline such that they are undetectable by the stage of the neutrophil metamyelocyte. This suggests that the NE gene is activated in promyelocytes, myelocytes, and metamyelocytes but is shut down prior to the departure of the mature neutrophil from the bone marrow. NE mRNA transcripts appear in marrow differentiation in a relatively similar fashion as transcripts of myeloperoxidase, an enzyme also found in azurophilic granules.

While it is clear that NE gene expression is tightly controlled, the mechanisms underlying this control are not well understood. Analysis of the 5′ flanking region of the NE gene demonstrates consensus promoter elements, including a CAAT box, a TATA box, and a GC box (14). This region also contains a number of internal repeats, including a 317-bp sequence containing six tandem repeats of 53 or 52 bp that are nearly identical. NE gene expression and myelocytic differentiation can be induced in HL-60 promyelocytic leukemia cells by exposure to dimethylsulfoxide, with the rate of NE gene transcription increasing 1.9-fold and NE transcript number increasing 2.5-fold over 5 days (15,17). In contrast, exposure to phorbol esters leads to a marked reduction in the rate of NE gene transcription and in the number of NE mRNA transcripts, accompanied by induction toward monocytic differentiation. Thus, NE gene expression is intimately linked to neutrophil differentiation, and it is restricted to immature neutrophil precursors.

The mature neutrophil elastase protein is a single-chain, 220-amino-acid glycoprotein with four intramolecular disulfide bonds linking eight half-cystine residues (16,18,19). The carbohydrate side chains account for about 20% of the molecular weight, and differences in the content of the carbohydrates due to variable glycosylation likely account for the isozyme character of elastase seen on gel electrophoresis (19,20). NE is a very basic protein; because of its high content of arginine residues, it has an isoelectric point of 9.4.

The catalytic site of NE includes the triad His^{41}–Asp^{88}–Ser^{173} (21). This triad (also called the "charge-relay system") functions by transfer of electrons from the carboxyl group of Asp^{88} to the oxygen of Ser^{173}, which then becomes a powerful nucleophile able to attack the carbonyl carbon atom of the peptide bond of the substrate (Fig. 2). This catalytic triad is highly conserved among all serine proteases. The other important component of the active center of NE is the substrate binding site, which is responsible for the substrate specificity of neutrophil elastase (21). Studies using synthetic substrates have demonstrated that the binding site specifically prefers substrates containing nonbulky amino acids, especially Val and Ala (13,20,21).

NE derives its name from its ability to cleave the peptide bonds of elastin, a rubber-like macromolecule that gives the lung elastic recoil and that is resistant to most proteases (22). NE will also cleave many other proteins that contain appropriate surface-exposed amino acid sequences. In the context of the extracellular matrices of the lung, other proteins cleaved by NE include collagen (types I–IV), fibronectin, laminin, and cartilage proteoglycans (1,20,22–24). In addition, NE can cleave coagulation factors (fibrinogen and Factors V, VII, XII, and XIII), plasminogen, IgG and IgM, complement factors C3 and C5, *Escherichia coli* cell walls, and gp120, the coat protein of the human immunodeficiency virus (7,20,25). It may also cleave other proteases found within neutrophil granules, including the latent form of gelatinase (6).

Neutrophils contain approximately 1 pg of NE per cell, although several studies have indicated that the NE content per neutrophil varies among individuals over approximately a 2.5-fold range (26). This may be