Leukocyte Elastase
Physiological Functions and Role in Acute Lung Injury

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Contents

The syndrome of acute lung injury, known in its most severe form as the acute respiratory distress syndrome (ARDS), is characterized by increased alveolocapillary membrane permeability and subsequent pulmonary edema. Acute lung injury can be initiated by any one of an extensive and heterogeneous list of pulmonary or systemic insults, the most frequent of which is sepsis (1). What these inciting factors have in common is the ability to initiate activation of an acute inflammatory response that apparently spirals out of control, leading to pulmonary parenchymal injury. Neutrophilic polymorphonuclear leukocytes (neutrophils, PMN), which normally pass through the lung relatively unimpeded, are sequestered and activated in the pulmonary microvasculature during the genesis of an inflammatory response. The activation of neutrophils leads to the release of multiple microbicidal products, including reactive oxygen species, cationic peptides, eicosanoids, and proteolytic enzymes that normally serve in host defense against invading microbial pathogens. Paradoxically, when released in an unregulated manner, these cytotoxic products may damage vicinal cells, leading to organ injury and dysfunction. In addition, the neutrophils release growth factors, cytokines, and chemokines, which may enhance the inflammatory response (2).

In this simplified construct of the pathogenesis of acute lung injury, it is important not to overlook an important detail of neutrophil-mediated tissue injury. Neutrophils do not cause damage while suspended in the bloodstream; instead, the release of cytotoxic agents occurs when neutrophils are adherent to endothelium or epithelium or in contact with extracellular matrix proteins in the interstitium (2). This concept highlights the importance of understanding the mechanisms by which neutrophils interact with endothelial and epithelial cells, as well as the surrounding extracellular matrix.

Human leukocyte elastase (HLE) is a serine protease found in the azurophilic granules of the neutrophil. It is also known as human neutrophil elastase, and has been assigned a unique number by the Enzyme Commission of the International Union of Biochemistry (3), based on its activity (E.C. 3.4.21.37). Its potential substrates include almost all components of the extracellular matrix, as well as proteins as diverse as clotting factors, complement, immunoglobulins, and cytokines (4, 5). Interest in HLE was engendered in part by the observation that in an inherited disease, α1-antitrypsin deficiency, unopposed action of HLE because of lack of a protease inhibitor predisposed to a premature and sometimes severe form of emphysema (6).

The requirement for neutrophils to migrate out of the vasculature and through the basement membrane, as well as the potent proteolytic repertoire of HLE have led to the supposition that HLE might be involved in the pathogenesis of inflammatory tissue injury such as occurs in acute lung injury and ARDS. Nonetheless, the role of HLE in acute lung injury remains far from clear. Indeed, HLE is only one of a myriad of proteases synthesized by leukocytes and other cells in the lung, and some of these (e.g., matrix metalloproteinases from macrophages) have also been implicated in acute lung injury (Table 1). In this article, we will review the genetics and molecular physiology of HLE, and discuss the current data regarding its role in the pathogenesis of acute lung injury.

GENETICS OF ELASTASE

The gene for HLE, ELA 2, is located within a 50-kilobase segment in the terminal region of the short arm of chromosome 19. This region also contains the genes for the related serine proteases azurocidin and proteinase 3 (7). High-level transcription of the gene is limited to the promyelocytic stage of granulocyte development, when HLE is produced and stored in cytoplasmic azurophilic granules. Indeed, mRNA for HLE

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cannot be detected in mature neutrophils isolated from the systemic circulation (8). The glycoprotein product contains 218 amino acids and four disulfide bridges, and is a member of the serine protease family (9). Such enzymes contain a conserved triad of amino acid residues within the catalytic domain; in the case of elastase, these include His-41, Asp-88, and Ser-173. The serine at the active site is highly nucleophilic and has a high affinity for small-uncharged amino acids.

Mutations in the HLE gene have been associated with cyclic neutropenia, a rare autosomal dominant disease characterized by oscillations in neutrophil (and other blood cell) production with 21-day periodicity (10). To our knowledge, there have been no reports on the incidence or natural history of acute lung injury or ARDS in such patients, although both acute lung injury and ARDS have been described in patients with neutropenia from other causes (11, 12).

Regulation of HLE expression at both the transcriptional and the translational levels is incompletely understood. Chromatin reorganization may be involved in the regulation of HLE transcription (13). The HLE gene promoter region also encodes binding sites for the transcription factors c-Myb, C/EBP, and PU.1; the appearance of distinct transcription factors during specific stages of granulocyte development may be important in determining the expression pattern of neutrophil genes. This may have implications for neutrophil granule proteins such as HLE, since the sorting of proteins into different granules is postulated to depend on the time of granule gene expression and biosynthesis during neutrophil development (14).

TABLE 1. SELECTED PROTEASES

Class	Enzyme	Source	MW	Potential Substrates (partial list)	Endogenous Inhibitors	Disease Association or Proposed Function	Reference
Serine proteases	Leukocyte elastase	PMN, (monocytes)	30	Elastin, other BM components	α_1-PI SLPI, elafin, α_2-MG	Antimicrobial defense α_1-antitrypsin deficiency, cyclic neutropenia, cystic fibrosis, ?Acute lung injury and sepsis	87
	Cathepsin G	PMN (monocyte, mast cell)	28.5	Elastin, other BM components	α_1-antiCT α_1-PI SLPI	Antimicrobial defense	87
	Proteinase 3	PMN (monocyte)	29	Elastin, other BM components	α_1-PI α_2-MG, elafin	Antimicrobial defense Wegener's granulomatosis, leukemia	87, 88
	Mast cell chymase	Mast cells	26	BM components, Angiotensin I, Substance P	α_1-CT, α_1-PI, chymostatin, eglin C, SLPI	Unknown ?chemoattraction of monocytes and PMN	89, 90, 91
	Tryptase	Mast cells	11–15	Neuropeptides coagulant proteins, stromelysin, proteinase-activated receptor-2	Unknown	Unknown ?neutrophil recruitment (tryptase βI)	89, 92
	Granzyme A	Lymphocytes, NK cells	60	Proteoglycans, BM components, myelin basic protein, pIL-1β, others	Antithrombin III, α_1-PI	Unknown ?induces apoptosis of target cells	93
	Granzyme B	Lymphocytes, NK cells	35	Pro-caspases	α_1-PI, PI-9	Induces apoptosis of target cells	93
Selected Matrix metalloproteases (MMP)	Macrophage elastase (MMP-12)	Macrophages	53	Elastin, other BM components	α_1-PI, α_2-MG, TIMP-1	Possible involvement in emphysema	91, 94, 95
	92-kD gelatinase (gelatinase B, MMP-9)	Macrophages, PMN, eosinophils	92	Elastin, other BM components	TIMP-1, 2	Trophoblast invasion in placental development, bone development, ?tumor invasion, ?acute lung injury	96, 97, 98
	Interstitial collagenase (MMP-1)	Macrophages, fibroblasts, Type 2 pneumocytes	48–50; 24–25	BM components	α_2-MG, TIMP-1	?Rheumatoid arthritis, ?emphysema	91, 97, 99
	Stromelysin-1 (MMP-3)	Fibroblasts, chondrocytes, monocytes	57	BM components	α_2-MG, TIMP-1 and TIMP-2	?Arthritis, glomerular disease, tumor invasion ?acute lung injury	91, 97, 98, 100
	Matrilysin (MMP-7)	Monocytes, epithelial cells (macrophages)	28	BM components, α_1-antitrypsin	TIMP-1	?tumor invasion?airway epithelial repair	91, 101
Cysteine proteinase	Cathepsin L	Macrophages	29	Elastin, others	Cysteine proteinase inhibitors from serum	Lysosomal function	91, 94, 102
	Cathepsin S	Macrophages, T cells	28	Elastin, others	Cysteine proteinase inhibitors from serum	Unknown, ?immune system regulation	91, 94, 103

Definition of abbreviations: Parentheses denote minor cellular sources; α_1-antiCT = α_1-antichymotrypsin; α_2-MG = α_2-macroglobulin; α_1-PI = α_1-proteinase inhibitor; BM = basement membrane, (components include type IV collagen, laminin, fibronectin, vitronectin); MW = molecular weight; SLPI = secretory leukoproteinase inhibitor; TIMP = tissue inhibitor of metalloproteinase.

The concentration of HLE in neutrophils exceeds 5 mM (15); each neutrophil contains approximately 400 HLE-positive granules, and the total cellular concentration of HLE has been estimated at 1 to 2 picograms (5). Although HLE is most abundant in neutrophils, small amounts are expressed by monocytes and T cells (16, 17). mRNA transcripts of HLE cannot be detected in either monocytes or macrophages, and these cells are believed to contribute little to the total "tissue burden" of HLE (18). When neutrophils are activated, HLE is rapidly released from cytoplasmic granules into the extracellular space, although some remains bound to the neutrophil plasma membrane (19).

IN VITRO SUBSTRATES OF ELASTASE—CLUES TO *IN VIVO* FUNCTION?

As alluded to earlier, the list of substrates susceptible to proteolysis by HLE is extensive. These include extracellular matrix proteins such as collagen, elastin, fibrin, fibronectin, the platelet IIb/IIIa receptor (20), and cadherins (21, 22). HLE is also capable of degrading a variety of soluble proteins, including coagulation factors, immunoglobulins, complement, and many protease inhibitors (4).

In addition to its role in the degradation of the extracellular matrix, HLE may also function as a negative regulator of inflammation. HLE is capable of degrading various proinflammatory cytokines such as IL-1β and TNFα (23). More recently, *in vitro* studies have demonstrated that HLE can degrade both IL-2 and IL-6 (16, 24). IL-2 can induce T cell activation and chemotaxis, and the peptide fragments produced by HLE have been shown to impair both T cell adherence and migration, providing potential mechanisms for modulation of inflammation (16). HLE is able to cleave complement receptor 1 (CR1) from the surface of erythrocytes, releasing soluble CR1 that can act as an inhibitor of complement (25). HLE can also proteolytically cleave CD14, the main receptor for bacterial lipopolysaccharide (LPS), on the surface of monocytes (26) and fibroblasts (27), leading to decreased surface expression of CD14 and decreased TNF-α (26) and IL-8 production (27) in response to LPS exposure. Vitamin D-binding protein (DBP) binds to the surface of neutrophils and enhances the chemotactic properties of C5a (i.e., cochemotactic activity); recently, it was demonstrated that elastase cleaves the binding site for DBP, causing intact DBP to be released into the extracellular media. Inhibition of elastase caused an accumulation of DBP on the neutrophil surface, and abolished its cochemotactic activity (28).

Other *in vitro* studies have suggested that HLE may also play a role in neutrophil adhesion. The integrin CR3 (also known as Mac-1 or CD11b/CD18) binds to ligands, including fibrinogen and intercellular adhesion molecule 1 (ICAM-1) and mediates the adhesion of neutrophils to the endothelial surface. *In vitro*, HLE can bind to CR3, suggesting that it might compete with other ligands for CR3 and decrease neutrophil adhesion; the implication is that HLE may be important in the release of integrins from their substrates. Indeed, a monoclonal antibody to HLE prevented detachment of neutrophils from fibrinogen-coated filters (29). In this study, PMN transmigration across such filters was impaired by the anti-HLE antibody, an effect the investigators attributed to the importance of HLE in the release of adherent PMN from substrates. In addition to binding to CR3, HLE can cleave cell-bound ICAM-1 (30) suggesting a role for HLE in regulating CR3 to ICAM-1 interactions, and hence neutrophil adhesion and migration.

Taken together, these and other observations suggest that HLE may be involved in the down-regulation of inflammation. Conversely, HLE has also been shown to have potentially proinflammatory effects. For example, it can enhance neutrophil migration by inducing the secretion of GM-CSF, IL-6, and IL-8 from epithelial cells (31, 32). HLE cleaves α1-antiprotease inhibitor, generating a fragment that is chemotactic for neutrophils (33). In addition, HLE can degrade interendothelial (VE-cadherin) (21) and interepithelial (E-cadherin) junctional proteins (22), potentially promoting endothelial and epithelial permeability and pulmonary edema.

Given the variety and heterogeneity of *in vitro* substrates for HLE, it becomes important to determine which of these diverse and seemingly paradoxical properties has physiological or pathological importance. This is perhaps best accomplished by an analysis of studies of animal models of acute lung injury, which will be discussed in more detail later.

REGULATION AND FUNCTION OF ELASTASE

Inhibition by and Evasion of Antiproteases

Host tissues are protected from unregulated proteolysis by HLE and other proteases by multiple proteinase inhibitors. These include α1-antiprotease (α_1AP, also known as α_1-proteinase inhibitor), secretory leukoprotease inhibitor (SLPI), α_2-macroglobulin, and eglin (34, 35). Nonetheless, the neutrophil has an elaborate repertoire of methods capable of circumventing these defenses. First, neutrophils are able to create a relatively sequestered "microenvironment" or "protected space" in the subjacent area encompassing the neutrophil and the surface to which it is adherent (36). Because of size constraints, most endogenous antiproteases are unable to penetrate this region and therefore cannot inhibit elastase. Second, antiproteases (e.g., α1AP, SLPI) are sensitive to inactivation by oxidants released from activated neutrophils, which oxidize a critical methionine residue in the active site (4, 37). Third, HLE that is bound to elastin is relatively resistant to inhibition by antiproteases (38). Finally, activated neutrophils have been shown to express HLE on the cell surface; this elastase is active and resistant to inhibition from antiproteases (19). Taken together, these observations suggest that despite the presence of potent inhibitors, HLE is able to act locally in the pericellular and subjacent regions of neutrophils. It is noteworthy that there is at least one protease inhibitor, secretory leukoprotease inhibitor (SLPI), that is able to gain access to the protected space. SLPI is a relatively small (11.7 kD) polypeptide found in mucus secretions that maintains activity against HLE in the protected space, and, to a lesser extent, against membrane-bound HLE (19).

Elastase Release by Neutrophils

Neutrophils can be stimulated to release elastase upon exposure to various cytokines and chemoattractants, including TNFα (39), IL-8, C5a (40), LPS (41), and a tripeptide derived from bacterial wall (*N*-formyl-methionyl-leucyl-phenylalanine, fMLP). (42). Calmodulin has also been demonstrated to enhance elastase release from LPS-stimulated neutrophils (41). The role of β_2-integrins in mediating elastase release is not clear; in one study, a monoclonal antibody to CD18 or CD11b (but not P-selectin) blocked HLE release from chemoattractant-stimulated neutrophils adherent to platelet monolayers (40). By contrast, another study reported that antibody cross-linking of CD18 on isolated human neutrophils caused a rise in intracellular Ca^{2+} and exocytosis of elastase (43). A third study reported that degranulation of cytokine-stimulated neutrophils adherent to endothelial cells was not affected by antibody block-

ade of E-selectin, β_2 integrins, or L-selectin. Unexpectedly, in this experiment, neutrophils suspended in supernatants from IL-1-stimulated endothelial cells demonstrated elastase secretion, and this secretion was only partially blocked by antibodies to IL-8. These observations led to the postulate that unknown paracrine factors secreted by endothelial cells resulted in neutrophil degranulation (39).

The intracellular signal transduction pathways involved in elastase release are also unclear, and probably depend on the stimulus to which the neutrophil is exposed. Leukotrienes (40), phospholipase C (43), and the mitogen-activated protein kinase (MAPK) family (42, 44), have all been implicated in elastase release.

Involvement of Elastase in Neutrophil Transvascular Emigration into the Interstitium

The basement membrane. It has been proposed that elastase-mediated degradation of the endothelial basement membrane facilitates neutrophil transit into the interstitium (45). This proposition has proven difficult to confirm and remains controversial. When neutrophil migration in response to fMLP was studied using Matrigel®, a basement membrane matrix derived from sarcoma cells, inhibition of elastase decreased migration through the matrix (46). This effect was postulated to be due to HLE activating progelatinase B, a metalloproteinase. Neutrophil migration was associated with a slight but significant increase in collagen degradation, supporting the notion that HLE facilitates migration by degrading the basement membrane.

Nonetheless, other studies have shown opposing results. First, neutrophil transendothelial migration can occur *in vivo* without changes in permeability to protein (47). In addition, a study using human umbilical vein endothelial cells grown on a collagen matrix demonstrated that neutrophil migration across the basement membrane in response to zymosan-activated plasma was not affected by inhibition of elastase (48). This was *despite* an increase in basement membrane permeability associated with neutrophil emigration. Similarly, a more recent study employing pulmonary artery endothelial cells (49) demonstrated that migration of fMLP-stimulated neutrophils across the basement membrane was not affected by low molecular weight serine protease inhibitors, either endogenous (SLPI) or synthetic (Pefabloc SC). Given their small size, these inhibitors would be expected to have access to the subjacent area between the neutrophil and its substrate.

The endothelium: role of intercellular cadherin. Although HLE may not be required for passage of neutrophils through the basement membrane, it may be still be involved in transendothelial migration. In a study using human umbilical vein endothelial cell monolayers (without a basement membrane), inhibitors of elastase prevented neutrophil transendothelial migration in response to low concentrations of platelet-activating factor (PAF) or fMLP. However, high concentrations of PAF, felt to cause endothelial cell retraction, led to PMN migration that was not prevented by inhibitors of elastase (50). Similarly, exposure to HLE induced an increase in permeability of cultured bovine pulmonary arterial endothelial cells (51). The mechanism of this increased permeability may involve endothelial cell retraction, through proteolytic destruction of interendothelial tight junctions. Indeed, as mentioned earlier, HLE may degrade both epithelial (22) and endothelial (21) cadherins.

It is possible that the importance of HLE in neutrophil migration across endothelial monolayers and the basement membrane depends in part on the chemotactic stimulus. This notion follows from the observation that neutrophil migration across endothelial cells can be categorized as either requiring the CD11/CD18 adhesion complex or not. Some stimuli (e.g., phorbol myristate acetate, endotoxin) are associated with CD11/CD18-dependent neutrophil migration, whereas others (e.g., *Streptococcus pneumoniae*, C5a) elicit CD11/CD18-independent migration (52). The existence of different mechanisms of neutrophil migration raises the possibility of selective involvement of HLE in these processes. However, it has recently been demonstrated *in vitro* that both CD18-dependent neutrophil migration to fMLP (49) and CD18-independent migration to IL-8 and LTB_4 do not require HLE (53).

The types of cells or preparation used in these studies may also be relevant in determining the importance of leukocyte elastase to neutrophil emigration. For example, important phenotypic differences have been identified between pulmonary microvascular and pulmonary arterial endothelial cells (54). *In vivo*, neutrophil migration occurs predominantly in the pulmonary capillaries and this may complicate interpretation of *in vitro* studies that use endothelium derived from pulmonary arteries. Thus, differences in experimental design may modulate the relative importance of leukocyte elastase. Interestingly, a study of pneumococcal pneumonia in rabbits suggested that neutrophil migration occurs through preexisting holes in the capillary basal laminae (obviating the need for elastase-induced degradation), underscoring the potential importance of species differences in experimental models (55).

Membrane-bound Elastase: Vectorial Migration of the Neutrophil?

The observation that neutrophils stimulated with TNF-α or IL-8 translocate active elastase to the plasma membrane (23) suggests a possible mechanism for focusing the immunomodulatory effects of HLE (56). For example, elastase bound to a specific area of the cell membrane could influence the direction of neutrophil migration: membrane-bound elastase is preferentially localized to the leading edge of the migrating cells and may facilitate transendothelial passage (57). In addition, any effects of elastase on chemoattractants would be focused in that region. Finally, as we have mentioned, given the observation that elastase is able to degrade ICAM-1, it is possible that elastase might facilitate neutrophil de-adhesion from the substrate prior to locomotion (30).

Cytotoxicity and Wound Healing

Another important issue is whether elastase is cytotoxic to endothelial cells. Although epithelial cells appear to be relatively resistant to the cytotoxic effects of elastase (58, 59), evidence suggests that endothelial cells are more vulnerable. An early study (60) using a human microvascular endothelial cell model observed that PMN stimulated with LPS in combination with fMLP or C5a caused marked endothelial cell lysis that was not attenuated by scavengers of oxygen free radicals. Exposure to purified HLE alone produced cell lysis with a similar time course of injury that was also unaffected by scavengers of reactive oxygen species. Injury caused by HLE alone or by stimulated neutrophils was inhibited by a serine protease inhibitor, suggesting an important role for leukocyte elastase in neutrophil-mediated endothelial cell injury. Leukocyte elastase has also been shown to cause endothelial cell detachment through digestion of cell surface proteins (61).

Finally, recent evidence suggests that unimpeded elastase activity may be implicated in delayed wound healing. Recombinant mice lacking the gene for secretory leukoprotease inhibitor (SLPI) demonstrated increased elastase activity and tissue inflammation, along with delayed closure of cutaneous wounds (62). Interestingly, the wounds in SLPI-null mice dis-

played increased levels of active TGF-β relative to the wounds of wild-type mice. A neutralizing antibody to TGF-β partially attenuated the inflammation and the delayed healing of wounds in SLPI-null mice. Because TGF-β1 is known to be bound to the extracellular matrix and is released by elastase (63), it is possible that the lack of SLPI results in increased amounts of active TGF-β through unopposed elastase activity. Although these results are intriguing, it is essential to remember that elastase is important in the normal host response to an infected wound. In another study, using a porcine skin wound model, the addition of a specific elastase inhibitor impeded the clearing of bacteria or fungi that had been inoculated at the site of injury (64). This apparent paradox between the potentially harmful and beneficial properties of leukocyte elastase is pivotal to our understanding of the role of HLE in acute inflammation, and will be discussed in some detail below.

In summary, current data suggest that leukocyte elastase is not required for PMN migration out of the pulmonary microvasculature and into the interstitium. The cytotoxic effects of leukocyte elastase on endothelial cells may result in increased permeability to neutrophils, but this is unlikely to be a prerequisite for neutrophil translocation.

EVIDENCE FOR THE ROLE OF ELASTASE IN ARDS IN HUMANS

One of the earliest studies to suggest a role for HLE in ARDS in humans compared elastolytic activity in lavage fluid of 23 patients with ARDS to a control group of 55 patients without the syndrome. Increased elastolytic activity, neutrophil predominance, and decreased α_1-antiprotease activity were detected in the patients with ARDS (65). However, among the patients with ARDS, there were no differences in clinical characteristics or outcome between those with high or those with low levels of elastase activity. Another study observed elevated elastolytic activity in patients with ARDS, and demonstrated that HLE and α_1-antiprotease were complexed in BAL fluid. Their data also suggested that α_1-antiprotease had been inactivated by oxidation (66). Two subsequent studies found that elastase activity, whether measured in the plasma (67) or in BAL fluid (68), was higher in patients who went on to develop ARDS than in patients who were at risk for, but did not develop, ARDS.

It is important to note that a correlation between elastase and the extent of lung injury has not been observed in all studies. For example, although elevated levels of elastase antigen was observed in patients with ARDS, elastase enzymatic activity was not increased (69). In another study, increased levels of elastase antigen as well as α_1-antiprotease and α_2-macroglobulin were observed in the BAL fluid of patients with ARDS when compared with healthy control subjects (35). This study also noted an important difference between "antigenic" elastase as measured by ELISA and its enzymatic activity; elastase was predominantly in a complex with α_1-antiprotease with minimal free (i.e., enzymatically active) elastase. Complexes of α_2-macroglobulin and HLE were not detected by ELISA, had no lytic activity against elastin, but retained proteolytic activity in assays using low molecular weight substrates. The investigators concluded that BAL fluid from patients with ARDS had little if any activity against elastin, and that the elastolytic activity detected in BAL fluid from earlier studies may have been an artifact of the assays that had been used.

In summary, observational studies from humans are contradictory, caused in part by technical aspects of the assays used to detect elastase antigen and/or enzymatic activity, and do not convincingly demonstrate a role for HLE in ARDS.

ANIMAL MODELS OF LUNG INJURY AND LEUKOCYTE ELASTASE

The complex and often conflicting conclusions from both *in vitro* experiments and observational data from humans highlight the importance of animal models of acute lung injury in the study of the role of leukocyte elastase. Such models may help to establish the relevance of *in vitro* observations about leukocyte elastase and to develop hypotheses concerning its role in the pathogenesis of acute lung injury in humans.

Early studies established that oxygen metabolites and leukocyte elastase could synergistically cause edematous lung injury in an isolated rat lung model (70). Administration of either catalase or an inhibitor of elastase decreased the injury. Of note, oxidant and elastase-mediated injury was prevented by an oxidant-resistant elastase inhibitor (Eglin C), but not by an oxidant-sensitive one (α_1-antiprotease). One interpretation of these studies is that oxidants can inactivate certain antiproteases such as α_1-antitrypsin, thus allowing the unconstrained activity of elastase with the potential for enhanced tissue injury.

Studies of Protease Inhibitors and Experimental Lung Injury

Many investigators have studied the effect of intravenous administration of synthetic inhibitors of neutrophil elastase in animal models of acute lung injury. These include thrombin-induced pulmonary microembolization and pulmonary edema, endotoxin-induced lung injury (administered parenterally or via inhalation), postperfusion lung injury (mimicking ARDS after cardiopulmonary bypass), and infusions of activated neutrophils into the pulmonary vasculature. Some models involved isolated perfused lungs, whereas others involved whole animals.

For example, in *Escherichia coli* endotoxin-induced lung injury in sheep, the leukocyte elastase inhibitor EI-546 decreased hypoxia and blood leukopenia, and blunted the rise in pulmonary pressures and pulmonary lymphatic flow resulting from endotoxin (71). The specific leukocyte elastase inhibitor ONO-5046 also attenuated the lung neutrophilia and pulmonary edema induced by endotoxin (72). In a similar model, the leukocyte elastase inhibitors SC-37698 and SC-39026 attenuated the decrease in lung compliance and the increase in lung lymph flow and lung lymph protein clearance associated with lung injury induced by endotoxin (73).

In a guinea pig model of acute lung injury induced by *E. coli* endotoxin, the leukocyte elastase inhibitor ONO-5046 decreased neutrophil count in BAL fluid, the lung wet to dry weight ratio, and alveolocapillary permeability when compared with control animals (74). In a hamster model of acute lung injury induced by endotoxin inhalation, the administration of the specific leukocyte elastase inhibitor sivelestat (even hours after administration of endotoxin) prevented the development of lung injury (75). In a piglet model of septicemia induced by *E. coli*, the elastase inhibitor eglin C reduced mortality and the accumulation of interstitial fluid in the lungs, and blunted the reduction in antithrombin III levels associated with sepsis (76).

Analogous results have been observed in animal models of postperfusion lung injury. In a dog model of postperfusion lung injury, ONO-5046 attenuated the rises in lung extravascular water, respiratory index, and plasma levels of IL-6 and IL-8. Indeed, compared with the marked alveolar and interstitial injury in the control arm, lung histology in the treatment group was virtually normal (77). Similar observations were reported in a pig model using a chemically modified tetracycline (CMT-3) that is a potent matrix metalloproteinase and elastase inhibitor (78).

When TNFα and activated neutrophils were infused into the pulmonary artery of isolated, perfused rabbit lungs, lung injury developed, characterized by increased alveolar permeability and pulmonary hypertension. ONO-5046-treated rabbit lungs had a lower permeability index, as well as attenuated and slower increases in PA pressure (79). Inhibitors of HLE have also had protective effects in IgG immune complex-induced lung injury (80) and thrombin-induced pulmonary edema (81) in rats.

These data support the notion that leukocyte elastase is important in the pathogenesis of acute lung injury. Nonetheless, the mechanisms by which leukocyte elastase could cause lung injury remain speculative. Some of the hypotheses have already been discussed; for example, HLE has been shown to degrade endothelial VE-cadherin (21), promoting microvascular permeability, as well as epithelial E-cadherin (22) that could predispose to alveolar flooding. HLE may also alter endothelial permeability because of its cationicity (82). HLE can itself be directly cytotoxic to endothelial and/or epithelial cells. Alternatively, HLE could play a crucial role in the modulation of the inflammatory response, either by influencing chemotaxis, cell adhesion, or mediators of inflammation such as cytokines and complement. However in some of the studies (74, 77), but not in others (21, 78), inhibition of elastase had no effect on neutrophil chemotaxis or on expression of adhesion molecules by neutrophils.

In addition, the relevance and applicability of the various animal models to human disease must be carefully considered. For example, lung injury induced by systemic administration of endotoxin to sheep (or other animals) differs from human ARDS in many aspects (72, 73): in animal models of lung injury, the animals are typically healthy before ARDS is induced, whereas most human patients who develop ARDS have severe comorbidities such as sepsis or disseminated intravascular coagulation. In addition, the inducing stimulus for acute lung injury in animal models is usually given only once and the experimental inhibitors are often given simultaneously. In contrast, human patients may have infection that evolves over many hours or days and rarely are pharmacologic interventions able to be given at the inception of the infection. Finally, interspecies variations in elastase activity, the content of neutrophil granules (e.g., lack of defensins in murine neutrophils) (83), the structure of the microvasculature, and differing potency and specificity of elastase inhibitors make it difficult to extrapolate directly the results of animal studies to humans.

Studies with Elastase Knockout Mice

Although it is easy to focus on the potentially destructive role of HLE in acute lung injury, it is important to recall that elastase has broad-spectrum antimicrobial activity *in vitro* (84) and may be critically important in host defense. Recent studies have suggested that this antimicrobial effect may involve degradation of outer membrane protein A on the surface of gram-negative bacteria such as *E. coli* (85). In related experiments, the responses of leukocyte elastase-deficient mice and wild-type control mice to infection were compared (15). When intraperitoneal *Klebsiella pneumoniae* was administered to both groups of animals, all of the elastase-deficient mice died within 48 h, compared with only half of the wild-type mice. The bacterial burden in the bloodstream and organs of the animals was significantly higher in the elastase-deficient mice. Interestingly, neutrophil number and recruitment into the peritoneal cavity and infected organs was similar between the two groups. The neutrophils from the elastase-deficient mice had less bactericidal activity, and on transmission electron microscopy appeared to have defective killing of internalized bacteria. Similar results were obtained with *E.coli* but not with *S. aureus*, underscoring the importance of elastase in the killing of gram-negative bacteria.

Subsequent studies demonstrated that elastase-deficient mice had normal neutrophil development and recruitment but were susceptible to infection with *Aspergillus fumigatus* (86). Interestingly, this study also demonstrated that elastase-deficient mice were resistant to normally lethal doses of LPS. Furthermore, mice that lacked both LE and another neutrophil protease (cathepsin G) were protected against alveolar damage induced by endotoxic shock. In concert, these observations highlight the important physiologic antimicrobial function of leukocyte elastase in the context of a regulated inflammatory response. However, they also indicate that under circumstances where the proinflammatory stimulus is excessive (e.g., systemic endotoxemia) or where counter-regulatory mechanisms are overwhelmed, leukocyte elastase may contribute to inflammatory tissue injury.

STUDIES OF ELASTASE INHIBITORS IN HUMAN LUNG INJURY AND SEPSIS

To date, there is very little information in the public domain about the effects of elastase inhibitors in the treatment of human acute lung injury. A phase II/III study of a small molecule pharmacologic elastase inhibitor in lung injury and sepsis is currently in progress (Dr. Charles Fisher, personal commu-

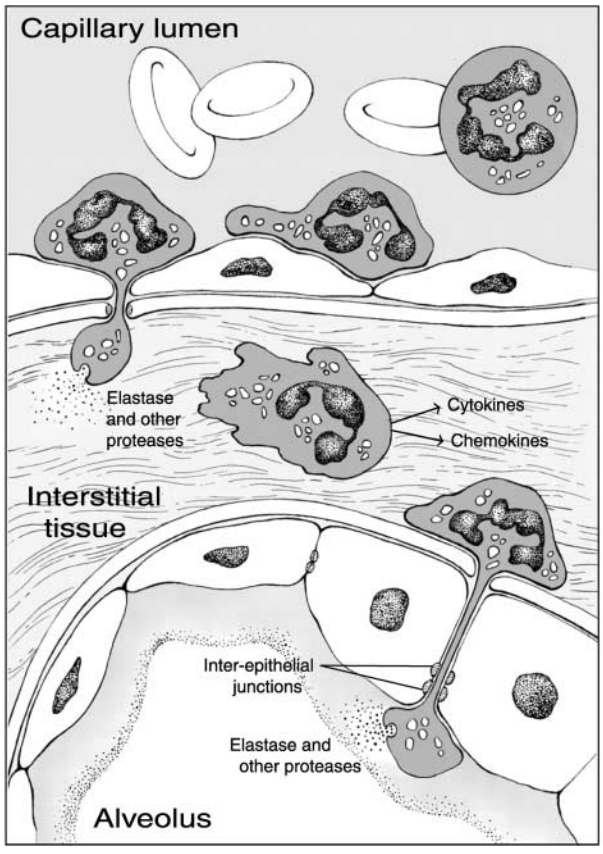

Figure 1. Elastase in the genesis of inflammatory tissue injury. There are multiple potential mechanisms whereby leukocyte elastase may contribute to the pathogenesis of lung injury, including (*1*) direct cytotoxicity to endothelial and epithelial cells, (*2*) degradation of endothelial and epithelial intercellular adhesion molecules, including (VE and E-cadherin), (*3*) direct modulation of the inflammatory response by effects on neutrophil adhesion molecules and cytokines, (*4*) modulation of the repair phase by effects on growth factors and cytokines such as TGFβ.

nication). The results of these and other studies using elastase inhibitors will be eagerly anticipated.

CONCLUSIONS AND UNANSWERED QUESTIONS

A Balance between Injury and Benefit: a Modulator of the Inflammatory Response?

A critical appraisal of the current literature concerning leukocyte elastase suggests that its role in inflammation and tissue injury is complex with both potentially injurious and beneficial effects (*see* Figure 1). The concept that leukocyte elastase is necessarily detrimental in sepsis and lung injury is simplistic. Indeed, animal studies suggest that although absence of leukocyte elastase may be protective against endotoxin-induced pulmonary edema, it can also be lethal in gram-negative sepsis by interfering with microbicidal responses essential to the innate immune response. It is important to make a distinction between *intracellular* elastase, which is required for effective killing of ingested (phagocytosed) bacteria, and *extracellular* elastase, which, in excessive amounts, has the potential to cause tissue damage in circumstances of unregulated inflammation.

Current evidence suggests that leukocyte elastase may *not* be required for the migration of neutrophils out of the vasculature into the pulmonary interstitium and eventually into the alveolar space. It can, however, be cytotoxic to endothelial cells and may increase vascular permeability and alveolar edema if present in excess. Leukocyte elastase may also be implicated in the regulation of wound healing, secondary to the release of TGF-β from the extracellular matrix.

Animal studies using diverse inhibitors of leukocyte elastase and various models of lung injury suggest that leukocyte elastase does indeed contribute to the pathogenesis of acute lung injury. Given its ability to degrade multiple cytokines, cell surface receptors, and complement components, it is also possible that leukocyte elastase serves as an important negative modulator of the inflammatory response. At present, however, there is a paucity of *in vivo* evidence to support this hypothesis.

Many unanswered questions remain regarding the role of leukocyte elastase in acute lung injury. First and foremost, the mechanism(s) by which leukocyte elastase contributes to acute lung injury remain unresolved. Whether this requires (*1*) elastase-mediated degradation of endothelial and/or epithelial cadherin, leading to increased alveolocapillary permeability and subsequent pulmonary edema, (*2*) modulation of the inflammatory response, or (*3*) some uncharacterized property of leukocyte elastase needs to be clarified. Secondly, a more detailed distinction needs to be made between the physiologic and pathologic importance of intracellular versus extracellular elastase. Indeed, this distinction may explain the discrepancy between the beneficial effects of elastase inhibition observed in animal models of lung injury (primarily targeting extracellular elastase) and the enhanced mortality in response to bacterial infection observed in elastase knock-out mice (complete deficiency of intracellular elastase). Finally, the importance of leukocyte elastase relative to other proteases in the genesis of lung injury remains uncertain. Clarification of this role will have important implications for the development of treatment strategies for acute lung injury, ARDS, and sepsis.

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References

1. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000; 342:1334–1349.
2. Downey GP, Dong Q, Kruger J, Dedhar S, Cherapanov V. Regulation of neutrophil activation in acute lung injury. *Chest* 1999;116(Suppl 1: S46–S54).
3. International Union of Biochemistry and Molecular Biology. Recommendations on Biochemical and Organic Nomenclature, Symbols and Terminology. Available at http://www.chem.qmw.ac.uk/iubmb/. Accessed May 3, 2001.
4. Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989;320: 365–376.
5. Vender RL. Therapeutic potential of neutrophil-elastase inhibition in pulmonary disease. *J Invest Med* 1996;44:531–539.
6. Eriksson S. Alpha$_1$-antitrypsin deficiency: lessons learned from the bedside to the gene and back again: historic perspectives. *Chest* 1989; 95:181–189.
7. Zimmer M, Medcalf RL, Fink TM, Mattmann C, Lichter P, Jenne DE. Three human elastase-like genes coordinately expressed in the myelomonocyte lineage are organized as a single genetic locus on 19pter. *Proc Natl Acad Sci USA* 1992;89:8215–8219.
8. Takahashi H, Nukiwa T, Basset P, Crystal RG. Myelomonocytic cell lineage expression of the neutrophil elastase gene. *J Biol Chem* 1988; 263:2543–2547.
9. Bode W, Meyer Jr. E, Powers JC. Human leukocyte and porcine pancreatic elastase: x-ray crystal structures, mechanism, substrate specificity, and mechanism-based inhibitors. *Biochemistry* 1989;28:1951–1963.
10. Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. *Nature Gen* 1999;23:433–436.
11. Ognibene FP, Martin SE, Parker MM, Schlesinger T, Roach P, Burch C, Shelmaher JH, Parrillo JE. Adult respiratory distress syndrome in patients with severe neutropenia. *N Engl J Med* 1986;315:547–551.
12. Vansteenkiste JF, Boogaerts MA. Adult respiratory distress syndrome in neutropenic leukemia patients. *Blut* 1989;58:287–290.
13. Wong ETL, Jenne DE, Zimmer M, Porter SD, Gilks CB. Changes in chromatin organization at the neutrophil elastase locus associated with myeloid cell differentiation. *Blood* 1999;94:3730–3736.
14. Borregaard N, Cowland JB. Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* 1997;89:3503–3521.
15. Belaaouaj A, McCarthy R, Baumann M, Gao Z, Ley TJ, Abraham SN, Shapiro SD. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. *Nat Med* 1998;4:615–618.
16. Ariel A, Yavin EJ, Hershkoviz R, Avron A, Franitza S, Hardan I, Cahalon L, Fridkin M, Lider O. IL-2 induces T cell adherence to extracellular matrix: inhibition of adherence and migration by IL-2 peptides generated by leukocyte elastase. *J Immunol* 1998;161:2465–2472.
17. Shapiro SD, Campbell EJ, Senior RM, Welgus HG. Proteinases secreted by human mononuclear phagocytes. *J Rheumatol* 1991; 18(Suppl 27:95–98).
18. Takahashi H, Nukiwa T, Basset P, Crystal RG. Myelomonocytic cell lineage expression of the neutrophil elastase gene. *J Biol Chem* 1988; 263:2543–2547.
19. Owen CA, Campbell MA, Sannes PL, Boukedes SS, Campbell EJ. Cell surface-bound elastase and cathepsin G on human neutrophils. A novel, non-oxidative mechanism by which neutrophils focus and preserve catalytic activity of serine proteinases. *J Cell Biol* 1995;131: 775–789.
20. Si-Tahar M, Pidard D, Balloy V, Moniatte M, Kieffer N, Van Dorsselaer A, Chignard M. Human leukocyte elastase proteolytically activated the platelet integrin $\alpha_{IIb}\beta_3$ through cleavage of the carboxyl terminus of the α_{IIb} subunit heavy chain. *J Biol Chem* 1997;272:11636–11647.
21. Carden D, Xiao F, Moak C, Willis BH, Robinson-Jackson S, Alexander S. Neutrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins. *Am J Physiol* 1998;275:H385–392.
22. Ginzberg H, Cherapanov V, Dong Q, Cantin A, McCulloch CAG, Shannon P, Downey GP. Neutrophil-mediated epithelial injury during transmigration: role of elastase. *Am J Physiol* 2001;281:705–725.
23. Owen CA, Campbell MA, Boukedes SS, Campbell EJ. Cytokines regulate membrane-bound leukocyte elastase on neutrophils: a novel mechanism for effector activity. *Am J Physiol* 1997;272:L385–L393.
24. Bank U, Küpper B, Reinhold D, Hoffman T, Ansorge S. Evidence for crucial role of neutrophil-derived serine proteases in the inactivation of interleukin-6 at sites of inflammation. *FEBS Lett* 1999;461:235–240.
25. Sadallah S, Hess C, Miot S, Spertini O, Lutz H, Schifferli J-A. Elastase and metalloproteinase activities regulate soluble complement receptor 1 release. *Eur J Immunol* 1999;29:3754–3761.
26. Le-Barillec K, Si-Tahar M, Balloy V, Chignard M. Proteolysis of monocyte CD14 by human leukocyte elastase inhibits lipopolysaccharide-mediated cell activation. *J Clin Invest* 1999;103:1039–1046.

27. Nemoto E, Sugawara S, Tada H, Takada H, Shimauchi H, Horiuchi H. Cleavage of CD14 on human gingival fibroblasts cocultured with activated neutrophils is mediated by human leukocyte elastase resulting in down-regulation of lipopolysaccharide-induced IL-8 production. *J Immunol* 2000;165:5807–5813.
28. DiMartino SJ, Shah AB, Trujillo G, Kew RR. Elastase controls the binding of the Vitamin D-binding protein (Gc-globulin) to neutrophils: a potential role in the regulation of C5a co-chemotactic activity. *J Immunol* 2001;166:2688–2694.
29. Cai T-Q, Wright SD. Human leukocyte elastase is an endogenous ligand for the integrin CR3 (CD11b/CD18, Mac-1, $\alpha_M\beta_2$) and modulates polymorphonuclear leukocyte adhesion. *J Exp Med* 1996;184: 1213–1223.
30. Champagne B, Tremblay P, Cantin A, St. Pierre Y. Proteolytic cleavage of ICAM-1 by human neutrophil elastase. *J Immunol* 1998;161: 6398–6405.
31. Bedard M, McCLure CD, Schiller NL, Francoeur C, Cantin A, Denis M. Release of interleukin-8, interleukin-6, and colony-stimulating factors by upper airway epithelial cells: implications for cystic fibrosis. *Am J Respir Cell Mol Biol* 1993;9:455–462.
32. Nakamura H, Yoshimura K, McElvaney NG, Crystal NG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J Clin Invest* 1992;89:1478–1484.
33. Banda MJ, Rice AG, Griffin GL, Senior RM. Alpha$_1$-proteinase inhibitor is a neutrophil chemoattractant after proteolytic inactivation by macrophage elastase. *J Biol Chem* 1988;263:4481–4484.
34. Sallenave J-M, Donnelly SC, Grant IS, Robertson C, Gauldie J, Haslett C. Secretory leukocyte proteinase inhibitor is preferentially increased in patients with acute respiratory distress syndrome. *Eur Respir J* 1999;13:1029–1036.
35. Wewers MD, Herzyk DJ, Gadek JE. Alveolar fluid neutrophil elastase activity in the adult respiratory distress syndrome is complexed to alpha-2-macroglobulin. *J Clin Invest* 1988;82:1260–1267.
36. Rice WG, Weiss SJ. Regulation of proteolysis at the neutrophil-substrate interface by secretory leukoprotease inhibitor. *Science* 1990; 249:178–181.
37. Boudier C, Bieth JG. Oxidized mucus proteinase inhibitor: a fairly potent neutrophil elastase inhibitor. *Biochem J* 1994;303:61–68.
38. Morrison HM, Welgus Hg, Stockley RA, Burnett D, Campbell EJ. Inhibition of human leukocyte elastase bound to elastin: relative ineffectiveness and two mechanisms of inhibitory activity. *Am J Respir Cell Mol Biol* 1990;2:263–269.
39. Topham MK, Carveth HJ, McIntyre TM, Prescott SM, Zimmerman GA. Human endothelial cells regulate polymorphonuclear leukocyte degranulation. *FASEB J* 1998;12:733–746.
40. Rainger GE, Rowley AF, Nash GB. Adhesion-dependent release of elastase from human neutrophils in a novel, flow-based model: specificity of different chemotactic agents. *Blood* 1998;92:4819–4827.
41. Houston DS, Carson CW, Esmon CT. Endothelial cells and extracellular calmodulin inhibit monocyte tumor necrosis factor release and augment neutrophil elastase release. *J Biol Chem* 1997;272:11778–11785.
42. Sue-a-Quan AK, Fialkow L, Vlahos CJ, Schelm JA, Grinstein S, Butler J, Downey GP. Inhibition of neutrophil oxidative burst and granule secretion by wortmannin: potential role of MAP kinase and renaturable kinases. *J Cell Physiol* 1997;172:94–108.
43. Walzog B, Seifert R, Zakrzewicz A, Gaehtgens P, Ley K. Cross-linking of CD18 in human neutrophils induces an increase of intracellular free Ca^{2+}, exocytosis of azurophilic granules, quantitative up-regulation of CD18, shedding of L-selectin, and actin polymerization. *J Leukoc Biol* 1994;56:625–635.
44. Patrick DA, Moore EE, Offner PJ, Meldrum DR, Tamura DY, Johnson JL, Silliman CC. Maximal human neutrophil priming for superoxide production and elastase release requires p38 mitogen-activated protein kinase activation. *Arch Surg* 2000;135:219–225.
45. Harlan JM. Leukocyte-endothelial interactions. *Blood* 1985;65:513–525.
46. Delclaux C, Delacourt C, d'Ortho M-P, Boyer V, Lafuma C, Harf A. Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. *Am J Respir Cell Mol Biol* 1996;14:288–295.
47. Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA. Effects of leukotriene B4 in the human lung: recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J Clin Invest* 1989;84:1609–1619.
48. Huber AR, Weiss SJ. Disruption of the subendothelial basement membrane during neutrophil diapedesis in an *in vitro* construct of a blood vessel wall. *J Clin Invest* 1989;83:1122–1136.
49. Mackarel AJ, Cottell DC, Russell KJ, FitzGerald MX, O'Connor CM. Migration of neutrophils across human pulmonary endothelial cells is not blocked by matrix metalloproteinase or serine protease inhibitors. *Am J Respir Cell Mol Biol* 1999;20:1209–1219.
50. Cepinskas G, Noseworthy R, Kvietys PR. Transendothelial neutrophil migration: role of neutrophil-derived proteases and relationship to transendothelial protein movement. *Circ Res* 1997;81:618–626.
51. Rodell TC, Cheronis JC, Ohnemus CL, Piermattei DJ, Repine JE. Xanthine oxidase mediates elastase-induced injury to isolated lungs and endothelium. *J Appl Physiol* 1987;63:2159–2163.
52. Doerschuk CM, Tasaka S, Wang Q. CD11/CD18-dependent and -independent neutrophil emigration in the lungs—how do neutrophils know which route to take? *Am J Respir Cell Mol Biol* 2000;23:133–136.
53. Mackarel AJ, Russell KJ, Brady CS, FitzGerald MX, O'Connor CM. Interleukin-8 and leukotriene-B$_4$, but not formylmethionyl leucylphenylalanine, stimulate CD18-independent migration of neutrophils across human pulmonary endothelial cells *in vitro*. *Am J Respir Cell Mol Biol* 2000; 23:154–161.
54. Stevens T, Creighton J, Thompson WJ. Control of cAMP in lung endothelial cell phenotypes: implications for control of barrier function. *Am J Physiol* 1999;277:L41–L50.
55. Walker DC, Behzad AR, Chu F. Neutrophil migration through preexisting holes in the basal laminae of alveolar capillaries and epithelium during streptococcal pneumonia. *Microvasc Res* 1995;50:397–416.
56. Vaday GG, Lider O. Extracellular matrix moieties, cytokines, and enzymes: dynamic effects of immune cell behavior and inflammation. *J Leukoc Biol* 2000;67:149–159.
57. Cepinskas G, Sandig M, Kvietys PR. PAF-induced elastase-dependent neutrophil transendothelial migration is associated with the mobilization of elastase to the neutrophil surface and localization to the migrating front. *J Cell Sci* 1999;112:1937–1945.
58. Chung Y, Kercsmar CM, Davis PB. Ferret tracheal epithelial cells grown *in vitro* are resistant to lethal injury by activated neutrophils. *Am J Respir Cell Mol Biol* 1991;5:125–132.
59. Kercsmar CM, Davis PB. Resistance of human tracheal epithelial cells to killing by neutrophils, neutrophil elastase, and *Pseudomonas* elastase. *Am J Respir Cell Mol Biol* 1993;8:56–62.
60. Smedly LA, Tonnesen MG, Sandhaus RA, Haslett C, Guthrie LA, Johnston RB, Henson PM, Worthen GS. Neutrophil-mediated injury to endothelial cells—enhancement by endotoxin and essential role of neutrophil elastase. *J Clin Invest* 1986;77:1233–1243.
61. Harlan JM, Killen PD, Harker LA, Striker GE, Wright DG. Neutrophil-mediated endothelial injury *in vitro*. *J Clin Invest* 1981;68:1394–1403.
62. Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, Hale-Donze H, McGrady G, Song X-Y, Wahl SM. Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. *Nat Med* 2000;6:1147–1153.
63. Taipale J, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-β 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J Biol Chem* 1995;270:4689–4696.
64. Cole AM, Shi J, Ceccarelli A, Kim Y-H, Park A, Ganz T. Inhibition of neutrophil elastase prevents cathelicidin activation and impairs clearance of bacteria from wounds. *Blood* 2001;97:297–304.
65. Lee CT, Fein AM, Lippmann M, Holtzman H, Kimbel P, Weinbaum G. Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory-distress syndrome. *N Engl J Med* 1981;304:192–196.
66. Cochrane CG, Spragg RG, Revak SD, Cohen AB, McGuire WW. The presence of neutrophil elastase and evidence of oxidation activity in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1983;127:S25–S27.
67. Donnelly SC, MacGregor I, Zamani A, Gordon MWG, Robertson CE, Steedman DJ, Little K, Haslett C. Plasma elastase levels and the development of the adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;151:1428–433.
68. Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, Dayer J-M. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. *Am Rev Respir Dis* 1992;145:1016–1022.
69. Idell S, Kucich U, Fein A, Kueppers F, James HL, Walsh PN, Weinbaum G, Colman RW, Cohen AB. Neutrophil elastase-releasing factors in bronchoalveolar lavage from patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1985;132:1098–1105.
70. Baird BR, Cheronis JC, Sandhaus RA, Berger EM, White CW, Repine

JE. O_2 metabolites and neutrophil elastase synergistically cause edematous injury in isolated rat lungs. *J Appl Physiol* 1986;61:2224–2229.
71. Kubo K, Kobayashi T, Koyama S, Koizumi T, Shinozaki S, Sekiguchi M, Sakai A, Ueda G. Effects of the neutrophil elastase inhibitor, EI-546, on endotoxin-induced lung injury in awake sheep (abstract). *Am Rev Respir Dis* 1991;143:A580.
72. Kubo K, Kobayashi T, Hayano T, Koizumi T, Honda T, Sekiguchi M, Sakai A. Effects of ONO-5046, a specific neutrophil elastase inhibitor, on endotoxin-induced lung injury in sheep. *J Appl Physiol* 1994; 77:1333–1340.
73. Gossage JR, Kuratomi Y, Davidson JM, Lefferts PL, Snapper JR. Neutrophil elastase inhibitors, SC-37698 and SC-39026, reduce endotoxin-induced lung dysfunction in awake sheep. *Am Rev Respir Dis* 1993;147:1371–1379.
74. Sakamaki F, Ishizaka A, Urano T, Sayama K, Nakamura H, Terashima T, Waki Y, Tasaka S, Hasegawa N, Sato K, Nakagawa N, Obata T, Kanazawa M. Effect of a specific neutrophil elastase inhibitor, ONO-5046, on endotoxin-induced acute lung injury. *Am J Respir Crit Care Med* 1996;153:391–397.
75. Kawabata K, Hagio T, Matsumoto S, Nakao S, Orita S, Aze Y, Ohno H. Delayed neutrophil elastase inhibition prevents subsequent progression of acute lung injury induced by endotoxin inhalation in hamsters. *Am J Respir Crit Care Med* 2000;161:2013–2018.
76. Welter HF, Siebeck M, Thetter O, Jochum M. Influence of the lysosomal elastase inhibitor eglin on the development of interstitial lung edema in *E.coli* bacteremia in pigs. *Prog Clin Biol Res* 1987;236A: 121–125.
77. Yamazaki T, Ooshima H, Usui A, Watanabe T, Yasuura K. Protective effects of ONO-5046-Na, a specific neutrophil elastase inhibitor, on postperfusion lung injury. *Ann Thorac Surg* 1999;68:2141–2146.
78. Carney DE, Lutz CJ, Picone AL, Gatto LA, Ramamurthy NS, Golub LM, Simon SR, Searles B, Paskanik A, Snyder K, Finck C, Schiller HJ, Nieman GF. Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. *Circulation* 1999;100:400–406.
79. Miyazaki Y, Inoue T, Kyi M, Sawada M, Miyake S, Yoshizawa Y. Effects of neutrophil elastase inhibitor (ONO-5046) on acute pulmonary injury induced by tumor necrosis factor alpha (TNFα) and activated neutrophils in isolated perfused rabbit lungs. *Am J Respir Crit Care Med* 1998;157:89–94.
80. Bless NM, Smith D, Charlton J, Czermak BJ, Schmal H, Friedl PH, Ward PA. Protective effects of an aptamer inhibitor of neutrophil elastase in lung inflammatory injury. *Curr Biol* 1997;7:877–880.
81. Saldeen T, Ahn C, Glass M. The effect of the neutrophil elastase inhibitor ICI 200,355 on development of pulmonary edema in the rat thrombosis model of ARDS (abstract). *Am Rev Respir Dis* 1991;143:A581.
82. Peterson MW, Stone P, Shasby DM. Cationic neutrophil proteins increase transendothelial albumin movement. *J Appl Physiol* 1987;62: 1521–1530.
83. Eisenhauer PB, Lehrer RI. Mouse neutrophils lack defensins. *Infect Immunol* 1992;60:3446–3447.
84. Gabay JE, Almeida RP. Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. *Curr Opin Immunol* 1993;5:97–102.
85. Belaaouaj AA, Kim KS, Shapiro SD. Degradation of outer membrane protein A in *Escherichia coli* killing by neutrophil elastase. *Science* 2000;289:1185–1187.
86. Tkalcevic J, Novelli M, Phylactides M, Iredale JP, Segal AW, Roes J. Impaired immunity and enhanced resistance to endotoxin in the absence of neutrophil elastase and cathepsin G. *Immunity* 2000;12:201–210.
87. Bank U, Ansorge S. More than destructive: neutrophil-derived serine proteases in cytokine bioactivity control. *J Leukoc Biol* 2001;69:197–206.
88. van der Geld YM, Limburg PC, Kallenberg CGM. Proteinase 3, Wegener's autoantigen: from gene to antigen. *J Leukoc Biol* 2001;69:177–190.
89. Caughey GH. Serine proteinases of mast cell and leukocyte granules: a league of their own. *Am J Respir Crit Care Med* 1994;150:S138–142.
90. Tani K, Ogushi F, Kido H, Kawano T, Kunori Y, Kamimura T, Cui P, Sone S. Chymase is a potent chemoattractant for human monocytes and neutrophils. *J Leukoc Biol* 2000;67:585–589.
91. BRENDA (enzyme database). Available at http://www.brenda.uni-koeln.de/. Accessed on June 4, 2001.
92. Huang C, De Sanctis GT, O'Brien PJ, Mizerd JP, Friend DS, Drazen JM, Brass LF, Stevens RL. Human mast cell tryptase betaI: evaluation of its substrate specificity and demonstration of its importance in bacterial infections of the lung. *J Biol Chem* 2001; May 2 [epub ahead of print]
93. Kam C-M, Hudig D, Powers JC. Granzymes (lymphocyte serine proteases): characterization with natural and synthetic substrates and inhibitors. *Biochim Biophys Acta* 2000;1477:307–323.
94. Shapiro SD. The macrophage in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:S29–S32.
95. Shapiro SD. Diverse roles of macrophage matrix metalloproteinases in tissue destruction and tumor growth. *Thomb Haemost* 1999;82:846–849.
96. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000;14:2123–2133.
97. McDonnell S, Morgan M, Lynch C. Role of matrix metalloproteinases in normal and disease processes. *Biochem Soc Trans* 1999;27:734–740.
98. Warner RL, Beltran L, Younkin EM, Lewis CS, Weiss SJ, Varani J, Johnson KJ. Role of stromelysin 1 and gelatinase B in experimental acute lung injury. *Am J Respir Cell Mol Biol* 2001;24:537–544.
99. Imai K, Dalal SS, Chen ES, Downey R, Schulman LS, Ginsburg M, D'Armiento J. Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. *Am J Respir Crit Care Med* 2001;163:786–791.
100. Nagase H, Woessner Jr JF. MINIREVIEW: matrix metalloproteinases. *J Biol Chem* 1999;274:21491–21494
101. Dunsmore SE, Saarialho-Kere UK, Roby JD, Wilson CL, Matrisian LM, Welgus HG, Parks WC. Matrilysin expression and function in airway epithelium. *J Clin Invest* 1998;102:1321–1331.
102. Pagano M, Esnard F, Engler R, Gauthier F. Inhibition of human liver cathepsin L by alpha 2 cysteine-proteinase inhibitor and the low-M_r cysteine proteinase inhibitor from human serum. *Biochem J* 1984;220: 147–155.
103. Villadangos JA, Bryant RA, Deussing J, Driessen C, Lennon-Dumenil AM, Riese RJ, Roth W, Saftig P, Shi GP, Chapman HA, Peters C, Ploegh HL. Proteases involved in MHC class II antigen presentation. *Immunol Rev* 1999;172:109–120.