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Neutrophil Elastase, Proteinase 3, and Cathepsin G as Therapeutic Targets in Human Diseases

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Abstract*——Polymorphonuclear neutrophils are the first cells recruited to inflammatory sites and form the earliest line of defense against invading microorganisms. Neutrophil elastase, proteinase 3, and cathepsin G are three hematopoietic serine proteases stored in large quantities in neutrophil cytoplasmic azurophilic granules. They act in combination with reactive oxygen species to help degrade engulfed microorganisms inside phagolysosomes. These proteases are also externalized in an active form during neutrophil activation at inflammatory sites, thus contributing to the regulation of inflammatory and immune responses. As multifunctional proteases, they also play a regulatory role in noninfectious inflammatory diseases. Mutations in the *ELA2/ELANE* gene, encoding neutrophil elastase, are the cause of human congenital neutropenia. Neutrophil membrane-bound proteinase 3 serves as an autoantigen in Wegener granulomatosis, a systemic autoimmune vasculitis. All three proteases are affected by mutations of the gene (*CTSC*) encoding dipeptidyl peptidase I, a protease required for activation of their proform before storage in cytoplasmic granules. Mutations of *CTSC* cause Papillon-Lefèvre syndrome. Because of their roles in host defense and disease, elastase, proteinase 3, and cathepsin G are of interest as potential therapeutic targets. In this review, we describe the physicochemical functions of these proteases, toward a goal of better delineating their role in human diseases and identifying new therapeutic strategies based on the modulation of their bioavailability and activity. We also describe how nonhuman primate experimental models could assist with testing the efficacy of proposed therapeutic strategies.

I. Introduction

Human polymorphonuclear neutrophils represent 35 to 75% of the population of circulating leukocytes and are the most abundant type of white blood cell in mammals (Borregaard et al., 2005). They are classified as granulocytes because of their intracytoplasmic granule content and are characterized by a multilobular nucleus. Neutrophils develop from pluripotent stem cells in the bone marrow and are released into the bloodstream where they reach a concentration of 1.5 to 5 $\times$ 10^9 cells/liter. Their half-life in the circulation is only on the order of a few hours. They play an essential role in innate immune defense against invading pathogens and are among the primary mediators of inflammatory response. During the acute phase of inflammation, neutrophils are the first inflammatory cells to leave the vasculature, where they migrate toward sites of inflammation, following a gradient of inflammatory stimuli. They are responsible for short-term phagocytosis during the initial stages of infection (Borregaard and Cowland, 1997; Hampton et al., 1998; Segal, 2005). Neutrophils use complementary oxidative and nonoxidative pathways to defend the host against invading pathogens (Kobayashi et al., 2005).

The three serine proteases neutrophil elastase (NE[1]), proteinase 3 (PR3), and cathepsin G (CG) are major components of neutrophil azurophilic granules and participate in the nonoxidative pathway of intracellular and extracellular pathogen destruction. These neutrophil serine proteases (NSPs) act intracellularly within phagolysosomes to digest phagocytized microorganisms in combination with microbicidal peptides and the membrane-associated NADPH oxidase system, which produces reactive oxygen metabolites (Segal, 2005). An additional extracellular antimicrobial mechanism, neutrophil extracellular traps (NET), has been described that is made of a web-like structure of DNA secreted by activated neutrophils (Papayannopoulos and Zychlinsky, 2009) (Fig. 1). NETs are composed of chromatin bound to positively charged molecules, such as histones and NSPs, and serve as physical barriers that kill pathogens extracellularly, thus preventing further spreading. NET-associated NSPs participate in pathogen killing by degrading bacterial virulence factors extracellularly (Brinkmann et al., 2004; Papayannopoulos and Zychlinsky, 2009).

In addition to their involvement in pathogen destruction and the regulation of proinflammatory processes, NSPs are also involved in a variety of inflammatory human conditions, including chronic lung diseases (chronic obstructive pulmonary disease, cystic fibrosis,

[1] Abbreviations: α1-PI, α1-protease inhibitor; ACT, α1-antichymotrypsin; ALI, acute lung injury; ANCA, anti-neutrophil cytoplasmic autoantibodies; AP, adaptor protein; ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; cANCA, cytoplasmic ANCA; CHS, Chediak-Higashi syndrome; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CG, cathepsin G; COPD, chronic obstructive pulmonary disease; DPPI, dipeptidyl peptidase I; E-64, (2*S*,3*S*)-3-[[[(1*S*)-1-[[[4-[(aminoiminomethyl)amino]butyl]amino]carbonyl]-3-methylbutyl]amino]carbonyl]-2-oxiranecarboxylic acid; FRET, fluorescence resonance energy transfer; HNE, human neutrophil elastase; IL, interleukin; LPS, lipopolysaccharide; MMP, matrix metalloprotease; mPR3, membrane-bound proteinase 3; NE, neutrophil elastase; NET, neutrophil extracellular trap; NFκB, nuclear factor κB; NSP, neutrophil serine protease; pANCA, perinuclear ANCA; PAR, protease-activated receptor; PDE4, phosphodiesterase E4; PI, protease inhibitor; PLS, Papillon-Lefèvre syndrome; PR3, proteinase 3; RBL, rat basophilic leukemia; RCL, reactive center loop; SDF, stromal cell-derived factor; Serpin, serine protease inhibitor; SLPI, secretory leukocyte protease inhibitor; TIMP, tissue inhibitor of metalloproteases; TLR, Toll-like receptor; TNF, tumor necrosis factor; WG, Wegener granulomatosis.

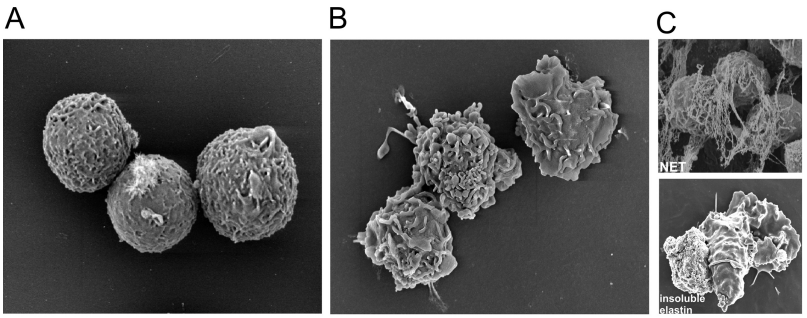


FIG. 1. Polymorphonuclear neutrophil. Quiescent (A) and chemically activated (B) neutrophils purified from peripheral blood. C, PMA-activated neutrophils embedded within NET and neutrophil spreading on insoluble elastin.

acute lung injury, and acute respiratory distress syndrome) (Lee and Downey, 2001; Shapiro, 2002; Moraes et al., 2003; Owen, 2008b). In these disorders, accumulation and activation of neutrophils in the airways result in excessive secretion of active NSPs, thus causing lung matrix destruction and inflammation. NSPs are also involved in other human disorders as a consequence of gene mutations, altered cellular trafficking, or, for PR3, autoimmune disease. Mutations in the *ELA2/ELANE* gene encoding HNE are the cause of human cyclic neutropenia and severe congenital neutropenia (Horwitz et al., 1999, 2007). Neutrophil membrane-bound proteinase 3 (mPR3) is the major target antigen of anti-neutrophil cytoplasmic autoantibodies (ANCA), which are associated with Wegener granulomatosis (Jenne et al., 1990). All three proteases are affected by mutation of the gene (*CTSC*) encoding dipeptidyl peptidase I (DPPI), which activates several granular hematopoietic serine proteases (Pham and Ley, 1999; Adkison et al., 2002). Mutations of *CTSC* cause Papillon-Lefèvre syndrome and palmoplantar keratosis (Hart et al., 1999; Toomes et al., 1999).

In this review, we focus on the physicochemical properties of HNE, PR3, and CG, drawing attention to recent advances in their physiopathological functions to better understand their role in human diseases. We propose and discuss new therapeutic strategies based on modulation of their activity. We also describe how nonhuman primate models of NSPs-related human diseases could help test the efficacy of therapeutic approaches.

II. Neutrophil Elastase, Proteinase 3, and Cathepsin G

A. History

Proteases are proteolytic enzymes that catalyze the splitting of proteins into smaller peptides or amino acids and are important in many ways to human health and biotechnology. Four major classes of proteases are distinguished on the basis of key residues involved in catalysis: serine proteases, cysteine proteases, aspartic acid proteases, and metalloproteases. A new type of catalysis that involves a threonine in the catalytic site has been identified more recently in studies of the proteasome. There are at least 500 to 600 different proteases in humans; most of them are serine, cysteine, and metalloproteases (López-Otín and Bond, 2008). The serine endopeptidases (EC 3.4.21.−) are characterized by the presence of a conserved serine residue as part of the catalytic center. In trypsin/chymotrypsin-type serine proteases, for example, this catalytic triad consists of the three residues Asp, His, and Ser (Polgár, 2005). The major clans found in humans include chymotrypsin-like, α/β-hydrolase, and signal peptidase. Serine proteases represent approximately one third of total proteases currently identified and are present in eukaryotes, prokaryotes, bacteria, and archaea (Hedstrom, 2002). They display diverse biological functions and include digestive enzymes of exocrine glands, clotting factors, and leukocyte granule-associated proteases, such as NE, PR3, and CG.

Although the activity of leukocyte proteases had been described early in the 20th century, HNE was identified only in 1968 by Janoff and Scherer (1968). The term "elastase" is used to describe an enzyme capable of releasing soluble peptides from insoluble elastin, which was the first substrate employed for characterizing its activities, but does not necessarily imply that its activities are always physiologically related to digestion of this target. Subsequently, another protease retaining enzymatic activity on synthetic substrates for chymotrypsin was described (Dewald et al., 1975; Rindler-Ludwig and Braunsteiner, 1975) and designated "cathepsin G" (Starkey and Barrett, 1976). A third protease, "myeloblastin," was first identified in 1978 and more extensively characterized 10 years later. This name was originally used by Bories et al. (1989) to describe a serine protease in promyelocytic leukemia cells HL-60. It was subsequently shown that myeloblastin was identical to a protease known independently as "proteinase 3" that had been studied for its role in emphysema (Baggiolini et al., 1978). The same molecule had been also described as a protein with antimicrobial activity (Campanelli et al., 1990a) and called azurophilic granule protein 7, and it was identified as the p29 autoantigen or ANCA auto-antigen in Wegener granulo-

matosis before it finally became referred to uniformly as "proteinase 3."

B. Biosynthesis, Processing, and Localization in Neutrophil

HNE, PR3, and CG are three homologous proteases that belong to the chymotrypsin superfamily of serine proteases. They evolved from a common ancestor through gene duplication. The genes encoding HNE, PR3, and CG consist of five exons and four introns (Zimmer et al., 1992; Caughey et al., 1993). The gene for HNE, formerly called *ELA2* and newly officially dubbed *ELANE*, is located in the same cluster as that of PR3 (*PRTN3*) and azurocidin in the terminal region of the short arm of chromosome 19p13.3 (Zimmer et al., 1992; Caughey et al., 1993). In contrast to its related family members, azurocidin has lost its catalytic serine and is devoid of proteolytic activity (Watorek, 2003). It primarily exhibits antimicrobial function. The CG gene (*CTSG*) is located on chromosome 14q11.2 in a cluster containing other genes for the related serine proteases chymase, granzyme H (Fellows et al., 2007), and granzyme B (Caughey et al., 1993). HNE, PR3, and CG genes are expressed in granulocytes, monocytes, and mast cells. High-level transcription of these HNE, PR3, and CG genes is limited to the promyelocytic stage of neutrophil maturation (Garwicz et al., 2005). PR3 is also found in basophiles (Braun et al., 1991). The main characteristics of HNE, PR3, and CG are presented in Table 1.

The synthesis of HNE, PR3, and CG is regulated first at the transcriptional level during granulocyte development and second at the post-translational level before they are stored in their proteolytically active mature form within neutrophil azurophilic granules. They are synthesized as an inactive prepro-protein containing a signal peptide and an amino-terminal prodipeptide (Rao et al., 1996; Garwicz et al., 1997, 1998; Gullberg et al., 1997). They also contain a C-terminal propeptide, the removal of which is not necessary for NSP activity. The processing and granule targeting of NSPs has been studied in transfected cells producing active proteases in a

TABLE 1
Main characteristics of elastase, proteinase 3, and cathepsin G

Characteristic	Elastase	Proteinase 3	Cathepsin G
EC number	3.4.21.37	3.4.21.76	3.4.21.20
Gene locus and gene structure	19p13.3, 5 exons 4 introns	19p13.3, 5 exons 4 introns	14q11.2, 5 exons 4 introns
Endogenous activator	Dipeptidyl peptidase I	Dipeptidyl peptidase I	Dipeptidyl peptidase I
Prodipeptide	Ser-Glu	Ala-Glu	Gly-Glu
Characteristics of mature forms			
Residues	218	222	235
Molecular mass	29–33 kDa	29–32 kDa	28.5 kDa
pI	~10.5	~9.5	~12
Number of glycosylation sites	2	2	1
Number of disulfide bridges	4	4	3
Optimal pH for activity	8.0–8.5	~8.0	~7.5
Substrate specificity	Small hydrophobic residue at P1 position: Val, Cys, Ala, Met, Ile, Leu, Ser	Small hydrophobic residue at P1 position: Val, Cys, Ala, Met, Ser, Leu	Aromatic or positively charged residues at P1 position: Phe, Tyr, Lys, Arg
Best FRET substrate	Abz-APEEIMRRQ-EDDnp	Abz-VADnorVADYQ-EDDnp	Abz-EPFWEDQ-EDDnp
Localization in neutrophil	Azurophilic granules	Azurophilic granules	Azurophilic granules
	Nuclear envelope	Specific granules	Neutrophil cell surface after priming
	Neutrophil cell surface after priming	Secretory vesicles	Neutrophil extracellular traps
	Neutrophil extracellular traps	On quiescent and activated neutrophil cell surface	
		Neutrophil extracellular traps	
Source	Neutrophil	Neutrophil	Neutrophil
	Monocyte	Monocyte	Monocyte
		Basophil	Mastocyte
Endogenous inhibitors	α2-Macroglobulin	α2-Macroglobulin	α2-Macroglobulin
	α1-PI/MNEI/PI9	α1-PI/MNEI	ACT/MNEI/α1-PI/PI6
	SLPI/Elafin/pre-elafin	Elafin/pre-elafin	SLPI
Biologicals functions	Degradation of ECM components	Degradation of ECM components	Degradation of ECM components
	Bactericidal properties	Bactericidal properties	Bactericidal properties
	Cleavage of inflammatory mediators	Cleavage of inflammatory mediators	Cleavage of inflammatory mediators
	Cleavage of receptors	Cleavage of receptors	Cleavage of receptors
	Cleavage of lung surfactant	Induction of endothelial cell apoptosis	Platelet activation
	Cytokine and chemokine induction	Negative feedback regulation of granulopoiesis	Induction of airway submucosal gland secretion
	Induction of airway submucosal gland secretion		
Pathological roles in human diseases	Inflammatory diseases	Inflammatory diseases	Inflammatory diseases
	Papillon-Lefèvre syndrome	Papillon-Lefèvre syndrome	Papillon-Lefèvre syndrome
	Hereditary neutropenia	Wegener's granulomatosis	

fashion similar to that of human neutrophils. Activation of NSPs occurs through cleavage of the amino-terminal peptide by signal peptidase, followed by the removal of an aminoterminal dipeptide by DPPI, a tetrameric cysteine protease also known as cathepsin C (EC 3.4.14.1). DPPI is a highly conserved lysosomal cysteine exopeptidase expressed mostly in the lung, spleen, kidney, and myeloid cells and belongs to the papain family (McGuire et al., 1993). It is composed of an oligomeric structure derived from four identical subunits and represents the only member of this family that is functional as a tetramer (Turk et al., 2001). DPPI is initially synthesized as a 55-kDa monomeric pro-enzyme rapidly processed to generate a mature form, but the regulation of its processing is still unknown. The proteolytically active enzyme adopts a papain like-structure made of a 23-kDa heavy chain (Leu207–Arg370) and a 7-kDa light chain (Asp371–Leu439) linked by disulfide bonds and an "exclusion" propeptide domain of 16 kDa (Asp1–Gly119) that remains bound to the mature active enzyme (Fig. 2A). This so-called exclusion domain transforms the framework of a papain-like endopeptidase into a dipeptidyl exopeptidase by blocking the active site beyond the S2 pocket (Turk et al., 2001) (Fig. 2B). In addition to its key role as an activator of hematopoietic granule serine proteases, DPPI also participates in lysosomal peptide degradation via its dipeptidyl peptidase activity. The enzyme cleaves two-residue units from the N termini of proteins until it reaches a stop sequence, typically an arginine or lysine in P2, a proline residue in P1 or P1′, or an isoleucine residue in P1. The C-terminal propeptide of HNE, PR3, and CG is normally excised by an as-yet-

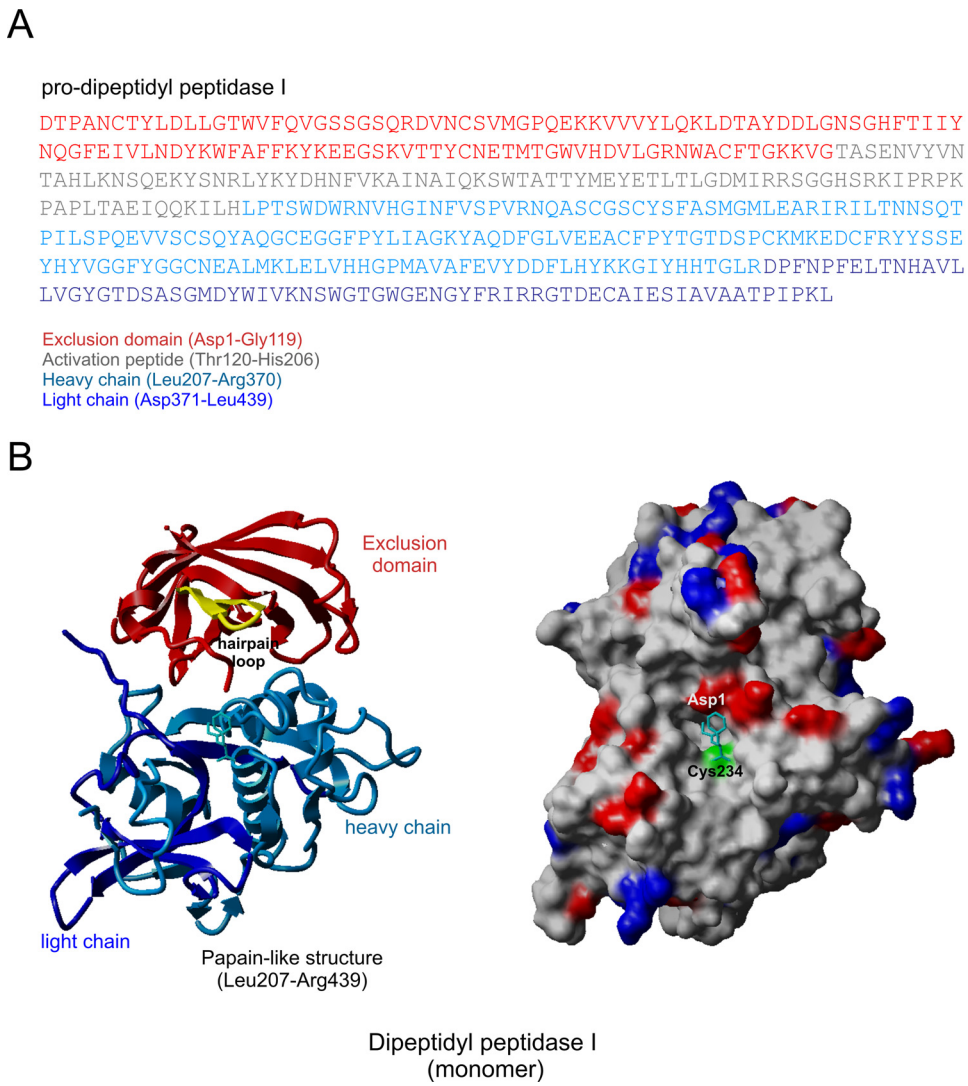


FIG. 2. Structure of human pro-dipeptidyl peptidase I. A, amino acid sequence of human pro-dipeptidyl peptidase I. Primary sequences corresponding to exclusion domain (Asp1–Gly119), activation peptide (Thr120–His206), heavy chain (Leu207–Arg370), and light chain (Asp371–Leu439) are colored in red, gray, light, and dark blue, respectively. B, ribbon representation and solvent-accessible surfaces of the DPPI functional monomer in complex with the inhibitor Gly-Phe-CHN_2 [Protein Data Bank 1K3B (Turk et al., 2001), 2DJF, and 2DJG (Mølgaard et al., 2007)]. Color codes in the ribbon plot are the same as in A except that Asp1 in the hairpin loop is colored yellow (left). Solvent-accessible surfaces with positive or negative electrostatic potential are colored dark blue and red, respectively (right). The side chain of the catalytic Cys234 is showed with a cyan stick. The figures have been created with Yasara (http://www.yasara.org).

unknown protease(s), although this does not seem to be required for either granule targeting or proteolytic activity (Gullberg et al., 1995; Capizzi et al., 2003).

Fully processed mature HNE, PR3, and CG isolated from azurophilic granules contain, respectively, 218 (Bode et al., 1986; Sinha et al., 1987), 222 (Campanelli et al., 1990b), and 235 (Salvesen et al., 1987; Hof et al., 1996) residues. They are present in several isoforms depending on their carbohydrate content, with apparent mass of 29 to 33 kDa upon SDS-polyacrylamide gel electrophoresis (Twumasi and Liener, 1977; Watorek et al., 1993). HNE and PR3 display two sites of N-glycosylation, whereas CG possesses only one. NSPs are stored mainly in neutrophil azurophilic granules, but HNE is also localized in the nuclear envelope, as revealed by immunostaining and electron microscopy (Clark et al., 1980; Benson et al., 2003), whereas PR3 is also found in secretory vesicles (Witko-Sarsat et al., 1999a). Upon neutrophil activation, granular HNE, PR3, and CG are secreted extracellularly, although some molecules nevertheless remain at the cell surface (Owen and Campbell, 1999; Owen, 2008a). The mechanism through which NSPs are sorted from the *trans*-Golgi network to the granules has not been completely defined, even though an intracellular proteoglycan, serglycin, has been identified as playing a role in elastase sorting and packaging into azurophilic granules (Niemann et al., 2007). Unlike HNE and CG, PR3 is constitutively expressed on the membranes of freshly isolated neutrophils (Csernok et al., 1990; Halbwachs-Mecarelli et al., 1995). Stimulation of neutrophils at inflammatory sites triggers intracytoplasmic granules to translocate to the phagosomes and plasma membrane, thereby liberating their contents. The first step of the translocation to the target membrane depends on cytoskeleton remodeling and microtubule assembly (Burgoyne and Morgan, 2003). This is followed by a second step of granule tethering and docking, which are dependent on the sequential intervention of SNARE proteins (Jog et al., 2007).

C. Structural Characteristics and Proteolytic Specificity

As found for other members of this family (i.e., the chymotrypsin/trypsin-type family), HNE, PR3, and CG display a three dimensional structure consisting of two homologous β-barrels and a C-terminal α-helix (Bode et al., 1986; Fujinaga et al., 1996; Hof et al., 1996) (Fig. 3A). Each barrel contains six antiparallel β-sheets connected through a linker segment. Residues of the catalytic triad [Ser195, Asp102, and His57 (chymotrypsin numbering)] are located at the junction of the two β-barrels, whereas the active site cleft runs perpendicular to this junction. This arrangement of amino acids in the active site presumably allows nucleophil attack by Ser195 on the carbonyl carbon (C = O) of the substrate scissile bond, thus setting off the catalysis process.

Upon processing of a dipeptide at the amino terminus of NSPs by DPPI, the free ammonium group of the first N-terminal residue (Ile16) forms an internal salt bridge with the side-chain carboxylate of Asp194 that renders the active site S1 pocket [Schechter and Berger (1967) nomenclature; Fig. 4A] accessible to substrate. Ile16 together with the three flexible surface loops (217–225 loop, 180 loop, and autolysis loop) of the C-terminal β-barrel form the activation domain of NSPs (Fig. 3A), the conformation of which differs after processing by DPPI. The main differences between the topologies of pro- and mature forms of related serine proteases have been previously documented for thrombin, prochymase, and progranzyme K (Hink-Schauer et al., 2002; Reiling et al., 2003; Jenne and Kuhl, 2006). HNE, PR3, and CG are highly cationic proteases as a result of their content of positively charged residues. HNE and PR3 display a cluster of positively charged residues located in the loops of the activation domain (Korkmaz et al., 2007). This positive cluster is disrupted by negatively charged residues in PR3. Four of the five hydrophobic residues forming the surface hydrophobic patch in PR3 are located on loop 217–225 (Fig. 3B). This most probably explains the specific binding of mature PR3 but not of its proform to the neutrophil membrane (Korkmaz et al., 2008b).

The three dimensional structure of the chymotrypsin family of serine proteases in complex with inhibitors provides useful information regarding the operation of the active site. Structures of HNE in complex with turkey ovomucoid third domain (Bode et al., 1986), secretory leukocyte protease inhibitor (SLPI) domain 2 (Koizumi et al., 2008), and synthetic chloromethyl ketone inhibitors (Wei et al., 1988; Navia et al., 1989) have been determined as that of CG in complex with Suc-Val-Pro-PheP-(OPh)2 (Hof et al., 1996) and β-ketophosphonate 1 (de Garavilla et al., 2005). The crystal structure of PR3 has been solved by molecular replacement using the crystallized HNE structure (Fujinaga et al., 1996). HNE, PR3, and CG display a common fold, but their distinct properties with respect to particular substrate cleavage specificity arises from the substitutions in the S1 pocket and also from the surface loops surrounding the active site (99 loop, 60 loop, 37 loop, and autolysis loop) defining the environment of the active site. Solvent-accessible surfaces in HNE, PR3, and CG show that their charge distributions differ significantly in the vicinity of the substrate binding region (Korkmaz et al., 2008c).

The S1 pocket in individual chymotrypsin-like serine proteases plays an important role in defining their substrate specificity. The HNE S1 pocket is hemispherical and hydrophobic because of the presence of Val190, Phe192 Ala213, Val216, Phe228, and the disulfide bridge Cys191–Cys220 (Bode et al., 1986). The S1 pocket of PR3 is also hemispheric but seems to be smaller than that of HNE because of the Val/Ile substitution at position 190 (Fujinaga et al., 1996). The S1 subsite of CG, which is larger than that of

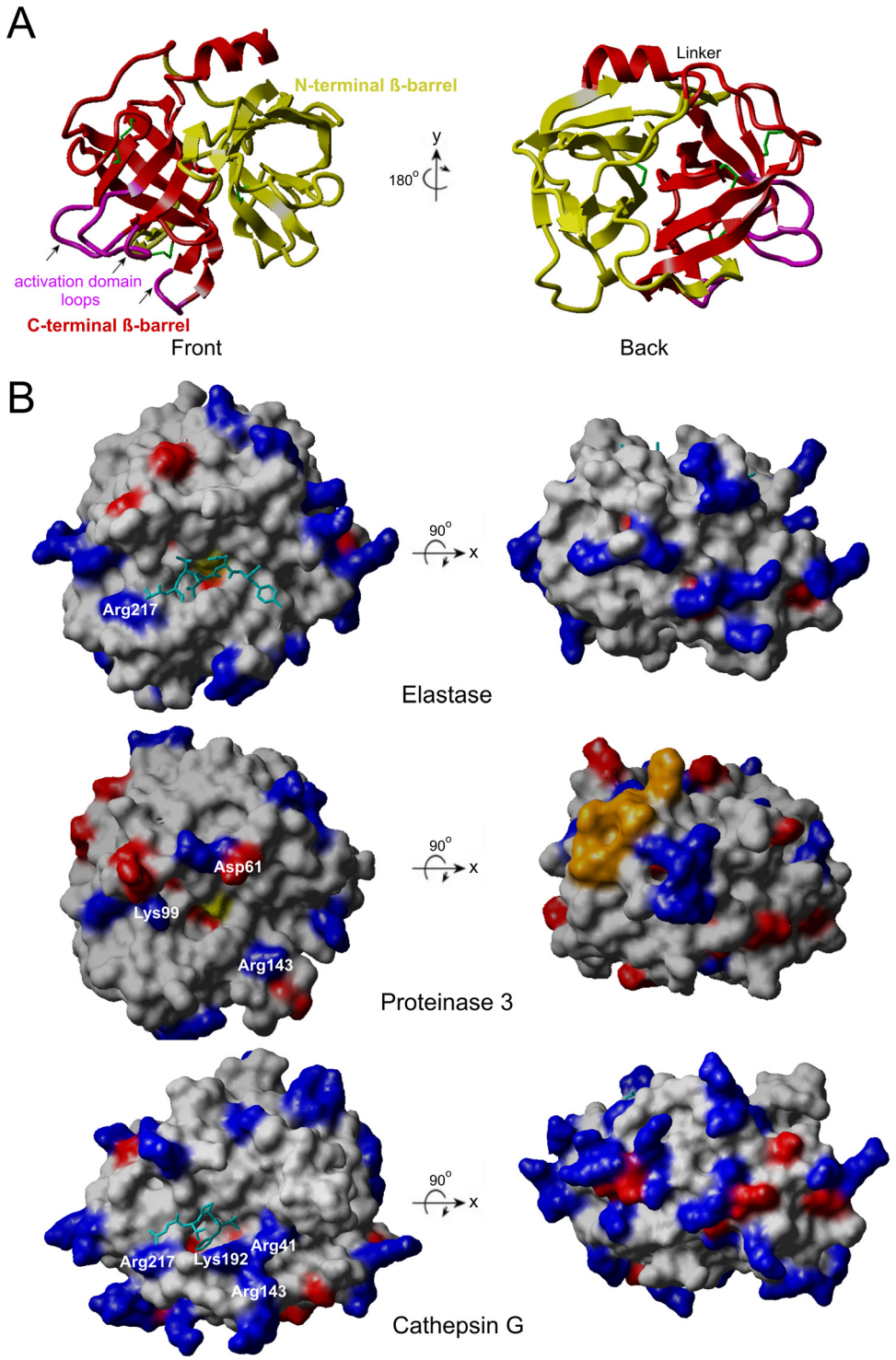


FIG. 3. Three-dimensional architecture of the trypsin/chymotrypsin family and structural differences between neutrophil elastase, proteinase 3, and cathepsin G. A, ribbon plot of neutrophil elastase showing the characteristics of the trypsin/chymotrypsin family, the two asymmetric β-barrels and the C-terminal α-helix, front view on the left, back view after a rotation of 180° around a vertical y-axis on the right. N- and C-terminal β-barrels are presented in red and yellow, respectively. The three flexible loops of the C-terminal β-barrels forming the activation domain are colored in pink and indicated by arrows. Disulfide bounds are depicted in green. B, the solvent accessible surface based on the atom coordinates of elastase [Protein Data Bank 1PPF (Bode et al., 1986)], proteinase 3 [Protein Data Bank 1FUJ (Fujinaga et al., 1996)], and cathepsin G [1CGH (Hof et al., 1996)] is colored to show its positive (blue) and negative (red) electrostatic potential. Surface-accessible residues forming a hydrophobic patch on proteinase 3 are depicted in orange. The loop of the inhibitor turkey ovomucoid third domain (P4–P3′) complexed to elastase and the irreversible phosphonate inhibitor Suc-Val-Pro-Phe^{P}-$(OPh)_2$ complexed to cathepsin G are given as a cyan stick model. The serine of the catalytic triad is yellow. Ribbon plot of neutrophil elastase and the molecular surfaces are generated with Yasara (http://www.yasara.org).

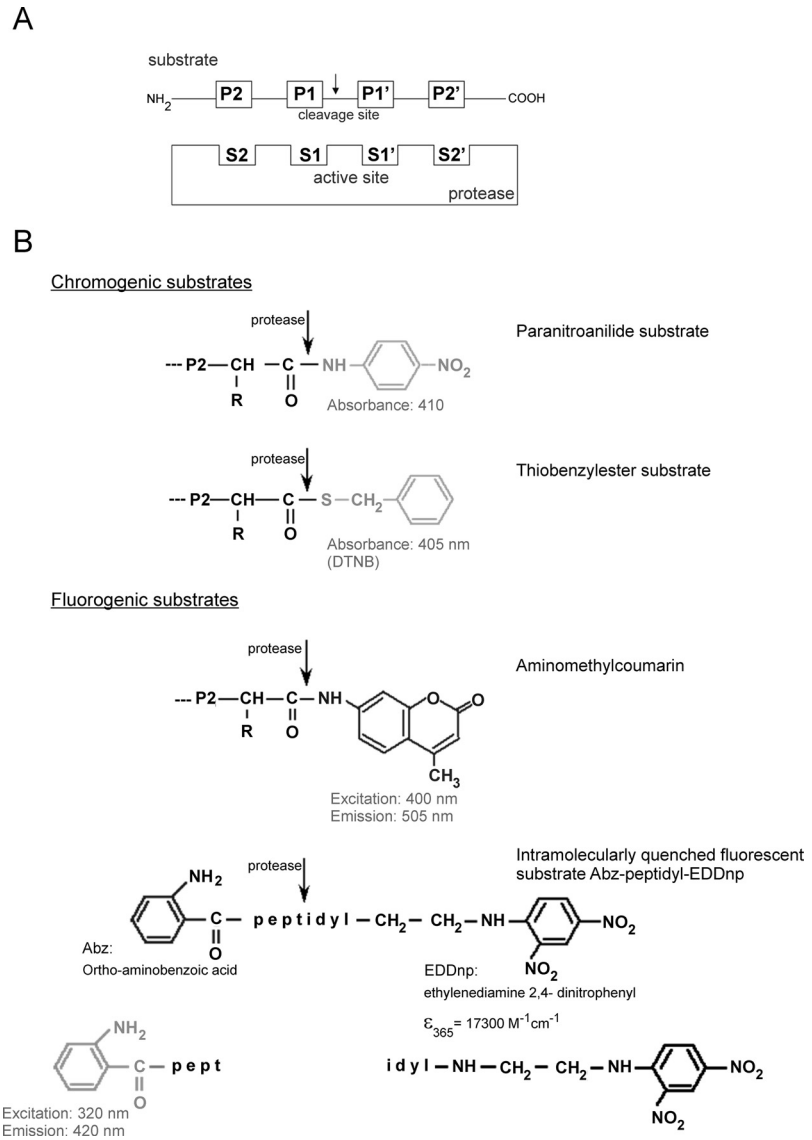


FIG. 4. Synthetic chromogenic and fluorogenic substrates of NSP. A, schematic diagram illustrating the Schechter and Berger (1967) nomenclature. This nomenclature defines a linear topology of the interactions between a protease and a substrate: S subsites on the protease accommodate P residues of the substrate upstream of the scissile bond, whereas S′ subsites accommodate P′ residues from the C-terminal part of the substrate, downstream of the cleavage site. Numbering starts with the first residue next to the cleavage site, P1 and P1′, and continues toward the N terminus (P2, P3, P4, etc.) and the C terminus (P2′, P3′, P4′, etc.). B, chromogenic substrates commonly used to measure the activity of NSPs, peptidyl-paranitroanilides (top), peptidyl-thiobenzylesters with chromogenic groups in gray. Paranitroanilide may be detected by direct measurement at 410 nm, but a coupled assay with a thiodisulfide reagent such as DTNP is required for measuring the absorbance of the released thiobenzyl group. Fluorogenic aminomethylcoumarin substrates contain a fluorescent group that is released by proteolytic cleavage. FRET substrates contain a fluorescent and a quenching group as a donor/acceptor pair [here a fluorescent *ortho*-aminobenzoyl (Abz) group and a *N*-(2,4-dinitrophenyl ethylenediamine (EDDnp) quenching group] at their N- and C-terminal ends, respectively. Fluorescence is released after cleavage of any peptide bond within the amino acid sequence (shown in gray). FRET substrates are convenient tools to investigate the protease specificity around the cleavage site. DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

HNE and PR3, is divided into two compartments with a negatively charged Glu226 at the bottom (Hof et al., 1996). The specificity of NSPs has been investigated using chromogenic (p-nitroanilide, $Anb^{5,2}$-NH_2, thiobenzylester) and fluorogenic [aminomethylcoumarin, fluorescence resonance energy transfer (FRET)] substrates (Castillo et al., 1979; Nakajima et al., 1979; McRae et al., 1980; Tanaka et al., 1985; Brubaker et al., 1992; Kam et al., 1992; Réhault et al., 1999; Wysocka et al., 2007; Korkmaz et al., 2008a; Carmona et al., 2009) (Fig. 4B). In agreement with structural data, HNE and PR3 preferentially accommodate small hydrophobic residues (Val, Cys, Ala, Met, Ile) (Brubaker et al., 1992; Koehl et al., 2003; Korkmaz et al., 2007; Wysocka et al., 2007) in the S1 pocket, whereas CG has both a chymotrypsin-like and a trypsin-like specificity, permitting both large, hydrophobic P1 residues (Phe, Leu, Met) and positive Lys or Arg residues (Tanaka et al., 1985). The S2 subsites of HNE and CG are bordered by Phe215, Leu99, and the flat side of the imidazole ring of the catalytic triad His57, which is quite hydrophobic. In contrast, the S2 subsite of PR3 is a deep polar pocket of increased polarity as a result of the Leu-to-

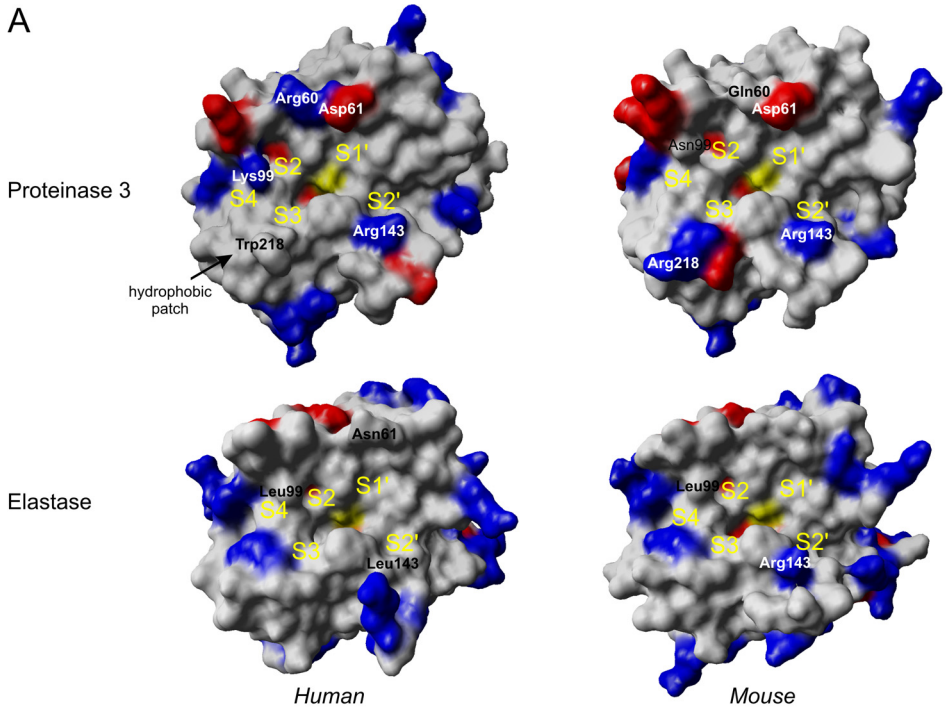
Lys substitution at position 99 (Fujinaga et al., 1996). The size and hydrophobic properties of the S2 subsite of HNE and CG account for the fact that medium-sized hydrophobic side chains, such as that of Pro, are preferred at the P2 position (Tanaka et al., 1985; Réhault et al., 1999). Indeed, most commercially available chromogenic and/or fluorogenic substrates used to measure HNE and CG display a proline at the P2 position. Because of the presence of Lys99, PR3 preferentially accommodates a negatively charged residue at P2 (Hajjar et al., 2006; Korkmaz et al., 2007). The S3 subsite of CG is formed by a Lys at position 192, which favors interaction with an acidic P3 residue (Tanaka et al., 1985). The residue at position 99 in all three proteases borders both the S2 and S4 subsites, which makes it smaller and more polar in PR3 than in HNE or CG because of the Leu-to-Lys substitution. Regarding the S′ subsites, the S1′ and S2′ subsites in HNE are relatively hydrophobic and lined with Cys42–Cys58 and Phe41, Leu143, respectively. The S2′ subsite in PR3 lies in the vicinity of the charged residues Asp61 and Arg143, neither of which is conserved in HNE (Fujinaga et al., 1996). The 60 loop in PR3 containing Asp61 is significantly displaced to bring the negatively charged side chain to the S1′ and S3′ sites, thereby making them smaller and more polar. These peculiarities have been exploited to develop the first specific synthetic substrates of human PR3 (Korkmaz et al., 2004, 2007). It is noteworthy that Asp61 and Arg143 are conserved in mouse and human PR3, whereas the Lys99–Arg60 doublet in human is replaced by Asn99–Gln60 in the mouse (Fig. 5A), which explains why mouse PR3 does not cleave human PR3 substrates bearing a negatively charged residue at P2 (Kalupov et al., 2009) (Fig. 5B). The P′-S′ specificity of CG has not yet been fully elucidated. X-ray structural analysis showed that CG displays two positively charged residues (Arg41 and Arg143) that reside in the immediate vicinity of the active S2′ site and that could contribute to the specificity of the protease (Hof et al., 1996; Réhault et al., 1999).

D. Plasma Membrane Association

Exposure of neutrophils to cytokines (TNF-α), chemoattractants (platelet-activating factor, formyl-Met-Leu-Phe, or IL-8), or bacterial lipopolysaccharide leads to rapid granule translocation to the cell surface with secretion of HNE, PR3, and CG into the extracellular medium (Owen and Campbell, 1999). A fraction of secreted HNE, PR3, and CG is detected at the surface of activated neutrophils (Owen et al., 1995a, 1997; Campbell et al., 2000). Resting purified neutrophils from peripheral blood express variable amounts of PR3 on their surface. A bimodal, apparently genetically determined, distribution has been observed with two populations of quiescent neutrophils that express or do not express the protease at their surface (Halbwachs-Mecarelli et al., 1995; Schreiber et al., 2003). The percentage of mPR3-positive neutrophils ranges from 0 to 100% of the total neutrophil population within individuals. Furthermore, the percentage of mPR3-positive neutrophils remains stable over time and is not affected by neutrophil activation (Halbwachs-Mecarelli et al., 1995).

The mechanism through which HNE and CG are associated with the outer surface of the plasma membrane of neutrophils mainly involves electrostatic interactions with the sulfate groups of chondroitin sulfate- and heparan sulfate-containing proteoglycans (Campbell and Owen, 2007). These two proteases are released from neutrophil cell surfaces by high concentrations of salt (Owen et al., 1995b, 1997; Korkmaz et al., 2005a) and after treatment with chondroitinase ABC and heparinase (Campbell and Owen, 2007). Membrane PR3 is not solubilized by high salt concentrations, which means that its membrane association is not charge dependant (Witko-Sarsat et al., 1999a; Korkmaz et al., 2009). Unlike HNE and CG, PR3 bears at its surface a hydrophobic patch formed by residues Phe166, Ile217, Trp218, Leu223, and Phe224 that is involved in membrane binding (Goldmann et al., 1999; Hajjar et al., 2008) (Fig. 3B). Several membrane partners of PR3 have been identified, including CD16/FcγRIIIb (David et al., 2005; Fridlich et al., 2006), phospholipid scramblase-1, a myristoylated membrane protein with translocase activity present in lipid rafts (Kantari et al., 2007), CD11b/CD18 (David et al., 2003), and human neutrophil antigen NB1/CD177 (von Vietinghoff et al., 2007; Hu et al., 2009), a 58- to 64-kDa glycosyl-phosphatidylinositol anchored surface receptor belonging to the urokinase plasminogen activator receptor superfamily (Stroncek, 2007). NB1 shows a bimodal distribution that superimposes with that of PR3 on purified blood neutrophils (Bauer et al., 2007). Active, mature forms of PR3 but not pro-PR3 can bind to the surface of NB1-transfected human embryonic kidney 293 cells (von Vietinghoff et al., 2008) and Chinese hamster ovary cells (Korkmaz et al., 2008b). Interaction involves the hydrophobic patch of PR3 because specific amino acid substitutions disrupting this patch in the closely related gibbon PR3 prevent binding to NB1-transfected cells (Korkmaz et al., 2008b). Decreased interaction of pro-PR3 with NB1-transfected cells is explained by the topological changes affecting the activation domain containing the hydrophobic patch residues. Together, these results support the hydrophobic nature of PR3-membrane interaction.

Because a significant fraction of HNE, PR3, and CG is detected at the surface of activated neutrophils, measuring and quantifying membrane-bound activities is of major interest for understanding their role during inflammation. The availability of sensitive and specific FRET substrates for each NSP has allowed the quantification of active proteases on the surface of stimulated neutrophils (Owen et al., 1995a, 1997; Korkmaz et al., 2005a, 2009). With the use of PR3-specific FRET substrate, it has been shown that constitutively expressed PR3 on the surface of quiescent blood neutrophils is



B

Human		PR3	HNE		*Mouse*		mPR3	mNE	
IL-8	AVLPR**SA KE**L-	+	-	(1)	MIP-2	AVVA **SE**LR-	+	+	(6)
IL-18	-MTDS**DC RD**N-	+	-	(2)	IL-18	-MTDS**DC RD**N-	-	-	(7)
SDF1-α	**K**PV SLSY-	-	+	(3)	SDF1-α	**K**PV SLSYR-	+	+	(8)
NFκB	-K**DC RD**GA-	+	-	(4)	NFκB	-GK**DC RD**GA-	-	-	(9)
p21	-IQ**EA RER**A-	+	-	(5)	p21	-LQ**EA RER**A-	-	-	(10)

FIG. 5. A, interspecies comparisons of the active site region of proteinase 3 and neutrophil elastase from men and mice. Values of electrostatic potential were mapped onto the solvent accessible surface of each protease with positively and negatively charged residues colored in blue and red, respectively. Positions of the subsites S4 to S2′ are shown in yellow. Key residues that lie in close vicinity to the substrate binding pockets are labeled by their three-letter codes. The hydrophobic patch in human PR3 is missing in the mouse homolog as the Trp218 residue is substituted by an Arg. B, sequences derived from natural human and mouse substrates that are cleaved by human and murine NE and PR3. Arrows indicate identified and potential cleavage sites. IL-8: (Padrines et al., 1994) (1); IL-18: putative cleavage sites [(2) and (7)]; SDF-1α: (Valenzuela-Fernandez et al., 2002) (3), putative cleavage site (8); NFκB: (Preston et al., 2002) (4), NFκB (Kalupov et al., 2009) (9); p21 (Dublet et al., 2005) (5), T. Kalupov et al., unpublished data (10); and MIP-2 (T. Dau and D. E. Jenne, unpublished data) (6). The structural models of murine proteases were derived from X-ray structures of human homologs by homology modeling (http://www.expasy.ch).

either enzymatically inactive or is unable to interact with substrates (Korkmaz et al., 2009).

III. Biological Functions of Elastase, Proteinase 3, and Cathepsin G

NSPs are currently viewed as multifunctional enzymes involved in pathogenic agent killing and in inflammatory process regulation (Pham, 2006). First recognized as degradative enzymes able to kill pathogens and cleave extracellular matrix components, NSPs have been attributed a potential role in chemotaxis and migration through cleavage of adhesion molecules at intercellular junctions (Cepinskas et al., 1999; Hermant et al., 2003). This function, however, remains debated because some studies demonstrated decreased neutrophil chemotaxis when using NSP inhibitors (Stockley et al., 1990; Lomas et al., 1995; Young et al., 2007), whereas others have revealed no alteration of neutrophil migration in response to neutrophil-specific chemotactic stimuli in animal models deficient in NSPs (MacIvor et al., 1999; Adkison et al., 2002; Allport et al., 2002).

A. Antimicrobial Roles in Infections

HNE, PR3, and CG participate in direct intracellular killing of phagocytosed bacteria in phagolysosomes in combination with myeloperoxidase and reactive oxygen

species generated by the NADPH oxidase complex (Kobayashi et al., 2005). In addition to intracellular killing, extracellular killing can occur through trapping of bacteria in NET composed of filamentous DNA structures decorated with cationic proteases, including NSP secreted by activated neutrophils (Brinkmann et al., 2004). HNE exerts its antimicrobial activity on Gram-negative bacteria by cleaving the outer membrane protein A of *Escherichia coli* (Belaaouaj et al., 2000) and other virulence factors of *Salmonella enterica*, *Yersinia enterocolitica*, and *Shigella flexneri* (Weinrauch et al., 2002). HNE also prevents the escape of *S. flexneri* from phagolysosomes in neutrophils (Weinrauch et al., 2002). Extracellular HNE and CG cleave the proinflammatory bacterial virulence factor flagellin (López-Boado et al., 2004) and degrade leukotoxin, a membrane pore-forming virulence factor of the Gram-negative bacteria *Actinobacillus actinomycetemcomitans*, which can lyse leukocytes and inhibit neutrophil functions (Tsai et al., 1979; Johansson et al., 2000). This pathogen has attracted attention because of its implication in severe destructive periodontal disease. However, the proteolytic activity of NSPs is not necessarily crucial for their antimicrobial activity. Their positive surface charges mediate strong binding to bacterial membranes. This binding alone may inhibit bacterial protein synthesis and induces membrane depolarization and disruption (Zasloff, 2002). Several peptides derived from the CG structure possess antimicrobial properties in vitro (Shafer et al., 1996, 2002). The antimicrobial properties of PR3 are also independent of its protease activity. PR3 efficiently kills both Gram-negative bacteria such as *E. coli* and Gram-positive bacteria such as *Streptococcus faecalis*, as well as fungi such as *Candida albicans.* PR3 also processes human cathelicidin (hCAP18) to its active LL-37 form in the extracellular environment after neutrophil activation (Sørensen et al., 2001). Human cathelicidin is an 18-kDa cationic anti-bacterial protein produced by epithelial cells of the gastrointestinal and respiratory tract and by sebocytes of the skin but also by blood cells, including neutrophils (Doss et al., 2010). Antimicrobial activity requires a proteolytic cleavage of hCAP-18 to liberate the active C-terminal 37-residue polypeptide that exerts broad antimicrobial activity against Gram-negative and -positive bacteria (Dürr et al., 2006).

Mice deficient in NE and CG have been employed as models for exploring their antibacterial functions in microbial infectious diseases. Previous studies showed that mice deficient in NE and CG are more susceptible to infection by Gram-negative and -positive bacteria (Reeves et al., 2002). In neutrophils from NE-deficient [NE(−/−)] mice infected by *S. flexneri*, 12% of bacteria escaped from the phagolysosome (Weinrauch et al., 2002).

B. Roles in Inflammatory Process Regulation

NSPs are abundantly secreted into the extracellular environment upon neutrophil activation at inflammatory sites. A fraction of the released proteases remain bound in an active form on the external surface of the plasma membrane so that both soluble and membrane-bound NSPs are able to proteolytically regulate the activities of a variety of chemokines, cytokines, growth factors, and cell surface receptors. Secreted proteases also activate lymphocytes and cleave apoptotic and adhesion molecules (Bank and Ansorge, 2001; Pham, 2006; Meyer-Hoffert, 2009). Thus, they retain pro- and anti-inflammatory activities, resulting in a modulation of the immune response at sites of inflammation.

1. Processing of Cytokines, Chemokines, and Growth Factors. TNF-α and IL-1β are inflammatory cytokines synthesized as inactive membrane-bound pro-forms and are converted to their active forms by the metalloprotease TNF-α converting enzyme (Black et al., 1997) and by the cysteine protease IL-1β-converting enzyme (caspase-1) (Black et al., 1988; Weber et al., 2010), respectively. PR3, however, can also cleave the proforms of TNF-α and IL-1β at Val-Arg bonds (Robache-Gallea et al., 1995; Coeshott et al., 1999). The effect of HNE on the processing of TNF-α is not clear; some studies claim that HNE degrades pro-TNF-α with a loss of activity; others suggest that HNE liberates soluble, biologically active TNF-α from its membrane-bound precursor. We showed that a synthetic FRET substrate derived from the pro-TNF-α sequence is efficiently cleaved by PR3 and HNE (Korkmaz et al., 2007). PR3, but not HNE, activates IL-1β. This is most probably due to a negatively charged residue at the P4 and a positively charged residue at the P1′ position in pro-IL-1β that electrostatically could interact with Lys99 and Asp 61 of the active-site region of PR3 but not with HNE (Korkmaz et al., 2007).

The pro-inflammatory cytokine IL-18 belongs to the IL-1 cytokine family and is an important regulator of the innate and acquired immune response. It is synthesized as an inactive precursor and can be processed by caspase-1, caspase-3, and PR3 to its active form, but only the caspase-1 and caspase-3 cleavage sites have been identified (Akita et al., 1997). By contrast to HNE, PR3 could cleave caspase 3 substrates because of its preference of aspartyl residues at P2 and P2′ (Korkmaz et al., 2007, 2008c). Caspase 3 cleaves after an Asp residue if another Asp is present at the P4 position (Stennicke et al., 2000; Wei et al., 2000). Thus, PR3 can cleave caspase-3 substrates two residues upstream of the caspase-3 cleavage site if the P2 position of a caspase 3 substrate is compatible with the S1 subsite of PR3. Based on this analysis, the predicted PR3 cleavage site in the pro-IL18 would be at the C-R bond in the -MTD^{71}SDC$\downarrow$RD^{76}NA- sequence, whereas that of caspase 3 has been identified after Asp76.

IL-8 is a major chemokine responsible for neutrophil degranulation and neutrophil migration to inflammatory sites (Baggiolini and Clark-Lewis, 1992; Baggiolini et al., 1994). It is secreted as a precursor by neutrophils, monocytes, endothelial cells, and fibroblasts in response to inflammatory stimuli. Full-length pro-IL-8 (77 residues) is converted by MMP-8, MMP-9, and PR3 to three truncated variants (70, 71, and 72 residues long) retaining chemotactic activity in human granulocyte lysates (Padrines et al., 1994; Van den Steen et al., 2000). PR3 (but also lysates of neutrophil granules) cleaves the full-length pro-IL-8 at the Ala-Lys bond (-AVLPR**SA**^{29} ↓ **KE**L-), liberating the most active form of IL-8 (IL-70). The FRET substrate derived from this sequence is cleaved by PR3 but not by HNE (B. Korkmaz, unpublished results). The Ser residue at P2 and positively and negatively charged residues at P1′ and P2′ (bold characters) explain the specific cleavage of pro-IL-8 by PR3.

The stromal cell-derived factor-1α (SDF-1α), also called CXCL12, is a chemokine constitutively produced by hematopoietic cells, but SDF-1 mRNA and protein have also been identified in the central nervous system in neuronal, astroglial, microglial, and endothelial cells. SDF-1α plays a critical role in cell migration because it is a chemotactic factor for T cells, monocytes, pre-B cells, dendritic cells, and hematopoietic progenitor cells (Barbieri et al., 2007). Gene inactivation of SDF-1α or of its receptor CXCR4 in mice impairs myelo- and lymphopoiesis (Ma et al., 1998; Odemis et al., 2005). In vitro, HNE and CG, but not PR3, process the highly flexible N-terminal end of SDF-1α, which results in decreased activity. Processing by HNE releases the N-terminal tripeptide Lys1-Pro2-Val3 (Valenzuela-Fernández et al., 2002), whereas that by CG occurs at the Leu5-Ser6 bond, liberating a pentameric peptide (Delgado et al., 2001).

Progranulin, also called proepithelin, is a heavily glycosylated growth factor of 90 kDa, ubiquitously expressed and involved in tissue regeneration, tumorigenesis, and inflammation (Bateman and Bennett, 1998; Zhu et al., 2002). It is also produced and secreted into the extracellular environment by TNF-α-activated neutrophils. Progranulin inhibits adhesion-dependent neutrophil activation by interfering with the secretion of neutrophil proteases and the production of reactive oxygen species (Zhu et al., 2002), but the mechanisms involved in these anti-inflammatory effects are not yet elucidated. Anti-inflammatory properties of progranulin are suppressed through proteolytic cleavage by HNE and PR3 (Zhu et al., 2002; Kessenbrock et al., 2008). Interestingly enough, processed granulin peptides possess pro-inflammatory properties, stimulating IL-8 release from granulocytes (Zhu et al., 2002). In a recent study, mice lacking both NE and PR3 showed a highly diminished, immune complex-mediated, neutrophil infiltration that was due to impaired progranulin degradation, thus emphasizing the crucial role of progranulin as an inflammation-suppressing mediator (Kessenbrock et al., 2008).

2. Processing and Activation of Cellular Receptors. NSPs contribute to immune regulation also through the cleavage and activation of specific cellular receptors. HNE, PR3, and CG can process the N-terminal extracellular domains of protease-activated receptors (PARs), which are a subfamily of related G-protein-coupled receptors (Ossovskaya and Bunnett, 2004; Vergnolle, 2009). These receptors are ubiquitously expressed in various tissues and cells and, more especially, in platelets and endothelial cells. Processing of PAR extracellular domains occurs through exposure of a tethered ligand that allows the autoactivation of the receptor and subsequent activation of an intracellular signaling cascade via phospholipase C (Ossovskaya and Bunnett, 2004; Vergnolle, 2009). Four PARs have been identified so far; three of them, PAR-1, PAR-3, and PAR-4, can be activated by thrombin. Besides thrombin, CG released from activated neutrophils can also activate PAR-4 at the surface of platelets and initiate their aggregation (Sambrano et al., 2000). All three NSPs cleave PAR-1, which impairs their activation by thrombin (Renesto et al., 1997). The sequence containing the CG cleavage site (-EPF^{55} ↓ WEDEE-) (Renesto et al., 1997) has been used to raise a sensitive FRET substrate for this protease (Attucci et al., 2002). PAR-2 is expressed on endothelial cells and can be activated by trypsin (SKGR^{34} ↓ SLIGKV) (Nystedt et al., 1994, 1995a,b) but also by the three NSPs (Uehara et al., 2002, 2003, 2004). PAR-2 activation results in the production and secretion of IL-8 and chemokine (C-C motif) ligand 2 (Uehara et al., 2003, 2004).

Toll-like receptors (TLRs) are transmembrane glycoproteins remarkably contributing to host defense against microbial infections and innate immune response. Thirteen mammalian TLRs have been identified so far. Most of them can recognize specific pathogen-associated molecular patterns presented by invading pathogens or danger-associated molecular patterns released by injured tissues (Akira et al., 2006; Creagh and O'Neill, 2006; Brikos and O'Neill, 2008; Buchanan et al., 2009). The LPS-sensitive TLR4 and EGFR can be directly up-regulated by HNE in lung epithelial cells, which results ultimately in an overproduction of pro-IL-8 mediated through the Myd88/IRAK/TRAF-6 pathway (Walsh et al., 2001; Devaney et al., 2003; Bergin et al., 2008). This HNE effect can be reduced by the addition of the serine protease inhibitor phenylmethylsulfonyl fluoride (Devaney et al., 2003). A new mechanism of HNE-induced pro-IL-8 expression involving activation of the metalloprotease meprin α has been identified (Bergin et al., 2008).

Receptors for the Fc region of IgG and integrin CD11b/CD18 at the neutrophil surface are involved in the recognition of immune complexes, which results in neutrophil activation through modulation of integrin clustering at the cell surface, cytoskeletal rearrangement, and intracellular ROS production (Raptis and Pham, 2005).

CG could enhance immune complex-receptor-mediated neutrophil activation as demonstrated using CG/NE double-deficient mice (Raptis et al., 2005), but the relevant CG target has not yet been identified.

3. Induction of Apoptosis by Proteinase 3. PR3 secreted by activated neutrophils during inflammation is involved in endothelial cell apoptosis (Yang et al., 2001; Preston et al., 2002; Pendergraft et al., 2004). The mechanism by which neutrophil-secreted PR3 enters the endothelial cell has not been elucidated but its molecular intracellular targets have been clearly identified. PR3 directly processes NFκB at the Cys-Arg bond in the sequence -KDC$^{95}\downarrow$RDGA- (Preston et al., 2002) and the cyclin-dependent kinase inhibitor p21 at the Ala-Arg bond in the sequence -IQEA$^{45}\downarrow$RER- (Dublet et al., 2005) (Fig. 5B). The proteolytic processing of NFκB and of p21 accelerates endothelial cell apoptosis. Apoptotic elimination of cells from inflammatory sites helps to resolve inflammation. As reported above for pro-IL-18, the positioning of the two Asp residues in the cleaved sequence of NFκB indicates that caspase 3 also cleaves this sequence but at a different site, located two residues downstream from the PR3 cleavage site. No preferential HNE cleavage site is present in this sequence, which explains the specific proapoptotic properties of PR3. The mouse homolog of human PR3, however, has a different specificity that impairs the cleavage of NFκB and p21 at the same sites (Kalupov et al., 2009) (Fig. 5B). Because human and mouse p21 and NFκB share the same sequence in this region, it is questionable whether these antiapoptotic properties of PR3 are biologically relevant.

Many other molecular targets of NSPs have been identified as membrane-bound and soluble substrates at inflammatory sites (for review, see Bank and Ansorge, 2001; Wiedow and Meyer-Hoffert, 2005; Pham, 2006, 2008; Meyer-Hoffert, 2009), and many others probably remain to be discovered. Proteolytic cleavage of these molecular targets may result in the activation of latent proforms, in the enhancement or abolishment of preexisting activities, or in anticipated degradation of inactive proforms that impair interaction with their physiological activators.

IV. Physiological Inhibitors of Elastase, Proteinase 3, and Cathepsin G

During phagocytosis and neutrophil turnover, HNE, PR3, and CG are released into the extracellular space as active proteases. The proteolytic activity of HNE, PR3, and CG seems to be tightly regulated in the extracellular and pericellular space to avoid degradation of connective tissue proteins including elastin, collagen, and proteoglycans (Janoff, 1985). Protein inhibitors that belong to three main families, the serpins, the chelonianins, and the macroglobulins, ultimately control proteolytic activity of HNE, PR3, and CG activities. The individual contributions of these families depend on their tissue localization and that of their target proteases. The main characteristics of HNE, PR3, and CG physiological inhibitors are presented in Table 2.

A. Serine Protease Inhibitors

Serpins are the largest and most diverse family of protease inhibitors; more than 1000 members have been identified in human, plant, fungi, bacteria, archaea, and certain viruses (Silverman et al., 2001; Mangan et al., 2008). They share a similar highly conserved tertiary structure and similar molecular weight of approximately 50 kDa. Human serpins belong to the first nine clades (A–I) of the 16 that have been described based on phylogenic relationships (Irving et al., 2000; Silverman et al., 2001; Mangan et al., 2008). For historical reasons, α1-protease inhibitor (α1-PI) was assigned to the first clade. Clade B, also known as the ov-serpin clan because of the similarity of its members to ovalbumin (a protein that belongs to the serpin family but lacks inhibitory activity), is the second largest clan in humans, with 15 members identified so far. Ov-serpin clan members are generally located in the cytoplasm and, to a lesser extent, on the cell surface and nucleus (Remold-O'Donnell, 1993).

Serpins play important regulatory functions in intracellular and extracellular proteolytic events, including blood coagulation, complement activation, fibrinolysis, cell migration, angiogenesis, and apoptosis (Potempa et al., 1994). Serpin dysfunction is known to contribute to

TABLE 2
Main characteristics of the endogenous inhibitors of elastase, proteinase 3, and cathepsin G

	Reactive site	Target Proteases	Source
Serpin			
α1-PI	Met358–Ser359	Elastase, proteinase 3, cathepsin G, trypsin, plasmin, thrombin, chymotrypsin	Plasma, neutrophil
ACT	Leu358–Ser359	Cathepsin G, chymase, chymotrypsin	Plasma
MNEI	Phe343–Cys344	Cathepsin G, chymase, kallikrein 3	Neutrophil, macrophage
	Cys344–Met345	Elastase, proteinase 3	
PI6	Arg341–Cys342	Cathepsin G	Placenta, granulocytes
PI9	Glu340–Cys341	Granzyme B, caspase 1	Lymphocyte T cells
	Cys341–Cys342	Elastase	Endothelial cells, epithelial cells
Chelonianin			
SLPI	Leu72–Met73	Elastase, cathepsin G, chymase, chymotrypsin	Epithelial cells, seminal plasma, neutrophil, macrophage
Elafin	Ala24–Met25	Elastase, proteinase 3	Bronchial secretions, skin, seminal plasma

diseases such as emphysema, thrombosis, angioedema, and cancer (Carrell and Lomas, 1997; Lomas and Carrell, 2002). Most inhibitory serpins target trypsin-/chymotrypsin-like serine proteases, but some, termed "cross-class inhibitors," have been shown to target cysteine proteases (Annand et al., 1999). The crystal structure of the prototype plasma inhibitor α1-PI revealed the archetype native serpin fold (Loebermann et al., 1984). All serpins typically have three β-sheets (termed A, B, and C) and eight or nine α-helices (hA–hI) arranged in a stressed configuration. The so-called reactive center loop (RCL) of inhibitory molecules determines specificity and forms the initial encounter complex with the target protease (Potempa et al., 1994; Silverman et al., 2001). Serpins inhibit proteases by a suicide substrate inhibition mechanism. The protease initially recognizes the serpin as a potential substrate using residues of the reactive center loop and cleaves it between P1 and P1′ This cleavage allows insertion of the cleaved RCL into the β-sheet A of the serpin, dragging the protease with it and moving it over 71 Å to the distal end of the serpin to form a 1:1 stoichiometric covalent inhibitory complex (Huntington et al., 2000). Such cleavage generates a ~4-kDa C-terminal fragment that remains noncovalently bound to the cleaved serpin. Displacement of the covalently attached active site serine residue from its catalytic partner histidine explains the loss of catalytic function in the covalent complex. The distortion of the catalytic site structure prevents the release of the protease from the complex, and the structural disorder induces its proteolytic inactivation (Huntington et al., 2000). Covalent complex formation between serpin and serine proteases triggers a number of conformational changes, particularly in the activation domain loops of the bound protease (Dementiev et al., 2006).

1. α1-Protease Inhibitor. α1-PI is a single-chain glycoprotein of 394 amino acids synthesized mainly by hepatocytes; it was originally named α1-antitrypsin because of its ability to irreversibly bind trypsin in vitro (Janciauskiene, 2001). It is the most abundant serpin present in human blood. Its primary function, however, seems to be in the lung parenchyma, where it protects alveolar tissues from destruction by NSPs. Its circulating level lies between 1.2 and 2 mg/ml in healthy persons (Jeppsson et al., 1978; Crystal et al., 1989), but it may increase during acute phases of inflammation and infection. The gene is located on the long arm of chromosome fourteen (14q32.1). More than 100 different genetically encoded variants of α1-PI have been described. At the protein level, α1-PI variants have been discriminated by isoelectric focusing (Hutchison, 1988) and are designated by a capital letter corresponding to their isoelectric point. The most common variant is the M type. Abnormalities of the S and Z types arise from single amino acid substitutions in the primary chain and result in decreased or undetectable α1-PI levels in serum (Hutchison, 1988). The crystal structure of α1-PI obeys the general structure of serpins with an exposed RCL that is cleaved at the Met358–Ser359 bond upon interaction with the target protease (Huntington et al., 2000). α1-PI is an excellent irreversible inhibitor of HNE that also inhibits related neutrophil proteases PR3 and CG. α1-PI inhibits HNE with a inhibitor/enzyme stoichiometry of 1, whereas that with PR3 and CG is slightly higher because of partial proteolytic inactivation of some inhibitor molecules occurring during the course of the interaction (Duranton and Bieth, 2003; Korkmaz et al., 2005b). The second-order constants of association of α1-PI with HNE, PR3, and CG are 6.5×10^7, 8.1×10^6, and $4.1 \times 10^5\ M^{-1} \cdot s^{-1}$, respectively (Beatty et al., 1980; Rao et al., 1991). Neutrophil membrane-bound HNE and PR3 are also inhibited by α1-PI to form soluble irreversible complexes (Korkmaz et al., 2005a, 2009). Conformational distortion of the hydrophobic 217–225 loop of PR3 involved in membrane interaction most likely triggers solubilization of mPR3 (Korkmaz et al., 2008b).

The association of α1-PI with target proteases is altered by their interaction with DNA, heparin, and other glycosaminoglycans found at inflammatory sites (Frommherz and Bieth, 1991; Frommherz et al., 1991; Belorgey and Bieth, 1998; Ying and Simon, 2000). Heparin promotes the formation of the Michaelis complex between HNE and α1-PI but prevents conversion into a covalently inactivated complex (Faller et al., 1993). DNA and heparin also decrease the rate constant of association between CG and α1-PI (Ermolieff et al., 1994; Duranton et al., 2000). The conformational change of α1-PI induced by complexation with a protease results in the appearance of new binding sites that are recognized by serpin-enzyme complex surface receptors (Perlmutter et al., 1990) or by receptors for low- and very low-density lipoproteins (Kounnas et al., 1996; Rodenburg et al., 1998), which allows for their clearance.

Proteolytic cleavage of the α1-PI reactive loop by exogenous or endogenous proteases and/or oxidation by biological oxidants of methionine residues in this loop alters its biological activity. MMP-8 (Knäuper et al., 1990; Michaelis et al., 1990), MMP-9 (Desrochers et al., 1992), leukolysine (Nie and Pei, 2004), MMP-7 (Zhang et al., 1994), cathepsin L (Johnson et al., 1986), and bacterial proteases produced by *Staphylococcus aureus*, *Serratia marcescens* (Rapala-Kozik et al., 1999), and *Pseudomonas aeruginosa* (Morihara et al., 1984) count among the proteases degrading α1-PI. RCL cleavages generate a ~4-kDa fragment similar to that generated by target proteases (Banda et al., 1988a). α1-PI contains 10 methionine residues (Vogt, 1995). Oxidation of two of them, Met358 and Met351, results in a marked loss of affinity of α1-PI for HNE and CG (Johnson and Travis, 1979; Beatty et al., 1980; Taggart et al., 2000). Oxidation of α1-PI is observed in vitro upon incubation with myeloperoxidase or activated neutrophils (Shock and Baum, 1988; Padrines and Bieth, 1993).

2. α1-Antichymotrypsin. α1-Antichymotrypsin (ACT) is a plasma glycoprotein synthesized by hepatocytes and secreted into the serum. Its serum concentration of approximately 0.25 mg/ml increases during acute inflammation by almost 5-fold (Calvin and Price, 1986). ACT contains 400 amino acids and has two glycosylation sites on Asn70 and Asn104, carbohydrate groups at these positions accounting for approximately 25% of the protein's mass. It displays a relatively high sequence identity with α1-PI (45%), suggesting that both inhibitors have evolved through duplication of a single gene (Bao et al., 1987). ACT can bind double-stranded DNA through several lysyl residues (Naidoo et al., 1995), a feature thought to be of relevance in controlling tumor progression of the thyroid gland (Lai et al., 1998). ACT is also recognized as a major constituent of amyloid deposits in the brains of patients with Alzheimer disease (Abraham et al., 1988).

ACT inhibits serine proteases of the chymotrypsin type. The main targets of ACT are CG ($k_{ass} = 5.1 \times 10^7$ $M^{-1} \cdot s^{-1}$), chymotrypsin, and mast cell chymase (Travis et al., 1978; Fukusen et al., 1987), with which it forms a covalent complex after cleavage of its inhibitory loop. The reactive site of ACT has been identified at the Leu358–Ser359 bond. ACT does not inhibit HNE and PR3 but is proteolytically degraded by HNE (Rubin et al., 1994). Neutrophil MMP-8 but not MMP-9 inactivates ACT by cleaving the inhibitory loop at the Ala362–Leu363 bond (Desrochers et al., 1992). Several other MMPs, including MMP-1 and MMP-2 (Mast et al., 1991), and bacterial proteases can inactivate ACT by cleaving its reactive center loop (Kress, 1983).

3. Monocyte Neutrophil Elastase Inhibitor, Proteinase Inhibitor 6, and Proteinase Inhibitor 9. Monocyte neutrophil elastase inhibitor (MNEI, now called SerpinB1), PI6, and PI9 are three cytoplasmic and/or nuclear serpins that belong to the family of ovalbumin and are potential inhibitors of one or more NSPs. The gene encoding serpinB1 (Zeng et al., 1998) is located on chromosome 6 in a cluster with those encoding PI6 and PI9 (Sun et al., 1998). SerpinB1 was first identified as a potent inhibitor of HNE present in the cytoplasm of neutrophils and monocytes (Remold-O'Donnell et al., 1989, 1992). Recombinant serpinB1 inhibits HNE ($k_{ass} = 3.4 \times 10^7$ $M^{-1} \cdot s^{-1}$), PR3 ($k_{ass} = 1.7 \times 10^7$ $M^{-1} \cdot s^{-1}$), and porcine pancreatic elastase but also chymotrypsin-like proteases [e.g., CG ($k_{ass} = 2.3 \times 10^6$ $M^{-1} \cdot s^{-1}$) and chymase] with a stoichiometry of inhibition close to 1 (Cooley et al., 2001). This duality is explained by the presence of two functional sites in the reactive center loop, one at Phe343–Cys344, allowing interaction with chymotrypsin-like protease, and the other at Cys344–Met345, which is cleaved by elastase-related proteases (Cooley et al., 2001).

PI9, which shares 49% sequence identity with serpinB1, is present in placenta, endothelial cells, and in lung (Sprecher et al., 1995; Buzza et al., 2001). PI9 protects host cells from apoptosis by inhibiting granzyme B (Sun et al., 1996). It also inhibits the cysteine protease caspase-1, which catalyzes the formation of interleukin-1β from its precursor (Annand et al., 1999). PI9 is the first example of a human serpin that can inhibit a cysteine protease. It also inhibits bacterial proteases and subtilisin A (Dahlen et al., 1997), and its recombinant form inhibits HNE with $k_{ass} = 1.5 \times 10^5$ $M^{-1} \cdot s^{-1}$ (Dahlen et al., 1999). The inhibition of HNE occurs through cleavage of the Cys341–Cys342 bond in the PI9-reactive loop.

PI6 was identified in 1993 as an inhibitor of trypsin, urokinase plasminogen activator, and plasmin (Coughlin et al., 1993) but has also been found in monocytes in a complex with CG (Scott et al., 1999). Cytoplasmic inhibition of CG may protect these cells against damage during biosynthesis. The relevance of this reaction is supported by the high rate constant for association with CG ($k_{ass} = 6.8 \times 10^6$ $M^{-1} \cdot s^{-1}$), (Scott et al., 1999).

B. Canonical Inhibitors

Canonical inhibitors include a still increasing number of families with different folds but sharing a canonical conformation of their inhibitory protease binding loop in the P3-P3′ region (Bode and Huber, 1992). This loop is a convex, extended, and solvent-exposed structure that is highly complementary to the concave active site of the enzyme. A huge number of canonical inhibitors have been isolated from various cells, tissues, and organisms, including plant seeds, avian eggs, and various body fluids (Krowarsch et al., 2003). The chelonianin family of canonical inhibitors includes the physiological endogenous inhibitors of NSPs, SLPI, elafin, and its precursor trappin-2 (Moreau et al., 2008).

1. Secretory Leukocyte Protease Inhibitor. SLPI is an 11.7-kDa nonglycosylated highly basic (pI > 11) protein consisting of 107 amino acids found in all body fluids, including tears, salivary glands, seminal fluid, bronchial secretions, cervical, and intestinal mucus (Si-Tahar et al., 2000; Doumas et al., 2005). Its wide distribution in the body explains why it was given different names: "bronchial inhibitor," "human seminal plasma inhibitor," "anti-LeukoProtease," "mucus protease inhibitor," and SLPI. In the lung, it is produced by epithelial cells of the trachea and bronchi, by alveolar type II cells, and by tracheal gland serous cells and Clara cells (Sallenave et al., 1997a; Sallenave, 2002). SLPI is present at highest concentrations in the upper airways. In the deep lung, SLPI concentration is low but it could play a physiological role, because it has been demonstrated by immunohistochemistry to be in contact with elastin fibers (Kramps et al., 1989). SLPI is also synthesized by phagocytes (neutrophils, monocytes, and alveolar macrophages) (Sallenave et al., 1997b). Its crystal structure obtained as a complex with bovine chymotrypsin shows two domains of similar architecture, each stabilized by four disulfide bridges (Grütter et al., 1988) but only the C-terminal domain is complexed to chymotrypsin.

SLPI is a reversible inhibitor of HNE [$k_{ass} = 6.4 \times 10^6$ $M^{-1} \cdot s^{-1}$ (Boudier and Bieth, 1989), $K_i = 0.1 \times 10^{-10}$ M (Gauthier et al., 1982)], CG [$k_{ass} = 1.5 \times 10^6$ $M^{-1} \cdot s^{-1}$ (Fath et al., 1998), $K_i = 4.2 \times 10^{-9}$ M (Smith and Johnson, 1985)], chymotrypsin, chymase, and bovine trypsin, but it does not inhibit PR3. Its reactive site extends on each side of the Leu72–Met73 bond located in its C-terminal domain. Heparin binding to SLPI induces a conformational change that accelerates inhibition of HNE (Faller et al., 1992). Fragments of native heparin (4.5 or 8.1 kDa) or *O*-butyryl (8 kDa) also accelerate the inhibition of CG by SLPI (Ermolieff et al., 1998).

Recently, a C-terminal domain of SLPI has been crystallized in a complex with HNE. X-ray data showed the importance of P1 Leu72 and of six hydrogen bonds in the primary contact region for inhibiting HNE and of P5 Tyr68, which forms a hydrogen bond with Arg217 of HNE for increasing the selectivity of SLPI toward this protease (Koizumi et al., 2008). The lack of PR3 inhibition by SLPI is explained by the presence of Ile190 (Val in HNE), which reduces the size of the S1 pocket of PR3, thus impairing accommodation of P1 Leu72 of the inhibitor (Fujinaga et al., 1996), and by the lack of a hydrogen bond between Tyr68 and Arg217 due to substitution of Arg217 by an Ile in the S5 pocket of PR3 (Koizumi et al., 2008). The unfavorable positive-positive contacts between PR3 and SLPI at two locations, one involving Arg36/Arg65 and the other Arg168/Lys99 of PR3, contribute also to the noninhibition of PR3 by SLPI (Zani et al., 2009).

Oxidation of Met73 in the SLPI inhibitory loop decreases the association rate constant for HNE by a factor of 20 (Boudier and Bieth, 1994), suggesting that the efficacy of the inhibitor is less affected by oxidation than that of α1-PI. The N-terminal domain of SLPI stabilizes the inhibitor thus favoring trypsin inhibition. This domain is also essential for activation of the inhibitor by heparin (Faller et al., 1992). Bacterial proteases and human cysteine proteases (cathepsin B, L, and S) can inactivate SLPI by proteolytic degradation (Sponer et al., 1991; Draper et al., 1998; Taggart et al., 2001). Unlike α1-PI, SLPI is able to inhibit HNE in vitro when linked to its natural substrate elastin (Hornebeck and Schnebli, 1982; Bruch and Bieth, 1986). Because of its lower molecular weight compared with α1-PI, SLPI can access the space between adherent neutrophil and extracellular matrix, thus protecting extracellular matrix proteins from proteolysis (Rice and Weiss, 1990).

2. Elafin. Elafin is a nonglycosylated 6-kDa inhibitor also called "skin-derived anti-leuko-protease" or "elastase specific inhibitor." Elafin was originally isolated from the skin of patients affected by psoriasis (Schalkwijk et al., 1990; Wiedow et al., 1990) but it is also present in lung secretions (Sallenave and Ryle, 1991). As with SLPI, it has a tracheobronchial origin, being synthesized by Clara cells and type II pneumonocytes (Sallenave, 2002).

Elafin is a basic inhibitor with a pI of 9.7, and it is stable at acidic pH (Wiedow et al., 1990). Its three-dimensional structure has been determined by solving the structure of a complex with porcine pancreatic elastase (Tsunemi et al., 1996). Similar to SLPI, it displays a compact structure maintained by four disulfide bridges, characteristic of whey acidic proteins. Elafin shares 40% of sequence identity with SLPI, and the active site residues are also similar. Elafin is released by proteolytic processing from a larger precursor made of two functional domains called trappin-2 or pre-elafin (Schalkwijk et al., 1999). The N-terminal domain of trappin-2 contains a repeated sequence GQDPVK acting as a transglutaminase substrate and therefore allows adsorption of the inhibitor on extracellular matrix proteins by interstitial transglutamination (Schalkwijk et al., 1999). Trappin immobilization could help maintain local inhibition compared with other soluble inhibitors of NSPs.

Elafin is a potent inhibitor of both HNE [$k_{ass} = 5 \times 10^6$ $M^{-1} \cdot s^{-1}$ (Ying and Simon, 2001), $K_i = 0.8 \times 10^{-10}$ M (Zani et al., 2004)] and PR3 [$k_{ass} = 4 \times 10^6$ $M^{-1} \cdot s^{-1}$ (Ying and Simon, 2001), $K_i = 1.2 \times 10^{-10}$ M (Zani et al., 2004)] but, unlike SLPI, does not inhibit CG. Elafin interacts with HNE and PR3 through its active site centered on the Ala24–Met25 peptide bond. The presence of an Ala residue at P1 is consistent with its specificity toward PR3 and HNE. Elafin/trappin-2 can also inhibit HNE and PR3 at the surface of activated neutrophils (Zani et al., 2009). Whereas membrane-associated HNE forms soluble complexes upon interaction with elafin, mPR3 complexes remain on the neutrophil surface, which indicates that HNE and PR3 are differently bound to neutrophil surfaces (Korkmaz et al., 2009). A recent in vitro study demonstrated that an excess of HNE at Val5 and Val9 cleaves recombinant elafin at Val5 and Val9. This does not affect its capacity to inhibit HNE but strongly impairs its ability to bind LPS and its immobilization by transglutamination (Guyot et al., 2008). This could greatly weaken its contribution to the defense of invading pathogens (Sallenave, 2002; Moreau et al., 2008).

C. α-Macroglobulins

Human α2-macroglobulin is a polyvalent homotetrameric inhibitor of 725 kDa present at a concentration of 2 mg/ml in serum (Petersen, 1993). It inhibits all classes of proteases, including NSPs (Travis, 1988). Because its high molecular mass impairs diffusion to inflammatory sites during neutrophil extravasation, its major role is probably restricted to controlling protease activity within the circulation. Its mechanism of interaction with proteases has been described in detail (Sottrup-Jensen, 1989). Its covalent association with proteases sterically shields their active sites from large molecular substrates, hence permitting enzymatic hy-

drolysis of only small synthetic substrates (Doan and Gettins, 2007).

V. Pathophysiology of Elastase, Proteinase 3 and Cathepsin G in Human Diseases

A. Chronic Inflammatory Lung Diseases

In many instances, the initiation and propagation of lung damage is a consequence of an exaggerated inappropriate inflammatory response, which includes the release of proteases and leukocyte-derived cytotoxic products (Owen, 2008b; Roghanian and Sallenave, 2008). Inflammation is a physiological protective response to injury or infection consisting of endothelial activation, leukocyte recruitment and activation, vasodilation, and increased vascular permeability. Although designed to curtail tissue injury and facilitate repair, the inflammatory response sometimes results in further injury and organ dysfunction. Inflammatory chronic lung diseases, chronic obstructive pulmonary disease, acute lung injury, acute respiratory distress syndrome, and cystic fibrosis are syndromes of severe pulmonary dysfunction resulting from a massive inflammatory response and affecting millions of people worldwide. The histological hallmark of these chronic inflammatory lung diseases is the accumulation of neutrophils in the microvasculature of the lung. Neutrophils are crucial to the innate immune response, and their activation leads to the release of multiple cytotoxic products, including reactive oxygen species and proteases (serine, cysteine, and metalloproteases). The physiological balance between proteases and antiproteases is required for the maintenance of the lung's connective tissue, and an imbalance in favor of proteases results in lung injury (Umeki et al., 1988; Tetley, 1993). A number of studies in animal and cell culture models have demonstrated a contribution of HNE and related NSPs to the development of chronic inflammatory lung diseases. Available preclinical and clinical data suggest that inhibition of NSP in lung diseases suppresses or attenuates the contribution of NSP to pathogenesis (Chughtai and O'Riordan, 2004; Voynow et al., 2008; Quinn et al., 2010). HNE could also participate in fibrotic lung remodeling by playing a focused role in the conversion of latent transforming growth factor-β into its biologically active form (Chua and Laurent, 2006; Lungarella et al., 2008).

1. Chronic Obstructive Pulmonary Disease. Chronic obstructive pulmonary disease (COPD) represents a group of diseases, including chronic bronchitis and emphysema, that are characterized by an airflow limitation that is not fully reversible (Barnes and Stockley, 2005). COPD represents an increasingly important cause of morbidity and mortality in the population. It is the fifth leading cause of death in the world, is expected to rise to third place by 2020, and is most often due to tobacco smoking or exposure to other airborne irritants or solvents, which trigger an abnormal inflammatory response in the lung. It is characterized by an increased number of neutrophils, macrophages, and T lymphocytes predominantly localizing in small airways and lung parenchyma (Barnes and Stockley, 2005; Owen, 2008b). One genetic cause of COPD, accounting for approximately 2% of cases, is α1-PI deficiency (Laurell and Eriksson, 1963), which is associated with the Z phenotype. In patients with α1-PI deficiency, the development of COPD is believed to be caused by the uncontrolled action of proteases on lung tissue. Intermittent infusions of active plasma-derived α1-PI in these patients increased the levels of α1-PI and anti-HNE capacities for a few days after infusion (Wewers et al., 1987). In smokers with emphysema, extracellular matrix undergoes significant physical and biochemical modifications. COPD is initiated by cigarette smoke or other irritants that activate respiratory tract macrophages (Barnes, 2004), which release neutrophil chemotactic factors, including IL-8 and leukotriene B_4, that induce neutrophil infiltration/accumulation in the lung and subsequent secretion of active NSPs (Barnes, 2004). Indeed, an increased level of NSP is observed in bronchoalveolar lavage (BAL) fluids of patients with COPD. The pathogenic roles of NSPs in COPD are attributed to their ability to break down connective tissue components and generate proinflammatory peptides from these components (Houghton et al., 2006; Weathington et al., 2006), to induce mucus secretion by submucosal glandular cells and goblet cells, and to express proinflammatory cytokines from airway epithelial cells (Sommerhoff et al., 1990; Takeyama et al., 1998; Witko-Sarsat et al., 1999b). It should be emphasized that NSPs are not the only proteases participating in lung damage in COPD; the role of other granular serine proteases, including MMPs, A disintegrin and metalloproteases, and cysteine proteases, has been reviewed recently by Owen (2008b).

Studies in animal models have shown that an imbalance between proteases and antiproteases in the lung favors increased proteolysis, thus contributing to COPD pathogenesis (Stockley, 1999; Barnes and Stockley, 2005; Owen, 2008b). An imbalance between oxidants and protective antioxidants resulting in oxidative stress could also contribute to COPD pathogenesis (Wood and Stockley, 2006). This is supported by the observation that oxidized, inactive α1-PI is present in BAL of smokers suffering from emphysema (Morrison et al., 1986a,b, 1987; Sethi and Rochester, 2000). Oxidation of α1-PI Met residues to Met-sulfoxides inactivates the inhibitor as shown by the analysis of BAL from smokers compared with nonsmokers (Gadek et al., 1979; Carp et al., 1982). Oxidation, however, cannot be responsible for the inactivation of ACT in BAL of patients with COPD. On the other hand, genetic alterations in the ACT gene are rare but have been reported. A single natural L55P substitution, for example, yields an ACT variant with little activity. Partial insertion of the reactive center loop into the β-sheet A (Chang and Lomas, 1998; Gooptu et al.,

2000) accounts for its dysfunction. BALs from COPD patients also contain oxidized SLPI and proteolytically processed fragments.

Experimental models have been developed to mimic human COPD (e.g., mouse) and use different injurious stimuli (e.g., cigarette smoke, instilled exogenous proteases) (Brusselle et al., 2006). In the mouse model of COPD, aerosolized human α1-PI, serpinB1, SLPI, reduced the severity and the extent of the elastase-induced tissue damages and airspace enlargement (Rees et al., 1999; Churg et al., 2003; Pemberton et al., 2006). We recently showed, however, a limitation of the mouse model by demonstrating the different substrate specificities of mouse and human HNE and PR3 (Kalupov et al., 2009). This would certainly impair testing human NSP inhibitors of putative therapeutic interest in the mouse model because of their different reactivity with target proteases in these two species (Wiesner et al., 2005).

2. Cystic Fibrosis. Cystic fibrosis (CF; MIM 219700) is an inherited complicated disease of the secretory glands, originally described in 1938 by Dorothy H. Andersen (Davis, 2006). It has an estimated prevalence of 1 in 2500 in Europe and the United States (Tizzano and Buchwald, 1992). It affects the lungs, pancreas, liver, intestines, sinuses, and reproductive system (Voynow et al., 2008). CF is caused by a mutation in a gene named cystic fibrosis transmembrane conductance regulator (CFTR) (Riordan et al., 1989). The product of this gene is a chloride ion channel involved in the movement of chloride ions out of epithelial cells toward the covering mucus (Riordan et al., 1989; Buchanan et al., 2009). The flow of chloride ions helps to control the movement of water in tissues, which is necessary for the production of thin, freely flowing mucus. As a consequence of CFTR mutations, cells that line the airways, pancreas, and other organs produce unusually thick and sticky mucous (Buchanan et al., 2009). Mucus clogging the airways and glands leads to the characteristic signs and symptoms of cystic fibrosis.

Excessive mucus production is frequently associated with bacterial lung infection, mainly by *S. aureus* and *P. aeruginosa* (Buchanan et al., 2009). The host system responds vigorously. The inflammatory process in CF airways is characterized by a massive influx of neutrophils, representing 95% of the cell population in BAL. High levels of elastinolytic activity are thus detected in sputum and BAL from patients with CF (Fick et al., 1984; Jackson et al., 1984; Suter et al., 1984; Bruce et al., 1985). The concentration of active NSPs in the sputum determined using specific and sensitive FRET substrate varies from 10^{-9} to 10^{-7} M (A. Gauthier, unpublished data) and overwhelms that of inhibitors found locally. In addition to their direct proteolytic action on the pulmonary matrix, airway NSPs stimulate massive neutrophil accumulation via secretion of the potent neutrophil chemoattractant IL-8 from airway epithelial cells and by interfering with mucus production. NSPs also induce detachment of the ciliated cells from their neighbors, causing epithelial disruption and impairing mucociliary clearance (Amitani et al., 1991).

In CF, the major lung problems are bacterial infections, inflammation, and airway obstruction (Amin and Ratjen, 2008), which occur despite the presence in CF lungs of cationic antimicrobial peptides such as lactoferrin, SLPI, lysozyme, defensins, and LL-37 (Rogan et al., 2006). Inactivation of LL-37 in CF sputum might contribute to the persistent susceptibility of CF patients to microbial colonization and infection. LL-37 inactivation in CF BAL is due to its association with glycosaminoglycans (Bergsson et al., 2009), DNA and/or filamentous F-actin (Bucki et al., 2007). Hypertonic saline disrupts glycosaminoglycans–LL-37 complexes thus promoting antimicrobial effects (Bergsson et al., 2009) but free LL-37 is then degraded by HNE and cathepsin D (Bergsson et al., 2009). The large amounts of DNA released by lysed neutrophils in the respiratory tract of patients with CF in response to infection can be disrupted through therapeutic DNase administration, thus resulting in the liquefaction of viscous mucus, a reduction of the viscoelasticity of purulent lung secretions, an airflow increase, and a consequently decreased risk of infection (Fitzgerald et al., 2005). DNase increases the amount of LL-37 peptide detected in the supernatant of CF sputum dissolved from DNA/actin bundles (Bucki et al., 2007). It also increases proteolytic activity by releasing positively charged NSPs embedded in the DNA network. Combining factors that disrupt DNA and GAGs complexes with an anti-protease cocktail could potentially help promote antimicrobial and antiproteolytic activities in lung secretions of patients with CF. As detailed in a recent study (Griese et al., 2008), neutrophils from patients with CF, COPD, or bronchiectasis are ineffective in eliminating bacteria and have poor bacteriocidal killing capacity. Reduction of CXCR1 on the patients' neutrophils was observed and was shown to be linked to the action of HNE. The cleaved CXCR1 fragments in turn were able to activate the epithelial TLR2 and to induce local IL-8 production (Hartl et al., 2007). The chemokine IL-8 is known to be critically involved in the recruitment of neutrophils to the lungs and is increased in all conditions of persistent neutrophilic airway inflammation. Neutrophils treated with IL-8 or TLR2 agonist release pro-MMP-9, which can be activated by plasmin or other proteases. Pericellular MMP-9 inactivates α1-PI and generates an inhibitor-free environment for HNE and PR3. PR3 in turn is able to remove an N-terminal heptapeptide from full-length IL-8 (residues 8–77 starting with KELRC) and enhances its agonist activity on CXCR2 in a positive feedback loop. Simultaneously, HNE can inactivate TIMP-1, an MMP-9 inhibitor (Itoh and Nagase, 1995), and further assists in this amplification cycle. In contrast to other pro-MMP-9–expressing cells, neutrophils externalize pro-MMP-9 without associated TIMP-1, which after activation in a TIMP-1–free environment can also cleave IL-8 but at a different site,

removing just six N-terminal residues (7–77). Although exopeptidolytic processing of IL-8 up to position 6 by aminopeptidase N (CD13) is quite unlikely out (Proost et al., 2007), the N-terminal pentapeptide of IL-8 could also be clipped off by MMP-8 (Tester et al., 2007). Hence, it remains to be clarified which of the various proteases (i.e., MMP-8, MMP-9, and PR3) are really relevant for this process in vivo.

3. Acute Lung Injury and Acute Respiratory Distress Syndrome. Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are inflammatory disorders of the lung most commonly caused by trauma, sepsis, and pneumonia, the latter two being responsible for approximately 60% of cases (Tsushima et al., 2009). These pathologic conditions are characterized by increased hypoxemia and alveolar-capillary permeability. ALI and ARDS, its most severe form, differ in the degree of hypoxemia. In ARDS, the alveolar-capillary barrier is compromised, resulting in the formation of a protein-rich edema, filling alveoli. Early in the initiation of ALI and ARDS, neutrophils massively accumulate in the vasculature of the lung. Neutrophils and their cytotoxic products, including oxidants and proteases, have central pathological functions in ALI and ARDS. Increased elastolytic activity (Lee et al., 1981), as well as increased levels of oxidative stress, reflected by increased exhalation of H_2O_2, have been observed in patients with ARDS (Repine, 1992). Increased levels of HNE in plasma and in BAL have also been observed with at-risk patients who later developed ALI (Suter et al., 1992; Donnelly et al., 1995). Pathologic effects of HNE are associated with microvascular injury, causing endothelial damage, increased capillary permeability, and interstitial edema. Oxidants derived from neutrophils and endothelial cells enhance the pathogenic function of HNE because of their ability to oxidatively inactivate endogenous inhibitors. After the formation of the protein-rich edema fluid, HNE can cleave fibrin and α1-PI to generate potent chemotactic peptides that further contribute to the inflammatory response in the lung (Banda et al., 1988b; Leavell et al., 1996). HNE may also potentiate the inflammatory response by increasing the expression and release of cytokines (Bédard et al., 1993) and by increasing mucin production (Voynow et al., 1999). In experimental animal models, intratracheal administration of exogenous HNE induces lung hemorrhage and ALI, whereas administration of pharmacological HNE inhibitors prevents lung injury, which further supports the role of NSPs in lung injury (Lee and Downey, 2001; Kawabata et al., 2002; Zeiher et al., 2002).

B. Anti-Neutrophil Cytoplasmic Autoantibody-Associated Vasculitides

ANCA-associated vasculitides encompasses a variety of diseases characterized by inflammation of blood vessels and by the presence of autoantibodies directed against neutrophil constituents. These autoantibodies are known as ANCAs (Kallenberg et al., 2006). In Wegener granulomatosis (WG), antibodies are mostly directed against PR3. WG is a relatively uncommon chronic inflammatory disorder first described in 1931 by Heinz Karl Ernst Klinger as a variant of polyarteritis nodosa (Klinger, 1931). In 1936, the German pathologist Friedrich Wegener described the disease as a distinct pathological entity (Wegener, 1936, 1939). WG is characterized by necrotizing granulomatous inflammation and vasculitis of small vessels and can affect any organ (Fauci and Wolff, 1973; Sarraf and Sneller, 2005). The most common sites of involvement are the upper and lower respiratory tract and the kidneys. WG affects approximately 1 in 20,000 people; it can occur in persons of any age but most often affects those aged 40 to 60 years (Walton, 1958; Cotch et al., 1996). Approximately 90% of patients have cold or sinusitis symptoms that fail to respond to the usual therapeutic measures and that last considerably longer than the usual upper respiratory tract infection. Lung involvement occurs in approximately 85% of the patients. Other symptoms include nasal membrane ulcerations and crusting, saddle-nose deformity, inflammation of the ear with hearing problems, inflammation of the eye with sight problems, and cough (with or without hemoptysis).

The cause of WG has not yet been elucidated, but infection with *S. aureus* could well contribute to its pathogenicity (Kallenberg, 2008). Deficiency in α1-PI also is a risk factor of WG. Studies of gene polymorphisms showed that the frequency of the α1-PI deficiency allele PI*Z is increased in WG and is related to its outcome (Esnault et al., 1993; Callea et al., 1997; Needham and Stockley, 2004). WG is characteristically associated with the presence of ANCA, directed against proteins of neutrophilic granulocytes and monocytes stored in cytoplasmic granules (Reumaux et al., 2004; Kallenberg et al., 2006; Kallenberg, 2008). The occurrence of this cytoplasmic staining pattern was first reported in 1982 in patients with necrotizing glomerulonephritis (Davies et al., 1982). Jenne et al. (1990) identified PR3 as the target antigen for ANCA. Though most patients with WG are positive for ANCAs, antibody titer in the serum of patients does not always correlate with the severity of the disease, which raises the question of the pathogenicity of ANCAs (Merkel et al., 2009). In addition to cytoplasmic ANCA (or cANCA, which usually recognizes PR3 in WG), perinuclear ANCA (pANCA) have been demonstrated by enzyme-linked immunosorbent assay and indirect immunofluorescence on ethanol-fixed neutrophils (Hoffman and Specks, 1998). pANCA are predominantly directed against myeloperoxidase but some are also directed against HNE and CG (Savige et al., 1998) and can additionally be found in a variety of inflammatory diseases, including sometimes Crohn disease, rheumatoid arthritis, and drug-induced vasculitis (Hoffman and Specks, 1998; Radice and Sinico, 2005). The precise subcellular localization resulting in the apparent perinuclear

distribution of pANCA remains uncertain (Yang, 1997; Hoffman and Specks, 1998).

Mouse monoclonal anti-PR3 antibodies and cANCA from WG patients recognize conformational epitopes on PR3 (Bini et al., 1992) that may vary during the course of the disease (Rarok et al., 2003). Four groups of mouse monoclonal anti-PR3 antibodies have been identified. Antibodies in the same group recognize similar epitopes on PR3 (Van Der Geld et al., 1999). Autoantibodies to HNE are responsible for a syndrome of midline destructive vasculitis, which is associated with use of cocaine via nasal insufflations (Wiesner et al., 2004).

Resting neutrophils from purified peripheral blood of healthy persons express PR3 on their membranes in a genetically determined manner, and this proportion of PR3 is increased in a stepwise fashion by neutrophil priming and subsequent activation (Schreiber et al., 2003). Circulating blood neutrophils from patients with active WG express PR3 on their cell membranes in larger quantities than healthy persons as a result of an ongoing inflammatory process (Rarok et al., 2002). Likewise, neutrophils from healthy volunteers can be primed with proinflammatory cytokines (e.g., TNF-α to increase the fraction of surface-exposed PR3 directly accessible to circulating cANCA) (Csernok et al., 1994). Neutrophils become fully activated upon cANCA binding to their membranes. They produce reactive oxygen species and release lysosomal proteases from their azurophilic granules to the pericellular environment. In recent work, Kessenbrock et al. (2009) showed that TNF-α-primed neutrophils stimulated by cANCA produce NET containing PR3 and myeloperoxidase. Deposition of NET in inflamed kidneys and the presence of circulating myeloperoxidase-DNA complexes suggest that NET formation contributes to focal necrotizing glomerulonephritis and enhances the autoimmune response against neutrophil components in individuals with small-vessel vasculitis.

C. Hereditary Neutropenias

Neutropenia is a hematological disorder characterized by an abnormally low number of neutrophils (Horwitz et al., 2007). The normal neutrophil count fluctuates across human populations and within individual patients in response to infection but typically lies in the range of 1.5 to 5×10^9 cells/liter. Neutropenia is categorized as severe when the cell count falls below 0.5×10^9 cells/liter. Hence, patients with neutropenia are more susceptible to bacterial infections and, without prompt medical attention, the condition may become life-threatening. Common causes of neutropenia include cancer chemotherapy, drug reactions, autoimmune diseases, and hereditary disorders (Berliner et al., 2004; Schwartzberg, 2006).

Cyclic neutropenia and severe congenital neutropenia are the two main forms of hereditary neutropenia (Ancliff, 2003; Horwitz et al., 2007). Their prevalence has been estimated at 1 in 200,000 in newborn. Cyclic neutropenia (MIM 162800) is an autosomal disease in which blood-cell production from the bone marrow oscillates with a 21-day periodicity. Circulating neutrophils vary between almost normal numbers and zero. Monocytes also cycle but do so in a phase that is opposite that of neutrophils (Berliner et al., 2004). During neutropenic periods, affected persons are at risk for opportunistic infections (Reimann and DeBerardinis, 1949). The periodic fluctuation between high and low neutrophil counts could be explained by a feedback mechanism through which mature neutrophils inhibit maturation of progenitor cells (Horwitz et al., 2003; Salipante et al., 2009). The first case of human cyclic neutropenia was reported by Leale (1910), but only in 1999 did Horwitz et al. (1999) reported that mutations of the elastase gene *ELA2*, since then renamed *ELANE*, were responsible for human cyclic neutropenia.

Severe congenital neutropenia (MIM 202700), also called Kostmann disease, refers to a noncyclic neutropenia present at birth (absolute neutrophil count below 0.5×10^9/liter). It was first reported as infantile agranulocytosis in a large Swedish family of consanguineous parents (Kostmann, 1956; Carlsson et al., 2006). A characteristic of severe congenital neutropenia is that the bone marrow displays a defect in maturation of developing myeloid cells at the promyelocyte stage. Mutations in *ELANE* are present in approximately 60% of severe congenital neutropenia cases, and approximately 50 different mutations scattered throughout the coding regions have been identified. Many of the mutations do not lead to loss of enzymatic activity (Horwitz et al., 2007). Bioinformatic analysis of different pathogenic missense mutations in *ELANE* suggests diverse deleterious effects on structural and physicochemical properties of HNE (Thusberg and Vihinen, 2006). Additional rare cases of severe congenital neutropenia arise from mutations in the genes encoding zinc finger transcription factor GFI1 (Person et al., 2003), glucose-6-phosphatase (Boztug et al., 2009), and HS1-associated protein X-1 (Klein et al., 2007). Patients with severe congenital neutropenia present symptoms of recurrent bacterial infections mostly in the skin, the respiratory system, and the oral cavity, whereas some patients may also present with severe generalized infections. Neutropenic patients often respond to granulocyte colony-stimulating factor, which stimulates neutrophil production in bone marrow. Bone marrow transplantation is warranted in patients who are unresponsive to granulocyte colony-stimulating factor or who develop leukemia.

The heterotetrameric adaptor protein complex 3 (AP-3) residing on the cytoplasmic surface of intracellular vesicles has been shown to function in the sorting of proteins to the endosomal/lysosomal system (Badolato and Parolini, 2007). Mutation of the gene encoding the AP-3 β subunit, which disrupts the complex, have been found to cause Hermansky Pudlak syndrome type 2 (MIM 203300) (Dell'Angelica et al., 1999; Huizing et al., 2002), a disorder

characterized by oculocutaneous albinism, platelet granular dysfunction, and neutropenic immunodeficiency (Huizing et al., 2008), and also to cause a canine form of cyclic neutropenia associated with pigmentation dilution known as "gray collie syndrome" (Lothrop et al., 1987). Benson et al. (2003) have argued that mutations in either the HNE or AP-3 gene perturbs intracellular trafficking of HNE, implying that mislocalization of HNE may be involved in the pathogenesis of the congenital neutropenias associated with *ELANE* mutations. Alternatively, mutations in *ELANE* may induce the unfolded protein response as a consequence of incorrect folding and targeting of the mutant proteins, with associated programmed cell death (Köllner et al., 2006; Grenda et al., 2007). Expression of mutant HNE, but not wild-type HNE, strongly induced BiP/GRP78 mRNA expression and XBP1 mRNA splicing, two classic markers of the unfolded protein response (Grenda et al., 2007).

D. Papillon-Lefèvre Syndrome

Papillon-Lefèvre syndrome (PLS; MIM 245000) is a hereditary skin disease characterized by a diffuse palmoplantar keratosis and severe periodontitis, leading to premature loss of both the deciduous and permanent teeth (Dhanrajani, 2009). Approximately 15 to 20% of patients are predisposed to recurrent infections. The combination of oral retinoids and antibiotics complemented with professional tooth cleaning are currently used in the treatment of severe periodontitis occurring with this disorder (Dhanrajani, 2009). PLS is an extremely rare inherited autosomal recessive disease with an estimated prevalence of 1 to 4 cases per million people, affecting children between the ages of 1 and 4 (Gorlin et al., 1964). It was first described by two French physicians, Papillon and Lefèvre (1924), as a form of "Mal de Meleda" and was only later classified as a distinct entity (Dekker and Jansen, 1956). More than 200 cases worldwide have been reported in the literature. Consanguinity of the parents was observed in approximately one third of the cases described. Both sexes are equally affected; no racial predominance seems to exist (Haneke 1979; Hattab et al., 1995). Loss-of-function mutations in the DPPI gene (*CSTC*) on chromosome 11q14-q21 are strongly correlated with PLS and the related Haim-Munk syndrome (Hart et al., 1999, 2000; Toomes et al., 1999).

The exact cause of periodontal disease in PLS has not been elucidated, but it has been attributed to a neutrophil defect resulting in decreased phagocytosis and chemotaxis (Brown et al., 1993; Bullon et al., 1993; Firatli et al., 1996). Association of neutrophil or myeloid abnormalities with severe periodontal destruction and the development of rapid periodontal infections in experimentally induced neutropenia in animals suggest that neutrophils are protective against periodontal destruction. The 116-kDa pore-forming leukotoxin is the prime virulence factor of the periodontopathogen *A. actinomycetemcomitans* and promotes its colonization while inducing granule exocytosis and lysis of primed neutrophils. NSPs degrade this toxin extracellularly (Johansson et al., 2000, 2001). Furthermore, they convert the neutrophil-derived hCAP-18 into LL-37, an antimicrobial peptide with activity against *A. actinomycetemcomitans* (de Haar et al., 2006). In PLS, the loss of DPPI activity within neutrophils results in a severe reduction in the activity and stability of NSPs (Pham et al., 2004; de Haar et al., 2004), leading to the generation of lower levels of LL-37 by neutrophils (de Haar et al., 2006) and reduced efficiency of degradation of leukotoxin produced by *A. actinomycetemcomitans* (de Haar et al., 2006).

Pham et al. (2004) have shown that neutrophils from most patients with PLS display normal microbicidal activity against *E. coli* and *S. aureus* despite their severe deficiency in NSPs, but neutrophils from certain patients were defective in microbial killing. This clinical observation suggests that NSPs are not essential antimicrobial molecules and may explain why only a few patients with PLS demonstrate severe recurrent systemic infections.

VI. New Strategies for Fighting Neutrophil Serine Protease-Related Human Diseases

Administration of therapeutic inhibitors to control unwanted proteolysis at inflammation sites has been tested as a therapy for a variety of inflammatory and infectious lung diseases (Chughtai and O'Riordan, 2004). Depending on the size and chemical nature of the inhibitors, they may be administered orally, intravenously, or by an aerosol route. Whatever the mode of administration, the access of therapeutic inhibitors to active proteases is often hampered by physicochemical constraints in the extravascular space and/or by the partitioning of proteases between soluble and solid phases. These solid structures are, for example, molecular components of the matrix or components of the cellular membranes, NET, proteoglycans, and possibly other macromolecular structures. One way to bypass this possible drawback of inhibitor administration would be to reduce the intracellular level of active NSPs within neutrophils by deregulating their intracellular trafficking, a process that may not affect neutrophil infiltration and accumulation at infectious sites and may therefore maintain their beneficial microbicidal properties. Reduction of neutrophil accumulation at inflammatory sites also seems to be another alternative for reducing extracellular NSP activities during noninfectious diseases, where the presence of neutrophils is a priori not beneficial (Fig. 6).

A. Therapeutic Inhibitors

CF, ALI, and ARDS are characterized by the presence of pathogens in the lung. Migration of neutrophils to sites of infection is beneficial because of their capacity to kill pathogens intracellularly after phagocytosis and ex-

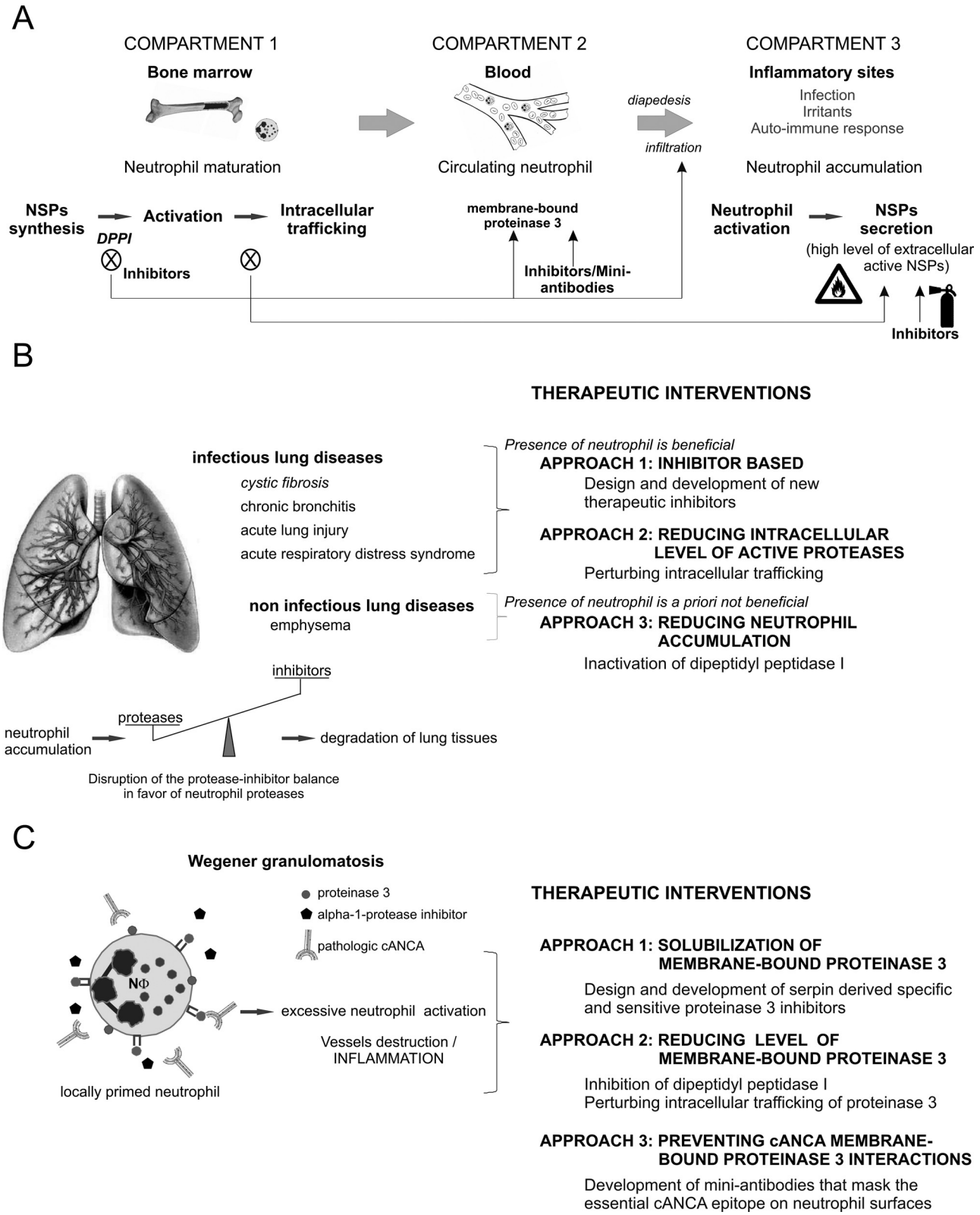


FIG. 6. A, interventional options for the control of neutrophil proteases. The different compartments in which the exposure to NSPs can be reduced and the biological impact of this intervention are shown. B, the different therapeutic approaches to rebalance the altered protease/antiprotease equilibrium in neutrophil-associated inflammatory lung diseases. C, specification for the treatment of Wegener granulomatosis, which is characterized by excessive ANCA-induced activation of locally primed PR3-positive neutrophils and subsequent small vessel destruction.

tracellularly upon release of NET. However, the presence of neutrophils at infection sites may also be deleterious when an excessive, uncontrolled amount of NSP is released into the extracellular medium and leads to lung matrix degradation. Supplementation with exogenous, natural, synthetic, or recombinant NSP inhibitors administered by the vascular route or by aerosols has been tried as a means for restoring the protease/inhibitor balance but without fully convincing results. This is not surprising, however, because 1) exogenous inhibitors do not target all three NSPs released from activated neutrophils, (Owen and Campbell, 1999; Owen, 2008b) and 2) proteolysis in the lung, for example, may occur even in the presence of active endogenous inhibitors as neutrophils adhere to extracellular matrix components and to the vascular endothelium and deposit NSPs in direct proximity to their protein substrates (Owen and Campbell, 1999; Owen, 2008b). Extracellular proteases may also escape inhibition through their association with cell membranes or with negatively charged macromolecular components such as DNA or proteoglycans. Thus, an ideal NSP inhibitor that would control proteolytic activities within given limits should be able to inhibit HNE, PR3, and CG with similar efficiency; would resist rapid oxidation and proteolysis; and would be of small size, thereby affording better access to NSPs when they are bound to extracellular matrices and to molecular or cellular components. It should also resist aerosolization and in vivo degradation. None of the inhibitors developed to fight chronic inflammatory diseases so far address all of these criteria. Nevertheless, replacement therapy by intravenous administration of α1-PI has long been shown to be efficient for α1-PI deficiency associated with emphysema (Wewers et al., 1987; Hubbard et al., 1988), and aerosolized administration of α1-PI or SLPI to patients with cystic fibrosis suppressed or significantly reduced HNE in the respiratory epithelial lining fluid and restored the anti-HNE capacity (McElvaney et al., 1991, 1993). α1-PI augmentation therapy also abrogates HNE-induced up-regulation of cathepsin B and MMP-2 expression, further demonstrating the central role of this protease as a target in many inflammatory diseases (Geraghty et al., 2007, 2008). A remaining problem, however, is that serpins may have impaired access to the microenvironment created by the contact between neutrophils and proteins of the extracellular matrix because of their large molecular weight and their potential sensitivity to oxidation and proteolysis. On the other hand, therapeutically applied inhibitors should not completely abrogate the putative physiological roles of proteases in the pericellular milieu of the neutrophil. This might happen with inhibitors that instantaneously and irreversibly inactivate the target proteases. Hence, competitive inhibitors that can dissociate from proteases, as after withdrawing inhibitor administration, seem to be more appropriate and better suited to curtail excessive proteolytic activity. Irreversible inhibitors such as serpins, however, may also preserve a basal low level of proteolytic activity provided the delay time for full inhibition is long enough to allow substantial substrate cleavage by the protease before its complete inactivation (Bieth, 1986). The delay time to reach a complete irreversible inhibition depends on the local concentration of the two partners, the association rate constant, and the specificity constant toward the biological substrate. In this way, partial oxidative inactivation of α1-PI in inflammatory biological fluids contributes to the maintenance of some local HNE activity and its pericellular functions (Luisetti and Travis, 1996). The chemical inhibitor sivelestat and the recombinant low-molecular-weight inhibitor depelstat are resistant to oxidative and proteolytic inactivation but are targeting only HNE (Kawabata et al., 1991; Attucci et al., 2006). Most synthetic and chimeric elastase inhibitors developed so far have not proceeded beyond phase II of development because of side effects in addition to poor efficacy (Tremblay et al., 2003). Polyvalent recombinant protein inhibitors that target all three NSPs have recently been developed (Zani et al., 2009). One is a recombinant chimera resulting from the fusion of the C-terminal domain of SLPI and elafin; the other is a trappin-2 variant in which the P1 residue Ala is replaced by Leu. These recombinant low-M_r proteins are tight-binding inhibitors of all three NSPs, demonstrating nanomolar or subnanomolar K_i values. They inhibit membrane-bound NSPs but do not resist oxidative inactivation because of the presence of a critical methionine residue at the P1′ position (Zani et al., 2009). Further investigation is needed to identify critical residues involved in the interaction of each protease with physiological, recombinant, and synthetic inhibitors. Specific and highly sensitive FRET substrates designed and developed to measure the activities of HNE, PR3, and CG at the surface of triggered neutrophils and in whole biological fluids are important tools for quantifying active HNE, PR3, and CG in lung diseases (Korkmaz et al., 2008a). These substrates, allowing measurement of proteolytic activities in both solid and soluble fraction of BAL fluid or sputum from patients with chronic inflammatory diseases, will no doubt aid the investigation of spatiotemporal efficacy of administered therapeutic inhibitors.

cANCA are directed against PR3 and are a diagnostic marker of WG, although cANCAs are not found in approximately 10% of patients with Wegener granulomatosis (Radice and Sinico, 2005). Their simultaneous interaction with mPR3 and Fc receptors is believed to trigger full activation and degranulation of neutrophils around small vessels in patients with active disease (Kallenberg, 2008). Unlike HNE and CG, PR3 is constitutively expressed on the surface of circulating neutrophils but is proteolytically inactive (Korkmaz et al., 2009). This prevents constitutive PR3 from interacting with circulating protease inhibitors but not with cANCAs, which, in turn, may activate neutrophils and favor expression of active NSPs at the cell

surface. Designing a specific PR3 inhibitor that removes active PR3 from the surface of activated neutrophils would probably help reduce ANCA binding to the cell surface and therefore small vessel inflammation. Unfortunately, such an inhibitor is not yet available. The main circulating PR3 inhibitor is α1-PI, which forms soluble protease-inhibitor complexes but inhibits PR3 at a rate approximately 100 times lower than that of HNE. This may result in a transient increase of active mPR3 on primed neutrophils with cANCA binding and subsequent neutrophil activation. Although it was found that heterozygotes for the PiZ variant of the α1-PI gene are at greater risk than the general population for developing WG (Elzouki et al., 1994), there is currently no evidence that AAT augmentation therapy is effective in the treatment of WG associated with AAT deficiency (Silverman and Sandhaus, 2009). In keeping with this, we recently found that pathogenic cANCAs react with membrane-bound PR3 faster than does α1-PI (Kuhl et al., 2010), which supports the idea that the inhibitor cannot significantly impair neutrophil triggering by cANCA.

Designing specific inhibitors capable of targeting a particular protease such as PR3 (for the treatment of WG) or polyvalent inhibitors (to improve control of multiple proteases in infectious and inflammatory disorders) requires a deeper investigation of the interaction between protease active sites and inhibitors at the molecular level. Determination of the three-dimensional structures of protease-inhibitor complexes and characterization of protease specificities through kinetic analysis using synthetic substrates has provided information indicating the residues and subsites that are involved in such interactions. A strategy for engineering α1-PI to become a specific and sensitive inhibitor of PR3 could consist of substituting the exposed reactive center loop of α1-PI, which could then interact with charged residues present in the active site of PR3. This approach might also help to elucidate the mechanism of degradation by PR3 of the α1-PI RCL (Duranton and Bieth, 2003). Such a hypothetical recombinant serpin-like inhibitor might be capable of rapidly removing active PR3 from the activated neutrophil membranes and could be helpful in modulating cANCA-neutrophil interactions in WG.

This approach could be extended by development of monoclonal humanized mini antibodies capable of masking the essential cANCA epitope on mPR3, which will impair neutrophil activation. It is noteworthy that monoclonal antibodies represent a promising class of biotherapeutics for the treatment of autoimmune diseases and cancers, and more than 20 mAbs have been approved for therapy or are in clinical trials (Lynch et al., 2009). Rituximab, a chimeric monoclonal antibody directed against CD20, which is present primarily on B cells and is currently used in the treatment of non-Hodgkin's lymphoma and rheumatoid arthritis (Plosker and Figgitt, 2003; Fleischmann, 2009), significantly decreases the level of anti PR3 autoantibodies, resulting in patient clinical remission (Ferraro et al., 2008) that is possibly related to a lower neutrophil activation.

B. Reducing Intracellular Levels of Active Elastase, Proteinase 3, and Cathepsin G

Impairment of access of therapeutic inhibitors to extracellularly active NSPs represents a possible limitation of an inhibitor-based approach to the treatment of infectious and inflammatory pulmonary diseases. This may occur both because of the composition of the extracellular medium (mucus, NET, proteoglycans, and other macromolecules) and because of the spatiotemporal localization of NSPs. Reducing the intracellular levels of active NSPs in mature neutrophils, without perturbing their functions and accumulation at infectious sites, in theory, represents an alternative approach for limiting uncontrolled proteolysis (Fig. 6). Potentially, this could be achieved through disruption of the intracellular trafficking of mature NSPs. A decrease in the immune reactive level of NSPs is observed when intracellular proteins involved in protein sorting and vesicle transport are mutated in human diseases. Mutations of the AP-3 cargo protein and of the CHS protein in Hermansky Pudlak syndrome-2 and Chediak-Higashi syndrome, respectively, result in decreased intracellular levels of HNE and CG (Ganz et al., 1988; Fontana et al., 2006). AP-3 cargo protein and the CHS protein are believed to be involved in vesicle formation and transport. A low HNE level is also observed in neutrophils from neutropenia patients with HS1-associated protein X-1 mutations (B. Korkmaz and M. S. Horwitz, unpublished results). The proteoglycan serglycin has recently been shown to play a role in the sorting of NE (Niemann et al., 2007). In serglycin knockout mice, NE is absent from mature neutrophils but serglycin inactivation has no effect on correct granular targeting and on intracellular quantities of CG and PR3. Furthermore, the lack of serglycin has no effect on neutrophil count and ultrastructure (Niemann et al., 2007), demonstrating that inactivation of a protein involved in the sorting of NE does not necessarily induce neutropenia, whereas dysfunctional AP3 and CHS proteins impair granule formation and protein targeting in general. Thus, there is cumulative evidence that the intracellular level of HNE and possibly of other related NSPs depends on the functionality of molecules involved in its intracellular trafficking.

Identifying and inactivating other molecular targets that deregulate protease trafficking without inducing neutropenia or altering neutrophil function or chemotaxis could possibly be exploited for a therapeutic strategy aiming at controlling the production and the activity of NSPs in chronic inflammatory lung diseases. It has been shown, however, that NSPs liberated at the inflammatory sites regulate local production of cytokines, leading to neutrophil infiltration and accumulation. In fact, neutrophil infiltration at sites of inflammation is severely diminished in PR3(−/−)/NE(−/−) mice un-

less only NE is deficient (Kessenbrock et al., 2008). In that case, neutrophil infiltration and accumulation is only marginally affected. Because both PR3 and NE in mice share similar active sites (Fig. 5) and are able to activate proinflammatory cytokines IL-8 and IL-1β, it can be concluded that only one is necessary to promote neutrophil recruitment at inflammatory sites. In humans, however, IL-8 and IL-1β are specifically activated by human PR3. This means that altered intracellular trafficking of HNE is not expected to modify neutrophil migration toward inflammatory sites. Using an animal (primate) model in which the NSP specificities are closer to those in humans should permit the testing of this hypothesis.

C. Reducing Neutrophil Accumulation at Inflammatory Sites

In noninfectious lung diseases and other inflammatory human diseases characterized by an excessive neutrophil accumulation, such as rheumatoid arthritis and inflammatory bowel diseases, containment of NSP activities could occur before proteases are released from neutrophils at the inflammatory site (Fig. 6). Strategies for impairing inflammatory cell recruitment to the lung have already been proposed and explored in vitro and in vivo. For example, inhibitors of phosphodiesterase E4 (PDE4) prevent hydrolysis of cAMP in inflammatory cells, thus reducing their chemoattractant and protease-secreting function (Fan Chung, 2006). By suppressing the release of inflammatory signals, PDE4 inhibitors display a high therapeutic potential in COPD and asthma (Schmidt et al., 1999; Giembycz, 2002). Intraperitoneal administration of the PDE4 inhibitor Rolipram in mice receiving an intranasal challenge of LPS significantly reduces neutrophil accumulation in the lungs (H. Korideck and J. D. Peterson, unpublished data). This observation holds true for mice undergoing long-term exposure to cigarette smoke and treated orally with the long-acting PDE4 inhibitor roflumilast (Martorana et al., 2005). Anti-oxidant supplementation, to block the transcription factor NF-κB (Szulakowski et al., 2006) and thus the production of several pro-inflammatory molecules causing lung inflammation, also reduces the accumulation of inflammatory cells, and the same result is obtained using inhibitors of chemokine receptors (Donnelly and Barnes, 2006).

DPPI is the major protease involved in the activation of NSPs; it also influences the total intracellular levels of these proteases, as observed in neutrophils from patients with PLS (Pham et al., 2004). In addition, mice deficient in DPPI demonstrate impaired neutrophil accumulation in their joints in response to noninfectious inflammatory stimuli but are protected against cartilage destruction in acute arthritis experimentally induced by immune complexes (Adkison et al., 2002). The decrease in neutrophil accumulation in DPPI($-/-$) mice is correlated with cytokine/chemokine dysregulation at inflammatory sites resulting from the absence of proteolytically active NSPs (Adkison et al., 2002; Akk et al., 2008). In patients with PLS, DPPI deficiency leads to the accumulation of neutrophils in the blood as they are hampered to enter the extravascular tissue. Taken together, these observations support the idea that DPPI inhibitors could be useful for the treatment of a number of important inflammatory diseases, including lung diseases. Using such inhibitors in infectious diseases, on the other hand, seems to be risky because efficient elimination of pathogens requires both efficient neutrophil recruitment to the site of microbial invasion and rapid destruction of microbes in phagolysosomes. As a significant number of PLS patients display a normal anti-microbial response to common pathogens, NSPs are presumably not essential to overcome invading pathogens (Pham, 2006). Hence, redundancy in antimicrobial defense systems provides a favorable window for the application of NSP inhibitors. In a therapeutic inhibitor-based approach, DPPI may be specifically targeted and inactivated in the bone marrow. DPPI inhibition can be achieved with general inhibitors of cysteine proteases such as diazomethyl ketones, dipeptide nitriles, vinylsulfones, and (2*S*,3*S*)-3-[[[(1*S*)-1-[[[4-[(aminoiminomethyl) amino]butyl]amino]carbonyl]-3-methylbutyl]amino]carbonyl]-2-oxiranecarboxylic acid (E-64) (Barrett et al., 1982; McGuire et al., 1992; Méthot et al., 2007). Among the existing inhibitors, Gly-Phe-diazomethylketone is one of the most effective compounds (Kam et al., 2004; Méthot et al., 2007). Dipeptide nitriles and vinyl sulfone inhibitors are nontoxic to cells and effective toward intracellular DPPI and are therefore potentially suitable for functional studies. Long-term administration of a dipeptide nitrile DPPI inhibitor transported by a poly(ethylene glycol)-based vehicle results in the nearly complete inhibition of DPPI and causes marked reduction of neutrophil accumulation at inflammatory site (Méthot et al., 2008). Specific, nontoxic inhibitors of DPPI may be delivered to the bone marrow by liposomal formulations originally developed for the treatment of leukemias (Gregoriadis et al., 1974; Kohlschütter et al., 2008). As a consequence of therapeutic DPPI inhibition, pro-NSPs would not be converted into active enzymes and that in turn would modulate the levels of inflammatory mediators, such that neutrophils will not infiltrate and accumulate at inflammatory sites. In vivo inhibition of NSP activation by targeting DPPI requires high fractional inhibition of this protease in the bone marrow (Méthot et al., 2008). Because mature neutrophils are released from the bone marrow into the peripheral circulation after approximately 11 to 14 days of development (Walker and Willemze, 1980), a continuous administration of DPPI inhibitor over this period would probably be required. Possible side effects of such a prolonged administration cannot be predicted yet, because severe periodontitis and hyperkeratosis, as found in DPPI-deficient patients with PLS, are not observed in mice successfully

treated with DPPI inhibitor to prevent NSP activation (Méthot et al., 2008).

Loss-of-function mutations in DPPI have provided important insight into its role in inflammation, but little information is available on intracellular NSP activation during neutrophil development. Hematopoietic cell lines that express DPPI and are able to activate and target NSPs in a fashion similar to that of human neutrophils represent good models for analyzing the processing of proenzymes by DPPI. The HL-60 (human promyelocytic leukemia cells) cell line contains DPPI and expresses HNE, PR3, and CG (Meagher and Cotter, 1988; Bories et al., 1989; Yoshimura and Crystal, 1992). But other mammalian hematopoietic cell lines [rat basophilic leukemia cells (RBL-1), murine 32D cells and the mast cell line-1] produce DPPI and can be transfected to produce active NSPs that will be stored in granules in a similar fashion as in human neutrophils (Gullberg et al., 1994, 1995; Specks et al., 1996; Garwicz et al., 1997; Li and Horwitz, 2001). Generally, processing and targeting is more rapid and efficient in RBL-1 cells than in other cell lines. Thus, RBL-1 cells have been successfully used to investigate NSP activation by DPPI and to study the influence of HNE mutations on the intracellular trafficking of the protease. Identification of the location of rat DPPI and its colocalization with active HNE by fluorescence microscopy may lead to a better understanding of where pro-NSPs are activated, a question remaining unanswered until now. To this end, chemical fluorescent tracers that irreversibly inactivate human NSPs could be used.

Recently, we showed that pharmacological inactivation of DPPI in RBL cells transfected with HNE impairs HNE targeting to the nuclear envelope and the same result was seen using neutrophils purified from patients with DPPI mutations (B. Korkmaz and M. S. Horwitz, unpublished results). PR3 transfected into RBL-1 cells is present in lysosomes but also on cellular surfaces. One might expect that inactivation of intracellular DPPI by cell permeable inhibitors could well reduce the interaction of PR3 with cellular membranes, because purified pro-PR3 displays less affinity to cellular surfaces than mature PR3 (Korkmaz et al., 2008b). This may be of major importance in WG, because it would allow for a reduction in membrane binding of pathogenic autoantibodies, which results in neutrophil priming and activation.

VII. Concluding Remarks

NSPs were first recognized as protein-degrading enzymes but have now proven to be multifunctional components participating in a variety of pathophysiological processes. Thus, they appear as potential therapeutic targets for drugs that inhibit their active site or impair activation from their precursor. Overall, the available preclinical and clinical data suggest that inhibition of NSPs using therapeutic inhibitors would suppress or attenuate deleterious effects of inflammatory diseases, including lung diseases. Depending on the size and chemical nature of inhibitors, those may be administered orally, intravenously, or by aerosolization. But the results obtained until now have not been fully convincing because of the poor knowledge of the biological function of each protease, their spatiotemporal regulation during the course of the disease, the physicochemical constraints associated with inhibitor administration, or the use of animal models in which NSP regulation and specificity differ from those in human. Two different and complementary approaches may help bypass these putative problems. One is to target active proteases by inhibitors at the inflammatory site in animal models in which lung anatomy and physiology are close to those in human to allow in vitro and in vivo assays of human-directed drugs/inhibitors. The other is to prevent neutrophil accumulation at inflammatory sites by impairing production of proteolytically active NSPs using an inhibitor of their maturation protease, DPPI. Preventing neutrophil accumulation at the inflammatory sites by therapeutic inhibition of DPPI represents an original and novel approach, the exploration of which has just started (Méthot et al., 2008). Thus pharmacological inactivation of DPPI in human neutrophils could well reduce membrane binding of PR3 and, as a consequence, neutrophil priming by pathogenic auto-antibodies in WG. In addition, it has been recognized that the intracellular level of NSPs depends on their correct intracellular trafficking. In the future, pharmacological targeting of molecules specifically involved in the correct intracellular trafficking of each NSP could possibly regulate their production and activity, a feature that could be exploited as a therapeutic strategy for inflammatory diseases.

Although rodent models of human diseases have largely contributed to progress in biomedical sciences for studying disease pathogenesis and identifying and testing novel therapeutic strategies, there are numerous examples showing that they cannot always provide fully reliable answers transferable to humans. For example, transgenic mice with a disrupted *CFTR* gene do not develop the typical manifestations of CF (Snouwaert et al., 1992) and mice exposed to cigarette smoke do not reproduce the exacerbation phases observed with COPD. Furthermore, we recently showed that NSPs in humans and mice demonstrate a slightly different specificity sufficient to prevent some putatively therapeutic human NSPs inhibitors from interacting with mouse proteases (Kalupov et al., 2009). Mouse cANCA serum generated by immunization of PR3(−/−)/NE(−/−) mice murine PR3 does not reproduce pathological features of WG when transferred to systemically lipopolysaccharide-primed wild-type mice (Pfister et al., 2004). The hydrophobic patch mediating membrane expression of human PR3, however, is substituted by highly hydrophilic residues on mouse PR3 (Korkmaz et al., 2008b). Sequence differences between human and mouse may

result in absent or highly reduced mouse PR3 expression at the membrane of neutrophils, which may explain the minor pathogenic effect of antibodies to mouse PR3 in experimental in vivo models. Unlike mouse PR3-directed ANCA, antibodies directed against mouse myeloperoxidase produce small-vessel vasculitis upon injection into wild-type mice (Xiao et al., 2002; Kain et al., 2010). Gene-targeted mice carrying the elastase mutation observed in patients with neutropenia (Grenda et al., 2007) or deficient in the DPPI gene do not present the same phenotypical characteristics as those observed in humans (Adkison et al., 2002). Using nonhuman primate models in which NSPs are closer to human homologs at both the structural and functional levels would probably help to circumvent these difficulties and permit exploration of new therapeutic strategies for NSP-related diseases. Nonhuman primates are already explored as experimental models in a variety of human diseases (diabetes, cardiovascular dysfunction, osteoporosis, reproductive senescence, neurobiological aging, and related cognitive decline (Lane, 2000; Brok et al., 2001). The first transgenic primate (*Macaca mulatta*) carrying a dysfunctional gene has been generated (Yang et al., 2008). This transgenic primate presents the symptoms of human Huntington disease, whereas the corresponding mutation of the same gene in mouse does not induce the brain changes and behavioral features observed in humans (Yang et al., 2008). This pioneering work should pave the way for further investigation in this direction particularly for analyzing the function of NSPs in primates with dysfunctional NSP genes.

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REFERENCES

Abraham CR, Selkoe DJ, and Potter H (1988) Immunochemical identification of the serine protease inhibitor alpha 1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell* **52:**487–501.

Adkison AM, Raptis SZ, Kelley DG, and Pham CT (2002) Dipeptidyl peptidase I activates neutrophil-derived serine proteases and regulates the development of acute experimental arthritis. *J Clin Invest* **109:**363–371.

Akira S, Uematsu S, and Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* **124:**783–801.

Akita K, Ohtsuki T, Nukada Y, Tanimoto T, Namba M, Okura T, Takakura-Yamamoto R, Torigoe K, Gu Y, Su MS, et al. (1997) Involvement of caspase-1 and caspase-3 in the production and processing of mature human interleukin 18 in monocytic THP.1 cells. *J Biol Chem* **272:**26595–26603.

Akk AM, Simmons PM, Chan HW, Agapov E, Holtzman MJ, Grayson MH, and Pham CT (2008) Dipeptidyl peptidase I-dependent neutrophil recruitment modulates the inflammatory response to Sendai virus infection. *J Immunol* **180:**3535–3542.

Allport JR, Lim YC, Shipley JM, Senior RM, Shapiro SD, Matsuyoshi N, Vestweber D, and Luscinskas FW (2002) Neutrophils from MMP-9- or neutrophil elastase-deficient mice show no defect in transendothelial migration under flow in vitro. *J Leukoc Biol* **71:**821–828.

Amin R and Ratjen F (2008) Cystic fibrosis: a review of pulmonary and nutritional therapies. *Adv Pediatr* **55:**99–121.

Amitani R, Wilson R, Rutman A, Read R, Ward C, Burnett D, Stockley RA, and Cole PJ (1991) Effects of human neutrophil elastase and Pseudomonas aeruginosa proteinases on human respiratory epithelium. *Am J Respir Cell Mol Biol* **4:**26–32.

Ancliff PJ (2003) Congenital neutropenia. *Blood Rev* **17:**209–216.

Annand RR, Dahlen JR, Sprecher CA, De Dreu P, Foster DC, Mankovich JA, Talanian RV, Kisiel W, and Giegel DA (1999) Caspase-1 (interleukin-1beta-converting enzyme) is inhibited by the human serpin analogue proteinase inhibitor 9. *Biochem J* **342:**655–665.

Attucci S, Gauthier A, Korkmaz B, Delépine P, Martino MF, Saudubray F, Diot P, and Gauthier F (2006) EPI-hNE4, a proteolysis-resistant inhibitor of human neutrophil elastase and potential anti-inflammatory drug for treating cystic fibrosis. *J Pharmacol Exp Ther* **318:**803–809.

Attucci S, Korkmaz B, Juliano L, Hazouard E, Girardin C, Brillard-Bourdet M, Réhault S, Anthonioz P, and Gauthier F (2002) Measurement of free and membrane-bound cathepsin G in human neutrophils using new sensitive fluorogenic substrates. *Biochem J* **366:**965–970.

Badolato R and Parolini S (2007) Novel insights from adaptor protein 3 complex deficiency. *J Allergy Clin Immunol* **120:**735–741.

Baggiolini M, Bretz U, Dewald B, and Feigenson ME (1978) The polymorphonuclear leukocyte. *Agents Actions* **8:**3–10.

Baggiolini M and Clark-Lewis I (1992) Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett* **307:**97–101.

Baggiolini M, Dewald B, and Moser B (1994) Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. *Adv Immunol* **55:**97–179.

Banda MJ, Rice AG, Griffin GL, and Senior RM (1988a) Alpha 1-proteinase inhibitor is a neutrophil chemoattractant after proteolytic inactivation by macrophage elastase. *J Biol Chem* **263:**4481–4484.

Banda MJ, Rice AG, Griffin GL, and Senior RM (1988b) The inhibitory complex of human alpha 1-proteinase inhibitor and human leukocyte elastase is a neutrophil chemoattractant. *J Exp Med* **167:**1608–1615.

Bank U and Ansorge S (2001) More than destructive: neutrophil-derived serine proteases in cytokine bioactivity control. *J Leukoc Biol* **69:**197–206.

Bao JJ, Sifers RN, Kidd VJ, Ledley FD, and Woo SL (1987) Molecular evolution of serpins: homologous structure of the human alpha 1-antichymotrypsin and alpha 1-antitrypsin genes. *Biochemistry* **26:**7755–7759.

Barbieri F, Bajetto A, Porcile C, Pattarozzi A, Schettini G, and Florio T (2007) Role of stromal cell-derived factor 1 (SDF1/CXCL12) in regulating anterior pituitary function. *J Mol Endocrinol* **38:**383–389.

Barnes PJ (2004) Mediators of chronic obstructive pulmonary disease. *Pharmacol Rev* **56:**515–548.

Barnes PJ and Stockley RA (2005) COPD: current therapeutic interventions and future approaches. *Eur Respir J* **25:**1084–1106.

Barrett AJ, Kembhavi AA, Brown MA, Kirschke H, Knight CG, Tamai M, and Hanada K (1982) L-trans-Epoxysuccinyl-leucylamido(4-guanidino)butane (E-64) and its analogues as inhibitors of cysteine proteinases including cathepsins B, H and L. *Biochem J* **201:**189–198.

Bateman A and Bennett HP (1998) Granulins: the structure and function of an emerging family of growth factors. *J Endocrinol* **158:**145–151.

Bauer S, Abdgawad M, Gunnarsson L, Segelmark M, Tapper H, and Hellmark T (2007) Proteinase 3 and CD177 are expressed on the plasma membrane of the same subset of neutrophils. *J Leukoc Biol* **81:**458–464.

Beatty K, Bieth J, and Travis J (1980) Kinetics of association of serine proteinases with native and oxidized alpha-1-proteinase inhibitor and alpha-1-antichymotrypsin. *J Biol Chem* **255:**3931–3934.

Bédard M, McClure CD, Schiller NL, Francoeur C, Cantin A, and Denis M (1993) 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* **9:**455–462.

Belaaouaj A, Kim KS, and Shapiro SD (2000) Degradation of outer membrane protein A in Escherichia coli killing by neutrophil elastase. *Science* **289:**1185–1188.

Belorgey D and Bieth JG (1998) Effect of polynucleotides on the inhibition of neutrophil elastase by mucus proteinase inhibitor and alpha 1-proteinase inhibitor. *Biochemistry* **37:**16416–16422.

Benson KF, Li FQ, Person RE, Albani D, Duan Z, Wechsler J, Meade-White K, Williams K, Acland GM, Niemeyer G, et al. (2003) Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. *Nat Genet* **35:**90–96.

Bergin DA, Greene CM, Sterchi EE, Kenna C, Geraghty P, Belaaouaj A, Belaaouaj A, Taggart CC, O'Neill SJ, and McElvaney NG (2008) Activation of the epidermal growth factor receptor (EGFR) by a novel metalloprotease pathway. *J Biol Chem* **283:**31736–31744.

Bergsson G, Reeves EP, McNally P, Chotirmall SH, Greene CM, Greally P, Murphy P, O'Neill SJ, and McElvaney NG (2009) LL-37 complexation with glycosaminoglycans in cystic fibrosis lungs inhibits antimicrobial activity, which can be restored by hypertonic saline. *J Immunol* **183:**543–551.

Berliner N, Horwitz M, and Loughran TP Jr (2004) Congenital and acquired neutropenia. *Hematology Am Soc Hematol Educ Program* 63–79.

Bieth J (1986) Possible biological functions of protein proteinase inhibitors, in *Cysteine Proteinases and Their Inhibitors* (Turk V ed) pp 693–703, Walter de Gruyter & Co, Berlin.

Bini P, Gabay JE, Teitel A, Melchior M, Zhou JL, and Elkon KB (1992) Antineutrophil cytoplasmic autoantibodies in Wegener's granulomatosis recognize conformational epitope(s) on proteinase 3. *J Immunol* **149:**1409–1415.

Black RA, Kronheim SR, Cantrell M, Deeley MC, March CJ, Prickett KS, Wignall J, Conlon PJ, Cosman D, and Hopp TP (1988) Generation of biologically active interleukin-1 beta by proteolytic cleavage of the inactive precursor. *J Biol Chem* **263:**9437–9442.

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, et al. (1997) A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* **385:**729–733.

Bode W and Huber R (1992) Natural protein proteinase inhibitors and their interaction with proteinases. *Eur J Biochem* **204:**433–451.

Bode W, Wei AZ, Huber R, Meyer E, Travis J, and Neumann S (1986) X-ray crystal

structure of the complex of human leukocyte elastase (PMN elastase) and the third domain of the turkey ovomucoid inhibitor. *EMBO J* **5:**2453–2458.
Bories D, Raynal MC, Solomon DH, Darzynkiewicz Z, and Cayre YE (1989) Down-regulation of a serine protease, myeloblastin, causes growth arrest and differentiation of promyelocytic leukemia cells. *Cell* **59:**959–968.
Borregaard N and Cowland JB (1997) Granules of the human neutrophilic polymorphonuclear leukocyte. *Blood* **89:**3503–3521.
Borregaard N, Theilgaard-Mönch K, Cowland JB, Ståhle M, and Sørensen OE (2005) Neutrophils and keratinocytes in innate immunity—cooperative actions to provide antimicrobial defense at the right time and place. *J Leukoc Biol* **77:**439–443.
Boudier C and Bieth JG (1989) Mucus proteinase inhibitor: a fast-acting inhibitor of leucocyte elastase. *Biochim Biophys Acta* **995:**36–41.
Boudier C and Bieth JG (1994) Oxidized mucus proteinase inhibitor: a fairly potent neutrophil elastase inhibitor. *Biochem J* **303:**61–68.
Boztug K, Appaswamy G, Ashikov A, Schäffer AA, Salzer U, Diestelhorst J, Germeshausen M, Brandes G, Lee-Gossler J, Noyan F, et al. (2009) A syndrome with congenital neutropenia and mutations in G6PC3. *N Engl J Med* **360:**32–43.
Braun MG, Csernok E, Gross WL, and Müller-Hermelink HK (1991) Proteinase 3, the target antigen of anticytoplasmic antibodies circulating in Wegener's granulomatosis. Immunolocalization in normal and pathologic tissues. *Am J Pathol* **139:**831–838.
Brikos C and O'Neill LA (2008) Signalling of toll-like receptors. *Handb Exp Pharmacol* **(183):**21–50.
Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, and Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. *Science* **303:**1532–1535.
Brok HP, Bauer J, Jonker M, Blezer E, Amor S, Bontrop RE, Laman JD, and 't Hart BA (2001) Non-human primate models of multiple sclerosis. *Immunol Rev* **183:** 173–185.
Brown RS, Hays GL, Flaitz CM, O'Neill PA, Abramovitch K, and White RR (1993) A possible late onset variation of Papillon-Lefèvre syndrome: report of 3 cases. *J Periodontol* **64:**379–386.
Brubaker MJ, Groutas WC, Hoidal JR, and Rao NV (1992) Human neutrophil proteinase 3: mapping of the substrate binding site using peptidyl thiobenzyl esters. *Biochem Biophys Res Commun* **188:**1318–1324.
Bruce MC, Poncz L, Klinger JD, Stern RC, Tomashefski JF Jr, and Dearborn DG (1985) Biochemical and pathologic evidence for proteolytic destruction of lung connective tissue in cystic fibrosis. *Am Rev Respir Dis* **132:**529–535.
Bruch M and Bieth JG (1986) Influence of elastin on the inhibition of leucocyte elastase by alpha 1-proteinase inhibitor and bronchial inhibitor. Potent inhibition of elastin-bound elastase by bronchial inhibitor. *Biochem J* **238:**269–273.
Brusselle GG, Bracke KR, Maes T, D'hulst AI, Moerloose KB, Joos GF, and Pauwels RA (2006) Murine models of COPD. *Pulm Pharmacol Ther* **19:**155–165.
Buchanan PJ, Ernst RK, Elborn JS, and Schock B (2009) Role of CFTR, Pseudomonas aeruginosa and Toll-like receptors in cystic fibrosis lung inflammation. *Biochem Soc Trans* **37:**863–867.
Bucki R, Byfield FJ, and Janmey PA (2007) Release of the antimicrobial peptide LL-37 from DNA/F-actin bundles in cystic fibrosis sputum. *Eur Respir J* **29:**624–632.
Bullon P, Pascual A, Fernandez-Novoa MC, Borobio MV, Muniain MA, and Camacho F (1993) Late onset Papillon-Lefèvre syndrome? A chromosomic, neutrophil function and microbiological study. *J Clin Periodontol* **20:**662–667.
Burgoyne RD and Morgan A (2003) Secretory granule exocytosis. *Physiol Rev* **83:** 581–632.
Buzza MS, Hirst CE, Bird CH, Hosking P, McKendrick J, and Bird PI (2001) The granzyme B inhibitor, PI-9, is present in endothelial and mesothelial cells, suggesting that it protects bystander cells during immune responses. *Cell Immunol* **210:**21–29.
Callea F, Gregorini G, Sinico A, Gonzales G, Bossolasco M, Salvidio G, Radice A, Tira P, Candiano G, Rossi G, et al. (1997) alpha 1-Antitrypsin (AAT) deficiency and ANCA-positive systemic vasculitis: genetic and clinical implications. *Eur J Clin Invest* **27:**696–702.
Calvin J and Price CP (1986) Measurement of serum alpha 1-antichymotrypsin by immunoturbidimetry. *Ann Clin Biochem* **23:**206–209.
Campanelli D, Detmers PA, Nathan CF, and Gabay JE (1990a) Azurocidin and a homologous serine protease from neutrophils. Differential antimicrobial and proteolytic properties. *J Clin Invest* **85:**904–915.
Campanelli D, Melchior M, Fu Y, Nakata M, Shuman H, Nathan C, and Gabay JE (1990b) Cloning of cDNA for proteinase 3: a serine protease, antibiotic, and autoantigen from human neutrophils. *J Exp Med* **172:**1709–1715.
Campbell EJ, Campbell MA, and Owen CA (2000) Bioactive proteinase 3 on the cell surface of human neutrophils: quantification, catalytic activity, and susceptibility to inhibition. *J Immunol* **165:**3366–3374.
Campbell EJ and Owen CA (2007) The sulfate groups of chondroitin sulfate- and heparan sulfate-containing proteoglycans in neutrophil plasma membranes are novel binding sites for human leukocyte elastase and cathepsin G. *J Biol Chem* **282:**14645–14654.
Capizzi SA, Viss MA, Hummel AM, Fass DN, and Specks U (2003) Effects of carboxy-terminal modifications of proteinase 3 (PR3) on the recognition by PR3-ANCA. *Kidney Int* **63:**756–760.
Carlsson G, Andersson M, Pütsep K, Garwicz D, Nordenskjöld M, Henter JI, Palmblad J, and Fadeel B (2006) Kostmann syndrome or infantile genetic agranulocytosis, part one: celebrating 50 years of clinical and basic research on severe congenital neutropenia. *Acta Paediatr* **95:**1526–1532.
Carmona AK, Juliano MA, and Juliano L (2009) The use of Fluorescence Resonance Energy Transfer (FRET) peptides for measurement of clinically important proteolytic enzymes. *An Acad Bras Cienc* **81:**381–392.
Carp H, Miller F, Hoidal JR, and Janoff A (1982) Potential mechanism of emphysema: alpha 1-proteinase inhibitor recovered from lungs of cigarette smokers contains oxidized methionine and has decreased elastase inhibitory capacity. *Proc Natl Acad Sci USA* **79:**2041–2045.
Carrell RW and Lomas DA (1997) Conformational disease. *Lancet* **350:**134–138.
Castillo MJ, Nakajima K, Zimmerman M, and Powers JC (1979) Sensitive substrates for human leukocyte and porcine pancreatic elastase: a study of the merits of various chromophoric and fluorogenic leaving groups in assays for serine proteases. *Anal Biochem* **99:**53–64.
Caughey GH, Schaumberg TH, Zerweck EH, Butterfield JH, Hanson RD, Silverman GA, and Ley TJ (1993) The human mast cell chymase gene (CMA1): mapping to the cathepsin G/granzyme gene cluster and lineage-restricted expression. *Genomics* **15:**614–620.
Cepinskas G, Sandig M, and Kvietys PR (1999) 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* **112:**1937–1945.
Chang WS and Lomas DA (1998) Latent alpha1-antichymotrypsin. A molecular explanation for the inactivation of alpha1-antichymotrypsin in chronic bronchitis and emphysema. *J Biol Chem* **273:**3695–3701.
Chua F and Laurent GJ (2006) Neutrophil elastase: mediator of extracellular matrix destruction and accumulation. *Proc Am Thorac Soc* **3:**424–427.
Chughtai B and O'Riordan TG (2004) Potential role of inhibitors of neutrophil elastase in treating diseases of the airway. *J Aerosol Med* **17:**289–298.
Churg A, Wang RD, Xie C, and Wright JL (2003) alpha-1-Antitrypsin ameliorates cigarette smoke-induced emphysema in the mouse. *Am J Respir Crit Care Med* **168:**199–207.
Clark JM, Vaughan DW, Aiken BM, and Kagan HM (1980) Elastase-like enzymes in human neutrophils localized by ultrastructural cytochemistry. *J Cell Biol* **84:**102–119.
Coeshott C, Ohnemus C, Pilyavskaya A, Ross S, Wieczorek M, Kroona H, Leimer AH, and Cheronis J (1999) Converting enzyme-independent release of tumor necrosis factor alpha and IL-1beta from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. *Proc Natl Acad Sci USA* **96:**6261–6266.
Cooley J, Takayama TK, Shapiro SD, Schechter NM, and Remold-O'Donnell E (2001) The serpin MNEI inhibits elastase-like and chymotrypsin-like serine proteases through efficient reactions at two active sites. *Biochemistry* **40:**15762–15770.
Cotch MF, Hoffman GS, Yerg DE, Kaufman GI, Targonski P, and Kaslow RA (1996) The epidemiology of Wegener's granulomatosis. Estimates of the five-year period prevalence, annual mortality, and geographic disease distribution from population-based data sources. *Arthritis Rheum* **39:**87–92.
Coughlin PB, Tetaz T, and Salem HH (1993) Identification and purification of a novel serine proteinase inhibitor. *J Biol Chem* **268:**9541–9547.
Creagh EM and O'Neill LA (2006) TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. *Trends Immunol* **27:**352–357.
Crystal RG, Brantly ML, Hubbard RC, Curiel DT, States DJ, and Holmes MD (1989) The alpha 1-antitrypsin gene and its mutations. Clinical consequences and strategies for therapy. *Chest* **95:**196–208.
Csernok E, Ernst M, Schmitt W, Bainton DF, and Gross WL (1994) Activated neutrophils express proteinase 3 on their plasma membrane in vitro and in vivo. *Clin Exp Immunol* **95:**244–250.
Csernok E, Lüdemann J, Gross WL, and Bainton DF (1990) Ultrastructural localization of proteinase 3, the target antigen of anti-cytoplasmic antibodies circulating in Wegener's granulomatosis. *Am J Pathol* **137:**1113–1120.
Dahlen JR, Foster DC, and Kisiel W (1997) Human proteinase inhibitor 9 (PI9) is a potent inhibitor of subtilisin A. *Biochem Biophys Res Commun* **238:**329–333.
Dahlen JR, Foster DC, and Kisiel W (1999) Inhibition of neutrophil elastase by recombinant human proteinase inhibitor 9. *Biochim Biophys Acta* **1451:**233–241.
David A, Fridlich R, and Aviram I (2005) The presence of membrane Proteinase 3 in neutrophil lipid rafts and its colocalization with FcgammaRIIIb and cytochrome b558. *Exp Cell Res* **308:**156–165.
David A, Kacher Y, Specks U, and Aviram I (2003) Interaction of proteinase 3 with CD11b/CD18 (beta2 integrin) on the cell membrane of human neutrophils. *J Leukoc Biol* **74:**551–557.
Davies DJ, Moran JE, Niall JF, and Ryan GB (1982) Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J Clin Res Ed* **285:**606.
Davis PB (2006) Cystic fibrosis since 1938. *Am J Respir Crit Care Med* **173:**475–482.
de Garavilla L, Greco MN, Sukumar N, Chen ZW, Pineda AO, Mathews FS, Di Cera E, Giardino EC, Wells GI, Haertlein BJ, et al. (2005) A novel, potent dual inhibitor of the leukocyte proteases cathepsin G and chymase: molecular mechanisms and anti-inflammatory activity in vivo. *J Biol Chem* **280:**18001–18007.
de Haar SF, Hiemstra PS, van Steenbergen MT, Everts V, and Beertsen W (2006) Role of polymorphonuclear leukocyte-derived serine proteinases in defense against Actinobacillus actinomycetemcomitans. *Infect Immun* **74:**5284–5291.
de Haar SF, Jansen DC, Schoenmaker T, De Vree H, Everts V, and Beertsen W (2004) Loss-of-function mutations in cathepsin C in two families with Papillon-Lefevre syndrome are associated with deficiency of serine proteinases in PMNs. *Hum Mutat* **23:**524.
Dekker G and Jansen LH (1956) Hyperkeratosis palmo-plantaris with periodontosis (Papillon-Lefèvre). *Dermatologica* **113:**207–219.
Delgado MB, Clark-Lewis I, Loetscher P, Langen H, Thelen M, Baggiolini M, and Wolf M (2001) Rapid inactivation of stromal cell-derived factor-1 by cathepsin G associated with lymphocytes. *Eur J Immunol* **31:**699–707.
Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, and Bonifacino JS (1999) Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. *Mol Cell* **3:**11–21.
Dementiev A, Dobó J, and Gettins PG (2006) Active site distortion is sufficient for proteinase inhibition by serpins: structure of the covalent complex of alpha1-proteinase inhibitor with porcine pancreatic elastase. *J Biol Chem* **281:**3452–3457.
Desrochers PE, Mookhtiar K, Van Wart HE, Hasty KA, and Weiss SJ (1992) Proteolytic inactivation of alpha 1-proteinase inhibitor and alpha 1-antichymotrypsin by oxidatively activated human neutrophil metalloproteinases. *J Biol Chem* **267:** 5005–5012.

Devaney JM, Greene CM, Taggart CC, Carroll TP, O'Neill SJ, and McElvaney NG (2003) Neutrophil elastase up-regulates interleukin-8 via toll-like receptor 4. *FEBS Lett* **544:**129–132.

Dewald B, Rindler-Ludwig R, Bretz U, and Baggiolini M (1975) Subcellular localization and heterogeneity of neutral proteases in neutrophilic polymorphonuclear leukocytes. *J Exp Med* **141:**709–723.

Dhanrajani PJ (2009) Papillon-Lefevre syndrome: clinical presentation and a brief review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **108:**e1–7.

Doan N and Gettins PG (2007) Human alpha2-macroglobulin is composed of multiple domains, as predicted by homology with complement component C3. *Biochem J* **407:**23–30.

Donnelly LE and Barnes PJ (2006) Chemokine receptors as therapeutic targets in chronic obstructive pulmonary disease. *Trends Pharmacol Sci* **27:**546–553.

Donnelly SC, MacGregor I, Zamani A, Gordon MW, Robertson CE, Steedman DJ, Little K, and Haslett C (1995) Plasma elastase levels and the development of the adult respiratory distress syndrome. *Am J Respir Crit Care Med* **151:**1428–1433.

Doss M, White MR, Tecle T, and Hartshorn KL (2010) Human defensins and LL-37 in mucosal immunity. *J Leukoc Biol* **87:**79–92.

Doumas S, Kolokotronis A, and Stefanopoulos P (2005) Anti-inflammatory and antimicrobial roles of secretory leukocyte protease inhibitor. *Infect Immun* **73:** 1271–1274.

Draper D, Donohoe W, Mortimer L, and Heine RP (1998) Cysteine proteases of Trichomonas vaginalis degrade secretory leukocyte protease inhibitor. *J Infect Dis* **178:**815–819.

Dublet B, Ruello A, Pederzoli M, Hajjar E, Courbebaisse M, Canteloup S, Reuter N, and Witko-Sarsat V (2005) Cleavage of p21/WAF1/CIP1 by proteinase 3 modulates differentiation of a monocytic cell line. Molecular analysis of the cleavage site. *J Biol Chem* **280:**30242–30253.

Duranton J, Belorgey D, Carrère J, Donato L, Moritz T, and Bieth JG (2000) Effect of DNase on the activity of neutrophil elastase, cathepsin G and proteinase 3 in the presence of DNA. *Febs Lett* **473:**154–156.

Duranton J and Bieth JG (2003) Inhibition of proteinase 3 by [alpha]1-antitrypsin in vitro predicts very fast inhibition in vivo. *Am J Respir Cell Mol Biol* **29:**57–61.

Dürr UH, Sudheendra US, and Ramamoorthy A (2006) LL-37, the only human member of the cathelicidin family of antimicrobial peptides. *Biochim Biophys Acta* **1758:**1408–1425.

Elzouki AN, Segelmark M, Wieslander J, and Eriksson S (1994) Strong link between the alpha 1-antitrypsin PiZ allele and Wegener's granulomatosis. *J Intern Med* **236:**543–548.

Ermolieff J, Boudier C, Laine A, Meyer B, and Bieth JG (1994) Heparin protects cathepsin G against inhibition by protein proteinase inhibitors. *J Biol Chem* **269:**29502–29508.

Ermolieff J, Duranton J, Petitou M, and Bieth JG (1998) Heparin accelerates the inhibition of cathepsin G by mucus proteinase inhibitor: potent effect of O-butyrylated heparin. *Biochem J* **330:**1369–1374.

Esnault VL, Testa A, Audrain M, Rogé C, Hamidou M, Barrier JH, Sesboüé R, Martin JP, and Lesavre P (1993) Alpha 1-antitrypsin genetic polymorphism in ANCA-positive systemic vasculitis. *Kidney Int* **43:**1329–1332.

Faller B, Cadène M, and Bieth JG (1993) Demonstration of a two-step reaction mechanism for the inhibition of heparin-bound neutrophil elastase by alpha 1-proteinase inhibitor. *Biochemistry* **32:**9230–9235.

Faller B, Frommherz K, and Bieth JG (1992) Heparin interferes with the inhibition of neutrophil elastase by its physiological inhibitors. *Biol Chem Hoppe Seyler* **373:**503–508.

Fan Chung K (2006) Phosphodiesterase inhibitors in airways disease. *Eur J Pharmacol* **533:**110–117.

Fath MA, Wu X, Hileman RE, Linhardt RJ, Kashem MA, Nelson RM, Wright CD, and Abraham WM (1998) Interaction of secretory leukocyte protease inhibitor with heparin inhibits proteases involved in asthma. *J Biol Chem* **273:**13563–13569.

Fauci AS and Wolff SM (1973) Wegener's granulomatosis: studies in eighteen patients and a review of the literature. *Medicine (Baltimore)* **52:**535–561.

Fellows E, Gil-Parrado S, Jenne DE, and Kurschus FC (2007) Natural killer cell-derived human granzyme H induces an alternative, caspase-independent cell-death program. *Blood* **110:**544–552.

Ferraro AJ, Drayson MT, Savage CO, and MacLennan IC (2008) Levels of autoantibodies, unlike antibodies to all extrinsic antigen groups, fall following B cell depletion with Rituximab. *Eur J Immunol* **38:**292–298.

Fick RB, Jr., Naegel GP, Squier SU, Wood RE, Gee JB, and Reynolds HY (1984) Proteins of the cystic fibrosis respiratory tract. Fragmented immunoglobulin G opsonic antibody causing defective opsonophagocytosis. *J Clin Invest* **74:**236–248.

Firatli E, Tüzün B, and Efeoğlu A (1996) Papillon-Lefèvre syndrome. Analysis of neutrophil chemotaxis. *J Periodontol* **67:**617–620.

Fitzgerald DA, Hilton J, Jepson B, and Smith L (2005) A crossover, randomized, controlled trial of dornase alfa before versus after physiotherapy in cystic fibrosis. *Pediatrics* **116:**e549–554.

Fleischmann RM (2009) Safety of biologic therapy in rheumatoid arthritis and other autoimmune diseases: focus on rituximab. *Semin Arthritis Rheum* **38:**265–280.

Fontana S, Parolini S, Vermi W, Booth S, Gallo F, Donini M, Benassi M, Gentili F, Ferrari D, Notarangelo LD, et al. (2006) Innate immunity defects in Hermansky-Pudlak type 2 syndrome. *Blood* **107:**4857–4864

Fridlich R, David A, and Aviram I (2006) Membrane proteinase 3 and its interactions within microdomains of neutrophil membranes. *J Cell Biochem* **99:**117–125.

Frommherz K and Bieth JG (1991) Effect of sulfated glycosaminoglycans on the inhibition of neutrophil elastase by alpha 1-proteinase inhibitor. *Adv Exp Med Biol* **297:**161–165.

Frommherz KJ, Faller B, and Bieth JG (1991) Heparin strongly decreases the rate of inhibition of neutrophil elastase by alpha 1-proteinase inhibitor. *J Biol Chem* **266:**15356–15362.

Fujinaga M, Chernaia MM, Halenbeck R, Koths K, and James MN (1996) The crystal structure of PR3, a neutrophil serine proteinase antigen of Wegener's granulomatosis antibodies. *J Mol Biol* **261:**267–278.

Fukusen N, Kato Y, Kido H, and Katunuma N (1987) Kinetic studies on the inhibitions of mast cell chymase by natural serine protease inhibitors: indications for potential biological functions of these inhibitors. *Biochem Med Metab Biol* **38:**165–169.

Gadek JE, Fells GA, and Crystal RG (1979) Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science* **206:** 1315–1316.

Ganz T, Metcalf JA, Gallin JI, Boxer LA, and Lehrer RI (1988) Microbicidal/cytotoxic proteins of neutrophils are deficient in two disorders: Chediak-Higashi syndrome and "specific" granule deficiency. *J Clin Invest* **82:**552–556.

Garwicz D, Lennartsson A, Jacobsen SE, Gullberg U, and Lindmark A (2005) Biosynthetic profiles of neutrophil serine proteases in a human bone marrow-derived cellular myeloid differentiation model. *Haematologica* **90:**38–44.

Garwicz D, Lindmark A, Hellmark T, Gladh M, Jögi J, and Gullberg U (1997) Characterization of the processing and granular targeting of human proteinase 3 after transfection to the rat RBL or the murine 32D leukemic cell lines. *J Leukoc Biol* **61:**113–123.

Garwicz D, Lindmark A, Persson AM, and Gullberg U (1998) On the role of the proform-conformation for processing and intracellular sorting of human cathepsin G. *Blood* **92:**1415–1422.

Gauthier F, Fryksmark U, Ohlsson K, and Bieth JG (1982) Kinetics of the inhibition of leukocyte elastase by the bronchial inhibitor. *Biochim Biophys Acta* **700:**178–183.

Geraghty P, Rogan MP, Greene CM, Boxio RM, Poiriert T, O'Mahony M, Belaaouaj A, O'Neill SJ, Taggart CC, and McElvaney NG (2007) Neutrophil elastase up-regulates cathepsin B and matrix metalloprotease-2 expression. *J Immunol* **178:** 5871–5878.

Geraghty P, Rogan MP, Greene CM, Brantly ML, O'Neill SJ, Taggart CC, and McElvaney NG (2008) Alpha-1-antitrypsin aerosolised augmentation abrogates neutrophil elastase-induced expression of cathepsin B and matrix metalloprotease 2 in vivo and in vitro. *Thorax* **63:**621–626.

Giembycz MA (2002) Development status of second generation PDE4 inhibitors for asthma and COPD: the story so far. *Monaldi Arch Chest Dis* **57:**48–64.

Goldmann WH, Niles JL, and Arnaout MA (1999) Interaction of purified human proteinase 3 (PR3) with reconstituted lipid bilayers. *Eur J Biochem* **261:**155–162.

Gooptu B, Hazes B, Chang WS, Dafforn TR, Carrell RW, Read RJ, and Lomas DA (2000) Inactive conformation of the serpin alpha(1)-antichymotrypsin indicates two-stage insertion of the reactive loop: implications for inhibitory function and conformational disease. *Proc Natl Acad Sci USA* **97:**67–72.

Gorlin RJ, Sedano H, and Anderson VE (1964) The syndrome of palmar-plantar hyperkeratosis and premature periodontal destruction of the teeth. A clinical and genetic analysis of the Papillon-Lefèvre syndrome. *J Pediatr* **65:**895–908.

Gregoriadis G, Wills EJ, Swain CP, and Tavill AS (1974) Drug-carrier potential of liposomes in cancer chemotherapy. *Lancet* **1:**1313–1316.

Grenda DS, Murakami M, Ghatak J, Xia J, Boxer LA, Dale D, Dinauer MC, and Link DC (2007) Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. *Blood* **110:**4179–4187.

Griese M, Kappler M, Gaggar A, and Hartl D (2008) Inhibition of airway proteases in cystic fibrosis lung disease. *Eur Respir J* **32:**783–795.

Grütter MG, Fendrich G, Huber R, and Bode W (1988) The 2.5 A X-ray crystal structure of the acid-stable proteinase inhibitor from human mucous secretions analysed in its complex with bovine alpha-chymotrypsin. *EMBO J* **7:**345–351.

Gullberg U, Andersson E, Garwicz D, Lindmark A, and Olsson I (1997) Biosynthesis, processing and sorting of neutrophil proteins: insight into neutrophil granule development. *Eur J Haematol* **58:**137–153.

Gullberg U, Lindmark A, Lindgren G, Persson AM, Nilsson E, and Olsson I (1995) Carboxyl-terminal prodomain-deleted human leukocyte elastase and cathepsin G are efficiently targeted to granules and enzymatically activated in the rat basophilic/mast cell line RBL. *J Biol Chem* **270:**12912–12918.

Gullberg U, Lindmark A, Nilsson E, Persson AM, and Olsson I (1994) Processing of human cathepsin G after transfection to the rat basophilic/mast cell tumor line RBL. *J Biol Chem* **269:**25219–25225.

Guyot N, Butler MW, McNally P, Weldon S, Greene CM, Levine RL, O'Neill SJ, Taggart CC, and McElvaney NG (2008) Elafin, an elastase-specific inhibitor, is cleaved by its cognate enzyme neutrophil elastase in sputum from individuals with cystic fibrosis. *J Biol Chem* **283:**32377–32385.

Hajjar E, Korkmaz B, Gauthier F, Brandsdal BO, Witko-Sarsat V, and Reuter N (2006) Inspection of the binding sites of proteinase3 for the design of a highly specific substrate. *J Med Chem* **49:**1248–1260.

Hajjar E, Mihajlovic M, Witko-Sarsat V, Lazaridis T, and Reuter N (2008) Computational prediction of the binding site of proteinase 3 to the plasma membrane. *Proteins* **71:**1655–1669.

Halbwachs-Mecarelli L, Bessou G, Lesavre P, Lopez S, and Witko-Sarsat V (1995) Bimodal distribution of proteinase 3 (PR3) surface expression reflects a constitutive heterogeneity in the polymorphonuclear neutrophil pool. *FEBS Lett* **374:** 29–33.

Hampton MB, Kettle AJ, and Winterbourn CC (1998) Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* **92:**3007–3017.

Haneke E (1979) The Papillon-Lefèvre syndrome: keratosis palmoplantaris with periodontopathy. Report of a case and review of the cases in the literature. *Hum Genet* **51:**1–35.

Hart TC, Hart PS, Bowden DW, Michalec MD, Callison SA, Walker SJ, Zhang Y, and Firatli E (1999) Mutations of the cathepsin C gene are responsible for Papillon-Lefèvre syndrome. *J Med Genet* **36:**881–887.

Hart TC, Hart PS, Michalec MD, Zhang Y, Firatli E, Van Dyke TE, Stabholz A, Zlotogorski A, Shapira L, Soskolne WA, et al. (2000) Haim-Munk syndrome and Papillon-Lefèvre syndrome are allelic mutations in cathepsin C. *J Med Genet* **37:**88–94.

Hartl D, Latzin P, Hordijk P, Marcos V, Rudolph C, Woischnik M, Krauss-Etschmann S, Koller B, Reinhardt D, Roscher AA, et al. (2007) Cleavage of CXCR1

on neutrophils disables bacterial killing in cystic fibrosis lung disease. *Nat Med* **13:**1423–1430.
Hattab FN, Rawashdeh MA, Yassin OM, al-Momani AS, and al-Ubosi MM (1995) Papillon-Lefèvre syndrome: a review of the literature and report of 4 cases. *J Periodontol* **66:**413–420.
Hedstrom L (2002) Serine protease mechanism and specificity. *Chem Rev* **102:**4501–4524.
Hermant B, Bibert S, Concord E, Dublet B, Weidenhaupt M, Vernet T, and Gulino-Debrac D (2003) Identification of proteases involved in the proteolysis of vascular endothelium cadherin during neutrophil transmigration. *J Biol Chem* **278:**14002–14012.
Hink-Schauer C, Estébanez-Perpiñá E, Wilharm E, Fuentes-Prior P, Klinkert W, Bode W, and Jenne DE (2002) The 2.2-A crystal structure of human pro-granzyme K reveals a rigid zymogen with unusual features. *J Biol Chem* **277:**50923–50933.
Hof P, Mayr I, Huber R, Korzus E, Potempa J, Travis J, Powers JC, and Bode W (1996) The 1.8 A crystal structure of human cathepsin G in complex with Suc-Val-Pro-PheP-(OPh)2: a Janus-faced proteinase with two opposite specificities. *EMBO J* **15:**5481–5491.
Hoffman GS and Specks U (1998) Antineutrophil cytoplasmic antibodies. *Arthritis Rheum* **41:**1521–1537.
Hornebeck W and Schnebli HP (1982) Effect of different elastase inhibitors on leukocyte elastase pre-adsorbed to elastin. *Hoppe Seylers Z Physiol Chem* **363:**455–458.
Horwitz M, Benson KF, Person RE, Aprikyan AG, and Dale DC (1999) Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. *Nat Genet* **23:**433–436.
Horwitz M, Li FQ, Albani D, Duan Z, Person RE, Meade-White K, and Benson KF (2003) Leukemia in severe congenital neutropenia: defective proteolysis suggests new pathways to malignancy and opportunities for therapy. *Cancer Invest* **21:**579–587.
Horwitz MS, Duan Z, Korkmaz B, Lee HH, Mealiffe ME, and Salipante SJ (2007) Neutrophil elastase in cyclic and severe congenital neutropenia. *Blood* **109:**1817–1824.
Houghton AM, Quintero PA, Perkins DL, Kobayashi DK, Kelley DG, Marconcini LA, Mecham RP, Senior RM, and Shapiro SD (2006) Elastin fragments drive disease progression in a murine model of emphysema. *J Clin Invest* **116:**753–759.
Hu N, Westra J, and Kallenberg CG (2009) Membrane-bound proteinase 3 and its receptors: relevance for the pathogenesis of Wegener's Granulomatosis. *Autoimmun Rev* **8:**510–514.
Hubbard RC, Sellers S, Czerski D, Stephens L, and Crystal RG (1988) Biochemical efficacy and safety of monthly augmentation therapy for alpha 1-antitrypsin deficiency. *JAMA* **260:**1259–1264.
Huizing M, Helip-Wooley A, Westbroek W, Gunay-Aygun M, and Gahl WA (2008) Disorders of lysosome-related organelle biogenesis: clinical and molecular genetics. *Annu Rev Genomics Hum Genet* **9:**359–386.
Huizing M, Scher CD, Strovel E, Fitzpatrick DL, Hartnell LM, Anikster Y, and Gahl WA (2002) Nonsense mutations in ADTB3A cause complete deficiency of the beta3A subunit of adaptor complex-3 and severe Hermansky-Pudlak syndrome type 2. *Pediatr Res* **51:**150–158.
Huntington JA, Read RJ, and Carrell RW (2000) Structure of a serpin-protease complex shows inhibition by deformation. *Nature* **407:**923–926.
Hutchison DC (1988) Natural history of alpha-1-protease inhibitor deficiency. *Am J Med* **84:**3–12.
Irving JA, Pike RN, Lesk AM, and Whisstock JC (2000) Phylogeny of the serpin superfamily: implications of patterns of amino acid conservation for structure and function. *Genome Res* **10:**1845–1864.
Itoh Y and Nagase H (1995) Preferential inactivation of tissue inhibitor of metalloproteinases-1 that is bound to the precursor of matrix metalloproteinase 9 (progelatinase B) by human neutrophil elastase. *J Biol Chem* **270:**16518–16521.
Jackson AH, Hill SL, Afford SC, and Stockley RA (1984) Sputum sol-phase proteins and elastase activity in patients with cystic fibrosis. *Eur J Respir Dis* **65:**114–124.
Janciauskiene S (2001) Conformational properties of serine proteinase inhibitors (serpins) confer multiple pathophysiological roles. *Biochim Biophys Acta* **1535:**221–235.
Janoff A (1985) Elastase in tissue injury. *Annu Rev Med* **36:**207–216.
Janoff A and Scherer J (1968) Mediators of inflammation in leukocyte lysosomes. IX. Elastinolytic activity in granules of human polymorphonuclear leukocytes. *J Exp Med* **128:**1137–1155.
Jenne DE and Kuhl A (2006) Production and applications of recombinant proteinase 3, Wegener's autoantigen: problems and perspectives. *Clin Nephrol* **66:**153–159.
Jenne DE, Tschopp J, Ludemann J, Utecht B, and Gross WL (1990) Wegener's autoantigen decoded. *Nature* **346:**520.
Jeppsson JO, Laurell CB, and Fagerhol M (1978) Properties of isolated human alpha1-antitrypsins of Pi types M, S and Z. *Eur J Biochem* **83:**143–153.
Jog NR, Rane MJ, Lominadze G, Luerman GC, Ward RA, and McLeish KR (2007) The actin cytoskeleton regulates exocytosis of all neutrophil granule subsets. *Am J Physiol Cell Physiol* **292:**C1690–C1700.
Johansson A, Claesson R, Belibasakis G, Makoveichuk E, Hänström L, Olivecrona G, Sandström G, and Kalfas S (2001) Protease inhibitors, the responsible components for the serum-dependent enhancement of Actinobacillus actinomycetemcomitans leukotoxicity. *Eur J Oral Sci* **109:**335–341.
Johansson A, Claesson R, Hänström L, Sandström G, and Kalfas S (2000) Polymorphonuclear leukocyte degranulation induced by leukotoxin from Actinobacillus actinomycetemcomitans. *J Periodontal Res* **35:**85–92.
Johnson D and Travis J (1979) The oxidative inactivation of human alpha-1-proteinase inhibitor. Further evidence for methionine at the reactive center. *J Biol Chem* **254:**4022–4026.
Johnson DA, Barrett AJ, and Mason RW (1986) Cathepsin L inactivates alpha 1-proteinase inhibitor by cleavage in the reactive site region. *J Biol Chem* **261:**14748–14751.
Kain R, Firmin DA, and Rees AJ (2010) Pathogenesis of small vessel vasculitis associated with autoantibodies to neutrophil cytoplasmic antigens: new insights from animal models. *Curr Opin Rheumatol* **22:**15–20.
Kallenberg CG (2008) Pathogenesis of PR3-ANCA associated vasculitis. *J Autoimmun* **30:**29–36.
Kallenberg CG, Heeringa P, and Stegeman CA (2006) Mechanisms of Disease: pathogenesis and treatment of ANCA-associated vasculitides. *Nat Clin Pract Rheumatol* **2:**661–670.
Kalupov T, Brillard-Bourdet M, Dadé S, Serrano H, Wartelle J, Guyot N, Juliano L, Moreau T, Belaaouaj A, and Gauthier F (2009) Structural characterization of mouse neutrophil serine proteases and identification of their substrate specificities: relevance to mouse models of human inflammatory diseases. *J Biol Chem* **284:**34084–34091.
Kam CM, Götz MG, Koot G, McGuire M, Thiele D, Hudig D, and Powers JC (2004) Design and evaluation of inhibitors for dipeptidyl peptidase I (Cathepsin C). *Arch Biochem Biophys* **427:**123–134.
Kam CM, Kerrigan JE, Dolman KM, Goldschmeding R, Von dem Borne AE, and Powers JC (1992) Substrate and inhibitor studies on proteinase 3. *FEBS Lett* **297:**119–123.
Kantari C, Pederzoli-Ribeil M, Amir-Moazami O, Gausson-Dorey V, Moura IC, Lecomte MC, Benhamou M, and Witko-Sarsat V (2007) Proteinase 3, the Wegener autoantigen, is externalized during neutrophil apoptosis: evidence for a functional association with phospholipid scramblase 1 and interference with macrophage phagocytosis. *Blood* **110:**4086–4095.
Kawabata K, Hagio T, and Matsuoka S (2002) The role of neutrophil elastase in acute lung injury. *Eur J Pharmacol* **451:**1–10.
Kawabata K, Suzuki M, Sugitani M, Imaki K, Toda M, and Miyamoto T (1991) ONO-5046, a novel inhibitor of human neutrophil elastase. *Biochem Biophys Res Commun* **177:**814–820.
Kessenbrock K, Fröhlich L, Sixt M, Lämmermann T, Pfister H, Bateman A, Belaaouaj A, Ring J, Ollert M, Fässler R, et al. (2008) Proteinase 3 and neutrophil elastase enhance inflammation in mice by inactivating antiinflammatory progranulin. *J Clin Invest* **118:**2438–2447.
Kessenbrock K, Krumbholz M, Schönermarck U, Back W, Gross WL, Werb Z, Gröne HJ, Brinkmann V, and Jenne DE (2009) Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med* **15:**623–625.
Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer AA, Rathinam C, Boztug K, Schwinzer B, Rezaei N, et al. (2007) HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). *Nat Genet* **39:**86–92.
Klinger H (1931) Grenzformen der periarteritis nodosa. *Frankf Ztschr Pathol* **42:**455–480.
Knäuper V, Reinke H, and Tschesche H (1990) Inactivation of human plasma alpha 1-proteinase inhibitor by human PMN leucocyte collagenase. *FEBS Lett* **263:**355–357.
Kobayashi SD, Voyich JM, Burlak C, and DeLeo FR (2005) Neutrophils in the innate immune response. *Arch Immunol Ther Exp* (*Warsz*) **53:**505–517.
Koehl C, Knight CG, and Bieth JG (2003) Compared action of neutrophil proteinase 3 and elastase on model substrates. Favorable effect of S'-P' interactions on proteinase 3 catalysts. *J Biol Chem* **278:**12609–12612.
Kohlschütter J, Michelfelder S, and Trepel M (2008) Drug delivery in acute myeloid leukemia. *Expert Opin Drug Deliv* **5:**653–663.
Koizumi M, Fujino A, Fukushima K, Kamimura T, and Takimoto-Kamimura M (2008) Complex of human neutrophil elastase with 1/2SLPI. *J Synchrotron Radiat* **15:**308–311.
Köllner I, Sodeik B, Schreek S, Heyn H, von Neuhoff N, Germeshausen M, Zeidler C, Krüger M, Schlegelberger B, Welte K, et al. (2006) Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response. *Blood* **108:**493–500.
Korkmaz B, Attucci S, Jourdan ML, Juliano L, and Gauthier F (2005a) Inhibition of neutrophil elastase by alpha1-protease inhibitor at the surface of human polymorphonuclear neutrophils. *J Immunol* **175:**3329–3338.
Korkmaz B, Attucci S, Juliano MA, Kalupov T, Jourdan ML, Juliano L, and Gauthier F (2008a) Measuring elastase, proteinase 3 and cathepsin G activities at the surface of human neutrophils with fluorescence resonance energy transfer substrates. *Nat Protoc* **3:**991–1000.
Korkmaz B, Attucci S, Moreau T, Godat E, Juliano L, and Gauthier F (2004) Design and use of highly specific substrates of neutrophil elastase and proteinase 3. *Am J Respir Cell Mol Biol* **30:**801–807.
Korkmaz B, Hajjar E, Kalupov T, Reuter N, Brillard-Bourdet M, Moreau T, Juliano L, and Gauthier F (2007) Influence of charge distribution at the active site surface on the substrate specificity of human neutrophil protease 3 and elastase. A kinetic and molecular modeling analysis. *J Biol Chem* **282:**1989–1997.
Korkmaz B, Jaillet J, Jourdan ML, Gauthier A, Gauthier F, and Attucci S (2009) Catalytic activity and inhibition of wegener antigen proteinase 3 on the cell surface of human polymorphonuclear neutrophils. *J Biol Chem* **284:**19896–19902.
Korkmaz B, Kuhl A, Bayat B, Santoso S, and Jenne DE (2008b) A hydrophobic patch on proteinase 3, the target of autoantibodies in Wegener granulomatosis, mediates membrane binding via NB1 receptors. *J Biol Chem* **283:**35976–35982.
Korkmaz B, Moreau T, and Gauthier F (2008c) Neutrophil elastase, proteinase 3 and cathepsin G: physicochemical properties, activity and physiopathological functions. *Biochimie* **90:**227–242.
Korkmaz B, Poutrain P, Hazouard E, de Monte M, Attucci S, and Gauthier FL (2005b) Competition between elastase and related proteases from human neutrophil for binding to alpha1-protease inhibitor. *Am J Respir Cell Mol Biol* **32:**553–559.
Kostmann R (1956) Infantile genetic agranulocytosis; agranulocytosis infantilis hereditaria. *Acta Paediatr Suppl* **45:**1–78.
Kounnas MZ, Church FC, Argraves WS, and Strickland DK (1996) Cellular internalization and degradation of antithrombin III-thrombin, heparin cofactor II-thrombin, and alpha 1-antitrypsin-trypsin complexes is mediated by the low density lipoprotein receptor-related protein. *J Biol Chem* **271:**6523–6529.

Kramps JA, Te Boekhorst AH, Fransen JA, Ginsel LA, and Dijkman JH (1989) Antileukoprotease is associated with elastin fibers in the extracellular matrix of the human lung. An immunoelectron microscopic study. *Am Rev Respir Dis* **140:** 471–476.

Kress LF (1983) Inactivation of human plasma alpha 1-antichymotrypsin by microbial proteinases. *Acta Biochim Pol* **30:**159–164.

Krowarsch D, Cierpicki T, Jelen F, and Otlewski J (2003) Canonical protein inhibitors of serine proteases. *Cell Mol Life Sci* **60:**2427–2444.

Kuhl A, Korkmaz B, Utecht B, Kniepert A, Schönermarck U, Specks U, and Jenne DE (2010) Mapping of conformational epitopes on human proteinase 3, the autoantigen of Wegener's granulomatosis. *J Immunol* **185:**387–399.

Lai ML, Rizzo N, Liguori C, Zucca G, and Faa G (1998) Alpha-1-antichymotrypsin immunoreactivity in papillary carcinoma of the thyroid gland. *Histopathology* **33:**332–336.

Lane MA (2000) Nonhuman primate models in biogerontology. *Exp Gerontol* **35:**533–541.

Laurell CB and Eriksson S (1963)) The electrophoretic alpha 1-globulin pattern of serum in alpha 1-antitrypsin deficiency. *Scand J Clin Lab Invest* **15:**132–140.

Leale M (1910) Recurrent furunculosis in an infant showing an unusual blood picture. *JAMA* **54:**1845–1855.

Leavell KJ, Peterson MW, and Gross TJ (1996) The role of fibrin degradation products in neutrophil recruitment to the lung. *Am J Respir Cell Mol Biol* **14:** 53–60.

Lee CT, Fein AM, Lippmann M, Holtzman H, Kimbel P, and Weinbaum G (1981) Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory-distress syndrome. *N Engl J Med* **304:**192–196.

Lee WL and Downey GP (2001) Leukocyte elastase: physiological functions and role in acute lung injury. *Am J Respir Crit Care Med* **164:**896–904.

Li FQ and Horwitz M (2001) Characterization of mutant neutrophil elastase in severe congenital neutropenia. *J Biol Chem* **276:**14230–14241.

Loebermann H, Tokuoka R, Deisenhofer J, and Huber R (1984) Human alpha 1-proteinase inhibitor. Crystal structure analysis of two crystal modifications, molecular model and preliminary analysis of the implications for function. *J Mol Biol* **177:**531–557.

Lomas DA and Carrell RW (2002) Serpinopathies and the conformational dementias. *Nat Rev Genet* **3:**759–768.

Lomas DA, Stone SR, Llewellyn-Jones C, Keogan MT, Wang ZM, Rubin H, Carrell RW, and Stockley RA (1995) The control of neutrophil chemotaxis by inhibitors of cathepsin G and chymotrypsin. *J Biol Chem* **270:**23437–23443.

López-Boado YS, Espinola M, Bahr S, and Belaaouaj A (2004) Neutrophil serine proteinases cleave bacterial flagellin, abrogating its host response-inducing activity. *J Immunol* **172:**509–515.

López-Otín C and Bond JS (2008) Proteases: multifunctional enzymes in life and disease. *J Biol Chem* **283:**30433–30437.

Lothrop CD, Jr., Coulson PA, Nolan HL, Cole B, Jones JB, and Sanders WL (1987) Cyclic hormonogenesis in gray collie dogs: interactions of hematopoietic and endocrine systems. *Endocrinology* **120:**1027–1032.

Luisetti M and Travis J (1996) Bioengineering: alpha 1-proteinase inhibitor site-specific mutagenesis. The prospect for improving the inhibitor. *Chest* **110:**278S–283S.

Lungarella G, Cavarra E, Lucattelli M, and Martorana PA (2008) The dual role of neutrophil elastase in lung destruction and repair. *Int J Biochem Cell Biol* **40:** 1287–1296.

Lynch CM, Hart BW, and Grewal IS (2009) Practical considerations for nonclinical safety evaluation of therapeutic monoclonal antibodies. *MAbs* **1:**2–11.

Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, Bronson RT, and Springer TA (1998) Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proc Natl Acad Sci USA* **95:**9448–9453.

MacIvor DM, Shapiro SD, Pham CT, Belaaouaj A, Abraham SN, and Ley TJ (1999) Normal neutrophil function in cathepsin G-deficient mice. *Blood* **94:**4282–4293.

Mangan MS, Kaiserman D, and Bird PI (2008) The role of serpins in vertebrate immunity. *Tissue Antigens* **72:**1–10.

Martorana PA, Beume R, Lucattelli M, Wollin L, and Lungarella G (2005) Roflumilast fully prevents emphysema in mice chronically exposed to cigarette smoke. *Am J Respir Crit Care Med* **172:**848–853.

Mast AE, Enghild JJ, Nagase H, Suzuki K, Pizzo SV, and Salvesen G (1991) Kinetics and physiologic relevance of the inactivation of alpha 1-proteinase inhibitor, alpha 1-antichymotrypsin, and antithrombin III by matrix metalloproteinases-1 (tissue collagenase), -2 (72-kDa gelatinase/type IV collagenase), and -3 (stromelysin). *J Biol Chem* **266:**15810–15816.

McElvaney NG, Doujaiji B, Moan MJ, Burnham MR, Wu MC, and Crystal RG (1993) Pharmacokinetics of recombinant secretory leukoprotease inhibitor aerosolized to normals and individuals with cystic fibrosis. *Am Rev Respir Dis* **148:**1056–1060.

McElvaney NG, Hubbard RC, Birrer P, Chernick MS, Caplan DB, Frank MM, and Crystal RG (1991) Aerosol alpha 1-antitrypsin treatment for cystic fibrosis. *Lancet* **337:**392–394.

McGuire MJ, Lipsky PE, and Thiele DL (1992) Purification and characterization of dipeptidyl peptidase I from human spleen. *Arch Biochem Biophys* **295:**280–288.

McGuire MJ, Lipsky PE, and Thiele DL (1993) Generation of active myeloid and lymphoid granule serine proteases requires processing by the granule thiol protease dipeptidyl peptidase I. *J Biol Chem* **268:**2458–2467.

McRae B, Nakajima K, Travis J, and Powers JC (1980) Studies on reactivity of human leukocyte elastase, cathepsin G, and porcine pancreatic elastase toward peptides including sequences related to the reactive site of alpha 1-protease inhibitor (alpha 1-antitrypsin). *Biochemistry* **19:**3973–3978.

Meagher LC and Cotter TG (1988) The degranulation response in differentiated HL-60 cells. *Clin Exp Immunol* **74:**483–488.

Merkel PA, Cuthbertson DD, Hellmich B, Hoffman GS, Jayne DR, Kallenberg CG, Krischer JP, Luqmani R, Mahr AD, Matteson EL, et al. (2009) Comparison of disease activity measures for anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis. *Ann Rheum Dis* **68:**103–106.

Méthot N, Guay D, Rubin J, Ethier D, Ortega K, Wong S, Normandin D, Beaulieu C, Reddy TJ, Riendeau D, et al. (2008) In vivo inhibition of serine protease processing requires a high fractional inhibition of cathepsin C. *Mol Pharmacol* **73:**1857–1865.

Méthot N, Rubin J, Guay D, Beaulieu C, Ethier D, Reddy TJ, Riendeau D, and Percival MD (2007) Inhibition of the activation of multiple serine proteases with a cathepsin C inhibitor requires sustained exposure to prevent pro-enzyme processing. *J Biol Chem* **282:**20836–20846.

Meyer-Hoffert U (2009) Neutrophil-derived serine proteases modulate innate immune responses. *Front Biosci* **14:**3409–3418.

Michaelis J, Vissers MC, and Winterbourn CC (1990) Human neutrophil collagenase cleaves alpha 1-antitrypsin. *Biochem J* **270:**809–814.

Mølgaard A, Arnau J, Lauritzen C, Larsen S, Petersen G, and Pedersen J (2007) The crystal structure of human dipeptidyl peptidase I (cathepsin C) in complex with the inhibitor Gly-Phe-CHN2. *Biochem J* **401:**645–650.

Moraes TJ, Chow CW, and Downey GP (2003) Proteases and lung injury. *Crit Care Med* **31:**S189–S194.

Moreau T, Baranger K, Dadé S, Dallet-Choisy S, Guyot N, and Zani ML (2008) Multifaceted roles of human elafin and secretory leukocyte proteinase inhibitor (SLPI), two serine protease inhibitors of the chelonianin family. *Biochimie* **90:**284–295.

Morihara K, Tsuzuki H, Harada M, and Iwata T (1984) Purification of human plasma alpha 1-proteinase inhibitor and its inactivation by Pseudomonas aeruginosa elastase. *J Biochem* (*Tokyo*) **95:**795–804.

Morrison HM, Burnett D, and Stockley RA (1986a) The effect of catalase and methionine-S-oxide reductase on oxidised alpha 1-proteinase inhibitor. *Biol Chem Hoppe Seyler* **367:**371–378.

Morrison HM, Kramps JA, Afford SC, Burnett D, Dijkman JH, and Stockley RA (1987) Elastase inhibitors in sputum from bronchitic patients with and without alpha 1-proteinase inhibitor deficiency: partial characterization of a hitherto unquantified inhibitor of neutrophil elastase. *Clin Sci* (*Lond*) **73:**19–28.

Morrison HM, Kramps JA, Dijkman JH, and Stockley RA (1986b) Comparison of concentrations of two proteinase inhibitors, porcine pancreatic elastase inhibitory capacity, and cell profiles in sequential bronchoalveolar lavage samples. *Thorax* **41:**435–441.

Naidoo N, Cooperman BS, Wang ZM, Liu XZ, and Rubin H (1995) Identification of lysines within alpha 1-antichymotrypsin important for DNA binding. An unusual combination of DNA-binding elements. *J Biol Chem* **270:**14548–14555.

Nakajima K, Powers JC, Ashe BM, and Zimmerman M (1979) Mapping the extended substrate binding site of cathepsin G and human leukocyte elastase. Studies with peptide substrates related to the alpha 1-protease inhibitor reactive site. *J Biol Chem* **254:**4027–4032.

Navia MA, McKeever BM, Springer JP, Lin TY, Williams HR, Fluder EM, Dorn CP, and Hoogsteen K (1989) Structure of human neutrophil elastase in complex with a peptide chloromethyl ketone inhibitor at 1.84-A resolution. *Proc Natl Acad Sci USA* **86:**7–11.

Needham M and Stockley RA (2004) Alpha 1-antitrypsin deficiency. 3: Clinical manifestations and natural history. *Thorax* **59:**441–445.

Nie J and Pei D (2004) Rapid inactivation of alpha-1-proteinase inhibitor by neutrophil specific leukolysin/membrane-type matrix metalloproteinase 6. *Exp Cell Res* **296:**145–150.

Niemann CU, Abrink M, Pejler G, Fischer RL, Christensen EI, Knight SD, and Borregaard N (2007) Neutrophil elastase depends on serglycin proteoglycan for localization in granules. *Blood* **109:**4478–4486.

Nystedt S, Emilsson K, Larsson AK, Strömbeck B, and Sundelin J (1995a) Molecular cloning and functional expression of the gene encoding the human proteinase-activated receptor 2. *Eur J Biochem* **232:**84–89.

Nystedt S, Emilsson K, Wahlestedt C, and Sundelin J (1994) Molecular cloning of a potential proteinase activated receptor. *Proc Natl Acad Sci USA* **91:**9208–9212.

Nystedt S, Larsson AK, Aberg H, and Sundelin J (1995b) The mouse proteinase-activated receptor-2 cDNA and gene. Molecular cloning and functional expression. *J Biol Chem* **270:**5950–5955.

Odemis V, Lamp E, Pezeshki G, Moepps B, Schilling K, Gierschik P, Littman DR, and Engele J (2005) Mice deficient in the chemokine receptor CXCR4 exhibit impaired limb innervation and myogenesis. *Mol Cell Neurosci* **30:**494–505.

Ossovskaya VS and Bunnett NW (2004) Protease-activated receptors: contribution to physiology and disease. *Physiol Rev* **84:**579–621.

Owen CA (2008a) Leukocyte cell surface proteinases: regulation of expression, functions, and mechanisms of surface localization. *Int J Biochem Cell Biol* **40:**1246–1272.

Owen CA (2008b) Roles for proteinases in the pathogenesis of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* **3:**253–268.

Owen CA and Campbell EJ (1999) The cell biology of leukocyte-mediated proteolysis. *J Leukoc Biol* **65:**137–150.

Owen CA, Campbell MA, Boukedes SS, and Campbell EJ (1995a) Inducible binding of bioactive cathepsin G to the cell surface of neutrophils. A novel mechanism for mediating extracellular catalytic activity of cathepsin G. *J Immunol* **155:**5803–5810.

Owen CA, Campbell MA, Boukedes SS, and Campbell EJ (1997) Cytokines regulate membrane-bound leukocyte elastase on neutrophils: a novel mechanism for effector activity. *Am J Physiol* **272:**L385–L393.

Owen CA, Campbell MA, Sannes PL, Boukedes SS, and Campbell EJ (1995b) 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* **131:**775–789.

Padrines M and Bieth JG (1993) Oxidized and Met358→Leu mutated alpha 1-proteinase inhibitor as substrates of *Pseudomonas aeruginosa* elastase. *Biochim Biophys Acta* **1163:**61–66.

Padrines M, Wolf M, Walz A, and Baggiolini M (1994) Interleukin-8 processing by neutrophil elastase, cathepsin G and proteinase-3. *Febs Lett* **352:**231–235.

Papayannopoulos V and Zychlinsky A (2009) NETs: a new strategy for using old weapons. *Trends Immunol* **30:**513–521.
Papillon M and Lefevre P (1924) [Two cases of symmetrically familial palmar and plantar hyperkeratosis (Meleda disease) within brother and sister combined with severe dental alterations in both cases.] *Bull Soc Fr Dermatol Syphiligr* **31:**82–87.
Pemberton PA, Kobayashi D, Wilk BJ, Henstrand JM, Shapiro SD, and Barr PJ (2006) Inhaled recombinant alpha 1-antitrypsin ameliorates cigarette smoke-induced emphysema in the mouse. *COPD* **3:**101–108.
Pendergraft WF, 3rd, Rudolph EH, Falk RJ, Jahn JE, Grimmler M, Hengst L, Jennette JC, and Preston GA (2004) Proteinase 3 sidesteps caspases and cleaves p21(Waf1/Cip1/Sdi1) to induce endothelial cell apoptosis. *Kidney Int* **65:**75–84.
Perlmutter DH, Glover GI, Rivetna M, Schasteen CS, and Fallon RJ (1990) Identification of a serpin-enzyme complex receptor on human hepatoma cells and human monocytes. *Proc Natl Acad Sci USA* **87:**3753–3757.
Person RE, Li FQ, Duan Z, Benson KF, Wechsler J, Papadaki HA, Eliopoulos G, Kaufman C, Bertolone SJ, Nakamoto B, et al. (2003) Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. *Nat Genet* **34:**308–312.
Petersen CM (1993) Alpha 2-macroglobulin and pregnancy zone protein. Serum levels, alpha 2-macroglobulin receptors, cellular synthesis and aspects of function in relation to immunology. *Dan Med Bull* **40:**409–446.
Pfister H, Ollert M, Fröhlich LF, Quintanilla-Martinez L, Colby TV, Specks U, and Jenne DE (2004) Antineutrophil cytoplasmic autoantibodies against the murine homolog of proteinase 3 (Wegener autoantigen) are pathogenic in vivo. *Blood* **104:**1411–1418.
Pham CT (2006) Neutrophil serine proteases: specific regulators of inflammation. *Nat Rev Immunol* **6:**541–550.
Pham CT (2008) Neutrophil serine proteases fine-tune the inflammatory response. *Int J Biochem Cell Biol* **40:**1317–1333.
Pham CT and Ley TJ (1999) Dipeptidyl peptidase I is required for the processing and activation of granzymes A and B in vivo. *Proc Natl Acad Sci USA* **96:**8627–8632.
Pham CT, Ivanovich JL, Raptis SZ, Zehnbauer B, and Ley TJ (2004) Papillon-Lefèvre syndrome: correlating the molecular, cellular, and clinical consequences of cathepsin C/dipeptidyl peptidase I deficiency in humans. *J Immunol* **173:**7277–7281.
Plosker GL and Figgitt DP (2003) Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. *Drugs* **63:**803–843.
Polgár L (2005) The catalytic triad of serine peptidases. *Cell Mol Life Sci* **62:**2161–2172.
Potempa J, Korzus E, and Travis J (1994) The serpin superfamily of proteinase inhibitors: structure, function, and regulation. *J Biol Chem* **269:**15957–15960.
Preston GA, Zarella CS, Pendergraft WF, 3rd, Rudolph EH, Yang JJ, Sekura SB, Jennette JC, and Falk RJ (2002) Novel effects of neutrophil-derived proteinase 3 and elastase on the vascular endothelium involve in vivo cleavage of NF-kappaB and proapoptotic changes in JNK, ERK, and p38 MAPK signaling pathways. *J Am Soc Nephrol* **13:**2840–2849.
Proost P, Mortier A, Loos T, Vandercappellen J, Gouwy M, Ronsse I, Schutyser E, Put W, Parmentier M, Struyf S, et al. (2007) Proteolytic processing of CXCL11 by CD13/aminopeptidase N impairs CXCR3 and CXCR7 binding and signaling and reduces lymphocyte and endothelial cell migration. *Blood* **110:**37–44.
Quinn DJ, Weldon S, and Taggart CC (2010) Antiproteases as therapeutics to target inflammation in cystic fibrosis. *Open Respir Med J* **4:**20–31.
Radice A and Sinico RA (2005) Antineutrophil cytoplasmic antibodies (ANCA). *Autoimmunity* **38:**93–103.
Rao NV, Rao GV, Marshall BC, and Hoidal JR (1996) Biosynthesis and processing of proteinase 3 in U937 cells. Processing pathways are distinct from those of cathepsin G. *J Biol Chem* **271:**2972–2978.
Rao NV, Wehner NG, Marshall BC, Gray WR, Gray BH, and Hoidal JR (1991) Characterization of proteinase-3 (PR-3), a neutrophil serine proteinase. Structural and functional properties. *J Biol Chem* **266:**9540–9548.
Rapala-Kozik M, Potempa J, Nelson D, Kozik A, and Travis J (1999) Comparative cleavage sites within the reactive-site loop of native and oxidized alpha1-proteinase inhibitor by selected bacterial proteinases. *Biol Chem* **380:**1211–1216.
Raptis SZ and Pham CT (2005) Neutrophil-derived serine proteases in immune complex-mediated diseases. *Immunol Res* **32:**211–215.
Raptis SZ, Shapiro SD, Simmons PM, Cheng AM, and Pham CT (2005) Serine protease cathepsin G regulates adhesion-dependent neutrophil effector functions by modulating integrin clustering. *Immunity* **22:**679–691.
Rarok AA, Stegeman CA, Limburg PC, and Kallenberg CG (2002) Neutrophil membrane expression of proteinase 3 (PR3) is related to relapse in PR3-ANCA-associated vasculitis. *J Am Soc Nephrol* **13:**2232–2238.
Rarok AA, van der Geld YM, Stegeman CA, Limburg PC, and Kallenberg CG (2003) Diversity of PR3-ANCA epitope specificity in Wegener's granulomatosis. Analysis using the biosensor technology. *J Clin Immunol* **23:**460–468.
Rees DD, Rogers RA, Cooley J, Mandle RJ, Kenney DM, and Remold-O'Donnell E (1999) Recombinant human Monocyte/Neutrophil elastase inhibitor protects rat lungs against injury from cystic fibrosis airway secretions. *Am J Respir Cell Mol Biol* **20:**69–78.
Reeves EP, Lu H, Jacobs HL, Messina CG, Bolsover S, Gabella G, Potma EO, Warley A, Roes J, and Segal AW (2002) Killing activity of neutrophils is mediated through activation of proteases by K+ flux. *Nature* **416:**291–297.
Réhault S, Brillard-Bourdet M, Juliano MA, Juliano L, Gauthier F, and Moreau T (1999) New, sensitive fluorogenic substrates for human cathepsin G based on the sequence of serpin-reactive site loops. *J Biol Chem* **274:**13810–13817.
Reiling KK, Krucinski J, Miercke LJ, Raymond WW, Caughey GH, and Stroud RM (2003) Structure of human pro-chymase: a model for the activating transition of granule-associated proteases. *Biochemistry* **42:**2616–2624.
Reimann HA and DeBerardinis CT (1949) Periodic (cyclic) neutropenia, an entity; a collection of 16 cases. *Blood* **4:**1109–1116.
Remold-O'Donnell E (1993) The ovalbumin family of serpin proteins. *FEBS Lett* **315:**105–108.
Remold-O'Donnell E, Chin J, and Alberts M (1992) Sequence and molecular characterization of human monocyte/neutrophil elastase inhibitor. *Proc Natl Acad Sci USA* **89:**5635–5639.
Remold-O'Donnell E, Nixon JC, and Rose RM (1989) Elastase inhibitor. Characterization of the human elastase inhibitor molecule associated with monocytes, macrophages, and neutrophils. *J Exp Med* **169:**1071–1086.
Renesto P, Si-Tahar M, Moniatte M, Balloy V, Van Dorsselaer A, Pidard D, and Chignard M (1997) Specific inhibition of thrombin-induced cell activation by the neutrophil proteinases elastase, cathepsin G, and proteinase 3: evidence for distinct cleavage sites within the aminoterminal domain of the thrombin receptor. *Blood* **89:**1944–1953.
Repine JE (1992) Scientific perspectives on adult respiratory distress syndrome. *Lancet* **339:**466–469.
Reumaux D, Duthilleul P, and Roos D (2004) Pathogenesis of diseases associated with antineutrophil cytoplasm autoantibodies. *Hum Immunol* **65:**1–12.
Rice WG and Weiss SJ (1990) Regulation of proteolysis at the neutrophil-substrate interface by secretory leukoprotease inhibitor. *Science* **249:**178–181.
Rindler-Ludwig R and Braunsteiner H (1975) Cationic proteins from human neutrophil granulocytes. Evidence for their chymotrypsin-like properties. *Biochim Biophys Acta* **379:**606–617.
Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, and Chou JL (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* **245:**1066–1073.
Robache-Gallea S, Morand V, Bruneau JM, Schoot B, Tagat E, Réalo E, Chouaib S, and Roman-Roman S (1995) In vitro processing of human tumor necrosis factor-alpha. *J Biol Chem* **270:**23688–23692.
Rodenburg KW, Kjoller L, Petersen HH, and Andreasen PA (1998) Binding of urokinase-type plasminogen activator-plasminogen activator inhibitor-1 complex to the endocytosis receptors alpha2-macroglobulin receptor/low-density lipoprotein receptor-related protein and very-low-density lipoprotein receptor involves basic residues in the inhibitor. *Biochem J* **329:**55–63.
Rogan MP, Geraghty P, Greene CM, O'Neill SJ, Taggart CC, and McElvaney NG (2006) Antimicrobial proteins and polypeptides in pulmonary innate defence. *Respir Res* **7:**29.
Roghanian A and Sallenave JM (2008) Neutrophil elastase (NE) and NE inhibitors: canonical and noncanonical functions in lung chronic inflammatory diseases (cystic fibrosis and chronic obstructive pulmonary disease). *J Aerosol Med Pulm Drug Deliv* **21:**125–144.
Rubin H, Plotnick M, Wang ZM, Liu X, Zhong Q, Schechter NM, and Cooperman BS (1994) Conversion of alpha 1-antichymotrypsin into a human neutrophil elastase inhibitor: demonstration of variants with different association rate constants, stoichiometries of inhibition, and complex stabilities. *Biochemistry* **33:**7627–7633.
Salipante SJ, Rojas ME, Korkmaz B, Duan Z, Wechsler J, Benson KF, Person RE, Grimes HL, and Horwitz MS (2009) Contributions to neutropenia from PFAAP5 (N4BP2L2), a novel protein mediating transcriptional repressor cooperation between Gfi1 and neutrophil elastase. *Mol Cell Biol* **29:**4394–4405.
Sallenave JM (2002) Antimicrobial activity of antiproteinases. *Biochem Soc Trans* **30:**111–115.
Sallenave JM and Ryle AP (1991) Purification and characterization of elastase-specific inhibitor. Sequence homology with mucus proteinase inhibitor. *Biol Chem Hoppe Seyler* **372:**13–21.
Sallenave JM, Si Tahar M, Cox G, Chignard M, and Gauldie J (1997a) Secretory leukocyte proteinase inhibitor is a major leukocyte elastase inhibitor in human neutrophils. *J Leukoc Biol* **61:**695–702.
Sallenave JM, Si Tahar M, Cox G, Chignard M, and Gauldie J (1997b) Secretory leukocyte proteinase inhibitor is a major leukocyte elastase inhibitor in human neutrophils. *J Leukoc Biol* **61:**695–702.
Salvesen G, Farley D, Shuman J, Przybyla A, Reilly C, and Travis J (1987) Molecular cloning of human cathepsin G: structural similarity to mast cell and cytotoxic T lymphocyte proteinases. *Biochemistry* **26:**2289–2293.
Sambrano GR, Huang W, Faruqi T, Mahrus S, Craik C, and Coughlin SR (2000) Cathepsin G activates protease-activated receptor-4 in human platelets. *J Biol Chem* **275:**6819–6823.
Sarraf P and Sneller MC (2005) Pathogenesis of Wegener's granulomatosis: current concepts. *Expert Rev Mol Med* **7:**1–19.
Savige JA, Paspaliaris B, Silvestrini R, Davies D, Nikoloutsopoulos T, Sturgess A, Neil J, Pollock W, Dunster K, and Hendle M (1998) A review of immunofluorescent patterns associated with antineutrophil cytoplasmic antibodies (ANCA) and their differentiation from other antibodies. *J Clin Pathol* **51:**568–575.
Schalkwijk J, Chang A, Janssen P, De Jongh GJ, and Mier PD (1990) Skin-derived antileucoproteases (SKALPs): characterization of two new elastase inhibitors from psoriatic epidermis. *Br J Dermatol* **122:**631–641.
Schalkwijk J, Wiedow O, and Hirose S (1999) The trappin gene family: proteins defined by an N-terminal transglutaminase substrate domain and a C-terminal four-disulphide core. *Biochem J* **340:**569–577.
Schechter I and Berger A (1967) On the size of the active site in proteases. I. Papain. *Biochem Biophys Res Commun* **27:**157–162.
Schmidt D, Dent G, and Rabe KF (1999) Selective phosphodiesterase inhibitors for the treatment of bronchial asthma and chronic obstructive pulmonary disease. *Clin Exp Allergy* **29 (Suppl 2):**99–109.
Schreiber A, Busjahn A, Luft FC, and Kettritz R (2003) Membrane expression of proteinase 3 is genetically determined. *J Am Soc Nephrol* **14:**68–75.
Schwartzberg LS (2006) Neutropenia: etiology and pathogenesis. *Clin Cornerstone* **8 (Suppl 5):**S5–S11.
Scott FL, Hirst CE, Sun J, Bird CH, Bottomley SP, and Bird PI (1999) The intracellular serpin proteinase inhibitor 6 is expressed in monocytes and granulocytes and is a potent inhibitor of the azurophilic granule protease, cathepsin G. *Blood* **93:**2089–2097.
Segal AW (2005) How neutrophils kill microbes. *Annu Rev Immunol* **23:**197–223.
Sethi JM and Rochester CL (2000) Smoking and chronic obstructive pulmonary disease. *Clin Chest Med* **21:**67–86.
Shafer WM, Hubalek F, Huang M, and Pohl J (1996) Bactericidal activity of a

synthetic peptide (CG 117–136) of human lysosomal cathepsin G is dependent on arginine content. *Infect Immun* **64:**4842–4845.
Shafer WM, Katzif S, Bowers S, Fallon M, Hubalek M, Reed MS, Veprek P, and Pohl J (2002) Tailoring an antibacterial peptide of human lysosomal cathepsin G to enhance its broad-spectrum action against antibiotic-resistant bacterial pathogens. *Curr Pharm Des* **8:**695–702.
Shapiro SD (2002) Proteinases in chronic obstructive pulmonary disease. *Biochem Soc Trans* **30:**98–102.
Shock A and Baum H (1988) Inactivation of alpha-1-proteinase inhibitor in serum by stimulated human polymorphonuclear leucocytes. Evidence for a myeloperoxidase-dependent mechanism. *Cell Biochem Funct* **6:**13–23.
Si-Tahar M, Merlin D, Sitaraman S, and Madara JL (2000) Constitutive and regulated secretion of secretory leukocyte proteinase inhibitor by human intestinal epithelial cells. *Gastroenterology* **118:**1061–1071.
Silverman EK and Sandhaus RA (2009) Clinical practice. Alpha1-antitrypsin deficiency. *N Engl J Med* **360:**2749–2757.
Silverman GA, Bird PI, Carrell RW, Church FC, Coughlin PB, Gettins PG, Irving JA, Lomas DA, Luke CJ, Moyer RW, et al. (2001) The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J Biol Chem* **276:**33293–33296.
Sinha S, Watorek W, Karr S, Giles J, Bode W, and Travis J (1987) Primary structure of human neutrophil elastase. *Proc Natl Acad Sci USA* **84:**2228–2232.
Smith CE and Johnson DA (1985) Human bronchial leucocyte proteinase inhibitor. Rapid isolation and kinetic analysis with human leucocyte proteinases. *Biochem J* **225:**463–472.
Snouwaert JN, Brigman KK, Latour AM, Malouf NN, Boucher RC, Smithies O, and Koller BH (1992) An animal model for cystic fibrosis made by gene targeting. *Science* **257:**1083–1088.
Sommerhoff CP, Nadel JA, Basbaum CB, and Caughey GH (1990) Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. *J Clin Invest* **85:**682–689.
Sørensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, and Borregaard N (2001) Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* **97:**3951–3959.
Sottrup-Jensen L (1989) Alpha-macroglobulins: structure, shape, and mechanism of proteinase complex formation. *J Biol Chem* **264:**11539–11542.
Specks U, Fass DN, Fautsch MP, Hummel AM, and Viss MA (1996) Recombinant human proteinase 3, the Wegener's autoantigen, expressed in HMC-1 cells is enzymatically active and recognized by c-ANCA. *FEBS Lett* **390:**265–270.
Sponer M, Nick HP, and Schnebli HP (1991) Different susceptibility of elastase inhibitors to inactivation by proteinases from Staphylococcus aureus and Pseudomonas aeruginosa. *Biol Chem Hoppe Seyler* **372:**963–970.
Sprecher CA, Morgenstern KA, Mathewes S, Dahlen JR, Schrader SK, Foster DC, and Kisiel W (1995) Molecular cloning, expression, and partial characterization of two novel members of the ovalbumin family of serine proteinase inhibitors. *J Biol Chem* **270:**29854–29861.
Starkey PM and Barrett AJ (1976) Human cathepsin G. Catalytic and immunological properties. *Biochem J* **155:**273–278.
Stennicke HR, Renatus M, Meldal M, and Salvesen GS (2000) Internally quenched fluorescent peptide substrates disclose the subsite preferences of human caspases 1, 3, 6, 7 and 8. *Biochem J* **350:**563–568.
Stockley RA (1999) Neutrophils and protease/antiprotease imbalance. *Am J Respir Crit Care Med* **160:**S49–S52.
Stockley RA, Shaw J, Afford SC, Morrison HM, and Burnett D (1990) Effect of alpha-1-proteinase inhibitor on neutrophil chemotaxis. *Am J Respir Cell Mol Biol* **2:**163–170.
Stroncek DF (2007) Neutrophil-specific antigen HNA-2a, NB1 glycoprotein, and CD177. *Curr Opin Hematol* **14:**688–693.
Sun J, Bird CH, Sutton V, McDonald L, Coughlin PB, De Jong TA, Trapani JA, and Bird PI (1996) A cytosolic granzyme B inhibitor related to the viral apoptotic regulator cytokine response modifier A is present in cytotoxic lymphocytes. *J Biol Chem* **271:**27802–27809.
Sun J, Stephens R, Mirza G, Kanai H, Ragoussis J, and Bird PI (1998) A serpin gene cluster on human chromosome 6p25 contains PI6, PI9 and ELANH2 which have a common structure almost identical to the 18q21 ovalbumin serpin genes. *Cytogenet Cell Genet* **82:**273–277.
Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, and Dayer JM (1992) 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* **145:**1016–1022.
Suter S, Schaad UB, Roux L, Nydegger UE, and Waldvogel FA (1984) Granulocyte neutral proteases and Pseudomonas elastase as possible causes of airway damage in patients with cystic fibrosis. *J Infect Dis* **149:**523–531.
Szulakowski P, Crowther AJ, Jiménez LA, Donaldson K, Mayer R, Leonard TB, MacNee W, and Drost EM (2006) The effect of smoking on the transcriptional regulation of lung inflammation in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **174:**41–50.
Taggart C, Cervantes-Laurean D, Kim G, McElvaney NG, Wehr N, Moss J, and Levine RL (2000) Oxidation of either methionine 351 or methionine 358 in alpha 1-antitrypsin causes loss of anti-neutrophil elastase activity. *J Biol Chem* **275:**27258–27265.
Taggart CC, Lowe GJ, Greene CM, Mulgrew AT, O'Neill SJ, Levine RL, and McElvaney NG (2001) Cathepsin B, L, and S cleave and inactivate secretory leucoprotease inhibitor. *J Biol Chem* **276:**33345–33352.
Takeyama K, Agustí C, Ueki I, Lausier J, Cardell LO, and Nadel JA (1998) Neutrophil-dependent goblet cell degranulation: role of membrane-bound elastase and adhesion molecules. *Am J Physiol* **275:**L294–L302.
Tanaka T, Minematsu Y, Reilly CF, Travis J, and Powers JC (1985) Human leukocyte cathepsin G. Subsite mapping with 4-nitroanilides, chemical modification, and effect of possible cofactors. *Biochemistry* **24:**2040–2047.
Tester AM, Cox JH, Connor AR, Starr AE, Dean RA, Puente XS, López-Otín C, and Overall CM (2007) LPS responsiveness and neutrophil chemotaxis in vivo require PMN MMP-8 activity. *PLoS One* **2:**e312.
Tetley TD (1993) New perspectives on basic mechanisms in lung disease. 6. Proteinase imbalance: its role in lung disease. *Thorax* **48:**560–565.
Thusberg J and Vihinen M (2006) Bioinformatic analysis of protein structure-function relationships: case study of leukocyte elastase (ELA2) missense mutations. *Hum Mutat* **27:**1230–1243.
Tizzano EF and Buchwald M (1992) Cystic fibrosis: beyond the gene to therapy. *J Pediatr* **120:**337–349.
Toomes C, James J, Wood AJ, Wu CL, McCormick D, Lench N, Hewitt C, Moynihan L, Roberts E, Woods CG, et al. (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nat Genet* **23:**421–424.
Travis J (1988) Structure, function, and control of neutrophil proteinases. *Am J Med* **84:**37–42.
Travis J, Bowen J, and Baugh R (1978) Human alpha-1-antichymotrypsin: interaction with chymotrypsin-like proteinases. *Biochemistry* **17:**5651–5656.
Tremblay GM, Janelle MF, and Bourbonnais Y (2003) Anti-inflammatory activity of neutrophil elastase inhibitors. *Curr Opin Investig Drugs* **4:**556–565.
Tsai CC, McArthur WP, Baehni PC, Hammond BF, and Taichman NS (1979) Extraction and partial characterization of a leukotoxin from a plaque-derived Gram-negative microorganism. *Infect Immun* **25:**427–439.
Tsunemi M, Matsuura Y, Sakakibara S, and Katsube Y (1996) Crystal structure of an elastase-specific inhibitor elafin complexed with porcine pancreatic elastase determined at 1.9 A resolution. *Biochemistry* **35:**11570–11576.
Tsushima K, King LS, Aggarwal NR, De Gorordo A, D'Alessio FR, and Kubo K (2009) Acute lung injury review. *Intern Med* **48:**621–630.
Turk D, Janjić V, Stern I, Podobnik M, Lamba D, Dahl SW, Lauritzen C, Pedersen J, Turk V, and Turk B (2001) Structure of human dipeptidyl peptidase I (cathepsin C): exclusion domain added to an endopeptidase framework creates the machine for activation of granular serine proteases. *EMBO J* **20:**6570–6582.
Twumasi DY and Liener IE (1977) Proteases from purulent sputum. Purification and properties of the elastase and chymotrypsin-like enzymes. *J Biol Chem* **252:**1917–1926.
Uehara A, Muramoto K, Takada H, and Sugawara S (2003) Neutrophil serine proteinases activate human nonepithelial cells to produce inflammatory cytokines through protease-activated receptor 2. *J Immunol* **170:**5690–5696.
Uehara A, Sugawara S, Muramoto K, and Takada H (2002) Activation of human oral epithelial cells by neutrophil proteinase 3 through protease-activated receptor-2. *J Immunol* **169:**4594–4603.
Uehara A, Sugawara Y, Sasano T, Takada H, and Sugawara S (2004) Proinflammatory cytokines induce proteinase 3 as membrane-bound and secretory forms in human oral epithelial cells and antibodies to proteinase 3 activate the cells through protease-activated receptor-2. *J Immunol* **173:**4179–4189.
Umeki S, Niki Y, and Soejima R (1988) Elastase/antielastase systems in pulmonary diseases. *Am J Med Sci* **296:**103–106.
Valenzuela-Fernández A, Planchenault T, Baleux F, Staropoli I, Le-Barillec K, Leduc D, Delaunay T, Lazarini F, Virelizier JL, Chignard M, et al. (2002) Leukocyte elastase negatively regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding and functions by amino-terminal processing of SDF-1 and CXCR4. *J Biol Chem* **277:**15677–15689.
Van den Steen PE, Proost P, Wuyts A, Van Damme J, and Opdenakker G (2000) Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4, and GRO-alpha and leaves RANTES and MCP-2 intact. *Blood* **96:**2673–2681.
Van Der Geld YM, Limburg PC, and Kallenberg CG (1999) Characterization of monoclonal antibodies to proteinase 3 (PR3) as candidate tools for epitope mapping of human anti-PR3 autoantibodies. *Clin Exp Immunol* **118:**487–496.
Vergnolle N (2009) Protease-activated receptors as drug targets in inflammation and pain. *Pharmacol Ther* **123:**292–309.
Vogt W (1995) Oxidation of methionyl residues in proteins: tools, targets, and reversal. *Free Radic Biol Med* **18:**93–105.
von Vietinghoff S, Eulenberg C, Wellner M, Luft FC, and Kettritz R (2008) Neutrophil surface presentation of the anti-neutrophil cytoplasmic antibody-antigen proteinase 3 depends on N-terminal processing. *Clin Exp Immunol* **152:**508–516.
von Vietinghoff S, Tunnemann G, Eulenberg C, Wellner M, Cristina Cardoso M, Luft FC, and Kettritz R (2007) NB1 mediates surface expression of the ANCA antigen proteinase 3 on human neutrophils. *Blood* **109:**4487–4493.
Voynow JA, Fischer BM, and Zheng S (2008) Proteases and cystic fibrosis. *Int J Biochem Cell Biol* **40:**1238–1245.
Voynow JA, Young LR, Wang Y, Horger T, Rose MC, and Fischer BM (1999) Neutrophil elastase increases MUC5AC mRNA and protein expression in respiratory epithelial cells. *Am J Physiol* **276:**L835–L843.
Walker RI and Willemze R (1980) Neutrophil kinetics and the regulation of granulopoiesis. *Rev Infect Dis* **2:**282–292.
Walsh DE, Greene CM, Carroll TP, Taggart CC, Gallagher PM, O'Neill SJ, and McElvaney NG (2001) Interleukin-8 up-regulation by neutrophil elastase is mediated by MyD88/IRAK/TRAF-6 in human bronchial epithelium. *J Biol Chem* **276:**35494–35499.
Walton EW (1958) Giant-cell granuloma of the respiratory tract (Wegener's granulomatosis). *Br Med J* **2:**265–270.
Watorek W (2003) Azurocidin—inactive serine proteinase homolog acting as a multifunctional inflammatory mediator. *Acta Biochim Pol* **50:**743–752.
Watorek W, van Halbeek H, and Travis J (1993) The isoforms of human neutrophil elastase and cathepsin G differ in their carbohydrate side chain structures. *Biol Chem Hoppe Seyler* **374:**385–393.
Weathington NM, van Houwelingen AH, Noerager BD, Jackson PL, Kraneveld AD, Galin FS, Folkerts G, Nijkamp FP, and Blalock JE (2006) A novel peptide CXCR

ligand derived from extracellular matrix degradation during airway inflammation. *Nat Med* **12:**317–323.
Weber A, Wasiliew P, and Kracht M (2010) Interleukin-1beta (IL-1beta) processing pathway. *Sci Signal* **3:**cm2.
Wegener F (1936) Über generalisieste, septische efäberkrankungen. *Verh Dtsch Pathol Ges* **29:**210.
Wegener F (1939) Über eine eigenartige rhinogene Granulomatose mit Beteiligung des Arterien systems und der Nieren. *Beitr Pathol* **102:**36–68.
Wei AZ, Mayr I, and Bode W (1988) The refined 2.3 A crystal structure of human leukocyte elastase in a complex with a valine chloromethyl ketone inhibitor. *FEBS Lett* **234:**367–373.
Wei Y, Fox T, Chambers SP, Sintchak J, Coll JT, Golec JM, Swenson L, Wilson KP, and Charifson PS (2000) The structures of caspases-1, -3, -7 and -8 reveal the basis for substrate and inhibitor selectivity. *Chem Biol* **7:**423–432.
Weinrauch Y, Drujan D, Shapiro SD, Weiss J, and Zychlinsky A (2002) Neutrophil elastase targets virulence factors of enterobacteria. *Nature* **417:**91–94.
Wewers MD, Casolaro MA, Sellers SE, Swayze SC, McPhaul KM, Wittes JT, and Crystal RG (1987) Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med* **316:**1055–1062.
Wiedow O and Meyer-Hoffert U (2005) Neutrophil serine proteases: potential key regulators of cell signalling during inflammation. *J Intern Med* **257:**319–328.
Wiedow O, Schröder JM, Gregory H, Young JA, and Christophers E (1990) Elafin: an elastase-specific inhibitor of human skin. Purification, characterization, and complete amino acid sequence. *J Biol Chem* **265:**14791–14795.
Wiesner O, Litwiller RD, Hummel AM, Viss MA, McDonald CJ, Jenne DE, Fass DN, and Specks U (2005) Differences between human proteinase 3 and neutrophil elastase and their murine homologues are relevant for murine model experiments. *FEBS Lett* **579:**5305–5312.
Wiesner O, Russell KA, Lee AS, Jenne DE, Trimarchi M, Gregorini G, and Specks U (2004) Antineutrophil cytoplasmic antibodies reacting with human neutrophil elastase as a diagnostic marker for cocaine-induced midline destructive lesions but not autoimmune vasculitis. *Arthritis Rheum* **50:**2954–2965.
Witko-Sarsat V, Cramer EM, Hieblot C, Guichard J, Nusbaum P, Lopez S, Lesavre P, and Halbwachs-Mecarelli L (1999a) Presence of proteinase 3 in secretory vesicles: evidence of a novel, highly mobilizable intracellular pool distinct from azurophil granules. *Blood* **94:**2487–2496.
Witko-Sarsat V, Halbwachs-Mecarelli L, Schuster A, Nusbaum P, Ueki I, Canteloup S, Lenoir G, Descamps-Latscha B, and Nadel JA (1999b) Proteinase 3, a potent secretagogue in airways, is present in cystic fibrosis sputum. *Am J Respir Cell Mol Biol* **20:**729–736.
Wood AM and Stockley RA (2006) The genetics of chronic obstructive pulmonary disease. *Respir Res* **7:**130.
Wysocka M, Kwiatkowska B, Rzadkiewicz M, Lesner A, and Rolka K (2007) Selection of new chromogenic substrates of serine proteinases using combinatorial chemistry methods. *Comb Chem High Throughput Screen* **10:**171–180.
Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, and Jennette JC (2002) Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* **110:**955–963.
Yang JJ, Preston GA, Pendergraft WF, Segelmark M, Heeringa P, Hogan SL, Jennette JC, and Falk RJ (2001) Internalization of proteinase 3 is concomitant with endothelial cell apoptosis and internalization of myeloperoxidase with generation of intracellular oxidants. *Am J Pathol* **158:**581–592.
Yang P (1997) Comparative evaluation of unfixed and fixed human neutrophils for determination of antineutrophil cytoplasmic antibodies by indirect immunofluorescence. *J Clin Pathol* **50:**677–680.
Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC, Snyder B, Larkin K, Liu J, Orkin J, et al. (2008) Towards a transgenic model of Huntington's disease in a non-human primate. *Nature* **453:**921–924.
Ying QL and Simon SR (2000) DNA from bronchial secretions modulates elastase inhibition by alpha(1)-proteinase inhibitor and oxidized secretory leukoprotease inhibitor. *Am J Respir Cell Mol Biol* **23:**506–513.
Ying QL and Simon SR (2001) Kinetics of the inhibition of proteinase 3 by elafin. *Am J Respir Cell Mol Biol* **24:**83–89.
Yoshimura K and Crystal RG (1992) Transcriptional and posttranscriptional modulation of human neutrophil elastase gene expression. *Blood* **79:**2733–2740.
Young RE, Voisin MB, Wang S, Dangerfield J, and Nourshargh S (2007) Role of neutrophil elastase in LTB4-induced neutrophil transmigration in vivo assessed with a specific inhibitor and neutrophil elastase deficient mice. *Br J Pharmacol* **151:**628–637.
Zani ML, Baranger K, Guyot N, Dallet-Choisy S, and Moreau T (2009) Protease inhibitors derived from elafin and SLPI and engineered to have enhanced specificity towards neutrophil serine proteases. *Protein Sci* **18:**579–594.
Zani ML, Nobar SM, Lacour SA, Lemoine S, Boudier C, Bieth JG, and Moreau T (2004) Kinetics of the inhibition of neutrophil proteinases by recombinant elafin and pre-elafin (trappin-2) expressed in Pichia pastoris. *Eur J Biochem* **271:**2370–2378.
Zasloff M (2002) Antimicrobial peptides of multicellular organisms. *Nature* **415:**389–395.
Zeiher BG, Matsuoka S, Kawabata K, and Repine JE (2002) Neutrophil elastase and acute lung injury: prospects for sivelestat and other neutrophil elastase inhibitors as therapeutics. *Crit Care Med* **30:**S281–S287.
Zeng W, Silverman GA, and Remold-O'Donnell E (1998) Structure and sequence of human M/NEI (monocyte/neutrophil elastase inhibitor), an Ov-serpin family gene. *Gene* **213:**179–187.
Zhang Z, Winyard PG, Chidwick K, Murphy G, Wardell M, Carrell RW, and Blake DR (1994) Proteolysis of human native and oxidised alpha 1-proteinase inhibitor by matrilysin and stromelysin. *Biochim Biophys Acta* **1199:**224–228.
Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, Wahl SM, Lacomis L, Erdjument-Bromage H, Tempst P, Wright CD, et al. (2002) Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. *Cell* **111:**867–878.
Zimmer M, Medcalf RL, Fink TM, Mattmann C, Lichter P, and Jenne DE (1992) 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* **89:**8215–8219.