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REVIEW

Intracellular and extracellular serpins modulate lung disease

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An imbalance between peptidases and their inhibitors leads to pulmonary disease. Imbalances occur in the adult and the neonate at risk for a specific set of lung pathologies. Serpins (serine peptidase inhibitors) make up the major source of antipeptidase activity in the lung. The purpose of this review is to describe the serpin mechanism of inhibition, their roles in the normal and pathological lung and their potential as therapeutic agents. *Journal of Perinatology* (2008) **28,** S127–S135; doi:10.1038/jp.2008.150

Introduction

The lung functions in the face of many physical challenges: exposure to oxygen and environmental toxins, airborn pathogens, continuous expansion and compression while breathing and maintenance of a delicate interface enabling gas exchange with the body's vascular system. As a result, tissue damage, inflammation, repair and remodeling are constant. These processes, from the induction of apoptosis and necrosis in acute injury to the defense mechanisms of inflammatory cells, coagulation and fibrinolysis, to extracellular matrix degradation and cell migration, are all peptidase driven. Peptidase inhibitors are required to regulate these processes and neutralize peptidases upon completion of their intended roles. Most pulmonary diseases are associated with an imbalance between peptidase and peptidase inhibitor activity (Table 1). This holds true in the neonate at risk for a specific set of dysfunctional lung pathologies, including meconium aspiration syndrome (MAS), respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD). The balance between peptidase and antipeptidase activities appears critical in the development and progression of these diseases of premature and full-term newborns. Serpins (serine peptidase inhibitors) make up the major source of peptidase inhibitors in the lung. Others peptidase inhibitors include Kunitz, Kazal and Bowman-Birk protein families. Unlike the other peptidase inhibitors, serpin inhibition occurs at a 1:1 stoichiometry. Importantly, peptidase inhibition by serpins is irreversible. The purpose of this review is to describe the general mechanism and regulation of serpin function in the lung. Critical roles in the normal and pathological lung, and serpins as potential therapeutic agents are examined.

Serpins are found in animals, plants, prokaryotes and viruses. They are distinguished from other peptidase inhibitors because their unique suicide substrate-like mechanism of inhibition (reviewed in Gettins[1,2]). A total of 37 serpin genes in humans are distributed within 9 clades (A–I). Clade A has 13 members including SERPINA1 (α1-antitrypsin, α1AT) and SERPINA5 (protein C inhibitor, PCI) that are secreted into the circulation. Clade B is made up of 13 serpins that are primarily intracellular. The remaining eleven serpins are dispersed across clades C–I and are secreted into the fluid phase. The tertiary structure of serpins is highly conserved and consists of three β-sheets (A–B), 7 to 9 α-helices (A–I) and extended reactive site loop (RSL) that acts as the bait for peptidase targets. The structure is critical to the unique suicide-substrate-like mechanism of peptidase inhibition.[3] Inhibitory serpins exist in a metastable state resembling a loaded mousetrap. Upon peptidase binding to the RSL, hydrolysis of the P1–P1′ peptide bond releases the RSL and allows the serpin to undergo a rapid conformational transition. With the peptidase covalently bound to the P1 residue, the RSL is inserted into β-sheet A as strand 4 and the peptidase is trapped in an inactive complex with the serpin.

This mechanism of inhibition has a side effect resulting in clinical diseases collectively referred to as serpinopathies.[4,5] Mutations that cause structural instability, specifically the opening of β-sheet A, can lead to the formation of serpin polymers. Under conditions of high serpin concentration, such as the endoplasmic reticulum of cells in the liver, the RSL of one molecule is inserted into β-sheet A of another. The prototype of this disease mechanism is the genetic deficiency of α1AT.[6] The two most common alleles, α1AT*S and α1AT*Z, result in formation of α1AT polymers in the liver and significantly diminished α1AT plasma levels. The consequence of this is twofold for the individual: hepatocyte cell death leading to cirrhosis,[7] and predisposition to emphysema, asthma and additional respiratory diseases.[8] Other serpins with

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naturally occurring alleles shown to develop polymers and subsequent serpinopathies are C1 inhibitor, antithrombin, α1-antichymotrypsin (ACT), heparin cofactor II and neuroserpin.

α1AT is the major source of protection against proteolytic damage in the lung

α1AT (SERPINA1) is the major peptidase inhibitor in plasma and provides the main source of antipeptidase activity in lung. α1AT is a 52 kDa glycoprotein produced in and secreted from the liver, as well as bronchial epithelial cells (BECs). Amino-acid residues *Met-Ser* at the P1−P1′ position within the RSL make α1AT a potent inhibitor of neutrophil elastase (NE), cathepsin G (catG) and proteinase-3 (Table 1).[9] In the lung, NE is capable of causing extensive damage because of its proteolytic activity against structural components collagen and elastin. During inflammation large amounts of this peptidase are delivered to the lung. Infiltrating neutrophils express catG on the cell surface and release peptidases including NE into the airway space. The primary role of α1AT is to maintain a local balance between peptidase activities required for inflammatory cell function and to protect the lung against peptidase-mediated tissue damage.[10]

α1AT deficiency is one of the most common inherited defects in Caucasians. Mutant alleles α1AT*S (*Glu*264*Val*) and α1AT*Z (*Glu*342*Lys*) account for most α1AT deficiencies, with α1AT*Z the more deleterious. Approximately 4% of northern Europeans carry the Z allele and approximately 1 in 2000 is homozygous (α1AT*ZZ) whereas 1 in 1000 is heterozygous for the two mutant alleles (α1AT*SZ). Individuals homozygous for the more common S allele (α1AT*SS) exhibit an ∼40% decrease in α1AT plasma levels. The α1AT*ZZ homozygous genotype results in a deficit of ∼85%. Individuals with this phenotype, or heterozygous for the two mutant alleles, are at risk for developing diseases associated with excess elastase activity such as emphysema.

Table 1 Serpins and their proposed functions in the lung

Serpin	*Peptidase targets*	*Function*	*Pulmonary diseases*
SERPINA1 *α1-antitrypsin*	Neutrophil elastase, cathepsin G, proteinase-3	Protect the lung against elastase activity	Empysema Chronic pulmonary obstructive disease
SERPINA5 *Peptidase C inhibitor*	Activated protein C, thrombin-thrombomodulin	Activate coagulation	
	Thrombin, factors Xa, XIa	Suppress coagulation	
	Urokinse-type plasminogen activator, tissue-type plasminogen activator	Suppress fibrinolysis	
SERPINC1 *Antithrombin III*	Thrombin, factors IXa, Xa, XIa, XIIa, kallikrein	Suppress coagulation	Sepsis/ALI
SERPINE1 *Plasminogen activator inhibitor-1*	Urokinse-type plasminogen activator, tissue-type plasminogen activator	Suppress fibrinolysis	ALI Idiopathic pulmonary fibrosis ARDS Asthma
SERPINB1	Neutrophil elastase, cathepsin G, proteinase-3	Protect the lung against elastase activity	
SERPINB2 *Plasminogen activator inhibitor-2*	Urokinse-type plasminogen activator, tissue-type plasminogen activator	Protect against cell death	
SERPINB3	Cathepsins K, L, S, V	Protection of cells against cytosolic lysosomal peptidases, Inhibit cell death	
SERPINB4	Cathepsin G, mast cell proteinase	Protect against cell death	
SERPINB6	Cathepsin G	Protect cells from granule peptidases	
SERPINB9	Granzyme B	Protect cytotoxic lymphocytes/maintain granzyme B granules	
SERPINB10	Trypsin, thrombin	Protect against cell death	
SERPINB12	Trypsin		
SERPINB13	Cathepsin K, L	Protect against cell death	

α1AT deficiency: COPD and emphysema

The proposed mechanism in the development of chronic obstructive pulmonary disease (COPD), including emphysema, is an imbalance of elastase and anti-elastase activity in the lung (reviewed in Elias *et al*.[11]). α1AT*ZZ and α1AT*SZ individuals are at a greater risk of developing COPD. For α1AT*ZZ individuals, plasma α1AT*Z levels of 15% translate into elastase-inhibitory activities in the lung far below this. First, protein encoded by the α1AT*Z allele exhibits an association rate with NE approximately fivefold slower than normal α1AT. Second, α1AT*Z protein maintains its inherent instability and continues to form polymers as it diffuses into the lung, leading to the detection of polymers in lungs of α1AT*ZZ individuals, and further loss of anti-elastase activity.[12,13] Finally, it was observed that α1AT*ZZ polymers served as a chemoattractant for human neutrophils.[14] This translates into increased inflammation and NE peptidase in conjunction with decreased α1AT function.

COPD development is largely influenced by environmental factors, of which the most common is tobacco smoke. It has been proposed that the P1-*Met* residue in the RSL renders α1AT sensitive to inactivation by oxidation from tobacco smoke exposure and the reactive oxygen burst released by neutrophils (reviewed in Carrell[10]). Accordingly, α1AT*ZZ individuals who smoke exhibit a rapid onset of emphysema, often by 30 years of age, and death by the age of 50.[15] However, most α1AT*ZZ individuals who avoid smoking live normal length lives with minimal complications. Replacement therapy for α1AT deficiency, with intravenous α1AT from pooled human plasma, results in longer survival. Data compiled by the National Heart, Lung and Blood Institute indicate that α1AT serum levels and lung function are improved with α1AT replacement therapy.[16] However, plasma is limited and therapy expensive. Gene therapy is an alternative toward which much progress been made in animal models.[17,18] Synthetic small molecule peptidase inhibitors are also being developed as a tool to counteract imbalances in peptidase activity (reviewed in Chughtai and O'Riordan[19]). On the basis of nature of α1AT deficit caused by polymerization, another therapeutic strategy is to prevent polymerization and facilitate α1AT secretion from the liver. This may be accomplished using small molecules interacting with α1AT directly or by enhanced chaperone function.[20–22]

Cystic fibrosis and α1AT

Cystic fibrosis (CF) presents another scenario where lung pathology and disease progression is associated with increased elastase activity. However, unlike α1AT deficiency, CF patients express normal amounts of α1AT. Decreased fluidity of mucus in the CF airway impairs mucociliary clearance, obstructs normal diffusion of innate immune components, as well as α1AT, and creates localized environments bacteria may colonize. Chronic infection recruits an excess of migrating and activated neutrophils that release their serine peptidases to the cell surface or directly into the airway space. The elevated peptidase levels overwhelm the available neutralizing activity of α1AT. CF progression is associated with exacerbations, frequently caused by increased bacterial load or viral infection (reviewed in Goss and Burns[23]). Inflammation is central to this event, including increases in interleukin (IL)-8, IL-6, IL-1β, tumor necrosis factor-α (TNFα), leukotriene B4 ($LKTB_4$) and free NE. Exacerbations lead to airway remodeling and decreased lung function.

α1AT replacement therapy to combat NE activity in CF lungs has been available for over two decades, however, there is little statistical data derived from clinical trials relating to its effectiveness.[24,25] Two recent papers described promising results with inhaled α1AT in CF patients. Both studies found decreased inflammation associated with lowered cytokines and neutrophil cell numbers.[26,27] Griese *et al*. compared peripheral lung versus the bronchial deposition of aerosolized prolastin (α1AT purified from plasma; Bayer Corporation, Clayton, NC, USA). Although no difference was observed between the two sites of treatment deposition, both groups receiving 4 weeks of daily prolastin exhibited significant decreases in IL-8, TNFα, IL-1β protein and $LKTB_4$ concentrations in sputum. In a second study, 4 weeks of treatment with recombinant α1AT (rα1AT) made in sheep produced significant decreases in neutrophil infiltration, and reduced complexes between NE and endogenous α1AT, suggesting that rα1AT was effective in neutralizing endogenous NE.[27] Favorable results have also been collected from nebulized α1AT therapy studies in animal models.[28] Together these results suggested that α1AT aerosol treatment would benefit CF patients. However, it will be important to examine in detail the mechanism by which α1AT inhibited inflammation and cytokine production.

α1AT and lung disease in the newborn

The premature infants lacking surfactant synthesis develop RDS. A direct result of underdeveloped lungs and respiratory system, therapy includes oxygen and mechanical ventilation, and surfactant replacement. Even with improved oxygen saturation monitoring and ventilation, unavoidable acute lung injury (ALI) adds to the severity of RDS. Infants born prematurely frequently go on to develop BPD. Prematurity is the primary risk factor for BPD, followed by oxygen toxicity and ventilation induced lung damage (reviewed in Jobe and Ikegami[29] and Chess *et al*.[30]). The 'new' BPD pathology consists of arrested alveolar development, resulting in decreased total alveoli, and abnormal vasculature localization observed in the lung periphery. Inflammatory cytokines have been associated with accelerated lung maturation.[29] Serine peptidases, released from inflammatory cells, caused epithelial cell injury and lung remodeling that is associated with RDS and BPD.[31] In the neonate the balance between elastase and elastase inhibitor activity

has been associated with lung injury and progression to BPD.[32] In addition to α1AT, low molecular mass inhibitor secretory leukocyte peptidase inhibitor (SLPI) was found to be important in neutralizing NE in tracheal aspirates of infants born prematurely.[33] Watterberg *et al*.[34] found that SLPI increases in the tracheal lavage of RDS, whereas neonates going on to develop BPD had a significantly higher elastase to elastase-inhibitor ratio. Sveger *et al*.[35] also reported significant correlations with BPD development and low levels of inhibitors α1AT and ACT (SERPINA3) in tracheobronchial aspirate of preterm infants at 3 to 4 days of age, and low SLPI at 7 to 8 days of age. Two trials assessed the effect of α1AT therapy in preterm infants on recovery from RDS and development of BPD.[36,37] A meta-analysis of the two trials found that although a trend for reduced risk of oxygen dependency after 28 days existed, no significant difference was observed between groups receiving α1AT therapy and placebo for risk of BPD or long-term neurodevelopmental abnormalities.[38]

Similar to endotoxin exposure, meconium aspiration leads to a rapid induction of cytokines, inflammation, decreased surfactant function, hypoxemia, pulmonary hypertension and excessive cell death in the airway of the newborn. In animal models, all of these events take place within 2 to 4 h of exposure to dilute human meconium.[39–42] Following the initial inflammatory response, MAS patients frequently undergo further pulmonary distress associated with oxygen toxicity upon intubation and mechanical ventilation. Zagariya *et al*.[43] hypothesized that α1AT in the lungs of neonates may attenuate meconium aspiration-induced lung injury. As has been observed in α1AT deficiency and CF patients, the absence of adequate anti-elastase activity associated with α1AT resulted in extensive pulmonary damage. Treatment with supplemental α1AT activity would seem to be a promising approach to arrest elastase-dependent lung damage following meconium aspiration.

Plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 (PAI-1, SERPINE1) is an inhibitor of the two plasminogen activators (PAs), urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA). uPA is expressed in specific tissues, whereas tPA functions as a soluble protein in the vascular system. The balance between PA and PAI-1 activities determines local fibrinolysis (Table 1). Diseases of acute inflammation, such as ALI and acute respiratory distress (ARDS) (reviewed in Ware and Matthay[44]), and fibrotic diseases like idiopathic pulmonary fibrosis (IPF)[45] share a common pathology of fibrin deposition in the alveolar compartment. To determine the best approach for treatment and prevention of these related diseases, it is important to identify the source of imbalanced fibrinolysis resulting in fibrin deposition. As the main inhibitor of fibrinolysis, PAI-1 is expressed by several cell types found in the lung and is modulated by inflammatory cytokines and tissue damage. Elevated levels of PAI-1 were observed in alveolar macrophages from patients with ARDS[46] and IPF.[45] An investigation of PAI-1, uPA and tPA levels in preterm infants with RDS also identified elevated ratios of PAI-1 to uPA protein in tracheal aspirate fluid.[47] Elevated PAI-1 in BAL fluid occurred in bacterial pneumonia.[48,49] Excessive PAI-1 levels were observed in ARDS and severe pneumonia cases requiring mechanical ventilation.[50] These findings supported the hypothesis that PAI-1 is a critical regulator of fibrin deposition in the alveolar space in response to ALI. Further support for this theory was gained from animal models of lung injury, including bacterial endotoxin,[51] and bleomycin[52] models of ALI. These studies established PAI-1, its activity and its regulation, as a critical target for therapies treating the family of diseases displaying poor lung function associated with fibrin deposition including ALI, ARDS and COPD.

PAI-1 has been identified as a potential mediator of host defense. In mouse lung infection models, PAI-1 deficiency did not affect the outcome of infection by Gram-positive *Streptococcus pneumonia*,[53] but was found to be critical in defense against Gram-negative *Klebsialla pneumonia*.[54] PAI-1-deficient animals exhibited increased mortality at 24 and 48 h, coinciding with increased bacterial dissemination, and elevated fibrinolysis. These results may be attributed to the ascribed function of PAI-1 in regulating fibrinolysis or a potential role of PAI-1 in mediating neutrophil recruitment signals, as was demonstrated *in vitro*.[55] In either case, these results suggest that PAI-1 function may prove to be a useful alternative target in the battle against bacterial pathogens.

Antithrombin III

Antithrombin III (SERPINC1, ATIII) regulates coagulation by inhibiting activated serine peptidases. ATIII can inhibit serine peptidases thrombin, factors IXa, Xa, XIa, XIIa and kallikrein (Table 1).[56] The peptidase inhibitor activity of ATIII is positively regulated by its cofactor heparin binding to the D helix. This induces a conformational switch putting the RSL in a more favorable position for serine peptidase binding.[57] Heparin molecules longer than 26 residues further accelerate peptidase inhibition through interactions with the peptidase. Defects in ATIII function primarily result in complications relating to thrombosis. However, a protective role of ATIII has been experimentally demonstrated in animal models of sepsis[58] and ischemia reperfusion of grafted lungs.[59] Recombinant ATIII (rATIII) at elevated levels resulted in reduced to complete inhibition of pulmonary vascular permeability associated with endotoxin treatment.[58] In a lung transplant model, lungs were stored for 28 h with normal saline, then transplanted into dogs receiving ATIII or vehicle alone. Transplant animals treated with ATIII exhibited no change in O_2 partial pressure, alveolar–arterial O_2 difference or pulmonary vascular resistance for up to 3 h.[59] These encouraging

results suggested that ATIII might be used to treat sepsis-related lung dysfunction, ARDS and ALI resulting from ischemia.

In humans, plasma ATIII levels normally decline with sepsis severity, and correlate with high mortality rate.[60] Small clinical trials suggested improved survival rates in patients receiving rATIII.[61–64] Eisele *et al*.[62] reported that rATIII therapy was associated with decreased lung dysfunction. However, Waydas *et al*.[63] found no difference in the duration of organ failure. A single large trial of 2314 patients with severe sepsis produced inconsistent results.[65] One group in this trial, receiving ATIII but not heparin, exhibited an increased 90-day survival rate compared with placebo. It was also observed that new pulmonary dysfunction was decreased in the group receiving rATIII therapy. Unfortunately, no critical trials have been performed to date specifically with ALI or ARDS patients to test ATIII or heparin therapy. Overall, improvement in fibrin deposition and lung function observed with ATIII in animal models of sepsis and ALI is promising but is unconfirmed in humans.

Peptidase C inhibitor

Peptidase C inhibitor (PCI, SERPINA5) has a broad peptidase inhibitor profile through which it modulates both the coagulation and fibrinolysis systems (Table 1) (reviewed in Church *et al*.[56] and Geiger[66]). PCI in plasma is the major inhibitor of the anticoagulant peptidase activated protein C (APC). PCI also inhibits the thrombin–thrombomodulin complex,[67] kallikrein, factors Xa, XIa and thrombin. PCI may downregulate fibrinolysis by inhibiting plasmin activator peptidases uPA and tPA. Like ATIII, the interaction between PCI and many of its target peptidases is modulated by heparin and other glycosaminoglycans.[68–70] The interaction between PCI and target peptidase kallikrein is inhibited by glycosaminoglycans. In humans, PCI is a plasma protein, present at a concentration of $\sim$100 nM and a half-life of $\sim$23 h.[71] PCI is found in many other body fluids and secretions, and in a wide range of tissues. The broad peptidase-inhibitory profile and widely distributed expression has made it difficult to assign specific biological functions to this serpin.

To determine the role of this serpin, PCI function was studied in the mouse. Expression of mouse PCI was limited to the male and female reproductive systems.[72] Homozygous-null PCI-knockout mouse appeared normal except male sterility was observed.[72] Limited endogenous PCI in the mouse allowed a unique approach to model human PCI pulmonary function. Hayashi *et al*.[73] expressed hPCI in mice from a transgene consisting of the human PCI gene contained within 25 kb of human genomic DNA. hPCI protein in these transgenic (Tg) animals was found to be an active inhibitor of APC, and expressed in a pattern similar to humans. This hPCI Tg animal system may be used as a tool to further explore PCI function in the lung under physiological and pathological conditions, as well as to test the therapeutic effect of human APC *in vivo*. Nishi *et al*.[74] used the hPCI Tg mouse to identify a role for PCI in pulmonary hypertension. Monocrotaline treatment was used to specifically induce pulmonary hypertension. A significant increase in right ventricular pressure was observed in treated wild-type (WT) control mice compared to hPCI Tg animals. BAL fluid levels of thrombin–antithrombin complex, monocyte chemoattractant protein-1, platelet-derived growth factor and IL-13, and the plasma level of TNFα were significantly increased in treated WT mice compared to hPCI Tg animals.[74] This study established that PCI in the lung is protective against monocrotaline-induce hypertension. Furthermore, it suggested that PCI fulfills both anti-inflammatory and anticoagulant activities in the lung.[74,75] To take advantage of PCI therapeutically it will be important to determine which activities as a serine peptidase inhibitor are protective in specific physiological conditions.

Clinical studies on the role of PCI are limited. Examination of 58 patients with interstitial lung disease (ILD) associated with diverse underlying pathologies discovered elevated PCI in the BAL fluid of each of them.[76] Groups with cryptogenic-organizing pneumonia, collagen vascular disease (CVD-ILD) and sarcoidosis exhibited elevated levels of PCI and thrombin-activatable fibrinolysis inhibitor (TAFI), supporting the hypothesis that PCI inhibition of APC results in elevated TAFI levels.[77]

The clade B serpins protect cells with an intracellular antipeptidase shield

In humans there are 13 clade B genes that encode serine and cysteine peptidase inhibitors. The intracellular serpins are expressed in a wide range of tissues including lung, and target a wide range of peptidases (Table 1). In the face of environmental insults such as bacterial and viral infection, and excessive peptidase levels associated with inflammation, the induction of cell death is a common yet critical step in ALI. There is increasing evidence that intracellular serpins function as a cytoprotective antipeptidase shield, limiting damage by the misdirected peptidases as well as the induction of necrosis and apoptosis (reviewed in Silverman *et al*.[78] and Scott[79]). SERPINB1 inhibits elastases expressed by the neutrophil.[80] SERPINB2, B3, B4, B10 and B13 have been implicated in blocking proapoptotic signals.[81,82] SERPINB6 and B9 inhibit peptidases stored in the cytolytic granules including catG and granzyme B (GzmB), respectively.[83,84] SERPINB12 inhibits trypsin and is expressed in the lung.[85] Together, the clade B serpin genes encode proteins with diverse antipeptidase activity, with critical intracellular roles in protecting cells from damage, and maintaining normal function in the lung.

SERPINB1 inhibits elastases in CF and BPD

SERPINB1 (monocyte/neutrophil elastase inhibitor) is an inhibitor of NE, catG and neutrophil peptidase-3 (pr-3) (Table 1).[80,86]

SERPINB1 protein was shown to exist at elevated levels and in a complex with NE in the lavage of CF patients compared to normal individuals.[87] Recombinant human SERPINB1 (rSERPINB1) was able to protect rat lungs against injury, including hemorrhage and epithelial permeability, from the instillation of NE or CF patient sputum preparations.[88] rSERPINB1 was shown to inhibit Surfactant-A degradation by the peptidase(s) in BAL fluid from CF patients.[89] Yasumatsu *et al*. probed SERPINB1 function in the established baboon BPD model.[90,91] As in humans, SERPINB1 was localized to bronchial and glandular epithelial cells, as well as mast cells, neutrophils and macrophages in the baboon lung.[92] SERPINB1 protein in baboon lung tissue was found in high molecular weight complexes with both NE and catG in BPD models but not gestational controls.[90] These results suggested that SERPINB1 function in the newborn lung is critical as an antipeptidase shield against elastases associated with inflammation and responsible for lung injury.

Plasminogen activator inhibitor 2 blocks apoptosis

Plasminogen activator inhibitor 2 (SERPINB2, PAI-2) inhibits uPA and tPA, and is expressed in macrophages and monocytes (Table 1). Its primarily intracellular and nuclear localization suggests, however, that PAI-2 has functions in addition to regulating fibrinolysis (reviewed in Medcalf and Stasinopoulos[93]). Several studies have demonstrated the ability of intracellular PAI-2 to inhibit apoptosis. TNFα-induced apoptosis was inhibited by ectopic PAI-2 expression in HeLa and fibrosarcoma cells.[94,95] Recently, PAI-2 was found to be required for macrophage survival following pathogen activation of the toll-like receptor-4 apoptosis pathway.[96] On the basis of these studies, PAI-2 appears to be a critical regulator of cell survival in cells of the host defense system where it is expressed.

SERPINB3 and SERPINB4 are serine and cysteine peptidase inhibitors

SERPINB3 and B4 are co-expressed in lung epithelium.[97] SERPINB3 inhibits the lysosomal cysteine peptidases, cathepsin L (catL), catK and catS.[98] SERPINB4 inhibits the serine peptidases catG and mast cell chymase (Table 1).[99] Ectopic SERPINB3 expression was cytoprotective against TNFα and natural killer (NK) cell-induced apoptosis,[81] and both SERPINB3 and B4 have been shown independently to protect cells against radiation.[82] The peptidase inhibitor activity of SERPINB3 and B4 may provide cellular protection in the environment of the lung during ALI and inflammation. SERPINB3 and B4 expression was induced in BECs in asthmatics and by IL-4 and IL-13.[100] SERPINB3 and B4 may provide protection against neutrophil and mast cell peptidases as well as localized bursts of reactive oxygen generated by neutrophils used to destroy pathogens. Cells exposed to reactive oxygen succumb to necrosis or apoptosis following lysosomal damage and release of its cysteine peptidases into the cytosol (reviewed in Lockshin and Zakeri[101] and Guicciardi *et al*.[102]). Cysteine peptidases are likely to be involved in development of BPD, as catK, catL, and catS were shown to be elevated in a baboon model of BPD.[103] SERPINB3 is predicted to provide protection to cells against cysteine peptidases. Maintenance of BEC by SERPINB3 and B4 would limit ALI and prevent epithelial permeability during infection.

SERPINB9 inhibits granzyme B

SERPINB9 is the only known inhibitor of GzmB in humans (Table 1).[84] SERPINB9 is expressed in cytotoxic lymphocytes (CTLs), dendritic cells (DCs) and NK cells of the monocyte and lymphocyte lineages.[104,105] Using a Serpinb9-deficient mouse model, Zhang *et al*.[106] demonstrated that Serpinb9 is required to protect CTLs from 'accidental death' induced by residual GzmB in the cytosol. Serpinb9 was found to be required for the maintenance of GzmB-containing granule integrity in CTLs. Finally, in the absence of Serpinb9, animals exhibited impaired clearance of lymphocytic choriomeningitis virus.[106] On the basis of these findings and the localization of SERPINB9-positive monocytes and DCs in lung tissue,[107] it is proposed that this serpin is required for normal host defense in the lung, particularly against bacterial and viral pathogens requiring cell contact-mediated killing by the immune system.

Summary and future directions

Lung function is dependent on several peptidase driven systems including the innate and adaptive immune systems, coagulation, fibrinolysis and tissue remodeling. As a result, pulmonary diseases are commonly linked to excessive peptidase activity. Serpins are the major regulators of peptidase activity in the lung. It follows that understanding the biological function and regulation of serpins will be critical in the development of therapies against pulmonary disease.

Serpins occupy two niches in the lung, regulation of extracellular peptidases and intracellular peptidases. The blood plasma serpins α1AT, PAI-1, ATIII and PCI, function extracellularly. Their localization within the lung makes them primary targets for the development of therapeutic agents, from simple replacement by inhalation, to recombinant gene transfer and development of small molecule inhibitors that mimic their activity. In the case of α1AT, this serpin inhibits a limited number of elastolytic peptidases. In diseases associated with excess elastase activity (empysema, CF), replacement therapy appears very promising. In contrast, ATIII and PCI are multi-peptidase inhibitors and their local active concentration *in vivo* is likely critical to properly balancing the function of specific peptidase

targets. To modulate the activity of these serpins therapeutically, we may take advantage of the endogenous mechanisms regulating their activity, interactions with glycosaminoglycans and conformational changes in structure. Alternatively, extensive success has been met using recombinant APC, a PCI target peptidase, in the treatment of sepsis and ALI.

The clade B serpins are mainly intracellular. They are predicted to form an anti-peptidase shield, protecting cells against exogenous and endogenous peptidase activity. This hypothesis is based upon their intracellular localization and broad tissue distribution, combined with the varied peptidase-inhibitory specificities of the clade B members. In their absence, cellular injury would lead to cellular stress and death. SERPINB9, the only inhibitor of GzmB in humans, is required for CTL survival, and therefore host defense against some viral infections. SERPINB3 and SERPINB4, expressed in BEC, are poised to protect the bronchial airways against both exogenous and endogenous serine and lysosomal cysteine peptidases. In lung epithelium, this antipeptidase shield is very important due to repeated exposure to neutrophil-derived serine peptidases and oxidative stress accompanying inflammation in response to pulmonary injury or infection. On the basis of the ability to block cellular damage, including that caused by lysosomal cysteine peptidases, and cell death, the intracellular clade B serpins are powerful agents to target in the development of therapeutics.

Disclosure

The authors have declared no financial interests.

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