well delineated, but, may in part involve cleavage of macrophage recognition molecules by NE, impairing apoptotic cell uptake (15). Bridging molecules, SP-A and -D are also decreased in the lungs of smokers (16), also potentially contributing to the defect. Of note, statins (17) and macrolides (18) were recently reported to enhance phagocytosis. This might help explain their anti-inflammatory properties and provide a mechanism of action to be pursued as therapy for COPD.

The neutrophilic response to cigarette smoke is clearly a false alarm with detrimental consequences. However, neutrophils are required to remove pathogens, particularly during acute airway infections that occur in patients with COPD. It has been very difficult to determine whether exacerbations themselves contribute to decline in lung function, although most evidence does point to an additional loss of FEV_1 with each exacerbation (19). It is also believed that the host response to a greater degree than the inciting infection is responsible for the lung damage. However, strategies to prevent neutrophils from gaining access to the infected airway seem riskier than removing the neutrophil quickly after pathogen clearance. The findings in this manuscript suggest that this might be difficult since *H. influenza* leads to neutrophil necrosis. A better understanding of the apoptotic versus necrotic response could lead to strategies to prevent necrosis and clear bacteria. In the meantime, limiting excess inflammation might be the simplest therapeutic strategy for COPD.

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Molecular Multitasking in the Airspace

α_1-Antitrypsin Takes on Thrombin and Plasmin

For the past 40 years, research focused on α_1-antitrypsin (A1AT) deficiency greatly enhanced the understanding of the pathobiology of chronic obstructive pulmonary diseases (COPD). The discovery that A1AT-deficient patients are at increased risk of developing COPD (1) led to the protease/antiprotease hypothesis of emphysema development (2), postulated to ultimately cause its hallmark irreversible alveolar destruction (3). Elegant investigations into pathological lung matrix proteolysis converged on implicating excessive alveolar inflammation as the potential source of extracellular proteolytic enzymes (proteases) (2). More recently, excessive alveolar cell apoptosis has also been linked to emphysema pathogenesis (4). These processes,

rather than operating in isolation, manifest complex interactions with each other and may therefore create feed-forward mechanisms that overwhelm the lung's repair capability (5, 6). For example, alterations in alveolar maintenance and excessive apoptosis may further enhance inflammation and exacerbate the protease/antiprotease imbalance (7). However, viable therapeutic options for the millions of patients with COPD have not yet emerged from these discoveries, signaling that substantial missing links in the pathogenesis of this disease still exist. As in the past, studies centered on A1AT function continue to provide fascinating and important novel knowledge into the pathogenesis of COPD. The findings of Churg and coworkers that A1AT blocks cigarette smoke and thrombin-dependent activation of TNF-α and MMP-12 in alveolar macrophages expand our understanding of biological functions of A1AT (49). Furthermore, their work has the potential to unveil both novel investigative leads connecting blood coagulation events to the pathogenesis of COPD and common targets between lung and cardiovascular diseases caused by cigarette smoke.

A1AT Deficiency and Lung Disease

Currently, the clinical entity of A1AT deficiency is defined by decreased serum levels of A1AT caused by the inheritance of two protease inhibitor (PI) deficiency alleles from the AAT gene locus (designated PI) on chromosomal segment 14q32.1. A1AT deficiency occurs predominantly in white persons of European origin and its frequency in Europe and North America is comparable to that of cystic fibrosis (~ 1 in 3,000; i.e., 100,000 A1AT-deficient patients in the United States) (8). The most common deficiency allele is PI*Z, characterized by a mutation leading to the replacement of glutamine in position 342 for lysine. A large majority of individuals with severe A1AT deficiency are PI type ZZ. This mutation destabilizes the conformational structure of A1AT, causing intracellular retention of polymerized A1AT-ZZ protein at synthesis sites, primarily in the liver, eventually resulting in abnormally low A1AT serum levels. Affected individuals may have no clinical manifestations, or may develop COPD with a high frequency of panacinar emphysema, or less commonly, liver disease. Other rare clinical manifestations of A1AT deficiency are panniculitis and a Wegener's-like vasculitis (8).

Since A1AT is an effective elastase inhibitor, emphysema is believed to occur as a result of increased and unopposed neutrophil elastase activity, destroying the elastin matrix of the lung. Although other serine protease inhibitors (serpins), including α-2 macroglobulin, inhibit elastase or proteinase-3 more efficiently than A1AT (9), it appears that A1AT accounts for most of the anti-elastase at the alveolar level (10). A1AT has biologically relevant nonserpin activities as it inhibits LPS-induced monocyte activation by a still undefined mechanism (11). Moreover, A1AT supplementation reduced *in vivo* inflammatory cell influx and NF-κB activation in the lung after exposure to cigarette smoke (12, 13). Furthermore, abnormal variants of A1AT may themselves have pathogenic effects in the lung, as oxidatively modified or polymerized A1AT activate monocytes or attract neutrophils, respectively (14, 15). Another noncanonical function of A1AT is that of apoptosis inhibition, exerted in lung microvascular endothelial cells (16, 17) or pancreatic B cells (18). The work of Churg and colleagues expanded the knowledge of the mechanisms by which A1AT may exert other activities beyond neutrophil elastase inhibition (49). They showed that A1AT inhibits proinflammatory activation of alveolar macrophages by neutralizing proteases of the coagulation cascade, particularly thrombin and plasmin, thus preventing activation of the protease activated receptors (PAR) by either cigarette smoke or thrombin (Figure 1). These findings highlight the potential involvement of thrombin-regulated coagulation in the pathogenesis of COPD, and the potential role played by A1AT in modulating this pathway in the context of its broader lung protective activities.

The Role of Thrombin in Inflammatory Responses

The activation of the coagulation cascade culminates in thrombin activation, platelet recruitment, and formation of fibrin plugs. There is a close homeostatic interaction between the coagulation cascade and inflammation, as thrombin and anti-clotting proteases may aid in host defense (19). Thrombin has been reported to activate NF-κB and therefore increase the expression of IL(s)-1, -6, and -8, and ICAM-1, to enhance endothelial cell adhesion of inflammatory cells, and to up-regulate a broad range of mediators of inflammation such as prostanoids and nitric oxide (20). In addition, thrombin promotes the recruitment of T cells, enhances mobilization of von Willebrand factor and P-selectin from endothelial cells, and increases endothelial cell permeability (21). Ultimately, these actions mediate inflammatory cell recruitment at an injury site within the realm of an appropriate homeostatic response. However, inappropriate activation of

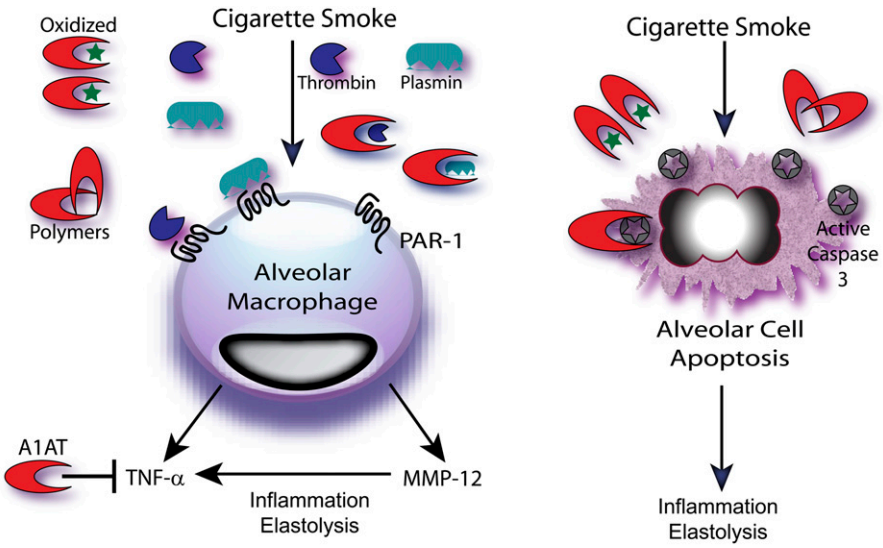

Figure 1. Interaction between A1AT and thrombin and plasmin in the setting of cigarette smoke–induced lung inflammation and alveolar cell apoptosis.

thrombin with a concomitant decrease in anticoagulant factors, as part of a so-called decompensated response (19), may lead to pathogenic clotting complications related to multi-organ failure in conditions such as sepsis, cardiovascular diseases, cancer, and inflammation (22).

One implication based on the report by Churg and coworkers is that cigarette smoke may activate proinflammatory responses mediated by thrombin (49). Whether this activation involves alveolar macrophage production of tissue factor or the participation of coagulation factors present in leaked plasma, as proposed by Churg and colleagues, remains to be elucidated. While plasma protein extravasation into the airway may be explained by increased capillary permeability via cigarette smoke–activated neutrophils (23), it is yet unknown whether alveolar macrophages are susceptible to activation when simply exposed to serum coagulation proteins. Nevertheless, coagulation cascade factors may not be needed for alveolar macrophage activation, since macrophages may themselves synthesize prothrombin, which could then be locally activated by MMP(s) (24).

The intracellular signal transduction triggered by thrombin occurs via ligation and activation of the protease-activated G-coupled receptors PAR(s) 1–4 (25). Thrombin activates PAR(s) 1, 3, and 4, while trypsin activates PAR(s) 1 and 2. PAR 2 is also activated by mast cell tryptase and neutrophil enzymes (elastase, cathepsins G, proteinase 3). PAR(s) 1 and 4 (in humans) and 3 and 4 (in rodents) mediate thrombin-induced platelet activation. Interventions aimed at disrupting the interaction between thrombin/plasmin with the PAR(s), such as with hirudin and aprotinin, were used by Churg and associates to confirm the up-regulation of TNF-α and MMP-12 caused by these serine proteases (49). Aprotinin (or Trasylol) is a bovine pancreatic trypsin inhibitor, which also blocks thrombin's access to the hirudin-like domain of PAR-1 (26). The leech anticoagulant hirudin, which shares a common sequence to PAR(s) 1 and 3, inhibits high-affinity thrombin binding to its receptors.

In the context of the experiments performed by Churg and colleagues, PAR(s) 1 and 2 might account for most of the inflammatory actions mediated by this group of receptors (49). The evidence, however, points toward PAR 1 as the mediator of cigarette smoke–induced macrophage activation, because plasmin, which also enhanced TNF-α and MMP-12 expression by cigarette smoke binds and activates PAR 1, whereas thrombin and plasmin cannot activate PAR 2. Interestingly, there is cell-type specificity in PAR 1 expression, with PAR 1 being expressed on human platelets, vascular cells, and leukocytes, while PAR 2 is expressed by airway epithelium, endothelial cells, and leukocytes. In the lung, PAR 1 has been involved in the pathogenesis of experimental acute lung injury and interstitial lung disease (27, 28), while PAR 2 was implicated in airway diseases, such as asthma (29). Serine proteases binding to the PAR(s) lead to receptor cleavage and generate a self-activating tethered ligand domain within the receptor. This ligand domain activates the receptor by interacting intramolecularly with the second extracellular domain. It is therefore possible to test the role of specific PAR(s) with receptor-specific activating peptides, as used by Churg and coworkers to confirm the role of PAR 1. Nevertheless, PAR 2 has also been linked to airway inflammation when activated by trypsin or mast cell tryptase, leading to activation of NF-κB (22). It remains to be determined whether PAR 2 activation contributes to macrophage activation by cigarette smoke. PAR 2 knockout mice (which are viable but have impaired leukocyte migration [30]), PAR 2–overexpressing mouse lines (22), siRNA(s) against PAR 2, and PAR 2 activating peptides represent useful experimental tools to address these questions.

Inappropriate activation of coagulation may complicate healing responses and contribute to tissue remodeling, with excessive production of growth factors (PDGF and connective tissue factor) and MMPs. Churg and colleagues demonstrate that thrombin and plasmin enhance MMP-12 expression, which has a central role in cigarette smoke–induced rodent emphysema (31). Other investigations have shown that thrombin may enhance MMP-2 and -9 expression as well (32), two other proteases implicated in the pathogenesis of human emphysema (33). Interestingly, MMP-12 can further propagate the inflammatory response through TNF-converting enzyme activity, further increasing levels of TNF-α (34), a cytokine with a causal role in experimental emphysema (35).

As with any central biological process, the homeostatic versus pathologic impact of thrombin may be determined by the balance of thrombin activation and its inhibitors. Activated thrombin binds to thrombomodulin on endothelial cells, leading to generation of activated protein C, a potent anti-inflammatory and anticlotting mediator. Since the endothelial cell protein C receptor (EPCR) exerts anti-inflammatory effects in endothelial and possibly mononuclear cells (36), and protects brain endothelial cells against hypoxia-induced apoptosis (37), decreased lung EPCR expression might also contribute to cigarette smoke inflammation and alveolar cell apoptosis in emphysema (5, 19) (Figure 1). We speculate cigarette smoke might impair the activated protein C/EPCR system on endothelial cells, enhancing thrombin's effect on alveolar cells.

The Role of A1AT in the Inhibition of Thrombin and Plasmin

The relevance of the findings reported by Churg and coworkers to cigarette smoke– and A1AT deficiency–associated COPD relies on the efficacy, specificity, and biological relevance of A1AT-mediated inhibition of thrombin and plasmin, as compared with other serine proteases (49). *In vitro*, A1AT has the highest and fastest (420 microseconds) inhibitory efficacy against neutrophil elastase and proteinase 3, interacting with $\sim$ 6-fold lesser efficacy against thrombin and plasmin (38). Based on kinetic studies, A1AT is predicted to inhibit the anti-clotting functions of plasmin, without affecting thrombin activity (39). Churg and colleagues used relatively high concentrations of A1AT (i.e., 500 μmol/liter) to effectively inhibit thrombin and plasmin activities (49). It is unclear whether such high concentrations exist physiologically in the lung interstitial and /or alveolar spaces, since lung epithelial lining fluid was reported to contain A1AT at concentrations of 4 μmol/liter (40; 41). It is likely that serum levels of A1AT increase under stressing conditions, but it is not known whether alveolar levels reflect changes in the serum, were basal levels of A1AT of 1.3 mg/ml (26 μmol/liter) increase 3- to 4-fold in response to systemic stressors such as fever and infection.

The mechanism by which A1AT inhibits serine proteases is via an exposed reactive center loop that presents the methionine-serine residues as a pseudosubstrate for the target proteinases. The proteinase cleaves at the P1-P1′ bond within the reactive loop linking the β-sheets and swings the trapped enzyme from the upper to the lower pole of the molecule, inserting the "trap and bait" as an extra strand in the β-sheet A (42). Although this interaction is essential for its antiproteinase activity, this conformational flexibility can also inhibit its function, as point mutations (such as the one causing the ZZ phenotype) facilitate the insertion of the reactive center loop into a β sheet of another A1AT molecule, resulting in the formation of polymers (43). As shown by Churg and coworkers, A1AT polymers, which are less effective protease inhibitors than the native protein, failed to inhibit cigarette smoke–induced TNF-α and MMP-12 activation in alveolar macrophages. Similarly, excessive reactive oxygen

species (ROS), such as found upon cigarette smoke exposure, significantly attenuate the antiproteolytic function of A1AT (44) via oxidation of the critical methionine residue at the reactive site and decrease affinity of A1AT for neutrophil elastase (45) (Figure 1). The prediction that oxidation of A1AT may also hamper its interaction with thrombin and plasmin (38) was confirmed by Churg and colleagues, who noted a weakening of the anti-inflammatory effect of oxidized A1AT on alveolar macrophages, providing further evidence for the detrimental effect of smoking on the protective mechanisms of A1AT against lung injury (49).

Conclusions

Can the local anti-inflammatory effects of A1AT be extended to other cell types or other organs? What are the sources of A1AT that may regulate its effect on alveolar macrophages? We have recently shown that A1AT is taken up by primary lung endothelial cells, where it interacts with intracellular activated caspase-3, inhibiting its function, as also seen in the murine lung *in vivo* (16, 17). These findings supported the existence of expanded noncanonical functions of A1AT. Furthermore, the lung endothelium may modulate the transit of A1AT from the serum to interstitial spaces or airway through transcellular or intercellular passage. These alternative functions of A1AT suggest the existence of receptor-mediated A1AT internalization in target lung cells.

Finally, is there any evidence that patients with A1AT deficiency have an increased incidence of clotting-related disorders? The inhibitory effect of A1AT on thrombin and plasmin was first proposed in 1968 when the serum of two A1AT-deficient patients failed to inhibit the fast thrombin clotting and the fast anti-plasmin activities (46). In contrast, the A1AT Pittsburgh variant with a methionine to arginine 358 mutation binds thrombin and even more avidly protein C, leading to increased risk of bleeding (47). While patients with the more common PiZ genotype appear not to be at increased risk of hemorrhage or thrombosis, there is evidence of a higher incidence of cardiovascular disease in patients with COPD compared with matched individuals without COPD (48). The work of Churg and colleagues may spark further research into the molecular basis of these important mechanistic links among smoking, cardiovascular disease, and COPD.

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