Alpha-1 Antitrypsin Investigations Using Animal Models of Emphysema

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Abstract

Animal models of disease help accelerate the translation of basic science discoveries to the bedside, because they permit experimental interrogation of mechanisms at relatively high throughput, while accounting for the complexity of an intact organism. From the groundbreaking observation of emphysema-like alveolar destruction after direct instillation of elastase in the lungs to the more clinically relevant model of airspace enlargement induced by chronic exposure to cigarette smoke, animal models have advanced our understanding of alpha-1 antitrypsin (AAT) function. Experimental *in vivo* models that, at least in part, replicate clinical human phenotypes facilitate the translation of mechanistic findings into individuals with chronic obstructive pulmonary disease and with AAT deficiency. In addition, unexpected findings of alveolar enlargement in various transgenic mice have led to novel hypotheses of emphysema development. Previous challenges in manipulating the AAT genes in mice can now be overcome with new transgenic approaches that will likely advance our understanding of functions of this essential, lung-protective serine protease inhibitor (serpin).

Keywords: alpha-1 antiproteinase; chronic obstructive pulmonary disease; emphysema

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Emphysema is a key component of the common group of airflow diseases collectively termed chronic obstructive pulmonary disease (COPD) (1, 2). The independent risk factors for developing COPD are a history of smoking, advanced age, and a history of asthma (3). Primarily occurring in those exposed to cigarette smoking (CS), emphysema has a strong genetic component, with only a subgroup of smokers developing emphysema (4). Of the genetic factors, deficiency in alpha-1 antitrypsin (AAT) remains one of the most common risk factors for developing emphysema. Individuals with AAT deficiency (AATD) are ninefold more likely to develop emphysema compared with those without this genetic deficiency, and lung disease is the most common cause of morbidity in individuals with AATD (5–7). Chronic exposures to CS, AATD, and other rare genetic diseases such as Marfan's syndrome (8) are associated with emphysematous lung changes; these changes have also been described in individuals with severe caloric restriction (9, 10), those of advanced age (11), and those infected with HIV (12, 13). We briefly discuss how each of these clinical scenarios has been modeled in the laboratory to better understand disease pathogenesis, and vice versa, that is, how basic science discoveries of emphysema-like phenotypes in animal lungs have led to discoveries that have informed our knowledge of human clinical phenotypes (Table 1).

The discovery of AAT as a serum antiprotease that is deficient in those with severe emphysema (14) and the identification of its antielastase properties have been intimately linked to the development of the elastase animal model of emphysema. The instillation of porcine pancreatic elastase or human neutrophil elastase into the lungs of either mice or hamsters culminated in the development of emphysema, an observation that underlies the most popular mechanistic paradigm, that of a protease/antiprotease imbalance leading to degradation of elastin fibers that compose the lung matrix (15). Validated by a similar phenotype in the tight skin mouse (16), mice defective in the fibrillin gene (17), the elastase model of

Table 1. Reviewed animal models of emphysema

Strain	Lung Phenotype	Potential Mechanism
Naturally occurring strains		
Tight skin mice (82–84)	Entire lung alveolar enlargement 4 d after birth suggestive of defect in postnatal alveolar maturation	Abnormal incorporation of mutant gene–duplicated fibrillin into microfibrils that renders them susceptible to elastolytic degradation
Pallid mice (26, 27, 85, 86)	Entire lung alveolar enlargement and elastin loss 8–10 mo after birth that can be exacerbated by CS, higher pulmonary compliance	Partial AAT serum deficiency, organelle defect, defect in AAT secretion
Exaggerated pathophysiological processes		
Caloric restriction (42, 87)	Reduction in alveolar number and surface area with 2 wk of two-thirds caloric reduction and reversal with diet restoration	Apoptosis and regeneration
Overexpression of inflammatory cytokines (e.g. IL-13) (68, 88)	Conditional overexpression of IL-13 in the lung, which induces airspace enlargement	Th2-type cytokine–induced inflammation associated with apoptosis and matrix proteolysis, as well as increased mucus secretion
VEGF inhibition (59, 60)	Alveolar enlargement and loss of elastic recoil within 1 mo after VEGF blockade	VEGF blockade leading to endothelial apoptosis
Klotho (45, 46)	Alveolar enlargement by 4 wk of age	Premature aging and airway apoptosis
SMP30 (47, 48)	Enhanced 2-mo CS-induced alveolar enlargement	Vitamin C metabolism; lack of protection from oxidative stress and apoptosis induced by CS and aging
TR (51)	Enhanced 6-mo CS-induced alveolar enlargement and weight loss in C57BL/6J background	TERT component TR deletion; short telomeres cause defects in epithelial repair.
Humanized PiZ AAT mouse (36)	CS exposure–exaggerated lung injury in PiZ-expressing mice	AAT polymers are directly pathogenic to the lung; CS-induced oxidative stress worsens AAT polymerization
Cigarette smoke injury		
Rat (23, 89, 90)	Alveolar enlargement after 2 mo of CS exposure	Inflammation, oxidative stress, and apoptosis
Guinea pig (24)	Alveolar enlargement and gas trapping after 3 mo of CS exposure	Inflammation and oxidative stress
Mouse (91, 92)	Alveolar enlargement with 6 mo of CS in C57BL/6, DBA/2, and A/J strains	Inflammation, oxidative stress, and apoptosis
Viral and bacterial antigens		
Poly(I:C) + CS (65)	Alveolar enlargement after 2 wk of CS followed by poly(I:C), but not with LPS	Poly(I:C) induction of IFNα/β, IL-12/IL-23 p40, and IFNγ
LPS (64)	Increased alveolar size with 3 mo of LPS	LPS induction of chronic inflammatory processes and recruitment of macrophages and $CD8^+$

Definition of abbreviations: AAT = alpha-1 antitrypsin; CS = cigarette smoke; IFN α/β = interferons alpha/beta; Poly(I:C) = polyriboinosinic:polyribocytidylic acid; LPS = lipopolysaccharides; TERT = telomerase reverse transcriptase; TR = telomerase RNA; VEGF = vascular endothelial growth factor.

emphysema remains popular because of a relatively rapid development of airspace enlargement, but it is criticized for the lack of physiological relevance of its disease course (18).

In contrast, CS exposure models have been better received because of the relevance of this environmental exposure to human disease. However, studies using this model require a relatively prolonged time, more than 4 months of exposure to CS, and result in a relatively mild phenotype. Numerous reports have described acute events after CS exposure, of which one of the earliest is oxidative stress with loss of epithelial and endothelial barriers, with leukocyte adhesion to the lung microvasculature within minutes after CS exposures (19). Chronic experimental CS exposure leads to mouse strain–specific and species-specific levels of lung injury. In common inbred strains of mice, such as C57Bl/6 and DBA2/J, chronic CS exposure causes airspace enlargement and is associated with various degrees of inflammation, oxidative stress, increased proteolysis, parenchyma cell apoptosis, markers of cellular stress, and elevated pulmonary static compliance (20). However, the degree of large airway pathology is relatively mild, only partially replicating the chronic airway remodeling seen in humans. Female sex and older age at the time of exposure are also phenotype modifiers in many mouse strains (21, 22). Many investigators have reported that CS exposure of larger animals, such as rats (23) and guinea pigs (24), has several advantages, including a more pronounced airway response to CS.

Several concerns about the CS model do persist, however, including the ability to accurately translate findings from another species to human disease, the difficulty of testing concurrent neoplastic risk from tobacco smoke exposure, and the difficulty

of modeling the unique elements of the human immune system. Nevertheless, with the increasing availability of regents and transgenic approaches in species besides mice, these models may become more attractive in the future for the preclinical validation of various targets and mechanisms. For example, nonhuman primates have been used recently to model human CS exposure (25). Studies of AAT in CS models have led to several important discoveries. For example, the relative levels of circulating AAT in particular mouse strains (26) and in the pallid mouse (27) are associated with distinct patterns of emphysema distribution and inflammation, oxidative stress, and apoptosis. These models can be useful in studying why the classical distribution of emphysema in individuals with AATD involves the whole lobule (panlobular), with a predisposition to the lower lobes of the lung. However, the multiple other systemic comorbid abnormalities that occur in the pallid mouse or in other mouse strains that develop spontaneous airspace enlargement, such as the blotchy (28) or beige mouse (29, 30), have reduced their usefulness in studying AAT biology.

A recent report characterized a new model of emphysema created by inbreeding C57Bl/6 mice, which led to spontaneous airspace enlargement (31). These mice exhibited a mild degree of endogenous AATD and diffuse bullous emphysematous changes evident from 4 weeks of age followed by lung inflammation, which was not evident until late, at 56 weeks of age (31). Because no major systemic abnormalities were reported, these mice may prove useful in studying the role of AATD in airspace enlargement. However, determining a true loss of function of AAT in mice has been challenging because there are three to five encoding genes (Serpina1a-1e) (32, 33), and efforts to generate individual knockouts, including the embryonically lethal Serpin1a-null mouse (34), have been unsuccessful. Nevertheless, new technologies for creating targeted mutant mice such as CRISPR-Cas9 will likely resolve this issue by offering easier target design and more efficient genomic modification over traditional methods.

CS has been found to decrease AAT activity in the lungs via oxidation of the molecule (35), which may induce a functional state of AATD. In addition, transgenic overexpression in mice of the most common human mutant AAT to cause severe emphysema, PiZZ (36), has yielded important insights into the proinflammatory properties of AAT polymers (37). This humanized transgenic mouse model suggest that in patients with AATD and emphysema, the lack of functional AAT is not the only cause of lung pathogenicity and that abnormal circulating AAT polymers are also driving detrimental inflammatory responses. Furthermore, protective effects of AAT gain of function have been demonstrated after either gene therapy using the well-tolerated adeno-associated virus to transduce human AAT (38, 39) or AAT protein augmentation therapy via intravenous injection (40, 41) or inhalation. Because AAT protein augmentation improved inflammation or airspace enlargement in mouse models of CS exposure, it is conceivable that supplementation therapy may be useful in select patients without AATD but with emphysema.

Other animal models have been created to model the clinical phenotypes associated with emphysema, such as that induced by caloric restriction, advanced age, or HIV infection. Of these, caloric restriction has resulted in a reversible phenotype (42) that lacks true destruction of the alveoli. In this context, the caloric restriction model may be useful in understanding the mechanism of autophagy and reversible cell injury that is caused by a lack of cell maintenance program rather than by alveolar destruction along with the role of AAT in these processes, because AAT levels are increased or unchanged during starvation (43, 44). Several models (e.g., the Klotho mutation mouse [45, 46], the senescence marker protein-30–null mouse [47, 48], the senescence-accelerated mouse [49], and the telomerase-null mouse [50, 51]) have deepened our understanding of the role of premature senescence in the development of emphysema. Although serpins such as plasminogen activator inhibitor-1 have been implicated in regulating senescence (52), little is known about the role of AAT in this process. Finally, little progress has been made in animals in modeling the effect of HIV on the human lung (53). Such investigations will shed light on the impact of AAT on HIV-associated loss of gas transfer surface, which has been described independently of CS history and greatly resembles the radiographic hallmarks of emphysema (54). A potentially important role for AAT is also hinted at by findings of low AAT levels (55) and increased fragments of AAT (56), suggesting excessive proteolysis in the plasma of HIV-infected patients (55). Furthermore, the reported protective effects of AAT against HIV replication and pathogenicity (57) (58) encourage future studies of augmentation therapy for this condition, especially when it affects the lung.

In addition to models of elastase and CS exposure, other models of emphysema have evolved from "bench" observations highlighting specific mechanisms of lung injury that replicate emphysema-like phenotypes in small animals. Among these are the vascular endothelial growth factor receptor (VEGFR) blockade models (59, 60), which led to the discovery of apoptosis as a central mechanism of emphysema development. These models revamped the vascular hypothesis of emphysema (61), which has been further cemented by observing a similar emphysematous phenotype after apoptosis induction specifically in lung microvascular endothelial cells (62, 63). The occurrence of airspace enlargement after repetitive exposures to certain LPS levels (64), the synergistic effect of coexposure to tobacco and viral antigens (65, 66), and models of autoantigen exposures (67) highlighted the importance of immune responses to emphysema pathogenesis. To this list of models one must add those that were developed via transgenic manipulations of multiple genes (e.g., for IL13, *see* Reference 68; for IFNγ, *see* Reference 69; for matrix metalloproteinase-1 (MMP1), *see* Reference 70; and so forth, as reviewed in Reference 71), which points to potential genes with susceptibility to, or which are putative therapeutic targets for, emphysema. Similar to the elastase model, these "nonphysiological" models of emphysema have advanced our understanding of the pathogenesis of airspace enlargement and parenchyma destruction that may be applicable to both AATD and nondeficient states and have revealed novel aspects of AAT biology. For example, the use of LPS in an elastase-deficient model led to the understanding that several immune modulatory effects of AAT occur independently of its antielastase function (72). The beneficial effects of AAT supplementation in the VEGFR model (73) increased the interest in defining the

antiapoptotic and vascular-protective effects of AAT in the lung and elsewhere, for example, in vasculitides. The endothelial cell antiapoptotic effects of AAT (73, 74) have been found to extend to CS-induced apoptosis (75), explained, at least in part, by inhibition of executioner caspases (76) or possibly by increasing vascular endothelial growth factor abundance (77) or inhibition of calpain activity (78). These previously unsuspected intracellular effects of AAT led to investigations into the trafficking of AAT across alveolar units, from its abundant intravascular localization to intraendothelial cell uptake, followed by secretion across the epithelium (79). This actively regulated process was found to be highly influenced by CS exposure (79–81) and, at least in part, to engage specific receptors of the scavenging receptor family that also participates in the clearance of lipoproteins (80).

In conclusion, animal models of disease have led to rapid progress in our understanding of emphysema pathogenesis in both individuals with AATD and AAT-sufficient individuals and have unveiled pleiotropic functions of AAT. There is a need to further improve these models, including developing AAT loss of function using modern gene-editing technologies, establishing COPD exacerbation models that better replicate human disease, and incorporating more efficient end points of disease activity assessment with improved throughput and sensitivity such as imaging and respiratory function measurements. When appropriately validated and extrapolated to human conditions, lessons learned from animal models of emphysema will spur the development of improved diagnosis for those at risk for its development. Animal models will also allow us to optimize AAT supplementation therapy and expand it to conditions other than AATD that could benefit from its ability to tame chronic inflammation, modulate immune responses, and confer vascular protection. ■

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