weakness, deconditioning, and impaired gas exchange, play a predominant role to reduced exercise tolerance. A recent study, however, has shown that in COPD there is a strong correlation (r = 0.81) between the resting inspiratory capacity and the exercise capacity. Accordingly, lung function impairment is probably an important cause of decreased exercise tolerance in many COPD patients. Indeed, because of expiratory FL, the maximal Vd decreased exercise tolerance in many COPD patients.

In conclusion, the NEP technique provides a simple and reliable tool for detecting expiratory FL both at rest and during exercise. The method does not require a body plethysmograph, does not depend on patient cooperation and coordination, and can be applied in any desired body posture.

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Animal Models for COPD*

Steven D. Shapiro, MD, FCCP

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**Abbreviations:** α₁-AT = α₁-antitrypsin; MMP = matrix metalloproteinase; MMP-12−/− mouse = macrophage elastase-deficient mice; MMP-12+/+ mice = wild-type mice; NE = neutrophil elastase; PPE = porcine pancreatic elastase

Animal models were critical in ushering in the modern era of COPD, after Gross et al.1 found that intratracheal administration of papain resulted in emphysema in experimental animals. This discovery, combined with the clinical finding by Laurell and Eriksson2 that patients with α₁-antitrypsin deficiency were at increased risk for emphysema, formed the scientific basis for the elastase-antielastase hypothesis for the pathogenesis of emphysema. Today, 35 years later, the elastase-antielastase hypothesis is still the prevailing theory for the development of emphysema, and animal models of COPD remain a critical experimental tool.

**Elastase-Induced Emphysema**

Since initial experiments of Gross et al., investigators have instilled a variety of proteinases into the lungs of many small and large animals. The administration of porcine pancreatic elastase (PPE; 1 to 4 mg/kg) has produced the most consistent and impressive airspace enlargement in rodents, guinea pigs, dogs, and primates.3,4 Instillation of PPE results in rapid and significant airspace enlargement, followed by acute neutrophil and subacute...

*From the Departments of Pediatrics, Medicine, and Cell Biology and Physiology, Washington University School of Medicine at St. Louis Children’s Hospital, St. Louis, MO.
Correspondence to: Steven D. Shapiro, MD, FCCP, Division of Allergy and Pulmonary Medicine, St. Louis Children’s Hospital, One Children’s Pl, St. Louis, MO 63110; e-mail: shapiro_s@kids.wustl.edu

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macrophage accumulation within the lung. Airspace enlargement continues over the first month after instillation and then stabilizes. Elastin content initially decreases, but appreciable elastin messenger RNA expression and elastic fiber deposition, albeit disorganized, is observed within weeks. A more recent study demonstrated by in situ hybridization that elastin messenger RNA was strongly expressed in the pleura, blood vessels, and airways following PPE administration. Within the alveolus, expression was observed primarily at the free margins of alveolar septa, with minimal expression in the walls of respiratory spaces.

Extracellular matrix repair must be partially effective since lesions are much more severe with the coincident application of β-aminopropanitrile, which prevents elastin and collagen cross-linking. Despite significant airspace enlargement, experimental animals survive. Whether they experience oxygen desaturation with exercise or demonstrate other significant physiologic abnormalities is not known.

The development of emphysema following instillation of other elastases including neutrophil elastase (NE) and proteinase-3, but not nonelastolytic enzymes such as bacterial collagenase, further supports the elastase-antielastase hypothesis. Elastase-induced emphysema remains a useful model of emphysema since it is relatively simple to perform and replicates many aspects of the disease. Of course, exposure to cigarette smoke may cause a variety of other abnormalities not observed in this model. Elastase instillation recently has been used to demonstrate that retinoic acid has the capacity to promote alveolarization and lung repair in adult male rats. This model will continue to be most useful in assessing the efficacy of therapeutic agents, particularly those with the capacity to repair lung damage, a critical need in the field.

**OTHER MODELS**

A variety of chemicals and irritants have been used to generate COPD in experimental animals. Interestingly, the administration of lipopolysaccharide results in neutrophil recruitment and airspace enlargement. Yet, humans tolerate bacterial pneumonia without residual emphysema. Whether humans have greater elastic fiber repair capacity or produce enough α1-AT to limit NE-mediated lung destruction is currently unknown. Cadmium chloride is a chemical agent that has been extensively used to generate airspace enlargement. However, airspace enlargement in this model appears to be secondary to fibrosis with subsequent tethering and enlargement of airspaces. While this has been viewed as a disadvantage of the model, we now appreciate that this mechanism also might be operative in humans with centrilobular emphysema.

Irritants such as pollutants, oxidants (nitrogen dioxide), and ozone also have been applied to experimental animals. These agents cause airway changes, such as injury to epithelial cells and loss of cilia, but little emphysema. Inorganic dusts, such as silica, have been associated with neutrophil and macrophage accumulation and with emphysema. Severe starvation also has been shown to cause emphysema in animals through unknown mechanism(s).

**NATURAL GENETIC MODELS OF EMPHYSEMA IN MICE**

Several mutant mouse strains develop airspace enlargement. These are usually developmental abnormalities rather than ones attributable to the destruction of mature lung tissue that characterizes emphysema. Tight skin (Tsk+/−) mice have a mutation in fibrillin-1, which is involved in elastic fiber assembly. These mice have abnormal airspace development and progressive alveolar enlargement with age. Pallid mice (pa/pa) develop mild emphysema late in life. Beige mice (bg) have a defect in the formation of primary neutrophil granules. It remains controversial whether they produce normal levels of serine proteinases and have the capacity to develop emphysema. In addition to these naturally occurring mutations, mice also provide unique opportunities to perform genetic engineering; specifically, manipulation of the mouse genome (see below).

**CIGARETTE SMOKE EXPOSURE**

Of course, the major environmental factor that predisposes patients to COPD is long-term cigarette smoking. A variety of animals has been exposed to cigarette smoke over the years, including dogs, rabbits, guinea pigs, and rodents. Guinea pigs are perhaps the most susceptible species, developing significant airspace enlargement within a few months of cigarette smoke exposure. Rats appear most resistant to emphysema, while, as mentioned, susceptibility in mice appears to be strain dependent.

**Murine Model of Cigarette Smoke-Related COPD**

We have begun to characterize the similarities and differences between mice and human lungs following chronic cigarette smoke exposure. Using smoking chambers similar to those described in the past for other species, we found that mice tolerate at least two cigarettes per day with carboxyhemoglobin levels of 10 to 14% following smoke exposure. C57BL/6-J mice tolerate cigarette smoke for at least 1 year, although their activity decreases and they occasionally die. Unlike humans, mice are obligate nose-breathers; yet, despite an intricate and extensive nasal sinus pathway, most of their epithelial cells are olfactory in nature without extensive cilia, so that they inefficiently filter tobacco smoke products. Mice have few submucosal glands, which are located exclusively in the trachea. They also contain Clara cells and epithelial cells but lack true goblet cells. In C57BL/6-J and A/J mice at least, ciliated epithelium extend throughout the airway with increased density in proximal airways. In response to 2 months of exposure to cigarette smoke, we observed a loss of ciliated epithelial cells and infiltration of immune and inflammatory cells (T cells, macrophages, neutrophils, and eosinophils), but saw no change in the number of Clara cells (S. Secaone, MD, and S.D. Shapiro, MD; unpublished observations; 1999). We also found that with prolonged cigarette smoke exposure (>6 months) small airways are occasionally obstructed with inflammatory cells and debris and that there are fewer alveolar attachments. Both of these changes in the small airways have...
been hypothesized to contribute to airflow obstruction in COPD.19,20 Mouse airways have much less extensive branching than those in humans and lack respiratory bronchioles. In alveolar spaces, we observed inflammatory cell recruitment and airspace enlargement in response to cigarette smoke, which is similar to humans. There is increased alveolar duct area and enlarged alveolar spaces18 (Fig 1). Whether these pathologic changes are associated with abnormal pulmonary function or gas exchange abnormalities awaits further study. We have also observed marked strain-dependent variability with respect to the findings discussed earlier, providing a unique opportunity to uncover COPD susceptibility genes.

GEOE TARGETING

Soon after the beginning of the next millennium, the sequence of all human genes will be known. However, the function of the proteins encoded by most of these genes will remain a mystery. Transgenic and gene-targeted mice provide powerful techniques that allow investigators to change single variables and, in essence, to perform controlled experiments in mammals, thus determining protein function in vivo. Introduction of a linear DNA fragment (transgene) into the pronucleus of one-celled embryos (or more recently into embryonic stem cells) allows the study either of the pattern of expression of that gene or of the biological consequences of overexpression of the protein encoded by the gene in specific tissues. More recently, gene targeting or targeted mutagenesis by homologous recombination in embryonic stem cells has allowed investigators to generate strains of mice that lack individual proteins, providing specific loss of function models.

Mice are used mainly because of their unique capacity to achieve germline transmission of genetic information. Other advantages of the mouse over other experimental animals include a rapid reproductive cycle, large litter sizes, extensive knowledge of mouse biology, abundance of mouse probes (such as antibodies and complementary DNAs), and lower cost. Importantly, evolutionary conservation has shown us that mice and other mammals are embarrassingly similar to humans. On the other hand, the applicability of these studies to understanding human biology and dissecting the mechanism of disease requires knowledge of similarities and differences with respect to protein profile between mouse and humans. With respect to COPD, long-term cigarette smoke exposure in mice results in many aspects of emphysema, and some aspects of large and small airway diseases that are observed in humans.

 gain-of-function (overexpression) Models

The overexpression of collagenase in the lung of transgenic mice that results in airspace enlargement is discussed in the study by D’Armiento et al.21 Whether collagen degradation alone is responsible for airspace enlargement in these mice remains unclear, but this surprising result raises questions about the intriguing role of collagen turnover in emphysema. Collagen turnover in emphysema is complex. Overall, there is net collagen synthesis in COPD with areas of increased deposition in small airways and depletion in alveolar walls.12 Further investigation is required to determine whether collagenase inhibition for emphysema will be beneficial or harmful, causing increased small airway fibrosis with increased tethering and enlargement of airspaces.

**Figure 1.** Cigarette smoke-induced airspace enlargement in mice. Scanning electron microscopy (×400) of age-matched C57BL/6J mice either not exposed (right) or exposed (left) to cigarette smoke for 6 months. Note the airspace enlargement in response to cigarette smoke. Alveolar pores also are increased in size and number (even when corrected for alveolar surface area).
Loss-of-Function (Underexpression) Models

Strains of mice deficient in individual candidate proteinases can be compared to determine their contributions to the development of emphysema in response to cigarette smoke. Macrophage elastase (MMP-12), nearly undetectable in healthy macrophages, is expressed in the alveolar macrophages of human cigarette smokers. MMP-12 also may be detected by immunohistochemistry and in situ hybridization in macrophages in patients with emphysema, but not in healthy lung tissue. To determine directly the contribution of macrophage elastase to emphysema, we generated macrophage elastase-deficient (MMP-12−/−) mice by gene targeting and subjected MMP-12−/− mice and wild-type (MMP-12+/+) littermates to chronic cigarette smoke exposure. In contrast to MMP-12+/+ mice, MMP-12−/− mice did not develop emphysema in response to long-term cigarette smoke exposure. Surprisingly, MMP-12−/− mice also failed to recruit macrophages into their lungs in response to cigarette smoke (Fig 2). Monthly intratracheal instillation of monocyte chemotactic protein-1 in MMP-12−/− mice exposed to tobacco smoke resulted in recruitment of MMP-12−/− alveolar macrophages but failed to cause airspace enlargement. Thus, MMP-12 is required for both macrophage accumulation and induction of emphysema resulting from chronic inhalation of cigarette smoke. Our current working model is that cigarette smoke induces constitutive macrophages to produce MMP-12 that cleaves elastin, generating fragments that are chemotactic for monocytes. This positive feedback loop perpetuates macrophage accumulation and lung destruction. The concept that proteolytically generated elastin fragments mediate monocyte chemotaxis is not original. Independent studies by Senior et al23 as well as by Hunninghake et al24 from the early 1980s demonstrated that elastase-generated elastin fragments were chemotactic for monocytes and fibroblasts. Gene targeting is merely reinforcing this as a major in vivo mechanism of macrophage accumulation in a chronic inflammatory condition.

Preliminary evidence using other proteinase-null mice demonstrate that uPA, an MMP activator, and MMP-9 are not involved in the development of cigarette smoke-mediated emphysema in mice. NE-deficient mice developed emphysema only two thirds as often as wild-type mice, suggesting a role for NE in this process (S.D. Shapiro, MD; unpublished manuscript; 1999). We suspect that NE also may be important in small airway changes associated with recurrent infections and neutrophil recruitment.

In summary, the exposure of gene-targeted mice to long-term cigarette smoke demonstrates that macrophage MMPs have the capacity to cause airspace enlargement. Moreover, neutrophils and macrophages have significant...

Figure 2. MME−/− mice fail to accumulate macrophages in response to cigarette smoke. MME+/+ and macrophage elastase-deficient (MME/null) mice were exposed to cigarette smoke for 6 months. Smoke-exposed mice and age-matched controls then were killed, their lungs were inflated and fixed, and their mid sagittal sections were stained for Mac-3. Note that exposure to cigarette smoke resulted in a fourfold increase in the number of alveolar and interstitial macrophages in MME+/+ mice. However, in MME/null mice, while there was an equal number of constitutive macrophages in the lung compared to MME+/+ mice, there was no significant additional recruitment in response to exposure to cigarette smoke.18

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interactions, with macrophage MMPs degrading α1-AT and NE degrading tissue inhibitors of metalloproteinases, each augmenting the proteolytic capacity of the other. In addition, NE may activate pro-MMPs into their active form. Finally, macrophages may be required to remove apoptotic neutrophils. Inefficient apoptosis leads to unopposed NE activity and emphysema. One cannot rule out the contribution of T-cells, eosinophils, mast cells, and structural cells of the lung.

**FUTURE UTILITY OF ANIMAL MODELS FOR COPD**

The usefulness of these studies in dissecting the pathogenesis of human disease is directly related to the similarities between human and mouse pathogenesis. Ultimately, as we learn more about mouse and human biology, differences may be as informative as similarities in determining biological pathways. With respect to emphysema, the mouse and human airspaces are quite similar. Mice have less airway branching and lack respiratory bronchioles. Nevertheless, in response to long-term cigarette smoke exposure, several strains of mice develop macrophage-predominant inflammation and airspace enlargement that is similar to those found in humans. Other models, such as elastase instillation, remain quick and useful, especially to begin to identify mechanisms of alveolar repair.

In the future, analysis of murine physiology would be of great assistance in assessing the mouse lesion. In addition, advances in imaging procedures such as CT scans, MRI, positron emission tomography scans, and, ultimately, optical coherence tomography will help to evaluate the pathologic and physiologic changes associated with COPD in mice. The ultimate goal is to utilize the knowledge gained from animal models in treating the many patients who have COPD.

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A Role for Collagenase (Matrix Metalloproteinase-1) in Pulmonary Emphysema*

S. Dalal, PhD; K. Inai, DMD, PhD; B. Mercer; Y. Okada, MD, PhD; K. Chada, PhD; and Jeanine M. D’Armiento, MD, PhD

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Abbreviations: dpc = days postcoitum; MMP = matrix metalloproteinase; RT-PCR = reverse transcriptase-polymerase chain reaction

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