THE PATHOPHYSIOLOGY OF COPD:
WHAT GOES WRONG AND WHY?

Steven Shapiro, MD†

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a growing worldwide problem. Although several effective therapies exist to prevent exacerbations and improve quality of life in these patients, there are no therapies to halt the devastating and progressive loss of lung function in COPD. While COPD is clearly a disease with a major cause—cigarette smoking—research now demonstrates this single cause is merely the entry point to a bewildering array of potential pathophysiologic pathways in the lungs. Not all COPD cases are alike. Many patients, for example, carry unique genetic susceptibilities to COPD-associated lung damage, and several specific genetic loci have been linked to an increased risk of early-onset COPD. In addition, several proteases, including the classic neutrophil elastase, and several matrix metalloproteinases are overexpressed in association with human COPD. Animal studies involving mice lacking the ability to produce these key proteases indicate these elastolytic enzymes may be released by macrophages and neutrophils in response to cigarette-smoke exposure. In addition to degrading extracellular matrix, these proteases may also lead to increased recruitment of inflammatory cells to the lung and cause fibrosis in small airways. Protease inhibitors may eventually be useful in halting the progression of emphysema. However, data showing multiple interactions of inflammatory cells and proteins in COPD emphasize the need for more animal research to untangle the underlying pathophysiology of this complex and heterogeneous disease. The 4 opportunistic cerebral mycoses that occur in Europe are cryptococcosis, which is the most frequent, and the rarer zygomycosis, candidiasis, and aspergillosis. Whereas the clinical picture and treatment of cryptococcosis are well known, management of the rarer fungal infections of the brain and central nervous system is also clinically challenging.


SMOKING CAUSES COPD, SO WHY BOTHER WITH PATHOPHYSIOLOGY?

Increased understanding of the pathogenesis of chronic obstructive pulmonary disease (COPD) will speed development of targeted and rational therapies for this heterogeneous and widespread disease. Already the fourth most common cause of death in the United States, COPD also is a leading contributor to disability and hospitalizations in developed countries. A COPD health crisis is looming in underdeveloped countries where the data are scant—but where rates of cigarette smoking continue to rise.

But why should busy clinicians care about the complex pathogenetic pathways leading from inhaled smoke to the reduced airflow and inflammation that characterize COPD? Both the global epidemiologist and the busy primary care physician accept the paramount importance of smoking cessation in current COPD....
prevention and treatment strategies. And although several treatments will limit exacerbations, none of the existing COPD medications modify the long-term decline in lung function. So why bother with pathophysiology?

Obviously, the ultimate goal of COPD pathophysiology research is to find interventions that do alter COPD decline. While several new therapies are on the horizon, research to date indicates that COPD is a complex multigenic disease not likely to yield to any single therapy. Emerging insights into the assortment of cells and cytokines involved in COPD lung inflammation and tissue destruction are helping researchers understand how both current and future interventions could at least reduce symptoms, limit the severity of exacerbations, and greatly improve the quality of life for millions of patients. Thus, an overview of COPD pathophysiology may interest clinicians wondering how the next generation of COPD therapies will work as well as practitioners struggling to optimize current COPD therapies for today's patients.

Identification of genetic susceptibility may also allow clinicians to counsel subsets of patients more intensively on the need to abstain from or to quit cigarette-smoke exposure. While smoking cessation is clearly the most important intervention goal, smoking cessation—even when prompted by evidence of genetic risk—is extremely difficult for patients. Those patients who do manage to quit smoking for a while, even after the diagnosis of a certain degree of disease severity, inflammation, and destruction, are likely to resume smoking.

A review of COPD pathophysiology may also help clinicians respond to that patient who asks why his "Uncle Charlie," who smoked a pack a day since he was a teenager, lived to be a very spry 97 years old, or why "Aunt Jane" and many people on her side of the family have severe early onset-COPD despite histories of moderate smoking. Reports show that a minority of smokers (the estimates range from about 15% to 40%) develop COPD, which strongly suggests differences in individual susceptibility (Figure 1). Are some patients naturally protected from COPD? This question is the starting point for researchers exploring the genetic and environmental interactions of COPD.

**Loss of Elastin, A Key Structural Protein in the Lung**

This article will explore several studies of COPD genetic susceptibility and then focus on the suspected pathophysiologic roles of inflammation, extracellular matrix proteins, proteases, and protease inhibitors. COPD inflammation research's origins are in the 1960's classic elastase:antielastase hypothesis that the balance between proteases and their inhibitors determined whether the lung was resistant or susceptible to airspace enlargement. The loss of elastin, due to gene-induced or smoking-induced loss of antielastase, causes collapse or narrowing of the smallest air passages and destruction and enlargement of alveoli.

The modern version of this hypothesis proposes that inflammatory cells (such as macrophages, neutrophils, and T cells) are recruited to the site of smoking injury where they produce a host of proteases that overwhelm the antiproteases and lead to matrix destruction and airspace enlargement and—in the absence of repair—clinical disease (Figure 2). The proteases play a direct role in destroying the lung matrix and apparently regulate the ongoing movement and activity of inflammatory cells in the airways and the alveoli. Somewhere within this ramping up of inflammatory activity, genes are thought to apply their leverage and increase COPD risk in certain patients. This theory can now be explored with animal studies.

**Figure 1. Natural History of COPD**

<table>
<thead>
<tr>
<th>FEV₁ = forced expiratory volume in 1 second.</th>
<th>Non-susceptible Smoker or Nonsmoker</th>
<th>Quit at age 45</th>
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<tbody>
<tr>
<td>Symptoms</td>
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<td>Disability</td>
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<tr>
<th>Age (years)</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-susceptible Smoker or Nonsmoker</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Susceptible Smoker</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
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involving genetically manipulated mice lacking 1 or more protease or inflammatory factor.

**Genetic Susceptibility**

Today, the only well-established genetic risk factor for COPD is a deficiency in the serum protease inhibitor called alpha1-antitrypsin (or alpha1-protease inhibitor). Patients lacking this endogenous enzyme are prone to an unopposed proteolytic destruction of the lung parenchyma, predisposing them to development of characteristic emphysema at an early age. Also, installation of proteolytic enzymes directly into the animal lung (without the inhibitor) reliably produces emphysema. However, only 1% to 2% of patients with emphysema have congenital alpha1-antitrypsin deficiency, so scientists are searching for other genetic risk factors that might interact with cigarette smoking to cause COPD.

For example, Silverman et al have identified families in New England with a history of cigarette smoking and severe early-onset COPD that is unrelated to alpha1-antitrypsin deficiency. First-degree relatives of individuals with early-onset COPD had decreased lung function. This familial aggregation pattern suggested that interactions between gene and environment (e.g., cigarette smoke) lead to COPD.

Silverman’s group then conducted a laboratory analysis of the distribution of DNA markers within these families to determine if a particular region of the genome was linked to the COPD phenotype. In this linkage analysis, the researchers evaluated the genome of 585 subjects, including 72 early-onset COPD probands, 320 first-degree relatives (48 parents, 147 siblings, and 125 children), and 162 other relatives. The LOD score, a statistical measure of linkage strength, was used to estimate the likelihood of a genetic susceptibility to COPD on a particular stretch of a chromosome. The scientists first confirmed there was a significant decline in lung function in the 287 first-degree relatives who smoked versus 48 control subjects who smoked (FEV1/FVC [% predicted 84.0 vs 94.3, P < .01]; FEV1 [% predicted] 77.0 versus 89.2, P < .02). A scan for short-tandem repeat polymorphic markers showed the strongest evidence for linkage at chromosomes 12 (LOD = 1.70) and 19 (LOD = 1.54) for moderate airflow obstruction, and at chromosomes 8 (LOD = 1.36) and 19 (LOD = 1.09) for mild airflow obstruction. When the analysis was limited to subjects who smoked, the LOD scores were even higher. Finally, when the researchers genotyped specific markers on chromosome 12, they found specific loci with LOD scores above 2 and 3—strongly suggesting a locus on chromosome 12p that contributes to susceptibility to early-onset COPD.7

These markers on chromosome 12p likely contain hundreds of genes potentially linked to COPD-related phenotypes. Association studies have uncovered several such candidate gene products, including those shown in the Table and others, including alpha1-antichymotrypsin, microsomal epoxide hydrolase, tumor necrosis factor (TNF)-alpha, cystic fibrosis transmembrane conductance regulator, ABH secretor, and vitamin D binding proteins.8 Detailed genetic mapping of all these candidates would be a way to identify the COPD genes but would require a huge investment (or tremendous luck). Thus, another complementary approach for identifying candidate genes involves animal studies of genomic physiology. By manipulating animal genes, controlled in vivo experiments can be designed to test the importance of the...
gain in gene function (ie, transgenic mice) or the loss of gene function (ie, gene targeting or “knockouts”). When tested in a disease model that simulates smoking, these animal experiments have been extremely useful in dissecting the pathogenesis of human COPD.

In a murine model of smoke-related COPD, for example, smoking-chamber exposure to the equivalent of 2 cigarettes per day leads to carboxyhemoglobin levels of 10% to 14% in mice. In addition, these mice display airway changes highly characteristic of COPD, including goblet cell hypertrophy, upper airway epithelial changes, loss of cilia, airspace enlargement, and inflammatory changes (eg, acute influx of neutrophils followed later by T cells and macrophages). Although mice provide only a simple model for a complex human disease, animal models remain a critical experimental tool in the search for the mechanisms of genetic susceptibility. By applying the knockout mice strategy to this model, the molecular basis for COPD susceptibilities in different strains of mice can be identified, providing a powerful means of “teasing out” the COPD disease pathogenesis.

INFLAMMATION AND PROTEOLYTIC DESTRUCTION IN COPD

Work in animal models verifies that neutrophils are acutely elevated in the hours following exposure to cigarette smoke. Over the next week or so following cigarette-smoke exposure, the number of CD+8 T-cells and macrophages increase in the airway walls, alveolar compartments, and vascular smooth muscle. Saetta et al have documented many of these same changes in humans. Other cells such as eosinophils, mast cells, and dendritic cells may also be involved.

What sparks and then sustains lung inflammation is still uncertain, but 2 types of lung chemokines may play a role in initiating inflammation following exposure to smoke. For example, CXC chemokines such as interleukin-8 can lead to neutrophil chemotaxis while the CC chemokines may stimulate monocyte migration. Meanwhile, proteins such as transforming growth factor (TGF)-beta may suppress the inflammation. Another intriguing question related to inflammation in smokers is how the inflammatory process can continue for extended periods after individuals with severe disease have quit smoking cigarettes. Some researchers have hypothesized that the inflammation is sustained by protease-induced elastin fragments, while others have proposed a latent adenovirus infection or bacterial colonization as the cause of sustained inflammation.

While early work on COPD-related inflammatory proteases focused on neutrophil elastase (NE), recent work indicates the macrophage and neutrophil also produce large quantities of other elastolytic proteinases (Figure 3). The neutrophil’s primary azurophil granules, for instance, contain serine proteinases, cathepsin G, and proteinase 3, while specific granules contain matrix metalloproteinases (MMPs) such as neutrophil collagenase (MMP-8) and gelatinase B (MMP-9). Researchers also are studying alveolar macrophages—known to be the predominant inflammatory cell in the lower airspace of patients with COPD—and their ability to produce MMP and cysteine proteinases. Some of these MMPs are elastolytic (eg, MMP-9 and MMP-12, also known as macrophage elastase), but their relative contributions to human emphysema are uncertain. Since many other cell types are capable of producing MMPs, the difficulty in sorting the roles of these inflammatory cell proteinases and the need for further animal testing is clear.

DECIPHERING THE ROLE OF MATRIX METALLOPROTEINASES

The MMPs have become a focus of inflammation research. This large family of matrix-degrading enzymes appears to be essential in development, tissue remodeling, and repair. Abnormal expression of MMPs has been associated with tumor cell progression, arthritis, pulmonary emphysema, and many other clinical conditions. Final publication of the Table. Candidate Genes for COPD

<table>
<thead>
<tr>
<th>Chromosome 12p:</th>
<th>MGST1, MGP</th>
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<tr>
<td>Chromosome 19p:</td>
<td>Leukotriene B4 omega-hydrolase (CYP4F2), Tropelastin</td>
</tr>
<tr>
<td>Chromosome 22q:</td>
<td>Heme oxygenase 1, TIMP3</td>
</tr>
<tr>
<td>Chromosome 2q:</td>
<td>IL8 Receptor A and B</td>
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IL8= interleukin-8
human genome revealed there are 23 human MMPs, with a shared capacity to degrade extracellular matrix and nonmatrix cell components. The MMPs are also structurally similar with a highly conserved and characteristic catalytic site at which zinc plays a critical on-off role. The MMPs are inhibited by a group of 4 tissue inhibitors of metalloproteinases (TIMPs).

Macrophage elastase, also known as MMP-12, is a macrophage-specific MMP with a broad spectrum of activity. Evaluation of archival human lung biopsies reveals MMP-12 expression in the lungs of patients who had stopped smoking many years previously. In the recent Lung Health Study, researchers also showed those patients whose lung function declined fastest tended to have polymorphisms in both MMP-1 (interstitial collagenase) and MMP-12 (P=0.0007).

Expanding on this human evidence that MMP-12 might contribute to loss of lung function, studies with knockout mice report that animals lacking the MMP-12 gene will grow and develop normally but will have a markedly diminished capacity to degrade extracellular matrix components and cannot penetrate simple basement membranes. What happens when these MMP-12 knockouts are exposed to cigarette smoke? In contrast to the nonknockouts, they do not develop chronic emphysema. Interestingly, the knockouts also fail to attract monocytes from the blood to the lung in response to the cigarette smoke. Thus, macrophage elastase appears to have a dual role in lung destruction. Apparently, the cigarette smoke induces constitutive macrophages (ie, those already in the lung) to produce MMP-12, which cleaves elastin to initiate the initial lung damage. But the fragments from the lung elastin are also chemotactic for monocytes, attracting more of these inflammatory precursor cells into the lung where they become activated and produce additional MMP-12, thus perpetuating a cycle of lung damage (Figure 4). This in vivo work supports the idea of a chemotactic gradient for monocytes created by the elastin fragments first proposed over 20 years ago.

Several other proteinases and inflammatory mediators, including interleukin-13, interferon gamma, surfactant protein D, and alpha V beta 6, may play a role in emphysema. The interactions of these mediators within animal models of COPD can be complex. For example, Sheppard et al recently demonstrated that mice missing the integrin alpha V beta 6 gene had a 100-fold increase in gene expression of MMP-12 (as compared to wild-type mice) as well as spontaneous emphysema. Sheppard theorizes that alpha V beta 6 binds and activates TGF-beta, known to inhibit MMP-12. Thus, the integrin knockouts have inactive TGF-beta and highly active MMP-12 and develop emphysema. However, when crossed with mice either overexpressing TGF-beta or underexpressing MMP-12, the emphysema does not develop.
Whether such interactions occur in humans is far from certain, but the uncertainty has not prevented researchers from testing MMP inhibitors-most were developed originally for anticancer applications-in animal models of lung macrophage recruitment and emphysema. Results with at least 1 company's MMP inhibitors have demonstrated that mice placed in smoking chambers for a 6-month period are protected from significant airspace enlargement. Initiating the MMP inhibitor after 3 months of smoking prevented the progressive enlargement seen in smoke-exposed controls.²⁴

Further work with MMP knockouts and inhibitors is warranted not only to identify potential clinical interventions but also to understand basic lung development and repair mechanisms. Obviously, a potently destructive enzyme such as MMP-12 is not produced in order to destroy the lung. The probable physiologic roles are inhibiting tumor progression and killing bacteria. Although different portions of the molecule provide these different functions (eg, the antimicrobial active site has nothing to do with the catalytic site), the broad actions of these MMPs dictate a careful and methodical approach in clinical development of MMP inhibitors. For example, while some MMPs may promote tumor progression, others generate angiogenic molecules of angiotensin and endostatin. In both cancer and emphysema research, more animal research is necessary to identify and exploit areas of highest clinical potential.

Our recent work in human COPD lung tissue also shows that eosinophils increase production of MMP-9. Animal studies involving MMP-9 knockout mice indicate that exposure to this protease is associated with pathologies such as atherosclerosis, aortic aneurysms, and bullous pemphigoid—but not emphysema. The animals capable of producing MMP-9 also developed subepithelial fibrosis (excess collagen) in the small airway in response to cigarette smoke while the MMP-9 knockouts did not. A possible explanation for this seemingly counterintuitive MMP mechanism (aren't proteases supposed to degrade matrix?) is that proteases such as MMPs activate latent TGF-beta or other profibrotic factors, or they promote a direct myofibroblast transformation and migration. Such protease-stimulated fibrosis could contribute to the obstruction and loss of elastic recoil in the small airway associated with cigarette smoking-related COPD.³¹

**Neutrophil Elastase**

Neutrophil elastase (NE) is the classic protease associated with emphysema. This potent elastase is abundant in the intracellular environment, with over 1 mg produced each day and huge concentrations residing within the primary neutrophil granules. This proteolytic enzyme is probably meant to stay within the cell. The high levels of alpha1-antitrypsin typically seen outside the cell very likely serve as a protective hedge against release of overexpressed NE.

Knockout mice lacking the NE gene grow and develop normally but also are protected against development of blisters related to autoimmune bullous pemphigoid,²⁵ against arthritis,²⁶ and against bleomycin-induced fibrotic lung injury.²⁷ Before focusing on the potential role of NE in emphysema, it may be useful to recall the presumed physiologic role of this ubiquitous elastolytic agent: antimicrobial activity. Intracellular NE is highly bactericidal against gram-negative organisms, such as in degrading the outer membrane proteins of gram-negative organisms and also some toxins (eg, from shigella)²⁸,²⁹. Researchers also have shown NE to have antifungal activity.³⁰ Thus, while the overall antimicrobial role of NE seems clear (eg, NE knockouts cannot fight off certain infections), the implications of this animal activity for NE and NE inhibitors in humans are vague. Human neutrophils, for example, have other means of defense (eg, defensins) as compared to mice. Thus, as with MMP inhibitors, more animal work with NE-specific inhibitors is required before advances in clinical testing for COPD can begin.

In tests of airspace enlargement following 6 months of exposure to cigarette smoke in knockout mice, we found that NE provided approximately 60% protection. The current hypothesis attributes this NE activity to cleaving of elastin and extracellular matrix and to recruitment of neutrophils and monocytes as well as to degradation of both TIMP-1 and the proform of MMP-12. Meanwhile, according to the evolving inflammatory model of emphysema, the macrophage simultaneously releases MMP-12, which also cleaves elastin and attracts more macrophages. In other words, aberrant expression of MMPs and NE may account both for the tissue destruction by degradation of extracellular matrix (ie, emphysema) and for the alteration of nonextracellular components (ie, fibroproliferation).
This review has highlighted a few of the innate effector molecules elaborated by the neutrophil and the macrophage that may contribute to COPD. The number of endogenous molecules with known lung activity increases daily, and the web of their potential interactions has become exceedingly complex. Understanding these complexities will provide clinicians with new tools to deal with the worldwide epidemic of COPD.

Conclusions

The traditional inflammatory model of COPD proposes that (1) cigarette smoke leads to inflammation, (2) inflammation leads to loss of the matrix, (3) without matrix for attachment, epithelial cells disappear, (4) then the entire alveolar unit disappears, and (5) the airspace is enlarged, as confirmed clinically and histopathologically. Alternative theories of COPD involving a noninflammatory-initiating event related to apoptosis have also been proposed.

It may be years or decades before we learn what cigarette smoke initiates the progressive chain reaction of lung damage now recognized as COPD. Whatever the initial trigger, researchers have already identified several of the key inflammatory cells and proteases involved in the active destruction and accumulation of the lung's extracellular matrix. For example, when MMP-12 and NE are overexpressed by the macrophage and by the neutrophil, lung damage characteristic of COPD advances rapidly. Proteinase inhibitors, while perhaps not capable of curing the disease, may soon be able to aid in halting the inflammation-driven progression of the disease. Improved genetic testing may soon assist clinicians in prescreening patients with COPD for more rational therapies aimed at each patient's specific pathophysiology. Ongoing basic research is necessary to define the exact mechanisms of alveolar development and repair and to lay the groundwork for even better treatments.

Discussion

Dr. Wise: Even for those diseases where we have identified a single causative gene—for example, phenylketonuria or alpha1-antitrypsin deficiency—we still struggle to develop targeted cures. How can we ever hope to create a cure for a complex disease such as COPD if, as the research seems to indicate, it is caused by a whole array of genes?

Dr. Shapiro: At this point, it's true that we're not sure where the gene studies are taking us. Certainly the hope is that identifying gene deficiencies—for metalloproteases in COPD or as with the recent discovery of ADAM-33 in asthma—will allow us to define disease pathways and eventually lead us to pharmacologic therapies we had not previously considered. Also, the genetic susceptibility studies by epidemiologists such as Silverman and others may eventually help in screening patients for risk. While we may still lack a treatment for these high-risk patients, perhaps the information could help to make a stronger case for smoking cessation. You are correct that both treatment and screening are more problematic in a multigenic disease such as COPD.

Dr. Tashkin: We know that many patients with advanced COPD have more small airway involvement than patients with emphysema. Did any of your wild-type mice in the smoking chambers develop small airway fibrotic changes in the absence of alveolar enlargement?

Dr. Shapiro: There are some strains of mice prone to the small airway fibrosis. In some cases, these are the same strains that have a tendency toward emphysema, but not always. The MMPs are proteins that could cause both the fibrosis and emphysema, but they could be biochemically separable events. It will be interesting to separate these in upcoming experiments.

Dr. Criner: Do any of your animal data support the old belief that people who have emphysema are also subject to extrapulmonary diseases?

Dr. Shapiro: In other labs, there have been reports of some systemic effect of cigarette smoke, for example in aortic aneurysms. We have done echocardiograms in the mice and did not see any significant cardiovascular changes, but the systemic changes have not been our focus. Also, our mice have rather mild emphysema. As the emphysema becomes more severe, systemic manifestations might become obvious.

Dr. Wise: Do exacerbations contribute to the progression of COPD?

Dr. Tashkin: There is some evidence of an association between exacerbations and rapid COPD progression. It is an old hypothesis that the COPD advances in stepwise decrements during exacerbations, but it's a hypothesis receiving renewed interest. If inflammation
causes these exacerbations, then inflammation may also affect the course of disease.

Dr Shapiro: And that should be something we could build into our animal models—for example, by causing airway infections in mice exposed to cigarette smoke.

REFERENCES


