Dysfunctional lung anatomy and small airways degeneration in COPD

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Abstract: Chronic obstructive pulmonary disease (COPD) is characterized by incompletely reversible airflow obstruction. Direct measurement of airways resistance using invasive techniques has revealed that the site of obstruction is located in the small conducting airways, i.e., bronchioles with a diameter < 2 mm. Anatomical changes in these airways include structural abnormalities of the conducting airways (e.g., peribronchiolar fibrosis, mucus plugging) and loss of alveolar attachments due to emphysema, which result in destabilization of these airways related to reduced elastic recoil. The relative contribution of structural abnormalities in small conducting airways and emphysema has been a matter of much debate. The present article reviews anatomical changes and inflammatory mechanisms in small conducting airways and in the adjacent lung parenchyma, with a special focus on recent anatomical and imaging data suggesting that the initial event takes place in the small conducting airways and results in a dramatic reduction in the number of airways, together with a reduction in the cross-sectional area of remaining airways. Implications of these findings for the development of novel therapies are briefly discussed.

Keywords: emphysema, small airways disease, airway mucus, innate immunity, adaptive immunity

Introduction
Chronic obstructive pulmonary disease (COPD) is characterized by persistent and usually progressive airflow limitation. In genetically susceptible individuals, inhaled noxious particles and gases induce an enhanced inflammatory response in the airways and result in structural changes (a process often referred to as “remodeling”) in airways and in lung parenchyma. Although COPD is associated with exacerbations and comorbidities that contribute to the overall severity in selected patients, the present article focuses on structural abnormalities and inflammation in the lung compartment under stable conditions. Our goal is to examine potential mechanisms leading to these structural abnormalities at the site of airflow limitation, i.e., the small conducting airways. Importantly, most of the data presented here were obtained in (ex) smokers. These data are relevant to the pathophysiology of cigarette smoke-induced COPD, but it is unclear at this time whether they also apply to COPD triggered by other environmental exposures (e.g., exposure to biomass).

Anatomical considerations
In humans, the lower conducting airways arise from the trachea and divide into 8–25 generations, depending on the length of the pathway followed down to
the terminal bronchioles (the smallest airways without
alveoli) and respiratory bronchioles, which open into the
gas-exchange apparatus (the alveoli). Small (conducting)
airways are usually defined as airways without cartilage
and with an internal diameter < 2 mm. These airways are
located from approximately the eighth generation of airway
to the respiratory bronchioles. In normal individuals, the
cross-sectional area of the airways increases rapidly from a
total of 2.5 cm² in the trachea to approximately 180 cm² at
the level of the terminal bronchioles. Resistance to airflow
in a tube (or an airway) varies inversely with the fourth power
of the radius, and resistance to airflow in parallel tubes (or
in branching airways) varies inversely with the fourth power
of the total cross-sectional area. These calculations explain
why most of the resistance to airflow in healthy humans is
located in the proximal airways (above the sixth division),
and why small conducting airways account for less than 10%
of airway resistance.

Site of airflow limitation: a long search for small airways

Direct measurement of airways resistance in small airways
using the wedge bronchoscope technique has revealed that
small airways resistance is multiplied by 4-40 in patients
with COPD, indicating that small conducting airways are the
main site of airflow limitation in patients with the disease.²,³
Although some of the relevant experiments were reported
more than 40 years ago,⁴ understanding of the mechanisms
of increased small airways resistance in patients with COPD
has been relatively slow. Measurement of small airways
dysfunction in living individuals remains a challenge, and
progress in this area has been impeded by technical limita-
tions related to the small size of these airways and to their
location deep within the thoracic cavity. Firstly, because
obstruction of a single small airway results in very little
change in the cross-sectional area of all small airways, abnor-
malities in numerous small airways may occur without any
change in conventional pulmonary function tests, eg, forced
expiratory volume in one second (FEV₁).⁵ Secondly, the
spatial resolution on computed tomography (CT) scan (the
most widely available lung tool to examine lung anatomy)
is around 0.6–1 mm, which allows direct assessment of
medium-sized airways (diameter > 2–2.5 mm), but not of
smaller airways. Thus, investigators have had to rely on
indirect assessment of the small airways by measuring areas
of mosaic lung attenuation or air trapping (on inspiratory and
expiratory CT scans, respectively) or by using sophisticated
physiological measurements (eg, nitrogen washout tests,
impulse oscillometry) of small airways dysfunction. These
indirect measurements have some usefulness in the clinical
setting, but have failed to provide significant insight into the
mechanisms of small airways dysfunction. Most progress in
this area has been made using pathological information and
more recently using very high-resolution imaging techniques
(eg, micro-CT) ex vivo.

Anatomical changes in small conducting airways

Small conducting airways appear to be the site of airflow
limitation in COPD, and the increase in expiratory resistance
to airflow implies a reduction in the total cross-sectional
area of the small airways. Theoretically, the reduction in
cross-sectional area of the small airways may be related to a
decrease in the number of small airways and/or a reduction in
the cross-sectional area of a large number of individual small
airways. Based on these calculations, it appears important to
examine both the number of small airways and the structural
abnormalities in the small airways of patients with COPD.
The number of small airways in the lungs of patients with
COPD has proven difficult to establish because principles of
unbiased stereology indicate that counting numbers of three-
dimensional structures requires knowledge of the reference
volume of the tissue analyzed,⁶ which is difficult to determine
using histological studies. Progress in lung imaging allows
determination of lung volume from volumetric high-resolution
CT scans. High-resolution CT also allows visualization
and reconstruction of the airways, but its spatial resolution
remains inappropriate for analysis of the small airways (see
above). Investigators have used high-resolution CT scans to
examine the number of medium-sized airways (as a surrogate
marker for small-sized airways) in patients with COPD.⁹,¹⁰
McDonough et al studied 58 high-resolution CT scans from
patients with COPD GOLD (Global Initiative for Chronic
Obstructive Lung Disease) stage 1–4 and compared them
with those of 20 smokers with normal lung function matched
for age and smoking history.¹⁰ They found reduced numbers
of medium-sized airways (internal diameter 2–2.5 mm) in
patients with COPD GOLD stage 1 and 2 as compared with
smokers who have normal lung function. The reduction in
numbers of medium-sized airways was even more pronounced
in GOLD stage 3 and 4 patients.¹⁰ These data suggested that
destruction of the small conducting airways is an important
feature in patients with COPD, although it is still possible that
medium-sized airways had undergone shrinkage and could not
be identified on CT scans due to their smaller size. Micro-CT
is an imaging technique with higher spatial resolution than
Emphysema

Emphysema is defined by “abnormal, permanent enlargement of airspace distal to the terminal bronchiole, accompanied by the destruction of their walls, and without obvious fibrosis.” Destruction of the alveolar wall has long been assumed to result from proteolytic degradation due to an altered protease/antiprotease imbalance. This concept was based on the description of emphysema in alpha1-antitrypsin deficiency and on development of animal models of emphysema through intratracheal instillation of proteases (pancreatic or neutrophil elastase). In recent years, the concept has emerged that destruction of the alveolar wall may also occur as a result of a failure of lung maintenance and repair programs, which are required in response to repeated injury caused by cigarette smoke. Thus, increased levels of apoptosis and proliferation of alveolar epithelial cells have been reported in patients with emphysema. The ability of alveolar cells to proliferate is a limited process, because repeated cell cycles may cause senescence, which has been reported in both epithelial and endothelial cells in the alveoli of patients with emphysema. A possible contribution of autoimmunity to the pathophysiology of emphysema has been suggested. Lee et al reported that an autoimmune response to elastin fragment involving T and B cell-mediated immunity against elastin was associated with emphysema. However, other studies did not find evidence for an autoimmune response against elastin. At this point, it is unclear whether autoimmunity is implicated in the pathogenesis of emphysema. Representative photomicrographs of small airway abnormalities and emphysema are presented in Figure 1.

Relationship between small airway abnormalities and emphysema

The classic understanding of the relationship between small airways disease and emphysema involves loss of alveolar attachment, which destabilizes the bronchioles due to loss of elastic recoil. In this scenario, alveolar destruction comes first and promotes premature airway closure during expiration. However, the sequence of events that leads from smoking exposure to structural abnormalities in the small conducting airways and adjacent alveoli that are found in patients with COPD remains speculative. An interesting hypothesis, which had been presented recently, suggests that narrowing and disappearance of small conducting airways occurs before the onset of emphysematous destruction. In this model, emphysema may result from loss of support at the distal acinus by terminal bronchioles, leading to collapse and folding of the alveolar walls. This hypothesis is based on histological data presented by McDonough et al; when these authors examined the numbers and thickness of terminal bronchioles in relation to the mean linear intercept (a marker of emphysema), they found that before emphysema can be detected microscopically, terminal bronchioles are...
reduced in numbers and those that remain had thickened airway walls.\textsuperscript{10}

A recent study by Galban et al examined high-resolution CT scans in a cohort of 194 subjects with COPD using parametric response mapping (PRM), a new voxel-wise image analysis technique, that enables differentiation of functional small airways disease (fSAD) from emphysema by analyzing individual voxels in inspiratory and expiratory CT scans.\textsuperscript{31} The authors found that PRM\textsuperscript{fSAD} can be detected in subjects with minimal emphysema, and that both emphysema and

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Representative photomicrograph of small airways disease and emphysema in a patient with chronic obstructive pulmonary disease.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Diagram depicting potential mechanisms leading to airflow limitation via structural abnormalities in the small conducting airways and alveoli.}
\end{figure}
fSAD contribute to airflow limitation in subjects with severe airflow limitation.\textsuperscript{31} Although both studies point toward the same attractive hypothesis, they had a cross-sectional design and the suggestion that small airways disease occurs before emphysematous destruction will require confirmation obtained in longitudinal studies. Although the pathological correlates of PRM\textsuperscript{fSAD} will need to be established, the use of PRM may allow for such a study design in living individuals.

**Inflammatory cells and mediators: relationship with structural abnormalities**

Cigarette smoke triggers an inflammatory response in the airway epithelium of the proximal and distal bronchi, in the alveoli, and in the pulmonary arteries.\textsuperscript{32} Recruitment of inflammatory cells in the airways and alveoli involves the secretion of chemokines by resident cells (eg, epithelial cells and alveolar macrophages) in response to cigarette smoke.\textsuperscript{33} Initial studies have identified phagocytes (eg, neutrophils and macrophages), which are part of the innate immune system, in the small airways and alveoli of both smokers and COPD subjects.\textsuperscript{34} The contribution of the adaptive immune system to the pathogenesis of COPD was later described.\textsuperscript{35} In particular, CD4\textsuperscript{+} and CD8\textsuperscript{+} T lymphocytes were found in small conducting airways and in the alveoli,\textsuperscript{36-38} and tertiary lymphoid structures (also called lymphoid follicles) containing B and T lymphocytes were reported around the small airways in subjects with severe airflow limitation.\textsuperscript{11} Dendritic cells are antigen-presenting cells which link the innate and adaptive immune systems. Recent studies have revealed that dendritic cell numbers are increased in the epithelium and adventitia of small conducting airways in subjects with COPD\textsuperscript{39} and have suggested that dendritic cell maturation is associated with the severity of airflow limitation.\textsuperscript{40} Eosinophils have been found in the airways during exacerbations of COPD, especially in the context of viral infection.\textsuperscript{41} More recently, mast cells have been identified in small airways smooth muscle and in alveoli.\textsuperscript{42,43} Mechanisms of recruitment and activation of these inflammatory cells have been discussed elsewhere.\textsuperscript{33,35}

Airways inflammation and remodeling both occur in the lungs of patients with COPD, and it is likely that inflammation contributes to the structural abnormalities. However, evidence linking these processes remains scarce. Loss of alveolar attachment in small conducting airways is correlated with leukocyte recruitment, suggesting a role for proteases secreted by leukocytes in this process.\textsuperscript{44} CD8\textsuperscript{+} T cells\textsuperscript{45} and neutrophils\textsuperscript{31} have been correlated with goblet cell hyperplasia in small conducting airway epithelium, suggesting that these cells contribute to hypersecretion of mucus.\textsuperscript{46} CD8\textsuperscript{+} T cells were also found in the alveoli, where they were suggested to contribute to alveolar destruction via release of proteolytic enzymes (eg, granzymes and perforins).\textsuperscript{47} Increased numbers of mast cells have been found in the alveoli in centrilobular emphysema, but not in panlobular emphysema, suggesting a distinct role in the pathogenesis of centrilobular emphysema.\textsuperscript{48} These findings suggest that therapies targeting inflammatory cell recruitment and/or activation may result in improvement in structural abnormalities, but this concept will require confirmation when appropriate drugs become available for clinical trials.

**Conclusion**

Small airways with an internal diameter < 2 mm represent the site of airflow limitation in patients with COPD. Abnormalities in the small airways include airway wall thickening due to epithelial modifications and to peribronchiolar fibrosis, and obstruction of small airway lumina by mucus exudates. Recent studies indicate that the number of small airways is reduced in subjects with COPD and suggest that small airways destruction may occur before emphysema. The pathogenesis of emphysema includes a protease/antiprotease imbalance and failure of lung maintenance and repair programs that may involve apoptosis, senescence, and autoimmunity. Airway inflammation by phagocytes (neutrophils, macrophages) and lymphocytes (especially CD8\textsuperscript{+} T lymphocytes) likely contributes to structural abnormalities in small airways and emphysema.

**Implications for treatment**

The airflow limitation characteristic of COPD occurs as a result of structural abnormalities in the small conducting airways and alveoli. The potential mechanisms leading to airflow limitation via structural abnormalities in the small conducting airways and alveoli is shown in Figure 2. Recent data suggest that the initial event leading to airflow limitation occurs in the small conducting airways, where inflammatory cells are recruited. Remodeling of the small conducting airways presumably occurs as a repair mechanism after epithelial injury but results in destruction of small airways, thickening of the airway wall, and hypersecretion of mucus in the small airways, promoting airflow limitation. Emphysema also promotes airflow limitation due to loss of elastic recoil of the small airways, but recent data also suggest that small airways disease may promote emphysema. Although some of these findings will
require confirmation in future longitudinal studies, the current evidence indicates that therapeutic intervention should be initiated as early as possible to prevent destruction of small conducting airways and emphysema. Smoking cessation is obviously the first therapeutic intervention, but is insufficient alone due to a self-perpetuating inflammatory and remodeling process in the airways. The effects of current therapies (eg, bronchodilators and inhaled steroids) on structural abnormalities in patients with COPD are unclear, but presumably limited. It is likely that novel therapies aimed at reducing airway inflammation and remodeling should be developed. These therapies would be optimally delivered by inhalation, which may then limit systemic side effects. Conventional inhaled therapies may not deposit in small airways owing to their granulometry, being composed of 3–5 μm particles which deposit mostly in the large airways. Targeting distal airways in COPD may be achieved by systemic administration of drugs and/or by using extra-fine particles, which deposit in both the proximal and distal airways. Future studies aimed at developing treatments for lung disease in COPD should take these findings into consideration.

Acknowledgment
The authors thank Professor Diane Damotte from the Department of Pathology, Université Paris Descartes, for providing photomicrographs of lung sections from a patient with COPD.

Disclosure
The authors report no conflicts of interest in this work.

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