passive immunization with neutralizing antibodies, or exogenous IP-10/CXCL10 was administered to the animals during bleomycin exposure, both treatment strategies resulted in marked attenuation of pulmonary fibrosis that was entirely attributable to a reduction in angiogenesis in the lung.\textsuperscript{17,18} These findings support the notion that angiogenesis is a critical biological event that supports fibroplasia and deposition of ECM in the lung during pulmonary fibrosis, and that angiogenic and angiostatic factors, such as CXC chemokines, play an important role in the pathogenesis of this process.

We have recently shown that IL-12 attenuates bleomycin-induced pulmonary fibrosis via induction IFN-\(\gamma\).\textsuperscript{19} Moreover, the beneficial effects of IL-12 can be inhibited by simultaneous administration of anti–IFN-\(\gamma\) antibodies.\textsuperscript{19} These findings provide further support for IFN-\(\gamma\) and thereby the IFN-inducible chemokines, IP-10/CXCL10 and MIG/CXCL9, as inhibitors of fibrosis. With the recent demonstration of the efficacy of IFN-\(\gamma\)-treatment of patients with IPF,\textsuperscript{20} the above-mentioned studies substantiate that IFN-\(\gamma\)-treatment of IPF may mediate its effect, in part, by shifting the imbalance of the expression of ELR\(+\) and ELR\(-\) CXC chemokines to favor an angiostatic environment leading to inhibition of dysregulated neovascularization/vascular remodeling, fibroproliferation, and deposition of ECM in patients with IPF.

## Conclusion

Angiogenesis is regulated by an opposing balance of angiogenic and angiostatic factors. CXC chemokines comprise a unique cytokine family that contains members that exhibit on a structural-functional basis either angiogenic or angiostatic biological activity. The mentioned studies have demonstrated that as a family, the CXC chemokines appear to be important in the regulation of angiogenesis associated with the pathogenesis of chronic inflammatory/fibroproliferative disorders. These findings support the notion that therapy directed at either inhibition of angiogenic or augmentation of angiostatic CXC chemokines may be a novel approach in the treatment of chronic fibroproliferative disorders.

## References


## Animal Models of Cigarette Smoke-Induced COPD*

Joanne L. Wright, MD; and Andrew Churg, MD

Objectives: To review the animal models of COPD, and to compare these data to those found in humans.

Results: Smoke-induced animal models can produce emphysema, although the lesions are not generally close mimics of human emphysema, as well as increases in mucous-secreting cells and vascular changes including pulmonary hypertension. There is considerable species-to-species variation in the degree and/or

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presence of these different abnormalities, so that care has to be used in selecting a species to study. Remarkably little information is available about the biochemical and molecular changes induced by cigarette smoke in animal models. Conclusions: Great insights into the pathology of chronic obstructive lung disease have been made using various animal models.

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Key words: animal models; cigarette smoke; emphysema; inflammation

Abbreviations: IL = interleukin; Th = T helper

This review article describes the development of our knowledge of the structural changes found in COPD using animal models, and compares the results in the models to those studies of human smokers.

EMPHYSEMA

Emphysema Types

There is much literature detailing the descriptions of emphysematous lung destruction in cigarette smokers. In general, the majority of the emphysema produced by smoke is of the centriacinar type, although panacinar emphysema may occur in conjunction with centriacinar emphysema.

There is also no doubt that chronic exposure of animals to cigarette smoke will produce emphysema, as defined by the National Heart, Lung, and Blood Institute. The length of time required to produce emphysema varies from animal to animal, but it generally requires at least 4 months depending on methods of exposure and cigarette dose. Although short-term exposures do not produce destruction, they are sufficient to potentiate elastase-induced emphysema. The early chronic exposure dog models had identifiable macroscopic disruption with destruction of the lung parenchyma adjacent to the respiratory bronchioles. Analysis of the majority of other models, however, demonstrates enlargement of the alveoli and alveolar ducts, in a more diffuse fashion. The pattern of airspace enlargement does therefore have some of the features of centriacinar emphysema, but does not have the distinctive focal lesions of centrilobular. Progression of emphysema with continued smoke exposure has been suggested by our laboratory using guinea pigs, and by March and colleagues comparing mice and rats.

Alveolar Fenestrae (Pores, Holes)

Scanning electron microscopic examination of the lungs of human smokers has demonstrated an increased number of pores (holes), with many of the larger holes having a fenestrated appearance. Two cigarette smoke-induced lung disease models, one in beagles and the other in guinea pigs, have also described and/or morphometrically documented increased numbers of pores, with apparent coalescence into larger holes. Both studies also remarked on the presence of alveolar macrophages in association with the holes. It is interesting to speculate whether these macrophages are part of the destructive pathogenesis, or whether they are simply using the holes as a transit pathway.

Evidence for Collagen and Elastin Destruction

In humans, there appear to be alterations in both collagen and elastin. Subjects with all types of emphysema have an identifiable increase in fibrous tissue, usually adjacent to the emphysematous foci. Elastic fibers have been morphometrically examined in the lungs of human smokers, although no alterations were identified in mild emphysema. By contrast, elastin peptides have been shown to be increased in the lavage, plasma, and urine of smokers with emphysema or COPD. In addition, an electron microscopic study by Fukuda et al demonstrated vacuolation of elastic fibers, accumulations of amorphous fibers, and clusters of irregularly arranged amorphous elastin. Some of the earlier animal studies have described focal areas of pulmonary fibrosis, but it is difficult to interpret these claims because of the high degree of coincident infections and bronchiectasis in these animals. Two morphometric analyses of lung elastin demonstrated a reduction in the elastic fiber perimeter in one study, and decreased length per unit volume that occurred prior to identifiable airspace enlargement, and then worsening at subsequent time periods in our study. These data would suggest elastin fiber loss or rearrangement. A formal electron microscopic-based morphometric analysis was performed in the guinea pig model and found early collagen fiber loss, with an increase in collagen after longer-term exposures. In that study, we could not identify alterations in the volume proportion of elastic fibers, but we also could not exclude elastic fiber reorganization.

Usefulness of Transgenic Models

Genetic manipulations should provide a new methodology to examine cigarette smoke-induced emphysema. For example, Hattamaki et al utilized a matrix metalloelastase knockout construct. Other potentially useful constructs exist, and those that will allow for gene expression modulation (activation and/or turn off) will be able to avoid conflicting or confusing relationships secondary to gene actions during lung development.

ALTERATIONS IN BRONCHIAL GLANDS

In human smokers, the bronchial glands are enlarged. Although the Reid index was initially developed to provide a correlation with chronic bronchitis, further studies found significant overlap between patients with and without mucus hypersecretion. Morphometric analysis of the
The study by Hernandes et al. described peribronchial inflammation in guinea pigs. Rubio et al. did not mention inflammation; they used image analysis morphometric techniques to demonstrate thickening of the small airway walls, but they did not identify whether any single wall airway compartment was affected, or if the entire wall was altered. We know of no study that has examined the inflammatory cell subtypes in an animal model, but the majority of the animal models have described the presence of foamy, and often pigmented, macrophages in the alveoli adjacent to the airways.

Alterations of the Pulmonary Vasculature

In humans, 6% of subjects with COPD will acquire pulmonary hypertension and, ultimately, cor pulmonale. Morphometric examination of the pulmonary vascular tree has demonstrated intimal and medial thickening of the muscular arteries, and muscularization of the small arteries/arterioles, which appeared to have a progressive increase in severity from nonsmokers to smokers without COPD to smokers with COPD. To our knowledge, the only model in which the vasculature has been examined is the guinea pig. We found that exposure to smoke increased the mean pulmonary arterial pressure, but that there did not appear to be any correlation between severity of increase and duration of exposure. We noted a significant arteriolization of the normally poorly muscularized vessels adjacent to the alveolar ducts, but no apparent alteration of the muscularized vessels. Using a sub-tic technique, we determined that the muscularized vessels in chronic smoke-exposed animals were not more reactive to vasoconstrictive agents than those in the control groups. To examine the capillary network, we performed methycrylate casting, and demonstrated that the capillary rings were dilated in the smoke-exposed animals, but that there was no evidence of capillary destruction. These experiments suggest that the increase in pulmonary arterial pressure in smoke-exposed animals is due to alterations in the small arterial vessels, rather than either spasm of the muscular arteries or destruction of the capillary network.

Alterations of Cytokines

The cytokine profile in smokers has recently become of great interest to investigators, largely because of the differentiation of the cytokine secretion profiles by the subset of CD4 lymphocytes that become activated. These subsets have been termed T-helper (Th)-1 and Th-2, and each has its characteristic cytokine secretion pattern.

Inflammation of bronchi and bronchioles was occasionally described in the early studies, but its significance is uncertain because of the presence of bronchiectasis, pneumonia, and perhaps other common infections found in animal colonies. In the study by Hernandes et al. however, the inflammation was described in association with lung destruction at the level of the respiratory bronchioles. Heckman and Dalbey described perivascular and bronchiolar inflammation in smoke exposed rats, while March et al. noted similar lesions in mice and rat, and Selman and colleagues described peribronchial inflammation in guinea pigs. Rubio et al. did not mention inflammation; they used image analysis morphometric techniques to demonstrate thickening of the small airway walls, but they did not identify whether any single wall airway compartment was affected, or if the entire wall was altered. We know of no study that has examined the inflammatory cell subtypes in an animal model, but the majority of the animal models have described the presence of foamy, and often pigmented, macrophages in the alveoli adjacent to the airways.

Epithelial Cell Alterations

Goblet cell metaplasia is a frequent finding in the large and small airways of cigarette smokers, but it has been difficult to find consistent results, probably due to sampling difficulties because the metaplastic cells are not randomly distributed, but occur in clusters. Metaplasia is also a frequent feature in asthmatic large and small airways.

In animals, secretory cell metaplasia as a response to cigarette smoke appears to be of greater intensity than that found in humans. Workers have used dog, rat, hamster models and found an increase in the number of secretory cells with a shift toward acid rather than neutral mucopolysaccharides. In the trachea, the smoke effect is generally present at all levels with the exception of the very proximal 3 mm. Secretory cell metaplasia has also been identified in the small noncartilagenous conducting airways of the guinea pig and hamster.

Airway Wall Alterations

In humans, the structure of the larger airways has been examined, with evidence of muscle thickening, particularly in those subjects with airflow obstruction, although there was a wide degree of variation in any group examined and there may also be variation from site to site. In examining biopsy specimens from the larger airways of cigarette smokers, it appears that when both chronic bronchitis and airflow obstruction exists, there are increases in the overall T-cell population, with some studies also finding a direct or proportionate increase in the CD8 subset. The negative correlation between CD8 levels and FEV1. The small airways of the lung are markedly altered, and there is much literature describing these changes and their correlations with pulmonary function test abnormalities. Studies have also performed cell typing on the inflammatory cells, and have demonstrated an increase in CD8 T lymphocytes in patients with airflow obstruction, but no definitive effect of smoke alone on the cell populations. In the most distal airways, one of the earliest findings in the lungs of cigarette smokers are the so-called smokers’ macrophages that are characteristically present near the respiratory bronchioles in alveolar airspaces. We know of no animal studies that have examined the structure of the large airway walls in detail.
When bronchial biopsy samples were obtained in current and ex-smokers with chronic bronchitis, and compared with data from nonsmokers, there were increased numbers of interleukin (IL)-2 receptor-positive T lymphocytes, which persisted in the ex-smoker group in those subjects with continued symptoms of chronic bronchitis.\(^4\) Sun et al\(^5\) examined the BAL fluid from subjects with chronic bronchitis due to smoking. They found an increased number of CD8 lymphocytes in the smokers, with increased levels of IL-8, tumor necrosis factor-\(\alpha\) and IL-2. Interestingly, in a bronchial biopsy-based study, patients with chronic bronchitis\(^6\) showed only occasional immunohistochemical staining for IL-4 and tumor necrosis factor-\(\alpha\), a profile more in keeping with a Th-2 type of reaction. Therefore, although there is some evidence that suggests that cigarette smoke-induced lung disease is associated with a Th-1 type of cytokine profile, these data are not clear, and information correlating the cytokine profiles and morphometric alterations are lacking.

Animal studies present in the literature\(^49,50\) have concentrated on the effects of very short-term (up to 14 days) exposure. Although smoke induced an increase in the numbers of polymorphonuclear leukocytes in the airway walls, and polymorphonuclear leukocytes and eosinophils in the BAL, there were no increases of either CD4 or CD8 cells in the airway walls, and no increase in BAL lymphocytes. Smoke exposure did increase bulk lung IL-8 messenger RNA, although reactivity was found in the airspaces, suggesting that alveolar macrophages were the source of the cytokine.

**Evidence of Cell Proliferation or Induction of Apoptosis**

There are few data in humans in regards to the balance between cell proliferation and cellular apoptosis in cigarette smokers. Kasahara et al\(^51\) demonstrated an increase in apoptotic cells in the lungs of smokers with emphysema, while there was no difference between the numbers of cells in nonemphysema smokers and nonsmokers. Interestingly, however, Yasuda and colleagues\(^52\) measured plasma sFas, which is an inhibitor of apoptosis and found an increase in those subjects with severe COPD.

In animals, we have shown that smoke induces cell proliferation in airway epithelium and walls, and in the pulmonary vasculature.\(^53\) Kasahara et al\(^54\) was able to induce apoptosis and subsequent emphysema in rats with an inhibitor of vascular endothelial growth factor receptors, but to our knowledge no studies have investigated the role of apoptosis in cigarette smoke-induced emphysema.

**Systemic Effects**

Human smokers, particularly those with COPD, often have significant weight loss, and those patients who lose larger amounts of weight appear to have a greater mortality than those with stable weight.\(^55\) Almost all of the models that have evaluated this parameter have demonstrated a failure of smoke-exposed animals to gain weight.\(^5,9,29,40\) We know of no study that has been constructed to determine whether the animals actually lose weight. In our guinea pig model, the smoke-exposed animals weighed approximately 85% of the control animals, and similar values have been found in the other studies. Although starvation will produce enlarged airspaces, the weight loss necessary to do so is more profound than that seen in association with cigarette smoke exposure.

**Conclusion**

This article has reviewed data from a wide variety of animal models of cigarette smoke-induced lung disease, and compared these results to the information available from human smokers. There is a basic degree of similarity among the studies, although certainly they differ in the degree of abnormalities produced, and the length of exposure time and protocols used to induce the lesions. The lesions of the lung parenchyma are not an absolute mimic of human emphysema, except perhaps in the dog models. The small airway lesions are similar in regards to secretory cell metaplasia, although the reaction may actually be greater than the human counterpart. Although they have not been examined in detail, the airway wall changes appear to be much milder than those found in humans. Secretory cell metaplasia of the large airways appears similar to that in humans. There is, however, a large difference between humans and animals in alteration of the bronchial glands, although this may simply be an anatomy difference, in that rodents tend to only have bronchial glands in the very proximal tracheal segment. There is very little information that addresses the differences or similarities between humans and animals in regards to the effect of cigarette smoke on cytokine profiles, cell proliferation, and apoptosis.

Finally, there appear to be species differences and strain differences that must be taken into consideration when selecting an appropriate model. For instance, it appears that guinea pigs will acquire vascular alterations with smoke that are not found in standard rat models; it is possible, however, that this represents a dose effect rather than a species effect. Despite the above-mentioned differences, investigators will be able to obtain useful information about the pathology and pathogenesis of lung lesions induced by cigarette smoke by studying animal models. Each animal species has its own strengths and weaknesses, but investigators should be able to choose among them and select the model most appropriate to test hypotheses.

**References**

1. Thurlbeck WM, Wright JL. Thurlbeck’s chronic airflow obstruction. 2nd ed. Hamilton, ON: B.C. Decker, 1999
12 Wright JL. The importance of ultramicroscopic emphysema in cigarette smoke induced lung disease. Lung 2001; 179:71–81
29 Lamb D, Reid L. Goblet cell increase in rat bronchial epithelium after exposure to cigarette and cigar tobacco smoke. BMJ 2001; 1:33–35
33 O'Shaughnessy TC, Ansari TW, Barnes NC, et al. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CDS+T lymphocytes with FEV1,. Am J Respir Crit Care Med 1997; 155:852–857
Airway Wall Remodeling Induced by Occupational Mineral Dusts and Air Pollutant Particles*

Andrew Churg, MD, and Joanne L. Wright, MD

Objectives: COPD has been reported in workers exposed to particulates, and there is increasing evidence that high levels of ambient particulate pollutants may also be associated with COPD. The studies here investigate the hypothesis that particulates, including air pollution particles, can induce airway wall fibrosis, a process that can lead to COPD. Design: Rat tracheal explants were exposed to various occupationally encountered dusts, air pollution particles, and model air pollution particles. In some experiments, iron was loaded onto the particle surface. Gene expression and nuclear factor (NF)-κB activation were measured after 7 days of air culture. Adhesion to and uptake of dusts by the tracheal epithelium were also evaluated. Results: Known fibrogenic dusts such as amosite asbestos produced increased gene expression of profibrogenic factors, transforming growth factor-β, and platelet-derived growth factor, and increased hydroxyproline in the explants, and the addition of iron increased these effects. The addition of iron also converted nonfibrogenic TiO$_2$ into a fibrogenic dust. Dusts with surface complexed iron activated NF-κB via an oxidant mechanism. However, an ultrafine TiO$_2$ with very low iron was also fibrogenic. In separate experiments, exogenous tumor necrosis factor-α increased dust adhesion to, and exogenous ozone increased dust uptake by, tracheal epithelial cells. Conclusions: Mineral dusts can directly induce fibrosis in the airway wall. Exogenous inflammatory cells and exogenous agents are not required, but they probably exaggerate the fibrogenic effects. An iron-mediated oxidant mechanism underlies the fibrogenic effects of some, but not all, of these dusts. Particle-induced airway wall fibrosis may lead to COPD.

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Key words: air pollution; COPD; mineral dusts

Abbreviations: AOS = active oxygen species; MB = membranous bronchiol; NF = nuclear factor; PM = ambient particulate pollutants; RB = respiratory bronchiol; TNF = tumor necrosis factor

There is increasing evidence that remodeling of the walls of the airways is associated with chronic airflow obstruction (COPD). This type of remodeling may take the form of increases in airway wall matrix, changes in matrix composition and organization, increases in muscle, and distortion of the airway wall with often subtle changes of physiologic properties. Airway wall remodeling on an inflammatory basis is believed to be fundamental to the development of COPD in smokers and asthmatics. The idea that occupational exposure to mineral dusts is associated with COPD is more controversial, but a number of epidemiologic reviews have concluded that high-level exposure to many different types of dust can produce COPD; this phenomenon has been best documented in workers with coal and silica exposure. Of interest, there is now a growing body of data that chronic exposure to high levels of ambient particulate pollutants (PM) can produce a variety of long-term effects on the lungs, and one of these effects also appears to be COPD. The morphologic and mechanistic basis of dust- and PM-associated COPD is uncertain. However, simple examination of histologic sections from the lungs of workers with occupational dust exposure shows that, in many cases, the small airways, typically the membranous bronchioles (MBs) and respiratory bronchioles (RBs), develop marked airway wall fibrosis with thickening of the airway wall, and narrowing and distortion of the airway lumen (illustrated in Wright et al). These lesions are easy to pick out since they are often marked by accumulation of pigmented dust in the airway walls, and have a very similar, quite stereotypic, morphology from case to case. Mathematical models

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Remodeling and Repair in Respiratory Diseases