Airway Wall Remodeling Induced by Occupational Mineral Dusts and Air Pollutant Particles

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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 pro-collagen, 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 TiO₂ into a fibrogenic dust. Dusts with surface complexed iron activated NF-κB via an oxidant mechanism. However, an ultrafine TiO₂ 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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Abbreviations: AOS = active oxygen species; MB = membranous bronchiole; NF = nuclear factor; PM = ambient particulate pollutants; RB = respiratory bronchiole; 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.

MORPHOLOGIC CHANGES IN THE AIRWAYS IN INDIVIDUALS WITH DUST OR PM EXPOSURE

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
suggest that the MBs and RBs are particular points of high dust deposition, and this is probably the primary reason for accumulation of dust in these airways. These lesions in fact greatly resemble the small airway changes induced by cigarette smoke (which in many respects behaves like a particle), although cigarette smoke lesions tend to be worse in the more proximal MBs, and dust lesions worse in the more distal RBs. Remarkably similar lesions have been reported in the lungs of workers from the Fresno, CA, region, a high PM area, and we have observed the same changes, as well as increases in airway wall muscle, in the lungs of never-smoking female residents of Mexico City, another high PM area (unpublished data).

**EXAMINATION OF FIBROGENIC PROCESSES IN A TRACHEAL EXPLANT MODEL**

In order to understand how deposition of particles leads to airway wall fibrosis, our laboratory has established a tracheal explant model of dust exposure. Two-millimeter rat tracheal explants can be maintained in air organ culture with basal feeding for long periods with preservation of both morphology and function. If such explants are first exposed to mineral particles or PM particles, the particles adhere to the apical epithelial surface and then are very slowly (over days) transported into and through the epithelial cells to the underlying interstitial tissues. Short (a few hours) time period experiments can thus be used to examine the factors that cause particles to adhere to the epithelial surface, and long (up to 7 days) experiments can be used to document the molecular and biochemical effects of the particles as they enter the tissues. The explants offer the major advantage that they contain both epithelial and mesenchymal elements in their normal anatomic arrangement, thus permitting up-regulation and down-regulation of responses in one compartment by the other, and also the major advantage that they allow one to examine a real end point (the development of fibrosis). As well, tracheal explants are free of exogenous inflammatory cells; thus, interpretation of findings is much simpler than in whole animal models.

We used reverse transcriptase-polymerase chain reaction and high-performance liquid chromatography analysis to show that exposure of tracheal explants to amosite asbestos caused increases in type I procollagen gene expression and increased tissue hydroxyproline, a marker of collagen content (Figs 1, 2). Of interest, the time course of increases in gene expression very clearly paralleled entry of dust into epithelial cells and in particular entry of dust into the interstitium. As well, in situ hybridization showed that the epithelium was producing transforming growth factor-β, emphasizing the importance of both compartments in the development of fibrosis. These experiments showed for the first time that particles could produce fibrosis through mechanisms intrinsic to the airway wall, and that, contrary to an often-expressed belief, exogenous inflammatory cells, particularly airspace macrophages, were not required for this process.

One mechanism frequently postulated to be the driving force in asbestos-induced fibrosis is the generation of active oxygen species (AOS) using redox active iron on the fiber surface. To further examine this question, we loaded increasing amounts of iron on the surface of amosite asbestos and investigated the effects of loaded dust in explants. Increasing amounts of surface iron were associated with increasing levels of gene expression of procollagen (Fig 2) and increasing amounts of tissue hydroxyproline (Fig 1). This process appeared to proceed through long-term activation of nuclear factor (NF)-κB and could be prevented by using the proteasome inhibitor MG-132 to prevent NF-κB activation. The broad-spectrum AOS scavenger, tetramethylthiourea, and the nonredox active iron chelator, deferoxamine, both prevented iron-mediated increases in procollagen production. Of interest, iron loading also increased gene expression of platelet-
derived growth factor and transforming growth factor-β, and this again was driven by AOS, but through an extracellular signal-regulated kinase pathway.

These experiments suggested that one general mechanism of dust-induced fibrogenesis might be related to levels of surface complexed iron, driven through an oxidant iron-mediated process, probably formation of hydroxyl radical, with oxidant induced NF-κB activation. Because amosite asbestos is intrinsically fibrogenic and contains large amounts of iron, we next investigated the effects of adding iron to an ordinarily nonfibrogenic, non-iron-containing particle. For these experiments, 0.12 μm TiO₂ was selected. This dust serves here as a model “fine” (as defined in terms of PM particles) relatively inert PM. When we added iron to the TiO₂, we once again observed increases in procollagen gene expression and hydroxyproline production. NF-κB activation was again seen with the iron-loaded dust. These findings provide support for observations in acute experimental systems that suggest that transition metals (iron, sometimes vanadium) are crucial factors in the toxicity of PM particles, and that PM particles with bioavailable surface iron (which includes most types of PM) are all potentially fibrogenic.

**ROLE OF PARTICLE SIZE IN AIRWAY WALL FIBROGENESIS**

One of the controversies in the epidemiology of air pollutant effects is the role of ultrafine particles, those particles with diameters < 0.1 μm. Ultrafine particles are numerically the largest fraction of PM. It has been claimed from animal experiments that such particles evoke particularly intense inflammatory infiltrates and also that they are fibrogenic, but these experiments produce complex responses and are hard to interpret. To examine this question, we exposed tracheal explants to fine (0.12 μm) or ultrafine (0.021 μm) TiO₂, again meant to serve here as model PM particles. Fine TiO₂ was not fibrogenic; however, ultrafine TiO₂ increased procollagen expression in a dose-response fashion over 7 days (Fig 3). These findings thus support the animal data and suggest that ultrafine PM particles may play a role in PM-induced airway wall fibrosis. What was particularly noteworthy in these experiments was that the fine TiO₂ used here had very low surface iron, implying that other mechanisms are at work.

**ROLE OF COEXPOSURES IN AIRWAY WALL FIBROGENESIS**

In the real world, exposure to combinations of toxic agents is a common and unavoidable event. We have used the tracheal explant model to investigate such interactions. We found that if explants were briefly exposed to cigarette smoke or to low levels (as low as 0.1 ppm) of ozone before dust exposure, the uptake of dust was increased in a smoke/ozone dose-response fashion (Fig 4). This process could be abrogated or abolished by AOS scavengers, indicating that oxidant damage to the epithelium can potentiate the effects of dust. We also observed that pretreatment of explants with TNF-α increased adhesion of dust particles, including PM particles, to the explant surface via an NF-κB–dependent mechanism (Fig 5).

Adhesion of particles to the surface is believed to be the first step in particle uptake; therefore, increased adhesion is presumed to result in increases in adverse effects downstream. Since TNF-α is frequently produced by airspace macrophages that encounter particles, interactions of particles with macrophages appear to establish a feedback loop that enhances the adverse effects of dust with epithelial cells. All of these observations suggest that the types of combinations of agents encountered in the...
environment tend to increase the potential for dust-induced remodeling of the airway wall.

CONCLUSION

Our studies show that mineral dusts and PM particles can induce airway wall remodeling and thus presumably COPD. Although our model uses large airways (tracheal explants), it very likely applies to the small airways, the crucial site of airway obstruction. These processes represent intrinsic reactions to dust and are particularly, although not exclusively, mediated by surface transition metals through an NF-κB-activation pathway. Coexposures to dust-evoked mediators such as TNF-α or to other pollutants, such as cigarette smoke or ozone, all appear to be able to potentiate the intrinsic reactions.

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Chronic Ethanol Ingestion Increases Susceptibility to Acute Lung Injury*

Role of Oxidative Stress and Tissue Remodeling

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Clinical studies have demonstrated that chronic alcohol abuse is an independent outcome variable in acute lung injury. The Emory Center for the Study of Acute Lung Injury is determining the mechanisms by which ethanol increases susceptibility to acute lung injury. We developed a rat model of chronic ethanol ingestion and demonstrated that ethanol predisposes rats to edematous lung injury elicited by endotoxemia or sepsis. Chronic ethanol ingestion in rats led to decreased levels of glutathione, an important antioxidant in the lung, and this defect was associated with alterations in epithelial cell permeability, decreased alveolar liquid clearance, decreased cell...