Chronic Exposure to High Levels of Particulate Air Pollution and Small Airway Remodeling

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Recent evidence suggests that chronic exposure to high levels of ambient particulate matter (PM) is associated with decreased pulmonary function and the development of chronic airflow obstruction. To investigate the possible role of PM-induced abnormalities in the small airways in these functional changes, we examined histologic sections from the lungs of 20 women from Mexico City, a high PM locale. All subjects were lifelong residents of Mexico City, were never-smokers, never had occupational dust exposure, and never used biomass fuel for cooking. Twenty never-smoking, non–dust-exposed subjects from Vancouver, British Columbia, Canada, a low PM region, were used as a control. By light microscopy, abnormal small airways with fibrotic walls and excess muscle, many containing visible dust, were present in the Mexico City lungs. Formal grading analysis confirmed the presence of significantly greater amounts of fibrous tissue and muscle in the walls of the airways in the Mexico City compared with the Vancouver lungs. Electron microscopic particle burden measurements on four cases from Mexico City showed that carbonaceous aggregates of ultrafine particles, aggregates likely to be combustion products, were present in the airway mucosa. We conclude that PM penetrates into and is retained in the walls of small airways, and that, even in nonsmokers, long-term exposure to high levels of ambient particulate pollutants is associated with small airway remodeling. This process may produce chronic airflow obstruction. *Key words:* **air pollution, chronic obstructive pulmonary disease, COPD, small airways disease.** *Environ Health Perspect* **111:714–718 (2003). doi:10.1289/ehp.6042 available via** *http://dx.doi.org/* **[Online 19 December 2002]**

Considerable attention has been devoted to the associations between levels of ambient particulate matter (PM) and acute cardiopulmonary mortality, hospital admissions, and exacerbations of respiratory and cardiac disease (Dockery 2001; Pope 2000). Less is known about chronic effects of high PM exposure, although such exposures have also been associated with increases in cardiopulmonary mortality, including increased rates of lung cancer (Beeson et al. 1998; Pope 2000; Pope et al. 2002). There is a limited amount of evidence to suggest that chronic exposure to high levels of PM may cause chronic airflow obstruction. Abbey et al. (1991, 1995, 1998, 1999) studied a cohort of nearly 4,000 nonsmoking Seventh Day Adventists in California. Significant risks for the development of new cases of chronic bronchitis and obstructive airways disease were associated with increased exposure to ambient PM ≤ 10 μm in diameter and PM < 2.5 μm in diameter (PM_{10} and $PM_{2.5}$, respectively) (Abbey et al. 1991, 1995). Increased symptom severity correlated with $PM_{2.5}$ and PM_{10} levels. In more recent analyses with this same cohort, long-term increases in PM_{10} concentrations were also associated with lung function decrements (Abbey et al. 1998) and increases in mortality from nonmalignant lung disease (Abbey et al. 1999).

Studies of women in developing countries exposed to very high levels of PM emitted from cooking with biomass fuels also suggest that chronic exposures are associated with the development of chronic airflow obstruction. A case–control study of Mexican women reported an increased risk of chronic bronchitis and chronic airflow obstruction associated with cooking with wood (Perez-Padilla et al. 1996). The risk of chronic bronchitis was linearly associated with hour-years of cooking with biomass fuels. A case–control study conducted in Colombia identified a similar risk in women who cooked with biomass fuels (Dennis et al. 1996).

Because smoke-induced pathologic abnormalities in the small airways are one of the causes of chronic airflow obstruction in cigarette smokers, the observation of chronic airflow abnormalities in individuals with chronic high PM exposures suggests that PM exposure might also produce morphologic changes in the small airways. We have previously reported (Brauer et al. 2001) on the bulk lung particle content in a series of women from Mexico City, a region of chronically high PM (3-year mean PM_{10} = 66 μg/m^3) and compared them with lungs of subjects from Vancouver, British Columbia, Canada, a city with low PM (1984–1993 average = 25 μg PM_{10} and 15 μg $PM_{2.5}$) (Brook and Dann 1997). In that study we observed that the lungs of the Mexico City subjects contained significantly greater particle loads, including numerous aggregates of ultrafine particles that appeared to be ambient combustion products. Because these women were never-smokers, had not cooked with biomass fuels, and had no known occupational particle exposures, their lungs are suitable for examining the morphologic effects of chronic exposure to high PM. In this article we examine the small airways.

Materials and Methods

Subject. The studies described in this article were approved by the University of British Columbia institutional review board. Portions of 50 autopsy lungs from women who were lifelong residents of Mexico City were obtained from the autopsy service of a cardiovascular referral hospital. None of the women had died of respiratory disease. Only women were selected for the Mexico City group to decrease the possibility of occupational particle exposures.

For this study, we randomly selected 20 of the 50 cases as a test group, and for comparison, we obtained 20 autopsy lungs from a general hospital autopsy service in Vancouver. Because of the random selection from the same pool of 50 autopsy lungs, 8 of the Mexico City subjects in the current study were the same as those previously analyzed in our article on PM retention in human lungs (Brauer et al. 2001), although in that report we dissected parenchymal tissue, whereas here we focus on airway tissue. In both Mexico City and Vancouver, occupational, smoking, and residential histories were obtained from interviews with relatives, using a standardized questionnaire. For Vancouver, lungs from both men and women (7 men, 13 women) were used because of low autopsy rates and difficulties in obtaining interviews with relatives. Subjects with any form of chronic lung disease were excluded from the study. All subjects in the study were never-smokers, had not worked in dusty occupations, had not cooked with biomass fuels, and were lifelong residents of Mexico City or residents of Vancouver for > 20 years prior to death.

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The age at death (mean ± SD) was 66 ± 9 years for the subjects from Mexico City. Most of the subjects from Mexico City were homemakers. None had a history of asthma or any other chronic lung disease. The causes of death were mostly cardiac failure or complications of myocardial infarction. The mean age at death in the patients from Vancouver was 76 ± 11 years. None had a history of chronic lung disease; some had minor degrees of terminal acute pneumonia. There was a wide variety of causes of death, including complications of Alzheimer disease, myeloma, peritoneal mesothelioma in a patient who had received abdominal radiation for a seminoma, renal failure, cerebrovascular accidents, peritonitis, and bowel infarctions.

Histologic preparations. The Mexico City lung tissue generally consisted of an approximately 6-cm cube of fairly central lung containing both parenchyma and large airways. Two 2 × 1 cm histologic sections were prepared from this tissue for each case. As comparable an area of lung as possible was selected for the lungs from Vancouver, and two histologic sections were again prepared. For grading, the sections were stained with Movat's Pentachrome stain. Because the lungs were not inflated in a standard fashion, we used a visual grading scheme (Wright et al. 1985) to evaluate airway wall fibrosis and airway wall muscle in the membranous bronchioles (MBs) and respiratory bronchioles (RBs). Using this scheme, the airways from the test slides were compared with a set of standard photographs and graded from 0 to 3, with a grade of 3 indicating severe abnormality. For the Mexico City lungs, 121 RBs and 126s MB were graded; for the Vancouver lungs, 96s RB and 81 MBs were graded. The grading was done in a blinded fashion. MBs and RBs were graded separately; the grades for all airways were then summed and expressed as a proportion of a possible maximum score for each case. Statistical analysis was conducted using the grade (fibrosis or muscle, type of airway) for each case. Because these were autopsy lungs, inflammation may reflect any number of causes, and we did not grade numbers of inflammatory cells.

Particle burden measurements. In order to determine whether PM particles penetrate into the airway walls, we randomly selected four lungs from the 50 Mexico City autopsy samples available to us for airway particle burden analysis. Again, because of random selection, two of the lungs analyzed in this study for airway particle content had been previously analyzed and reported in our study on parenchymal particle retention in Mexico City lungs (Brauer et al. 2001). For each case, airway mucosa, defined as all tissues between the cartilage and the lumen, was microdissected from mainstem, segmental, and subsegmental bronchi, as well as carinas of these airways, and in some cases from MBs. Details of the dissection procedure have been published elsewhere (Churg et al. 1999). The tissues were then dissolved in bleach and the particles were collected and examined in an analytical electron microscope as previously described (Churg et al. 1999). Particles were identified by morphology and chemical composition, and concentrations were expressed as particles per gram dry tissue.

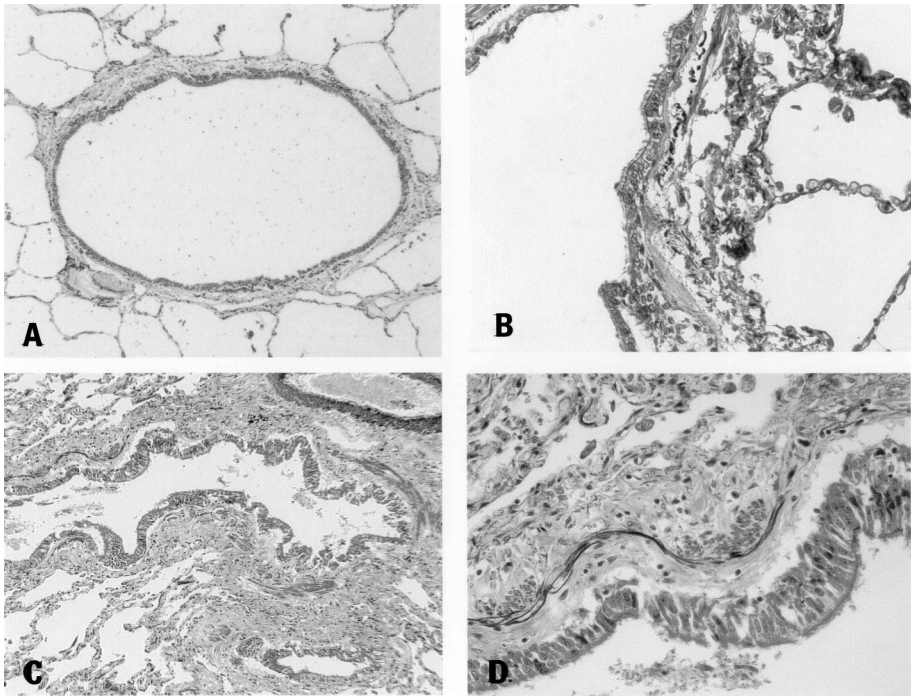

Figure 1. MBs in representative images of a lung from Vancouver (*A,B*) and representative images of a lung from Mexico City (*C,D*). Note the greatly thickened airway wall and the marked increase in fibrous tissue and muscle in the airway walls in the Mexico City lung (*C,D*). Magnification: ×50 for (*A*) and (*C*); ×200 for (*B*) and (*D*).

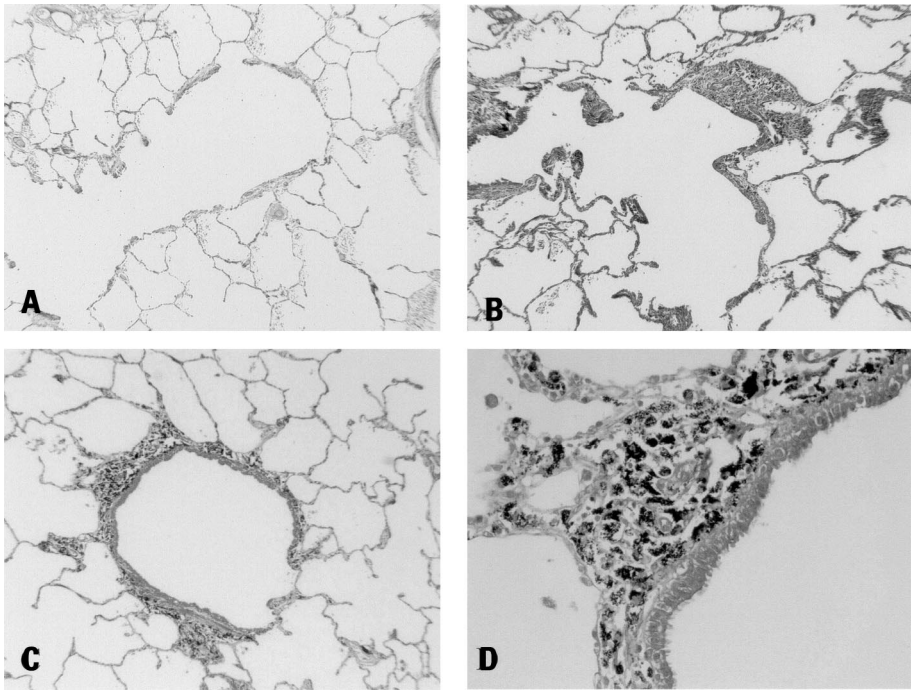

Figure 2. RBs in representative images of lungs from Vancouver and Mexico City. (*A*) Lung from Vancouver; the airway wall is very thin with almost no muscle present. (*B*) Lung from Mexico City; there is marked thickening and distortion of the airway wall along with dust deposition. (*C,D*) RBs from another lung from Mexico City showing extensive dust deposition in the wall. Magnification: ×50 for (*A–C*); ×200 for (*D*).

Statistics. Amounts of fibrous tissue and muscle were compared between specimens from Mexico City and Vancouver, and also between men and women in the Vancouver cases, by one-way analysis of variance using SYSTAT (Systat Inc., Evanston, IL, USA).

Results

On histologic inspection, both the MBs and RBs in the lungs from Mexico City were abnormal, with variably increased amounts of muscle and fibrous tissue compared with the lungs from Vancouver (Figures 1 and 2). The MBs in the Mexico City lungs generally showed no increases or only minimal increases in pigmented dust in the walls, but many of the RBs contained considerable amounts of pigmented dust (Figure 2C,D). No dust or only very small amounts were seen in the RBs of Vancouver lungs (Figure 2A). Many of the airways from Mexico City also showed lumenal distortion (Figure 1C).

Because the men from Vancouver might, despite the screening process, have had more dusty occupations or be otherwise different from women, a statistical comparison of the histologic grades between the Vancouver men and women was carried out. No statistical differences were found, and thus all 20 Vancouver cases were used for comparison with the Mexico City cases.

Table 1 shows the mean grades of all cases per location for muscle and fibrous tissue by airway type. Significantly greater amounts of both components were seen in the Mexico City lungs for both MBs and RBs. The differences are presented graphically as box plots showing the value for each case in Figure 3.

The electron microscopic analysis revealed the presence of chained carbonaceous or carbon/sulfur aggregates of spherical ultrafine particles in the airway mucosa in all four cases from Mexico City. We have previously shown (Brauer et al. 2001) that these particle aggregates are similar to particle aggregates sampled from ambient air in Mexico City. Particles of this type were found in 20 of the 25 airway samples examined and in all locations including the mainstem bronchi, segmental and subsegmental bronchi, and the MBs; concentrations of particles ranged from 10 to > 1,000 × 10^6/g dry tissue (Figure 4). The geometric mean size of the individual particles making up the aggregates varied from 0.040 to 0.067 µm, and of the aggregates themselves from 0.34 to 0.54 µm (Table 2). These are similar in size to the carbonaceous aggregates found in the parenchyma in the Mexico City lungs in our previous study (Brauer et al. 2001).

Discussion

Although there is epidemiologic evidence indicating that chronic exposure to PM is associated with chronic airflow obstruction, the anatomic basis of this effect is unknown. The traditional view of the effects of ambient PM on lung structure can be found in standard texts such as Spencer's *Pathology of the Lung* (Spencer 1985), which states that anthracosis (i.e., black particles inhaled from the air and retained in the lung) is not associated with lung pathology. However, more recent studies suggest that, in fact, ambient PM does produce airway disease. Souza et al. (1998) evaluated forensic (violent death) autopsy lungs from 34 residents of low PM regions of Brazil and 50 residents of São Paolo, a high PM region (mean annual PM_{10} approximately 80–100 µg/m^3 between 1991 and 1995). Histories were obtained from relatives. About 90% of subjects were male. The overall mean age at death was approximately 28 years, and 61% were smokers (average pack-years about 7). Small airways were graded visually for four parameters (anthracosis, inflammation, wall thickness, and mucus hypersecretion); in addition, the gland/wall ratio in the large airways was determined. No differences were seen between high-PM and low-PM subjects in gland/wall ratio, regardless of smoking status. The other four measures were generally larger

Table 1. Grading of changes in small airways (mean ± SD of grade for all cases per location).

	Mexico City	Vancouver	Significance
RBs			
Mural fibrosis grade	38 ± 17	7 ± 6	$p < 0.001$
Mural muscle grade	45 ± 20	6 ± 8	$p < 0.001$
MBs			
Mural fibrosis grade	58 ± 9	23 ± 11	$p < 0.01$
Mural muscle grade	69 ± 18	33 ± 18	$p < 0.01$

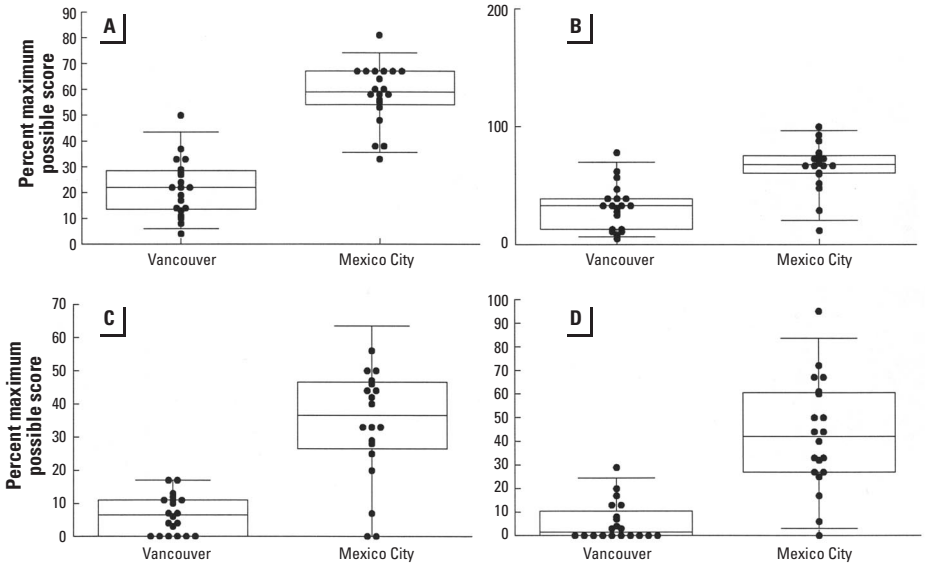

Figure 3. Box plots of airway fibrosis and muscle grades by case. (*A*) MBs, fibrosis grades. (*B*) MBs, muscle grades. (*C*) RBs, fibrosis grades. (*D*) RBs, muscle grades.The box shows the 25th and 75th percentiles, the horizontal line is the median value, and the whiskers show the 5th and 95th percentiles. For each measure, the Mexico City lungs show significantly greater abnormalities than the Vancouver lungs.

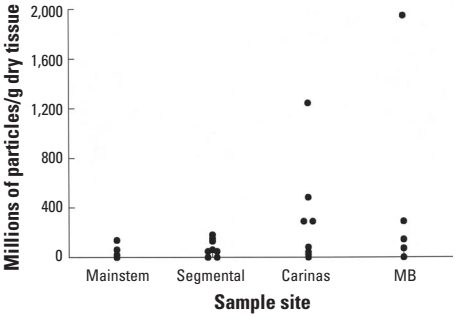

Figure 4. Plot showing the concentrations of carbonaceous aggregates in the mainstem bronchi, segmental/subsegmental (labeled "segmental") bronchi, large airway carinas, and MBs over all the samples examined.

Table 2. Sizes of carbonaceous aggregates (µm).[a]

Case	Size of individual particles in aggregates	Size of aggregates
1	0.067 (2.35)	0.54 (1.73)
2	0.040 (1.60)	0.34 (1.74)
3	0.051 (2.04)	0.53 (1.62)
4	0.048 (1.98)	0.50 (1.70)

[a]Values are geometric mean (geometric SD)..

in smokers and somewhat larger comparing high-PM to low-PM subjects in either the smoking or nonsmoking groups. There was a clear correlation between anthracosis score, used as a surrogate of PM exposure, and either airway wall inflammation or airway wall thickness.

The findings of Souza et al. (1998) would suggest that long-term residence in high PM regions leads to chronic structural changes in the airways, changes that, in cigarette smokers, have been shown to correlate with clinical airflow obstruction (Wright et al. 1992). However, in that study more than half the subjects were smokers and more than half had occupational dust exposures. Although the statistical analysis still indicated an effect of PM levels after adjustment for smoking and occupational dust exposure, the authors' graphical representations of their data suggest that the PM effects, apart from anthracosis, were small.

Pinkerton et al. (2000) examined the autopsy lungs of 42 Hispanic males from the Fresno County Coroner's Office; this region of California has high PM (during the time the samples were collected, mean PM_{10} levels in Fresno were 43.5 μg/m^3 and mean $PM_{2.5}$ levels were 22 μg/m^3). The median age of the subjects was 33 years, and approximately half were smokers. Most had worked in local farming operations or in blue-collar occupations. Lungs were inflated with fixative and carefully microdissected along two paths into the left upper lobe. Histologic samples were taken from airway generations 2, 4, 6, 9, and distal parenchyma containing terminal bronchioles and RBs.

Pinkerton et al. (2000) found histologic evidence of cigarette smoke injury (chronic bronchitis and small airways disease) in about half the subjects. Few abnormalities were noted in the larger airways, but the small airways showed both carbonaceous dust and birefringent particles in most cases. Particle loads were highest in the walls of generation 1 RBs and in MBs. The most intriguing observation was that the amount of dust (pigment) correlated with the degree of fibrosis in these airways. The study of Pinkerton et al. (2000) was very carefully designed and undertaken and was intended to show that exposure to PM caused abnormalities in the small airways, but the inclusion of a high percentage of subjects who were smokers or had occupational dust exposure (farming is known to be a very dusty process in this region) may confound the results. Smoking produces lesions similar in location and appearance [pigmentation, inflammation, and fibrosis of the walls of the membranous and to a certain extent the RB (Wright et al. 1992)] to those caused by dusts, although smoke-induced lesions tend to be more proximal in the bronchioles and dust-induced lesions more distal. Synergistic interactions between smoke and dust may amplify dust effects as indicated in animal models (Tron et al. 1987); thus, determining what effects really are chronic ambient PM effects is not straightforward.

In this study we have attempted to overcome these limitations by selecting a group of subjects who were never-smokers, did not have a history of occupational dust exposure, and, for the women from Mexico City, did not cook with biomass fuels. Cooking with biomass fuels produces very high PM exposures (Brauer et al. 1996; Smith 1993), has been associated with chronic airways disease (Dennis et al. 1996; Perez-Padilla et al. 1996), and can even produce a form of pneumoconiosis when cooking occurs in enclosed spaces.

It is possible that other confounders such as differences in exposure to environmental tobacco smoke might exist between the Vancouver and Mexico City subjects. Given that airway abnormalities in cigarette smokers are, in a broad sense, proportional to the amount of smoking, it is extremely unlikely that such exposures could produce the types of airway lesions that were observed. Nevertheless, the available data did not allow us to accurately characterize environmental tobacco smoke exposure or other potential confounders such as genetic differences, and this must be recognized as a limitation of the available data.

Because of difficulties in obtaining autopsies and interviews for our Vancouver control group, we were forced to use both men and women and to use a population that was, on average, 10 years older than the test group. Although this may be considered a limitation of our analysis, it is most likely that this age difference would decrease the ability to detect differences between the Mexico City and Vancouver groups, as dust and airway abnormalities tend to accumulate with age. An additional issue is that the autopsy lungs from the two sites were not saved in their entirety, and we could not exactly match sample sites. However, given the marked differences between the Mexico City and Vancouver lungs, it is unlikely that sampling variations, variations that are effectively random, could explained the differences observed.

PM is not the only air pollutant present in higher concentrations in Mexico City compared with Vancouver. The concentrations of ozone, nitrogen dioxide, sulfur dioxide, and carbon monoxide are also considerably higher (Brauer M. Unpublished data). Thus, one could argue that our findings might relate to a pollutant other than PM. However, because essentially identical types of airway lesions are found in workers occupationally exposed to mineral dusts in the absence of other pollutants (Wright et al. 1992), we believe that PM is the primary pollutant responsible for these effects. It is possible that synergisms between PM and other pollutants might play a role in augmenting these lesions, particularly since experimental data suggest that combinations of particles plus ozone lead to greater particle uptake by epithelial cells and greater inflammatory responses compared with particles alone (Adamson et al. 1999; Churg et al. 1996). It is also possible the other pollutants that adhere to particles may play a role in the observed differences in airway lesions.

The present results suggest, therefore, that chronic exposure to high levels of PM produces distinctly visible small airway lesions, in particular, increases in fibrous tissue, muscle, and, in the RBs, pigmented dust. The morphologic changes seen in the lungs from Mexico City are very similar to those found in cigarette smokers and in workers with high-level occupational exposure to many kinds of dusts (Wright et al. 1992), and in both of these groups, airway remodeling of this type appears to be associated with chronic airflow obstruction. Our findings thus provide an anatomic basis for the functional abnormalities detected in persons with chronic exposures to elevated PM concentrations.

In our previous analysis of bulk lung samples from our Mexico City test population, we found numerous chain aggregates of ultrafine carbon or carbon plus sulfur particles (Brauer et al. 2001); the same particles were found in Mexico City air and are very similar in terms of morphology, size, and composition to published descriptions of diesel exhaust particles (Harrison et al. 1999). We have now shown that exactly the same particles are present in the airway walls. Because the current approach is a bulk analysis of airway tissue, we cannot determine exactly where in the airway walls these particles reside, but the light microscopic images of black pigment in the walls of the airways, particularly the RBs, suggest that they are in the connective tissue beneath the basement membrane, rather than in the epithelium. Thus, these electron microscopic data confirm for the first time that this visible pigment is PM that has entered and been retained in airway walls.

Tissue penetration appears to require high local PM levels, as we have never observed these particles in extensive electron microscopic examinations of the airways of lung samples from Vancouver residents (Churg et al. 1999). Our intention here was only to demonstrate that PM particles enter and are retained in the airway walls, and hence we did not sample a larger number of lungs nor pursue in detail the question of particle concentration by airway location and size.

Depending on particle type, entry of particles into the airway wall may be associated with a subsequent fibrogenic response. We have previously shown in a tracheal explant

system using a model air pollution particle that this is a direct effect of the particle and does not require added inflammatory cells (Dai et al. 2002). We suggest, therefore, that PM particles are fibrogenic in individuals exposed to high levels for long periods, and that the resulting airway wall remodeling may be associated with chronic airflow obstruction.

References

Abbey DE, Burchette RJ, Knutsen SF, McDonnell WF, Lebowitz MD, Enright PL. 1998. Long-term particulate and other air pollutants and lung function in nonsmokers. Am J Respir Crit Care Med 158:289–298.

Abbey DE, Mills PK, Petersen FF, Beeson WL. 1991. Long-term ambient concentrations of total suspended particulates and oxidants as related to incidence of chronic disease in California Seventh-Day Adventists. Environ Health Perspect 94:43–50.

Abbey DE, Nishino N, McDonnell WF, Burchette RJ, Knutsen SF, Lawrence Beeson W, et al. 1999. Long-term inhalable particles and other air pollutants related to mortality in nonsmokers. Am J Respir Crit Care Med 159:373–382.

Abbey DE, Ostro BE, Petersen F, Burchette RJ. 1995. Chronic respiratory symptoms associated with estimated long-term ambient concentrations of fine particulates less than 2.5 microns in aerodynamic diameter ($PM_{2.5}$) and other air pollutants. J Expo Anal Environ Epidemiol 5:137–159.

Adamson IYR, Vincent R, Bjarnason SG. 1999. Cell injury and interstitial inflammation in rat lung after inhalation of ozone and urban particulates. Am J Respir Cell Mol Biol 20:1067–1072.

Beeson WL, Abbey DE, Knutsen SF. 1998. Long-term concentrations of ambient air pollutants and incident lung cancer in California adults: results from the AHSMOG study. Adventist Health Study on Smog. Environ Health Perspect 106:813–823.

Brauer M, Avila-Casado C, Fortoul TI, Vedal S, Stevens B, Churg A. 2001. Air pollution and retained particles in the lung. Environ Health Perspect 109:1039–1043.

Brauer M, Bartlett K, Regalado-Pineda J, Perez-Padilla R. 1996. Assessment of particulate concentrations from domestic biomass combustion in rural Mexico. Environ Sci Technol 30:104–109.

Brook JR, Dann TF. 1997. The relationship among TSP, PM_{10}, $PM_{2.5}$, and inorganic constituents of atmospheric particulate matter at multiple Canadian locations. J Air Waste Manage Assoc 47:2–19.

Churg A, Brauer M, Keeling B. 1996. Ozone enhances the uptake of mineral particles by tracheobronchial epithelial cells in organ culture. Am J Respir Crit Care Med 153:1230–1234.

Churg A, Brauer M, Vedal S, Stevens B. 1999. Ambient mineral particles in the small airways of the normal human lung. J Environ Med 1:39–46.

Dai J, Xie C, Churg A. 2002. Iron loading makes a non-fibrogenic model air pollutant particle fibrogenic in rat tracheal explants. Am J Respir Cell Mol Biol 26:685–693.

Dennis R, Maldonado D, Norman S, Baena E. 1996. Woodsmoke exposure and risk for obstructive airways disease among women. Chest 109:115–119.

Dockery DW. 2001. Epidemiologic evidence of cardiovascular effects of particulate air pollution. Environ Health Perspect 109(suppl 4):483–486.

Harrison R, Jones M, Collins G. 1999. Measurements of the physical properties of particles in the urban atmosphere. Atmos Environ 33:309–321.

Perez-Padilla R, Regalado J, Vedal S, Pare P, Chapela R, Sansores R, et al. 1996. Exposure to biomass smoke and chronic airway disease in Mexican women. Am J Respir Crit Care Med 154:701–706.

Pinkerton KE, Green F, Saki C, Vallyathan V, Plopper CG, Gopal V, et al. 2000. Distribution of particulate matter and tissue remodeling in the human lung. Environ Health Perspect 108:1063–1069.

Pope CA III. 2000. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk. Environ Health Perspect 108(suppl 4):713–723.

Pope CA III, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, et al. 2002. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. JAMA 287:1132–1141.

Smith K. 1993. Fuel combustion, air pollution exposure and health: the situation in developing countries. Annu Rev Energy Environ 18:529–566.

Souza MB, Saldiva PHN, Pope CA, Capelozzi VL. 1998. Respiratory changes due to long-term exposure to urban levels of air pollution. Chest 113:1312–1318.

Spencer H. 1985. Pathology of the Lung. 4th ed. Oxford, UK:Pergamon Press, 457.

Tron V, Wright JL, Harrison N, Wiggs B, Churg A. 1987. Cigarette smoke makes airway and early parenchymal asbestos-induced lung disease worse in the guinea pig. Am Rev Respir Dis 136:271–276.

Wright JL, Cagle P, Churg A, Colby TV, Myers J. 1992. Disease of the small airways. Am Rev Respir Dis 146:240–262.

Wright JL, Cosio M, Wiggs B, Hogg JC. 1985. A morphologic grading scheme for membranous and respiratory bronchioles. Arch Pathol Lab Med 109:163–165.