Rodent Models of Cardiopulmonary Disease: Their Potential Applicability in Studies of Air Pollutant Susceptibility

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The mechanisms by which increased mortality and morbidity occur in individuals with preexistent cardiopulmonary disease following acute episodes of air pollution are unknown. Studies involving air pollution effects on animal models of human cardiopulmonary diseases are both infrequent and difficult to interpret. Such models are, however, extensively used in studies of disease pathogenesis. Primarily they comprise those developed by genetic, pharmacologic, or surgical manipulations of the cardiopulmonary system. This review attempts a comprehensive description of rodent cardiopulmonary disease models in the context of their potential application to susceptibility studies of air pollutants regardless of whether the models have been previously used for such studies. The pulmonary disease models include bronchitis, emphysema, asthma/allergy, chronic obstructive pulmonary disease, interstitial fibrosis, and infection. The models of systemic hypertension and congestive heart failure include: those derived by genetics (spontaneously hypertensive, Dahl S, renin transgenic, and other rodent models); congestive heart failure models derived by surgical manipulations; viral myocarditis; and cardiomyopathy induced by adriamycin. The characteristic pathogenic features critical to understanding the susceptibility to inhaled toxicants are described. It is anticipated that this review will provide a ready reference for the selection of appropriate rodent models of cardiopulmonary diseases and identify not only their pathobiologic similarities and/or differences to humans but also their potential usefulness in susceptibility studies. — *Environ Health Perspect* 106(Suppl 1):111–130 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-1/111-130kodavanti/abstract.html*

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Introduction

The potential for preexistent disease to alter adverse responses to toxicant exposure is widely acknowledged but poorly understood. The variation in susceptibility to the health effects of toxicants due to normal host attributes such as species, age, and gender (Figure 1) generally is studied in animals and considered in estimations of human health risk (*1–4*). A solid database on human susceptibilities related to preexistent disease is lacking, in large part because involving diseased subjects in clinical research is likely to be complicated by their inherent variability and by ethical concerns. Studies involving animal models of human diseases, on the other hand, offer more control over both host and environmental variables but the results require careful interpretation when extrapolated to the human situation. Animal models of human diseases have been used extensively in investigations of disease pathogenesis and potential pharmacologic interventions. However, their application in ascertaining altered susceptibility to toxicants has been spotty and has yet to gain popularity. In this review the discussion of rodent models of human cardiopulmonary diseases is focused on their use in toxicology and the criteria for judging their appropriateness (where extrapolation to the human situation is critical) as well as their limitations.

The Need for Studies of Air Pollutant Exposure Effects in Susceptible Animals

Several historic episodes of relatively high levels of air pollution have resulted in excess mortality, particularly among elderly individuals with preexistent cardiopulmonary disease [reviewed in Pope et al. (*5*)]. Recently, more modest levels of air pollution, notably particulate matter (PM), have similarly been linked with increased acute and long-term mortality among individuals with cardiopulmonary (but not other) impairments, even after controlling for the confounding host factors of age, gender, education level, smoking status, and occupational exposure history (*5–11*). Likewise, increased acute morbidity (e.g., inhaled bronchodilator use, school absenteeism, lung dysfunction, and hospital admissions) has also been linked to air PM levels, especially among young asthmatics (*12–14*). These findings thus have drawn renewed attention to the issue of susceptible groups (*5–11*).

Of particular interest to the public health community is that the protection of susceptible subpopulations is specifically mandated in the Clean Air Act of 1971 (*15*), and that health effects have been associated with PM levels previously considered harmless, i.e., below contemporary (as established in 1988) National Ambient Air Quality Standards for PM ≤ 10 μm in aerodynamic diameter (*15*). This revitalized interest is clearly being driven by PM (*15,16*), but other criteria (i.e., ozone [O_3], NO_2, CO, SO_2) and air toxic pollutants (e.g., phosgene, acrolein) may also impose higher risk to susceptible individuals. In

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Abbreviations used: BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease, Dahl R, Dahl salt-resistant; Dahl S, Dahl salt-sensitive; PM, particulate matter; RAIV, rat adapted influenza virus; ROFA, residual oil fly ash; SH, spontaneously hypertensive; Th1, T-helper type 1; WKY, Wistar Kyoto rats.

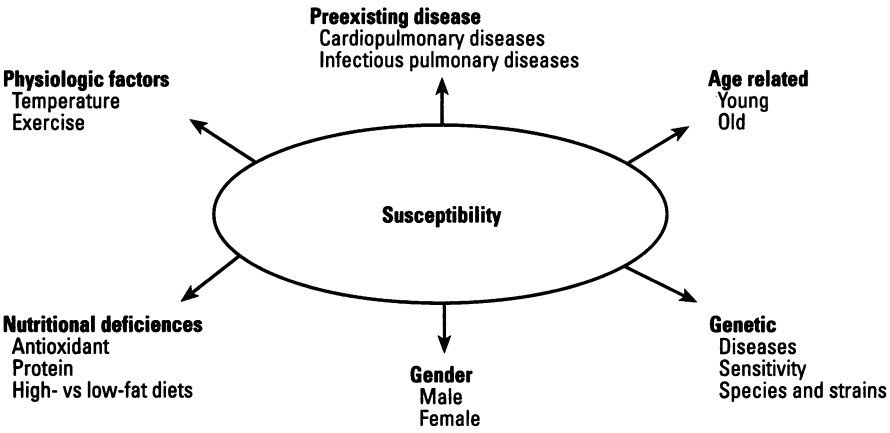

Figure 1. Host factors that influence the susceptibility to inhaled toxicant-induced injury.

fact some data exist on various animal disease models to support this possibility (*17,18*).

Because of growing concern regarding the impact of air pollutants on susceptible subpopulations, there is a need to understand which disease subgroups are specifically vulnerable and what plausible biologic mechanisms likely are responsible for the increased susceptibility. It is not apparent from current epidemiology whether threshold levels exist for pollutant effects in individuals with cardiopulmonary disease. Also there are no data available within these groups to indicate dose–response relationships. Epidemiology is even more limited in its ability to identify meaningful biologic indices of response or underlying mechanisms. Thus, toxicologic studies involving exposure of various animal models of cardiopulmonary disease may be critical in addressing questions of disease-specific susceptibility to air pollutants. What the animal model approach offers is the ability to define and reasonably titrate the attribute of interest in an otherwise well-defined study situation in which subject genetics, husbandry, and personal exposure scenarios are well controlled. Studies of acute air pollutant effects in such models thus should be relatively straightforward.

Air pollution exposures in the real world, however, are likely to be encountered episodically throughout life and may pose with each exposure a variable acute (or perhaps cumulative) threat to cardiopulmonary compromised individuals. Because many cardiopulmonary diseases in animal models are not progressive or chronic in nature, overlying lifetime exposure studies would offer challenges to inhalation toxicologists. However, some models that are progressive (e.g., hypertension) were used in the 1970s and 1980s to study chronic air pollutant exposures (O_3, SO_2, CO, acrolein, and a mixture of urban air pollutants) with resultant mortality (*19–23*). Unfortunately these studies have not been replicated or followed more thoroughly to investigate the mechanisms, but they nevertheless support the feasibility of investigating the influence of increased susceptibility on chronic air pollutant responses in the experimental laboratory setting.

Of a more acute nature, recent studies aging adult rodent models of allergical airways disease [(reviewed by Selgrade and Gilmour (*17*) and Gilmour (*18*)] have in general complemented epidemiologic findings of exacerbated asthma due to air pollutants (*12–14*). While it is important to understand the susceptibility of young asthmatics to air polution, it may be necessary to involve younger rodents to mimic the maturity of the immune system. An approach using young animal models may be critical to investigations of asthmagenesis itself—an approach not amenable to human study. Likewise, animal studies provide an avenue for investigation of susceptibility factors in very young and very old groups, to which researchers may have limited access in a clinical setting.

Speculative Mechanisms of Increased Air Pollutant Susceptibility due to Underlying Cardiopulmonary Disease

Human cardiopulmonary diseases are diverse; they vary in etiology, the primary organ involved, and the degree of host compensation. There is also a spectrum of severity and time period of onset (chronicity). The epidemiologic evidence neither elucidates whether air pollution-associated deaths among individuals with cardiopulmonary disease are related to the chronicity of the preexistent condition nor provide clues about how sudden deaths relate to the severity of underlying disease.

Recent studies in animals compromised by monocrotaline-induced pulmonary vasculitis/hypertension and bleomycin-induced pulmonary injury/inflammation suggest that these conditions render the animal model more susceptible to PM-induced cardiac dysfunction (*24–28*) by mechanisms not yet understood. Formal speculations about the cause of PM effects in humans such as that proposed by Seaton et al. (*29*) on blood clotting events and by Peters et al. (*30*) on increased plasma viscosity by pollutants have not as yet been verified or shown to distinguish susceptible subgroups. Nevertheless, a number, or perhaps cascade, of events provoked by pollutant (in this case PM) inhalation can be hypothesized: *a*) enhanced oxidant production, which causes increased damage to an already-inflamed lung resulting in hypoxemia; *b*) neural irritant reflexes involving pulmonary afferents and cardiac efferents leading to cardiac arrhythmias; *c*) compromised host compensatory mechanisms or loss of reserve, which allow persistent and more critical dysfunction or injury to occur; *d*) activation of tissue-specific mediators or circulating blood elements in the lung leading to acute vascular spasm or platelet occlusion in atherosclerotic vessels; and *e*) disabled host defenses, which can lead to acute infection. Although to date no supportive experimental evidence exists for these speculative mechanisms, it is feasible that animal models could be instrumental in elucidating these or alternative proposals.

Scope of the Review

This review provides essential descriptive information on selected rodent cardiopulmonary disease models we believe may be suitable for inhalation toxicology studies (Table 1). Primary criteria for review are each model's appropriateness relative to the human condition and its ready availability or ease of generation via experimental manipulation. Because most rodent models have been developed to mimic either cardiac or pulmonary disease, they are divided accordingly for discussion. However, an attempt is made to integrate what is known or might be construed about the health status of the cardiopulmonary system as a

Table 1. Selected human cardiopulmonary diseases and their most extensively used rodent models.[a]

Primary organ	Human disease type	Rodent species used	Experimental manipulations	Air pollutant studied (reference)
Lung	Chronic bronchitis	Rat, hamster	SO_2	Aerosol, PM (*58,59*)
		Guinea pig	Polymyxin B	—
	Emphysema	Rat, hamster, mouse	Pancreatic elastase	Aerosol, PM, NO_2, SO_2, O_3 cigarette smoke, O_2, ammonium sulfate (*77,86–95*)
		Mouse	Genetic	—
	Asthma/allergy	Mouse, guinea pig, Brown Norway rat	Ovalbumin	O_3, NO_2, SO_2, PM (*17,18,132–136*)
		Mouse, Brown Norway rat	Dust mite	NO_2 (*101*)
	COPD	Hamster	Elastase + SO_2	—
	Pulmonary fibrosis	Rat, hamsters, mouse	Bleomycin	PM (*25,147,158,159*)
	Pulmonary infections, viral	Mouse, rat	Influenza, respiratory syncytial virus	O_3, phosgene, PM (*17,183,189–194*)
	Pulmonary infections, bacterial	Mouse, rat	*S. zooepidemicus, H. influenzae, S. aureus, P. aeruginosa*	PM, O_3, NO_2, phosgene,SO_2, metals (*17,193,202–207*)
Cardiopulmonary, vascular	Pulmonary	Rat	Monocrotaline	PM (*24,26–28*)
		Rat	Hypoxia	—
	Systemic hypertension	Rat	Genetic	Acrolein, O_3, CO, SO_2, PM (*20–23, 26*)
	Hypertension	Rat	Genetic/salt sensitive	—
		Rat, mouse	Renin transgenic	—
	Hypertension	Rat	Genetic	—
	Congestive heart failure	Rat	Surgical (aortic coarctation)	Mixture of SO_2, NO_2, O_3, and CO (*19*)
		Rat	Surgical (myocardial infarction)	—
	Myocarditis	Rat	Autoimmune (cardiac myosin), viral	—
	Cardiomyopathy	Rat	Adriamycin	—
		Hamster	Genetic	—

[a]This table does not include all available rodent cardiopulmonary disease models; only the more popular models are included. Also, although the table does not give an exhaustive list of all air pollutant studies, the major studies and in some cases the review papers are listed.

whole and how this information relates to susceptibility. Models of pulmonary diseases discussed include: various chronic obstructive pulmonary disease (COPD)-like models (emphysema, bronchitis, and asthma), interstitial fibrosis, and infectious lung disease. The cardiopulmonary vascular disease models considered include: pulmonary vasculitis/hypertension, systemic hypertension, and congestive heart failure. Although animals with targeted genetic disruptions as models of cardiopulmonary disease is a growing area of research (*31–33*), this review considers only a limited number of transgenic animals that appear appropriate as bona-fide disease models. Whether a transgenic or knockout model can be regarded as a true model of cardiopulmonary disease depends on the relation between the genetic target being modified and resulting phenotypic pathology. In most cases it appears these animals are best used for studies of specific mechanisms arising from targeted genetic alterations because they frequently have phenotypic lesions distinct from those of the complex pathology of the human disease (*31,33*).

To the extent possible, the models of cardiopulmonary disease are discussed in the context of their potential use in studies of susceptibility to air pollution effects regardless of whether they have previously been used for such a purpose. The goal is to present their relevant pathophysiologic characteristics and potential use rather than to catalog the existing database of every application. Understanding differences and similarities between the human disease and that in its animal models will enable investigators to make more appropriate inferences about susceptibility factors and mechanisms that relate to humans. Because the information provided in this review on cardiopulmonary disease models has been gathered from a multidisciplinary perspective, every detail pertaining to the specific phenotypic disease cannot be provided. Readers are encouraged to consult cited literature for additional information.

Critical Considerations and Limitations for the Use of Rodent Models of Cardiopulmonary Diseases

Selection criteria for an animal disease model should be based on the model's intended application. Choices for susceptibility studies related to pollutants would differ from those for basic investigations of pathogenesis or pharmacologic manipulation. Toxicologists seeking to extrapolate their data to humans often are faced with difficult dosimetric issues as well as species and strain variations in response, problems that exist even when the studies are conducted in healthy animals. However, using animal disease models for risk-based toxicity studies imposes the added complication

of extrapolating from diseased animals to diseased humans. Thus, the difficulties inherent in disease models in general beyond those of standard rodent toxicology include: differences in disease causation; qualitative differences in disease pathogenesis; acute or subacute onset or induction of disease in the animal versus the generally chronic pathogenesis in the human; underlying differences in host responsiveness; and the potential confounding of compensatory and defense mechanisms, e.g., the ability of rat lung to regenerate after an acute injury. However, because the difficulties in experimentation with human disease patients are so formidable, animal disease models often offer the only reasonable approach to studying those individuals most impaired.

The etiology, progression, and chronicity of cardiopulmonary disease can vary considerably between humans and rodents (*34,35*). For example, although the cause of the disease in humans can be multifactorial or even unknown, progressing insidiously over time, diseases in rodent models typically are induced by acute experimental insult. The obvious question then is whether the model must or even can exhibit all the characteristics of the human disease of interest. Ideally, one would like to know the character and kinetics of the lesion and the related inflammatory and healing processes operant in both the model and the human. However, our understanding of human disease mechanisms frequently is rudimentary and although it is advancing rapidly as molecular and immunochemical tools become available, the decision about whether to use a model remains based largely on its descriptive end-stage pathophysiology.

Selection of the outcome measure(s) in the assessment of responses in the models should take into account the pathophysiology intrinsic to the model. Intuitively, one might suspect that the probability of interactions will be greatest when the disease lesion and the toxicity outcomes are similar (e.g., edema) and will simply worsen when disease and pollutant challenges are combined. However, such an interaction may not be known a priori and may be model dependent. Thus, the basic pathophysiologic characterization of the model and some knowledge of the effects of the pollutant in normal animals may be helpful. For example, in rat models of bleomycin-induced pulmonary inflammation/injury (*25*) and monocrotaline-induced pulmonary vasculitis/hypertension (*26*), the bronchoalveolar lavage fluid (BALF) markers of residual oil fly ash (ROFA)-induced pulmonary edema and inflammation were of limited value, as these responses were expressed both dramatically and variably. However, the relative pathology in both these models, although of a gross nature, yielded a clearer separation of the exaggerated ROFA effects (i.e., in terms of interstitial thickening and presence of inflammatory cells; Figure 2). Toxicologic evaluations should include relevant biomarker(s) of response that may not be so readily obscured by the disease. At present there is not sufficient experience with the toxicologic application of these models to generalize on the most sensitive or appropriate biomarkers of response. Rather, the strategy for end point selection needs to be developed conceptually with model characterization.

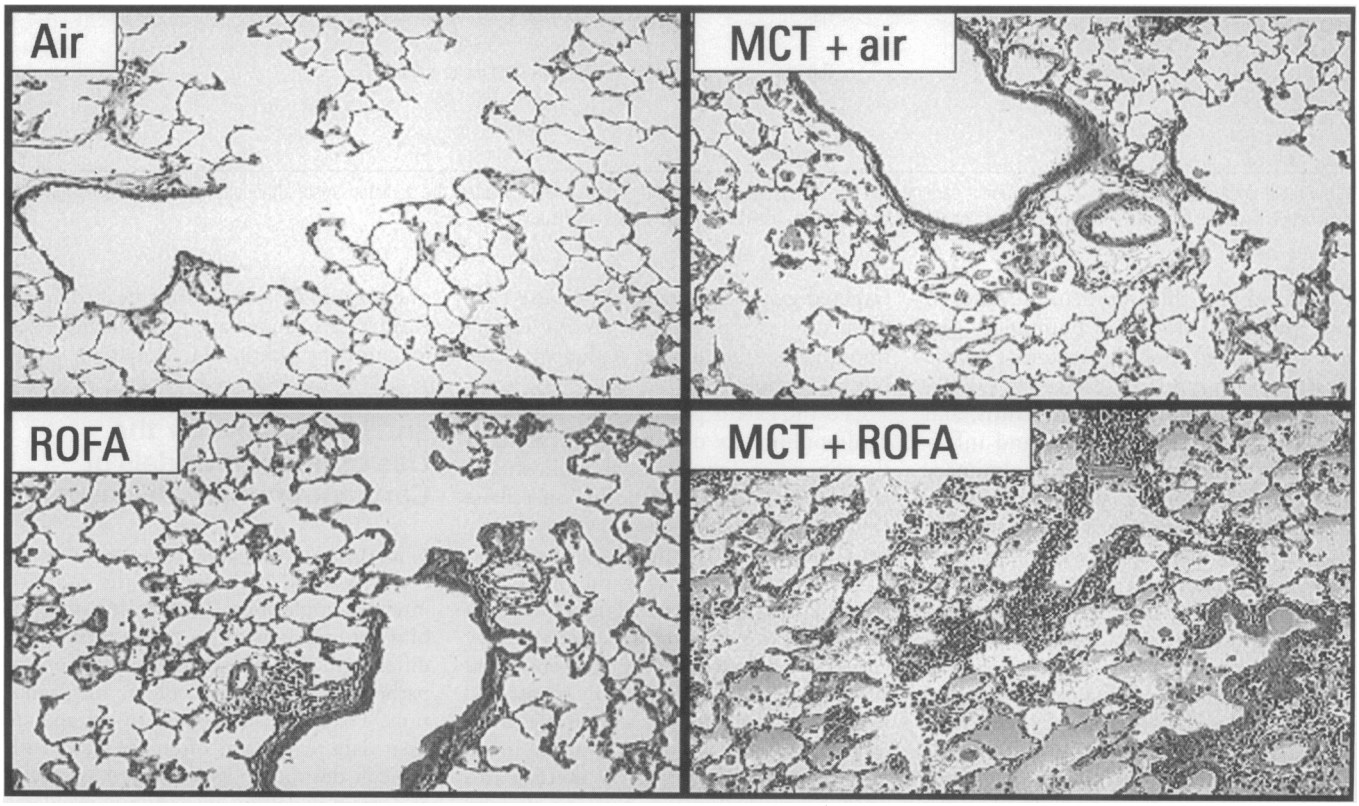

Figure 2. Pulmonary pathology from inhaled ROFA in a rat model of monocrotaline-induced pulmonary vasculitis/hypertension. Male SD rats were treated with monocrotaline (60 mg/kg, ip), and 10 days later exposed to ROFA (15 mg/m^3, 6 hr/day for 3 consecutive days). One day after ROFA exposure, rats were sacrificed and paraffin lung sections stained with hematoxylin and eosin. Monocrotaline (MCT) pretreated rats exposed to ROFA had extensive edema, thickening, and inflammatory response. Relatively small changes of inflammation and parenchymal thickening were noted in normal (saline treated) rats exposed to ROFA or in monocrotaline-treated rats exposed to air.

An animal disease model may be derived through different procedures. For example, rodent models of pulmonary vasculitis/hypertension can be induced by either prolonged hypoxia or acute treatment with the alkaloid, monocrotaline (*36,37*). The models, however, are distinct in important ways (see model description for details) and each has similarities and dissimilarities with regard to primary pulmonary hypertension in humans. Thus, when using ostensibly similar models in air pollution studies, one must consider the potential impact of specific variables in associating susceptibility with the dominant defect (in this case pulmonary hypertension).

Rodent Models of Cardiopulmonary Diseases

Pulmonary Diseases

Most human pulmonary diseases are the result of prolonged or repeated injury to the airway and/or alveolar parenchymal tissues and appear to involve chronic inflammation with coexistent remodeling. Human lung disease is frequently multidimensional, e.g., chronic bronchitis or COPD with concomitant pulmonary bacterial or viral infection (*38,39*). In some cases more advanced disease is initiated through mechanisms that are more classically immune mediated (e.g., chronic allergic asthma). However, over the course of the disease, stereotypic nonimmune mechanisms with their ensuing pathology frequently become involved. The end result is a complex of pathologic lesions and physiologic deficits that together may underlie the phenotypic susceptibility. Descriptions of selected pulmonary disease models potentially useful in air pollution susceptibility studies are discussed below.

Bronchitis. Human chronic bronchitis long has been associated with heavy cigarette smoking and exposure to occupational or environmental irritants, but it can also result from recurring airway infections (a frequent confounder of smoker's bronchitis) (*40*). Symptoms include increased cough and protracted sputum production (i.e., daily production for over 3 months) with underlying pathologic airway inflammation and epithelial damage, mucus cell hyperplasia and hypersecretion, airflow obstruction, and in advanced cases, airway fibrosis (*41,42*). Airway hyperresponsiveness to nonspecific pharmacologic bronchoconstrictors is also common. Based on this pathophysiology in humans, the criteria for an animal model of chronic bronchitis would include mucus hypersecretion, airway cell metaplasia, airway inflammation and possibly fibrosis, and increased airway responsiveness.

Among the animal models of bronchitis, those in the rat and dog produced by subchronic SO_2 exposure have been the most extensively studied (*43–47*). Hamsters are less sensitive to SO_2-induced bronchitis but they also have been employed as models (*48,49*). The SO_2 exposures required to generate the model in most animals are high (150 to 600 ppm), not meant to be relevant to ambient air SO_2 exposure, and must continue for 4 to 6 weeks. Occasionally, aerosolized sodium meta bisulfite has been used, but SO_2 is more readily handled in the laboratory (*46,47,50,51*). Protracted exposure to this irritant results in pathologic changes to the airways; changes similar to those of chronic bronchitis in humans. The lesions in the rat include: increased numbers of epithelial mucus-producing (goblet) cells extending to peripheral airways and associated mucus hypersecretion, loss of cilia, modest airway inflammation, increased proinflammatory cytokine expression, and thickening of airway epithelium (*47,51*). One difference between the rat and human, however, is the anatomical absence of submucosal glands in the rat. Goblet cells proliferate from a small baseline number in rats relative to that in normal humans (*52,53*). Similar to humans, however, rat models exhibit increased airway responsiveness to inhaled bronchoconstricting agonists (*47*).

Because the rat model of bronchitis exhibits good homologies to the human disease in pathology and airway reactivity (*47,50*) and is well characterized, it would likely be an appropriate choice for the study of susceptibility to air pollution effects. However, it should be noted that with bronchitis, when the cause or stimulant (e.g., smoking in humans, SO_2 in rats) is removed, the pathology slowly reverses (*50,54*), although the time course and extent of reversal differs significantly between the rodent and human. The reasons for this difference are unclear but may relate to a more sustained signal in the human perpetuating the lesion and the possible involvement of infection. Alternatively, efficient hypertrophic/hyperplastic parenchymal growth in response to an injury in the rat (*55*) may contribute to the reversibility of bronchitis upon discontinuation of SO_2 exposure. When applying this model to susceptibility studies, disease etiology and reversibility thus may be important considerations in the study design.

Lipopolysaccharide (endotoxin) has also been used in rats to induce a form of bronchitis (*56*). Repeated instillation or inhalation of endotoxin induces an increase in the amount of intraepithelial mucosubstances and marked hypertrophy/hyperplasia of epithelial cells lining the main axial pathways within the lung (*56*). These changes are similar to the secretory epithelial alterations in bronchial airways of human patients with chronic bronchitis as well as in the animal SO_2 model (*40,47*). The mucus hypersecretion occurs from surface goblet cells in the airways, as the rat lacks the equivalent of human submucosal glands (*52,53,56*). The essential differences between the endotoxin and SO_2 models could be the degree of neutrophilic inflammation (greater in the endotoxin model) and in airway mucus hypersecretion (greater in the SO_2 model). The endotoxin model, however, has the advantage of ease of generation, both in terms of equipment and time (~2 weeks) required (*56*). Repeated endotoxin exposure may be used to sustain the bronchitic lesion, but definitive study in this regard is lacking.

The guinea pig exposed to SO_2 develops significant bronchoconstriction that can be quite stressful (*54*). Lower more tolerable levels of SO_2 apparently are inadequate to initiate pathology resembling the chronic bronchitis of the rat model or humans. Nevertheless, at the higher concentrations of SO_2 (similar to those in the rat), acute bronchitis can be induced in guinea pigs, but associated mortality complicates the utility of a model (*54*).

Bronchitis also has been successfully induced in guinea pigs by intranasal administration of polymyxin B sulfate (a mast cell-degranulating agent) at a concentration of 5 mg/ml/kg twice a week for a total of 3 weeks (*57*). An eosinophilic bronchitis results and is confined largely to the upper respiratory tract because of the limited distribution of polymyxin; however, goblet cell pathology in this model is not well defined. The near-exclusive eosinophil cell influx (no lymphocyte and neutrophil influx) and the lack of airway hyperresponsiveness to histamine (though cough receptor sensitivity is increased) (*57*) also distinguishes this model from human bronchitis and the SO_2 rat model.

The pattern of deposition of various gases and particles in the bronchitis has only partially been examined. In the case of PM, pulmonary distribution in the rat bronchitis model indicates increased overall deposition and focal hot spots, especially in areas most affected by the disease (*58*). The biologic reaction to this alteration in

tissue dosimetry remains to be investigated, but recent preliminary findings in bronchitic rats suggest that the rat model is hyperresponsive to ROFA PM and may experience significant mortality (*59*). The role of hot spot deposition and focal injury in this outcome is not known and is but one area of needed investigation.

Emphysema. The pathology of emphysema features permanent enlargement of airspaces distal to the terminal bronchiole because of destruction of alveolar walls (*60,61*). In humans emphysema is most often associated with smoking (*61–63*) and is also seen in individuals with a severe genetic deficiency in serum levels of α-1 antiprotease (*64*), particularly if they smoke. In the population at large, emphysema frequently coexists with bronchitis, may or may not be progressive, and is attended by significant functional impairments in lung mechanics, gas exchange, and pulmonary hypertension (*61–64*).

The prevailing theory of the mechanism of the disease is that an underlying inflammatory state involving the lung parenchyma leads to an excess total lung elastase burden relative to its inactivating protein (α-1 antiprotease) with eventual matrix degradation (*60,61*). Because elastic fiber damage is important in the pathogenesis of emphysema, proteases and in particular elastases have been extensively used to induce emphysema in rodents (*60,65–67*). Destruction of structural elements of the lung results in air space enlargement, which generally is panlobular and most closely resembles the pathology in α-1 antitrypsin-deficient individuals. Smoking- or other toxicant-induced emphysema typically is centrilobular and focuses on the respiratory bronchiole regions of the acinus, which correspond to the site of primary toxicant deposition (*68,69*). Interestingly, hyperoxia, mineral dust, and cadmium oxide can also induce airspace enlargement but without the substantial alveolar degeneration apparently due to scar retraction of focal sites pulling open the airspaces. This lesion is termed paracicatricial emphysema (*61,68,70*) and is considered distinct from the classic smoker's disease. The criteria for demonstrating emphysema in rodent models include destructive anatomical changes or remodeling, a decrease in alveolar surface area, and lung functional impairment (*60,61*). The coexistence of these criteria is important in defining a model as emphysema because the rodent lung is capable of hypertrophic enlargement and may develop enlarged airspaces in the absence of evidence of alveolar damage [e.g., chronic hyperoxia (*71*)].

Elastase-induced emphysema models have been used in lung research for more than two decades (*61,65,72*). Several reviews detail various approaches to types of enzymes used, animal species, structural, biochemical, and physiologic alterations, the time course of disease development, and the degree of reversibility of some of the structural changes (*60,61,65,67,72*). Basic information on the induced and genetic rodent models of emphysema is discussed below. Some critical considerations regarding the time course as well as reversibility of the lesion are also discussed.

ELASTASE-INDUCED EMPHYSEMA. The hamster has been the most common rodent emphysema model because of its innate sensitivity to elastolytic enzymes (*60,73*). The rat, mouse, and rabbit also have been used as models despite their lower sensitivity to elastase (*60,74–76*). Most studies use porcine pancreatic or human neutrophil elastase (*72,73*) to induce disease, although early models (*72*) used an unpurified cocktail of vegetable enzymes (papain). Pancreatic trypsin also has been used in some studies (*66*) but is considerably less effective than elastase. In the rat the porcine pancreatic elastase typically is instilled intratracheally at a dose of 75 to 150 U elastase activity per 100 g body weight. The resulting emphysema is dose dependent; higher doses offer no advantage, as mortality can result from severe pulmonary hemorrhage (*76*). More severe disease without increasing mortality can be attained, however, by exposing animals to cigarette smoke or by inhibiting elastin cross-linking (feeding animals with β-aminopropionitrile, an inhibitor of lysyl oxidase, in the diet) following elastase instillation (*77,78*). Morphologic alterations produced by elastase treatment resemble human panacinar emphysema when examined by light microscopy. Physiologic changes associated with this treatment include increases in total lung capacity, static lung compliance, functional residual capacity, residual volume, and a diminution in maximum expiratory flow and CO diffusion (*60,61,65*).

Immediately after intratracheal instillation of elastase, degradation of elastin, rupture of the alveolar epithelium, pulmonary edema, hemorrhage, and modest infiltration of neutrophils occur, with airspace enlargement noted within hours of the treatment (*61,72,76*). Synthesis of new elastin and repair begin almost immediately and normal total lung elastin and collagen levels are restored in 3 to 4 weeks; however, distortion and derangement of alveolar structure is progressive through this period and these changes are largely permanent. As the lesion matures inflammation recedes and the structural alterations stabilize (*73,74,76*). Pulmonary hypertension develops, but this has not been well studied in rodents (*65*).

GENETICALLY DERIVED MODELS OF EMPHYSEMA. Spontaneously occurring genetic models of emphysema are also available. The blotchy mouse exhibits spontaneous panlobular emphysema due to an impairment of copper absorption (essential for action of copper–dependent lysyl oxidase involved in elastin cross-linking) (*79*). Mechanistically, however, the model is not considered relevant to the most common form of human emphysema resulting from protease–antiprotease imbalance (*72*). Emphysema in tight-skin (*80*) and pallid mice (*81*), on the other hand, results from protease–antiprotease imbalance and thus is considered more relevant to the human disease. Homozygous pallid mice with C57B1/6J backgrounds exhibit severe genetic α-1 antiprotease deficiency and eventually develop emphysema (*81*). A progressive burden of excess elastolytic activity can be observed from the first month of life. Unlike the exogenous elastase model, total lung elastin appears to decrease over time and inflammation is not evident in this model as determined by BALF analysis (*81*). Thus, this model may represent a tool to investigate disease-related susceptibility in the absence of coexistent inflammation.

Other models of emphysema have been developed in rodents by exposing them to cadmium salts (*68,82*), NO_2 (*83*), hyperoxia (*71*), and tobacco smoke (*69*), and through starvation (*84*) and copper-deficient diets (*85*). Some of these treatments are associated with enlargement of air spaces without elastin damage or protease involvement and may exhibit coexisting fibrosis (*60,61,68,82*). These models have been used primarily to study pathogenesis and not as disease models per se, but the unique pathogenesis of each may be amenable to examination for subsequent exposure interactions.

A number of studies have been conducted that examine susceptibility of emphysematous animals to inhaled pollutants (*77,86–91*). In the hamster and rat models of elastase-induced emphysema, exposure to hyperoxia, O_3, or a mixture of olefin–O_3–SO_2 reaction products did not

reveal unusual susceptibility in terms of the resulting pulmonary inflammation and lung dysfunction (*86,90,92*). Similarly, SO_2 or ammonium sulfate did not affect emphysematous rats pretreated with elastase any differently than normal rats (*91*). However, in the guinea pig model of elastase-induced emphysema, exposure to ammonium sulfate aerosol resulted in more pronounced lung functional changes (*88*), suggesting that interactive effects may be species dependent. In other studies cigarette smoke and NO_2 have been shown to augment elastase-induced emphysema in Long Evans rats (*77,89*), whereas exposure to NO_2 or diesel exhaust had no such effect in elastase-treated Fischer 344 rats (*93,94*), suggesting that interactive effects may also be strain dependent. Interestingly, inhaled PM had decreased overall deposition in the emphysematous lungs of hamsters but showed focal hot spot deposition in central airway bifurcations as has been predicted by theoretical deposition estimates (*95*). Although it is less likely that air pollution episodes may have a major impact on acute exacerbation of emphysema, the loss of functional reserve in this model may diminish its ability to accommodate even mild toxicant effects. Thus, more studies will also be required to understand the chronic impact of inhaled toxicants on the pathogenesis of the disease, especially with coexistent inflammation or bronchitis.

Asthma/Allergy. The incidence of asthma in developed countries has risen steadily over the past two decades (*96*). Asthma is usually episodic but leads eventually to chronic and sometimes severe life-threatening disease. Asthma is a complex pulmonary disease, if not actually a group of related diseases with similar signs and symptoms. Atopic or allergic asthma is more prevalent than the nonatopic form, especially in children (*97–100*); the allergy typically involves environmental antigens [e.g., dust mites (*101*)]. Reexposure of sensitized individuals results in pulmonary inflammation that may be modulated by T-helper lymphocytes (*102*). The cells exhibiting the T-helper cell type 2 phenotype (Th2) are proposed to upregulate IgE production and release cytokines such as IL-4 and IL-5 as part of the pathogenesis of asthma (*103*). Serum levels of cytophilic antibodies (e.g., IgE) are considered reasonable indicators of allergic sensitization in asthmatic humans and many animal models (*97,99,100*). Asthma is characterized by inflamed airway walls that are prone to constrict suddenly and vigorously and secrete thick mucus that can plug airway lumens. Local airway cell damage and edema, thickening of the reticular layer beneath the basement membrane, varying degrees of smooth muscle hypertrophy (*104,105*), eosinophilic and lymphocytic inflammation, as well as airway fibrosis are common features of human asthma (*97*). The twitchy airway behavior is frequently quantified by enhanced nonspecific airway reactivity to pharmacologic bronchoconstrictor agonists such as acetylcholine, methacholine, or histamine (*97–100*). Although a causal link between airway inflammation and airway hyperreactivity remains uncertain, it is generally accepted that there is at least an association between inflammation, airway damage, and hyperreactivity (*106,107*).

The most popular rodent models of allergic airway disease/asthma involve ovalbumin-sensitized and -challenged mice, Brown Norway rats, and guinea pigs (*108–116*). Other less frequently used animal models include sensitized dogs, monkeys, and sheep (*117–120*).

MOUSE MODEL. The B6D2F1/J and BALB/c strains of mice frequently are used as models because of their well-characterized allergic responses. Sensitization typically involves single or multiple intraperitoneal or subcutaneous injections of ovalbumin (*112,115,116,121–123*). In general, adjuvants such as *Bordetella pertussis* vaccine or aluminum hydroxide are used; however, in some specific studies the use of adjuvant has been curtailed because of nonspecific T-cell responses associated with the adjuvant (*115*). The mice may be challenged 1 to 3 weeks later with one or more booster doses of intratracheally instilled or aerosolized ovalbumin. Subsequently, serum levels of IgE, pulmonary inflammatory responses, and airway hyperresponsiveness to one of several agonists or the allergen are used to define the baseline model. Eosinophilia can persist several days after challenge depending upon the challenge protocol used (*115,121–124*). To address questions of interaction with pollutants, alterations in the baseline responses or kinetics of response can be ascertained but these are highly protocol dependent. Laboratory standardization is critical.

BROWN NORWAY RAT MODEL. Among rats, the Brown Norway strain has the most marked eosinophilia and clear serum IgE responses to allergens (*125,126*). The eosinophilia and bronchial hyperresponsiveness after allergen challenge in Brown Norway rats appears to be quite analogous to reactions in atopic human asthmatics (*127,128*). In sensitized dogs and sheep, allergen exposure causes an increase in bronchial responsiveness associated with neutrophilia (*117,118*); however, in Brown Norway rats, as in humans, eosinophilia has been correlated with bronchial hyperresponsiveness to agonist bronchoconstrictors (*109,128*). Ovalbumin remains a common allergen in this model (*109,110,126,128*), but recently more relevant antigens (e.g., dust mites) have been implemented in the model with good success (*101*).

GUINEA PIG MODEL. As with the murine and rat models, ovalbumin is the most common allergen used in the guinea pig (*114,129*). Two to four weeks after a parenteral sensitization the animals are challenged with aerosolized (~1%) ovalbumin. Because guinea pigs are sensitive to atopic bronchoconstriction, antihistaminic or β-adrenergic receptor blocker drugs are sometimes administered before challenge to protect animals from lethal anaphylactic bronchoconstriction (*130*). Lung pathology generally includes airway edema, perivascular and peribronchiolar eosinophilia, and epithelial damage. Cytotoxic basic proteins (markers of eosinophil activation) and other cell biochemical markers are assessed in BALF to determine the degree of inflammation (*130*). These end points and the assessments of lung function are generally used to ascertain the severity of the reaction to allergen challenge.

Each of these models has advantages and disadvantages with respect to similarities to human asthma and the availability of study tools to characterize the model and its responses. For example, unlike any other commonly used small laboratory animal species, normal guinea pigs have a highly sensitive bronchoconstrictive reflex (*114,131*) similar to that of asthmatic humans. However, most guinea pig-specific immunologic reagents and antibodies for cytokine and cell marker assays generally are not available. Murine models, on the other hand, can be better studied because of well characterized genetics and a wide availability of specific cytokine antibodies and other markers, but they are less sensitive to bronchoconstriction than the guinea pig. The murine models offer an added advantage over the guinea pig model in that the major allergic cytophilic antibody class in the mouse is IgE as in the human and the rat (*112,116*), whereas the guinea pig functions via the IgG_1 subclass antibody (*114,131*). Another general advantage of mouse models is the growing

availability of transgenic and gene knockout animals. Based on available information it is clear that there is no ideal rodent model of human asthma and that selection of any model should be approached on the basis of the question being investigated.

Effects of exposure to air pollutants on pulmonary allergic responses have been reviewed recently by Selgrade and Gilmour (*17*) and Gilmour (*18*). The Brown Norway rat model shows interaction between dust mite allergy and NO_2 exposure, which suggests its potential utility for air pollutant studies (*101*). Prior exposure of naive rodents to either O_3, SO_2, or NO_2 appears to enhance allergic sensitization (i.e., adjuvantlike effect), serum antibody titers, and bronchoconstrictive responsiveness to agonists (*18,132*). There is some experimental evidence to indicate that intratracheally instilled diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine production in mice (*133*). Occupationally, exposure to diesel exhaust has been linked to increased incidence of asthma (*134,135*). More experiments are needed to evaluate the host immune responses to diesel exhaust emissions in environmentally relevant scenarios. Just as air pollutant exposure before sensitization enhances the severity of the inflammatory response to subsequent allergen challenge in rodents, O_3 exposure has resulted in increased bronchial responsiveness to antigen in asthmatic humans (*136*). In general these findings are consistent with epidemiologic studies demonstrating increased hospital admissions for asthma during high air pollution episodes (*12–14*), although the underlying mechanisms are not clear. These studies suggest the potential usefulness of animal models to study the interaction of allergic airways disease and air pollutant exposure.

Chronic Obstructive Pulmonary Disease. Chronic obstructive lung disease is a collective description for lung diseases represented by chronic and relatively irreversible expiratory airflow dysfunction due to some combination of bronchitis, emphysema, and/or asthma (*38,137*). Airflow in asthma is generally considered reversible, but in chronic forms of asthma much of this reversibility can be lost (*138*). Also, the chronic hypoxemia of COPD is usually absent in nonaggravated asthma (*138*). COPD may first appear at about 40 to 60 years of age and typically progresses, especially with continued cigarette smoking or exposure to irritants (*38,137*). A form of COPD can also develop in the young, e.g., in patients with cystic fibrosis (*139*). In most COPD patients airway infection coexists (*38,39*) because of impaired mucociliary clearance and phagocytic function and excess mucus production. In the advanced disease, the presence of hypoxemia and hypercapnia promotes pulmonary hypertension and right heart enlargement (*cor pulmonale*). The ideal animal model of COPD should exhibit signs of emphysema with an airway inflammatory component (e.g., bronchitis). Such models combining SO_2 and elastase as well as a viral infection have been attempted in hamsters, resulting in pathology more closely resembling human advanced COPD (*48,49*); however, detailed characterization beyond pathology was not pursued. It is likely that similar approaches can be adapted to refine an appropriate COPD model for toxicologic application. Rat models of SO_2-induced bronchitis or monocrotaline-induced pulmonary hypertension (introduced elsewhere in this review) occasionally have been referred to as COPD models as they exhibit some COPD-like characteristics. However, unlike the acute manifestation of the disease induced in rats by artificial means, human COPD is usually the result of life-long processes (*137*).

Limited studies with human subjects have indicated that COPD patients retain a larger dose of inhaled PM in their lungs than healthy individuals (*140,141*), and thus the cumulative focal dose may be more toxic for them than for healthy individuals. On the other hand, the effects of acute O_3 exposure on forced expiratory volume in 1 second and forced vital capacity have been variable in COPD patients (*142*). Clearly, more thorough investigations need to be conducted. Because the responses to air pollutant exposure in COPD patients or animal models may be complicated by differential host sensitivity/compensatory repair capability as well as the total and regional doses of a toxicant, interpretations of challenge response data in COPD subjects must be done with care.

Pulmonary Fibrosis. Human pulmonary fibrosis long has been associated with environmental and occupational exposure to various metals, minerals, or organic dusts (*143,144*). Typically, the condition develops over many years. Idiopathic or drug-induced pulmonary fibrosis, on the other hand, can develop fairly rapidly (within months), but such cases are relatively rare (*143,145*). The etiology of nonoccupational fibrotic disease is unclear but involves inflammation, which may be partially autoimmune in origin (*143,145*). The pathologic manifestation of either disease is characterized by widespread fibrous or diffuse lesions and bridging of foci by collagen, which ultimately leads to decreased compliance and occasionally to airway obstruction. The toxicant-induced forms are frequently more regional, preferentially distributed to certain lung lobes, and may have a granuloma character (e.g., silicosis). A number of animal models of pulmonary fibrosis have been described, but for studies of clinically relevant pathogenesis, the most extensively used and best characterized rat model is that induced by the antineoplastic agent bleomycin (*146*).

Bleomycin is recommended for the treatment of certain malignant diseases; however, its clinical use is constrained by increased risk of pulmonary fibrosis with cumulative dose. The drug has been used widely in the rat, mouse, and hamster for fibrosis model development. Although hamsters are more sensitive to bleomycin and the resulting lesion is more widely distributed through the lung (*147,148*), mouse strains exhibit differential sensitivity to bleomycin and are being used to exploit molecular mechanisms of fibrosis (*149,150*). Emphasis in this review is on the rat model because of its greater potential application to studies of toxic inhalants.

Typically, rats are instilled intratracheally with 2 to 5 U/kg body weight pharmaceutical-grade bleomycin sulfate (*151–153*). Events preceding fibrosis include pulmonary edema and inflammation through the first 3 to 7 days, with the development of focal alveolar fibrotic lesions starting at about 1 week and progressing to a plateau level approximately 3 weeks postinstillation (*151,152*). Extensive alveolar and airway remodeling occurs during bleomycin-induced fibrogenesis starting from 2 days to 3 weeks and involves expression and production of proinflammatory cytokines and growth factors and fibroblast-mediated matrix synthesis (*148,151,154*).

A concern with this model in rats is that the lesions are focal, are largely peribronchiolar, and can be quite severe. Moreover, there is evidence that in response to injury compensatory hypertrophic and hyperplastic lung tissue growth occurs in the nonfibrotic lung areas, with some functional restoration, i.e., in terms of diffusing capacity (*155*). To overcome the problem

of focality of fibrotic lesions, some investigators have modified the treatment protocol to include exposing the rats to hyperoxia following lower doses of bleomycin (*155,156*). This treatment regimen results in a more diffuse lesion and may result in a better model of diffuse fibrosis than that from instillation of bleomycin alone. Although the morphology and severity of fibrotic lesions in these rat models compare reasonably well with those of idiopathic fibrosis in humans, the lesions in the rat are not progressive and inflammation wanes.

Other rat models of environmentally or occupationally related fibrosis have been developed by intratracheal instillation of silica or asbestos fibers (*144*). The mechanisms by which asbestos and silica cause fibrosis are reasonably well understood. They involve prolonged presence of the particles, protracted cytotoxicity, inflammation, and a network of cytokines and growth factors (*144,157*).

Application of the bleomycin-induced fibrosis model to pollution research has been limited to studies of regional deposition and clearance of inhaled submicroscopic particles in the rat, mouse, and hamster (*147,157–159*). In a rat model of bleomycin-induced fibrosis, we recently showed that modest mortality in response to ROFA instillation occurs only when the model exhibited active inflammation, though the dose of ROFA required to cause this response was rather high (*25*). Thus, the susceptibility of the fibrosis model may depend on the pathogenic state of the disease. Silica- and asbestos-induced fibrosis models have only rarely been used to study air pollutant-induced susceptibility (*160*); however, they provide fertile opportunities to address long-term interaction studies because of their progressive nature and their relevance to occupational and environmental exposures.

Pulmonary Infection. Respiratory infections are very common and rank as one of the most common causes of death. A variety of microorganisms infect the upper and lower respiratory tract in humans and depending on the infectious organism, disease symptoms and severity vary. Infections, although quite common in all individuals, typically are cleared quickly in healthy people depending on the virulence of the agent or organism. However, in individuals with underlying immunologic impairment or lung diseases such as cystic fibrosis, asthma, or COPD, the residence time for an organism in the lung is extended, allowing even less virulent types to proliferate (*161–165*). Common community-acquired bacterial infections that occur in humans include nontypable *Haemophilus influenzae*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Legionella species*, *Chlamydia pneumoniae*, and *Streptococcus pneumoniae* (*161–163*). *Pseudomonas aeruginosa* and *Streptococcus aureus* infections are common in cystic fibrosis patients (*139*), whereas nontypable *Hemophilus influenzae* plays a prominent role in exacerbations of COPD (*165–167*). Respiratory viral pathogens include rhinovirus, respiratory syncytial virus, influenza, and parainfluenza viruses; these generally are self-limiting and resolve in healthy individuals who usually mount antiviral immune responses (*168,169*). A variety of infectious agents have been used to develop infection models in rodents. The review by O'Reilly (*161*) cites the relevance of several animal models of bacterial infections to humans. The commonly used rodent models of viral and bacterial infections are described below.

VIRAL INFECTION MODELS. Viral infections, although common in all individuals, occur more frequently during early childhood (*170,171*). These acute viral infections cause epithelial necrosis, increased bronchial epithelial and endothelial permeability, and inflammatory cell influx. The immune cascade is thought to involve the T-helper type 1 (Th1) cell, as evidenced by increased production of interferon-γ and interleukin-2 (*96,102,172*). Although initial clearance of virus is mediated by $CD8^+$ cytotoxic T lymphocytes (*172*), under certain conditions viral infections can evoke T-helper type 2-like responses associated with allergy and atopy (*96,173,174*). This has been thought to alter sensitivity to aeroallergens in the young (*175–177*). Although most individuals with COPD and asthma recover from acute infection, severe or multiple infections have been implicated in the worsening of chronic disease and its resulting airway dysfunction. Following an acute episode of viral bronchitis, asthmatic children frequently develop repeated episodes of wheezing and symptom exacerbation that last for weeks and are easily induced by challenges previously having little or no impact (*178–180*).

Viral infection models most frequently use mice and rats. Infection models typically are achieved by intranasal inoculation, intratracheal instillation, aerosol dispersion, or intravenous infusion. Assessments focus on proliferation and clearance of the infectious organisms and associated pulmonary injury or pathology (*176,177,181–183*). Lebrec and Burleson (*183*) have described three influenza models: first, a model with a highly virulent, lethal strain (influenza A/Hong Kong/8/68, H3N2 virus) adapted to B6C3F1 and CD mice; second, a CD mouse model involving a less virulent strain (A/Port Chalmers/1/73, H3N2); and third, a similar (A/Port Chalmers/1/73, H3N2) nonlethal rat-adapted influenza virus model (RAIV). In contrast to the lethal mouse model that terminates in extensive pneumonia and lung consolidation, the A/Port Chalmers/1/73, H3N2 models exhibit airway epithelial damage, immune responses including interferon induction, neutrophilic, and lymphocytic influxes, and eventual antibody formation in keeping with the Th1 concept (*183,184*). Airway reactivity associated with the RAIV model subsides and recovery apparently is complete in about 2 weeks (*185*).

The BALB/c mouse model of human respiratory syncytial virus yields an airway injury that is similar to but milder than the human infection; it peaks during the first week postinoculation and the mice recover quickly during ensuing weeks (*181,186*). Using the genetically immunosuppressed cotton rat does not seem to enhance the response to this virus despite rather high doses of inoculum (*187*). Only the guinea pig developed significant complications and overt clinical disease related to this infection, thus more closely mimicking human infection (*182*).

A neonatal rat model of parainfluenza type 1 (Sendai virus) infection results in the usual epithelial desquamation initially and persistent airway pathology including hyperresponsiveness even 16 weeks after challenge (*175*). However, this model evokes considerable airway remodeling with the protracted infection, which is not common to most viruses. Also, Sendai virus may impose additional critical problems regarding general animal husbandry because it is quite infectious. This model may be more applicable to determining interactions of air pollutants in young hosts as a model of severe childhood respiratory infections.

Rodent models of viral infection have a history of use in studies of air pollution (*17,183,188–193*). In a murine influenza model, prolonged O_3 exposure after virus inoculation reduced virus replication and antibody titer (*189*) while potentiating the postinfection alveolitis and parenchymal changes (*190*). Prior exposure to O_3 in mouse models of influenza infection yielded variable responses in terms of viral

pathogenesis and O_3 exposure protocols (*17*), indicating the complexity of interpretation and extrapolation to the human situation. More recently, the RAIV model has been used to study the effects of coexposure to pollutants such as phosgene, O_3, and PM (*191,192,194*). The interactive effects observed in these studies were also exposure and infection protocol dependent. However, studies to determine how pollutants interact with viral infections in rodent models clearly are feasible and may aid in understanding the effects of pollutants on infectious diseases.

BACTERIAL INFECTION MODELS. Bacterial infections of the respiratory tract, although not as common as viral infections, can be quite pathogenic in humans. Those with underlying diseases such as COPD, cystic fibrosis, asthma, or various types of immune deficiency may be at particular risk of persistent and progressive bacterial infection (*161*). Bacterial pathogenesis in healthy individuals includes an initial proliferative phase and phagocytic cell-mediated clearance of the organisms. Efficient macrophage function and mucociliary clearance are critical to the initial defense against bacteria. Any impairments in these functions greatly increase the risk of protracted or severe infection and resultant lung injury. Because human bacterial infections are frequently associated with persistent pulmonary diseases, various experimental approaches are being taken to develop chronic infection models (*161*).

In general the mouse and rat models develop signs similar to those in humans, but the pathology in the rodents tends to be relatively mild, perhaps because human predisposing factors are absent (*17,195–198*). As with viral infections, bacterial infections usually are generated by intranasal inoculation or aerosol dispersion. To mimic the more chronic bacterial infections experienced by humans with other underlying diseases, bacteria were encased in agar beads to prevent the rapid clearance of inoculum (*161,162*). This has been attempted in the rat using nontypable *Hemophilus influenzae* (*162*) and *Pseudomonas aeruginosa* (*199*). Pulmonary epithelial mucosal damage induced by hexamethylphosphoramide or cobra venom factor promotes colonization of *Hemophilus influenzae*, which suggests that such intervention may also be useful in retaining the bacteria for a longer period to promote long-term infection (*200,201*). Models of chronic infections in mice are produced by experimental immunosuppression with dexamethasone pretreatment to mimic humans receiving glucocorticoid therapy (*196*). Empirically, immunosuppressed mice (e.g., CB-17 +/+ and CB-17 scid/scid, SCID) develop spontaneous fungal (*Pneumocystis carinii*) but not bacterial infections and thus have been used in mechanistic studies of such infections (*195*). These chronic infection models may be particularly important in considerations of host susceptibility in those already compromised with diseases such as COPD.

Bacterial infectivity models long have been used in air pollution studies, particularly to show increased bacterial infection-related mortality (*17,193*). In the mouse model of *Streptococcus zooepidemicus* infection, prior exposure to either O_3, NO_2, phosgene, or SO_2 enhances infection-associated mortality and pathology (*202–206*). Preexposure to a variety of PM and metal salts has also resulted in increased infection-related mortality in this model (*207*). Impairment of alveolar macrophage function by air pollutant exposure and consequent diminished clearance of bacteria are suggested to cause the decreased resistance (*193*). Thus it appears that air pollution exposure can alter host responsiveness and result in more persistent and virulent infections. Models of bacterial infectivity generally involve preexposure to the pollutant followed by bacterial challenge with the aim of assessing subsequent infection. The converse approach of bacterial infection followed by the pollutant has not been widely explored, however. Thus, the possibility of such a response in the model or the human is questionable and requires more study.

Cardiopulmonary Vascular Diseases

Cardiopulmonary diseases typically are diseases that involve not only the basic parenchyma of the heart and lungs but also their respective vasculature. It is also important to recognize that disease in either the lung or the heart may affect the other because of their intimate hemodynamic relationship. Indeed, the loss of vascular integrity may result from or cause organ dysfunction. On the other hand pulmonary vascular diseases can contribute secondarily to heart disease as the entire cardiac output must be pumped through the pulmonary vasculature (*208–210*). Chronic pulmonary disease frequently involves pulmonary hypertension and right ventricular hypertrophy, which when advanced can indirectly alter left ventricular function and contribute to blood gas abnormalities. Conversely, left-sided cardiac disease can also alter pulmonary venous pressure (*211–213*). Each of these conditions may sensitize the lung and/or heart to the effects of inhaled toxicants. In the context of this review, we consider pulmonary and systemic vascular diseases (including pulmonary and systemic hypertension) and congestive heart failure models.

Pulmonary Vasculitis/Hypertension. Pulmonary vasculitis/hypertension refers to an increase in pulmonary arterial pressure because of pulmonary vascular remodeling, pulmonary capillary bed damage, or disease. The etiology of human idiopathic or primary pulmonary hypertension is unclear but appears to involve selective damage or alteration to the pulmonary vasculature; it is relatively rare, almost always fatal, and affects mostly young women (*214*). A mild, nonpathogenic, and nonprogressive pulmonary hypertension occurs in native highlanders as part of a compensatory response to the chronic alveolar hypoxia due to an altitude-associated reduction in oxygen pressure (*215*). However, pulmonary hypertension in humans is most frequently a secondary development to airway and vascular pathology associated with COPD, chronic asthma, or cystic fibrosis (*208,210*). Loss of the pulmonary vascular bed from disease and inadequate gas exchange appears to play a significant role. Pulmonary hypertension can also be found in a variety of congenital heart diseases and in acquired left-sided heart diseases (*216*).

Two rat models of pulmonary vasculitis/hypertension have been widely used to study the pathobiology of this syndrome: parenteral treatment with monocrotaline (*217–219*) and chronic inhalation hypoxia (*220–223*). Although each of these models exhibits functional pulmonary hypertension, they offer the advantage of distinctly different mechanisms of induction and differences in the character of the syndrome, which may allow segregation of the mechanisms of interaction with pollutants.

MONOCROTALINE-INDUCED PULMONARY VASCULITIS/HYPERTENSION. Monocrotaline-induced vascular disease has been well characterized in the rat and in a number of other animal species (*217–219,224–227*). The proposed mechanism of injury includes selective pulmonary endothelial damage by the pyrrole metabolite of monocrotaline with induction of progressive pulmonary arteriolar muscularization (*219*). As impedance to blood flow through the lung increases, pulmonary artery hypertension develops (*218,224,228*). A single intraperitoneal or

subcutaneous injection of monocrotaline (50–60 mg/kg) produces pulmonary hypertension in the rat starting at 10 to 15 days posttreatment (*217–219,225*). This in turn causes right ventricular hypertrophy (as measured by weight of right ventricle/[left ventricle + septum]; RV/[LV+S]). At about 1 month there may be some mortality—depending in part on rat strain—because of the progression of pulmonary hypertension and inflammation (*217–219*). However, animals that do not die within the first 1 or 2 months can live with pulmonary hypertension for a long period (*217–219*). Pulmonary and vascular inflammation in surviving animals have not been characterized.

In addition to remodeling of arterioles and perhaps venules, pulmonary parenchymal changes including alveolar thickening, edema, and inflammation with frank endothelial and epithelial cell damage is evident (*229,230*). Hypoxemia, perhaps as a result of damage to the pulmonary vasculature and increased edema, is also apparent in this model (*231*) and oxygen therapy decreases pulmonary vascular resistance if given 10 to 21 days after treatment (*232*). Despite pulmonary hypertension no major impact on systemic circulation or hematocrit has been noted (*226,233*). Hepatotoxicity may become apparent at very high doses of monocrotaline (120–140 mg/kg); therefore, it is recommended that doses not exceed 50 to 60 mg/kg body weight to avoid these systemic effects (*217–219,224*).

HYPOXIA-INDUCED PULMONARY HYPERTENSION. Hypobaric and nitrogen dilution hypoxia have been used to generate pulmonary hypertension in rats. A continuous 3-week exposure of rats to a low F_IO_2 (10–12%, in nitrogen) with normobaria or to a simulated altitude of 16,000 ft (hypobaric chambers, P_{Bar} = ~250 mmHg) results in progressive lung vascular muscularization and pulmonary hypertension (*36,223,234–238*). Unlike the monocrotaline model, little if any pulmonary edema and inflammation are present in the established model (*36,239,240*). Acute hypoxia induces pulmonary hypertension through an increase in pulmonary arteriolar constriction, which causes resistance to blood flow through the lung. Erythropoietin-mediated polycythemia follows with an increase of atrial natriuretic peptide (a cardiac hormone) in the blood (*236,241*). The hypoxia also stimulates production of vascular endothelial growth factor and expression of its receptor (*223*) as well as expression of fibronectin and laminin (*220*), interleukin-1α (*242*), nitric oxide synthase (*240*), interleukin-6 (*243*), and endothelin (*222*), all of which are linked to vascular remodeling.

Although the pathogenesis of the lesions is significantly different between the monocrotaline and hyopxia models, the lesions in each model result in functional pulmonary hypertension and vascular remodeling reminiscent of the idiopathic human disorder (*36,37*). Chronic hypoxia typically yields a largely reversible pulmonary hypertensive condition, whereas monocrotaline results in a progressive, irreversible, and frequently fatal disorder. In comparison, human primary pulmonary hypertension is typically insidious and exhibits little nonvascular alveolar remodeling or inflammation, but it is generally progressive and fatal (*244*). The greatest relevance of these models for air pollution studies lies not in their similarity to human pulmonary hypertension but in their distinctive hypertensive/inflammation character, which may differentiate the role of inflammation in the secondary pulmonary hypertensive state common in advanced lung diseases.

To date, the application of the monocrotaline model to air pollution studies has been limited to PM. This model is uniquely sensitive to inhaled (*27*) or intratracheally instilled anthropogenic ROFA PM (*24,26,28*). The hypoxia model of pulmonary hypertension has yet to be used for similar studies. This collective data set on PM-associated susceptibility in the monocrotaline model has been the major impetus for the rekindling of interest in using diseased animal models of susceptibility for studies of air pollution. The underlying mechanisms and whether other air pollutants will yield analogous interactions remain to be determined.

Systemic Hypertension and Congestive Heart Failure. Together hypertension and heart diseases constitute the leading cause of death in the United States (*245,246*). Although diet and lifestyle are thought to play critical roles in development and progression of systemic hypertension and other vascular diseases such as atherosclerosis, genetic susceptibility may be the most important determinant of the condition. Both epidemiologic and experimental studies using animal models indicate that while cardiac disease can occur independently, long-lasting systemic hypertension can hasten atherosclerotic cardiovascular disease and culminate in an infarct with cardiac injury or failure (*247–259*). Animal models of hypertension and congestive heart failure have been used extensively to investigate basic disease pathogenesis (*247–251*) with the rat as the model of choice in the majority of studies. Several rodent hypertension models have been developed by breeding over several generations to segregate phenotypic traits (*247*). However, alternative cardiac models can be induced by surgical or pharmacologic manipulations of the cardiac vasculature.

MODELS DERIVED BY GENETIC MANIPULATIONS. *SPONTANEOUSLY HYPERTENSIVE RATS.* The spontaneously hypertensive (SH) rat represents a model of essential or primary hypertension derived from an outbred strain of the Japanese Wistar Kyoto (WKY) rat. It is the most widely used genetic model in hypertension research (*247,260*). Blood pressure begins to rise at approximately 5 weeks of age and increases progressively to about 180 mm Hg by 10 weeks of age regardless of gender (*247,260–262*). After prolonged hypertension, complications arise that are similar to those in humans—cerebral and myocardial lesions (e.g., infarction, hemorrhage) and nephrosclerosis (*247,252,263*). Alterations in the extracellular matrix of cardiac and vascular tissues progress over this same time period (*257,264*).

Hypertension in SH rats resembles human primary hypertension in that there is a genetic component, though no specific pathogenic mechanisms are yet known. There are other similarities: the course of pathogenesis, the major cardiovascular complications of hypertension, increased peripheral resistance, salt sensitivity, and finally the effectiveness of antihypertensive therapy (*260*). A potential shortcoming of the SH rat for studies involving the genetics of hypertension, however, is the appropriate genotype control for the SH rat; the WKY rat, the parental stock of the SH rats, is often used (*247,260*), but there appear to be confounding genotype differences. How this difference would complicate interpretations of air pollutant effects is not known.

DAHL SALT-SENSITIVE AND DAHL–IWAI RATS. Salt intake can exacerbate essential hypertension in humans, particularly if there is familial trend (*265*). Two Sprague–Dawley-derived rat strains show genetically determined differential sensitivity to salt-induced hypertension. The Dahl salt-sensitive (Dahl S) rat develops hypertension in 4 to 15 weeks when fed as little as 4% salt in its diet, whereas the Dahl salt-resistant (Dahl R) rat does not (*266*).

There is evidence that the sympathetic nervous system is involved in this model, as is kidney tubular fibrosis (*267*). However, unlike most human hypertension the renin system does not appear to be affected by salt-induced hypertension in the Dahl S rat (*247,268*). Studies involving these rats can be controlled by the paired S and R rat genotype (inbred Dahl–Iwai S/R and outbred SS/R/jr strains) and the Sprague–Dawley parentage (*269*).

RENIN TRANSGENIC RATS. A transgenic Sprague–Dawley rat model has been developed that harbors the mouse *renin-II* gene. Fulminant hypertension develops by 12 to 14 weeks of age (*247,270–275*). The hypertension is associated with secretion of adrenal steroids and increased angiotensin-converting enzyme activity (*270*). Myocardial remodeling (*274*) and defects in sympathetic neural control in the left ventricle have also been noted (*276*). This model has an advantage over many other hypertension models because of the involvement of the renin system as seen in human hypertension. To date, however, the model has not been widely used in hypertension research, and associated lung attributes have not been investigated.

OTHER GENETICALLY DERIVED MODELS OF HYPERTENSION AND CONGESTIVE HEART FAILURE. In addition to their use as models of hypertension, several SH rat and other substrains have been generated that develop congestive heart failure (*247,252,255,277,278*). SH rats, if allowed to age more than 21 months, also develop congestive heart failure and associated complications (*252,263*). One SH rat-derived model that is genetically obese (spontaneously hypertensive heart failure/*Mcc-fa^{cp}*) exhibits the features of chronic left ventricular failure including myocardial fibrosis, electrocardiogram abnormalities, pulmonary and hepatic congestion, dyspnea, and cyanosis as well as right-heart hypertrophy (*247*). Other cross-bred models have evidence of atherosclerosis when fed a high cholesterol diet (SHRSP/Izm) (*247,255,279*) or when in possession of a corpulent gene (*LA/N-cp*) (*280,281*). Several other strains of rats exhibiting hypertension and heart failure have also been used in cardiovascular research (*247,261,282,283*). Again, the extent of pulmonary studies in these rats is quite limited, but the models appear to provide opportunities to explore risks to inhaled toxicants associated with chronic cardiac diseases.

A number of earlier studies with the Dahl S/Dahl R strains chronically exposed to O_3, SO_2, and CO showed greater mortality in the Dahl S than in the Dahl R rat strain (*20–23*). However, only with CO was there a relationship with salt or hypertension, which suggests a genetically linked response with O_3 and SO_2. Our recent studies have shown marked basic differences in BALF end points (e.g., inflammation and glutathione) for WKY and SH rats (*26*). The responses to inhaled ROFA in terms of inflammation and glutathione levels in BALF were also markedly different for SH and WKY rats (*26*). How this may relate to increased susceptibility to inhaled air pollutants is not known, but clearly systemic hypertension as a predisposing condition to PM-induced increased morbidity remains to be further investigated.

MODELS DERIVED BY SURGICAL MANIPULATIONS. *AORTIC COARCTATION AND OTHER RENIN-DEPENDENT MODELS.* Rat models of renin-dependent hypertension can be developed by restricting renal blood flow. Approaches include partial ligation of the abdominal aorta below the right and above the left renal artery to unilaterally restrict blood flow (*284–287*) or clamping the left renal artery using a variation of the Goldblatt method (*288*). This approach has also been applied successfully to other rodents (*289*). Hypertension develops in several weeks, is progressive, and leads eventually to congestive failure. This model of hypertension may be compared to secondary hypertension associated with the renin angiotensin system in humans.

MYOCARDIAL INFARCTION AND ARTERIAL STENOSIS MODELS. The most widely used rat model of congestive heart failure is derived by surgical ligation of the left coronary artery (*290*). The degree of cardiac hypertrophy resulting from coronary artery ligation and infarct is often assessed by measuring the absolute and relative weights of the heart chambers and the dissected infarct areas. Though this model has high mortality (25–45%) within 24 to 48 hr of surgery, the surviving animals with large infarcts begin progression to congestive heart failure, which occurs within 4 to 8 weeks (*291–295*). This could be a relevant model of human cardiac failure secondary to ischemic heart disease with its ensuing pulmonary complications. A balloon-injury rat carotid artery stenosis model has also been developed (*296–298*). The resulting lesion seems comparable to human arteriosclerotic lesions.

MYOCARDITIS. Viral and giant cell myocarditis in humans, although uncommon, is an inflammatory heart disease that is frequently fatal (*299,300*). It is postulated that in case of giant cell or hypersensitivity myocarditis, presence of the multinucleated giant cells in the heart may be due to an autoimmune response. Viral myocarditis is characterized by the presence of inflammatory cells and myocardial dysfunction. In both cases congestive heart failure develops as a result of myocardial fiber injury and loss. The Lewis rat model of myocarditis is based on T-cell-mediated autoimmunity stimulated by immunizing rats with cardiac myosin in Freund's complete adjuvant (*301,302*). Myocarditis, characterized by pericardial effusion, enlargement, and discoloration of the heart and appearance of the multinucleated giant cells, develops over 4 to 6 weeks (*301*). The model leads to congestive failure as in the human disease. Mice (and less frequently hamsters and monkeys) infected with Coxsackie B virus have been most often used in developing a model of myocarditis (*299*). Despite the fact that myocarditis is rare in humans, these rat models may be easily developed and used for specific mechanistic studies involving congestive heart failure without marked chronic systemic alterations.

Susceptibility studies of air pollutants in surgically derived rodent models of hypertension/congestive heart failure are rare. One study conducted using the Goldblatt hypertension rat model demonstrated a dose-dependent increase in mortality from a 32-week exposure to a mixture of gaseous air pollutants (SO_2, NO_2, CO, and ammonium sulfate aerosol) at environmentally relevant levels and above (*19*). To our knowledge no follow-up systematic studies have been conducted to determine the cause of this unusual sensitivity. Because ischemic heart diseases appear to be a risk factor of PM-induced mortality according to epidemiologic studies (*5*), rodent models may help elucidate possible cardiopulmonary mechanisms involved in susceptibility.

CARDIOMYOPATHY. *CARDIOMYOPATHY INDUCED BY PHARMACOLOGIC MANIPULATION.* Clinical use of the anthracycline antibiotic drug adriamycin in cancer chemotherapy poses a risk of irreversible cardiomyopathy (*303*). The risk in humans increases with cumulative dose. Rodents develop similar cardiopathology when treated over several weeks (*304–306*). Cardiomyopathy is particularly severe in the left ventricle. No reduction in basal heart rate and aortic flow occurs; however, coronary artery flow is reduced (*305–308*). Increased lipid peroxidation in cardiac mitochondria is thought to underlie the pathogenesis (*308*). Brain and kidney

effects become significant, especially at higher concentrations of adriamycin (*309,310*), but pulmonary effects have not been studied in detail. Increases in angiotensin-converting enzyme activity have been noted in the lung (*309*); this may be secondary to left ventricular myopathy. This model of congestive cardiomyopathy offers an advantage of ease of induction and has demonstrated characteristic alterations in cardiac myocytes similar to the pathologic findings in human idiopathic dilated heart muscle disease (congestive cardiomyopathy). However, as with any drug-induced model, the possible involvement of other damaged organs in the susceptibility state is a potential confounder.

Genetic Predisposition Toward Cardiomyopathy. Cardiomyopathy characterized by focal cardiac lesions develop in a strain of Syrian hamster at 40 to 50 days after birth and progresses with time. As a result congestive heart failure and death occur within 1 year of birth (*209,311–314*). There is also evidence of some degree of skeletal muscle degeneration in this model. The cardiomyopathy is characterized by ventricular dilation, focal areas of fibrosis, myocytolitic necrosis, and cellular hypertrophy. Alterations in lung natriuretic peptide receptors have been noted in this model of cardiomyopathy (*209*). These findings also occur frequently in congestive heart failure in humans.

These models of cardiomyopathy have not been used for susceptibility studies involving air pollutants; however, they represent yet another tool to investigate mechanisms of susceptibility when damage to myocardium is not caused secondarily by systemic or other organ failures. The potential utility of cardiac disease models in the study of air pollutant effects has to date been underappreciated. Because heart diseases are the predominant ailment of aging humans, the public health significance of a demonstrated cardiac-related susceptibility would be dramatic. Animal models of cardiac diseases, although used only sparingly in the past, represent potentially useful tools to address the questions that would be difficult to explore in cardiac disease human patients. One specific advantage with cardiac disease models is that as with humans, they are largely chronic in nature and thus may be specifically useful in chronic pollution exposure studies. It remains to be seen which animal model affords the best instrument for these studies and to what extent coexisting pulmonary impairments may be involved.

Conclusion

The issue of susceptibility as it relates to the threshold of response and the basic risk of adverse effects associated with air pollution is gaining wide public health and political interest. By the nature of their illnesses, many perceived susceptible humans are not available for direct study. Thus, animal models of disease provide opportunities to explore basic issues of susceptibility, mechanism, and risk, albeit with the limitations of extrapolation. With careful preparation of the models and conservative interpretation of the data, animal models may provide toxicologists with another tool to investigate environmental cardiopulmonary disease.

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