ANIMAL MODELS OF HUMAN LUNG DISEASE

Animal models of chronic obstructive pulmonary disease

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Because COPD is closely related to the underlying anatomy of the lung, a good animal model should have pulmonary anatomy similar to that of humans. The fundamental mechanisms behind COPD should be similar in animal models and humans. The ideal model would allow the investigator to produce the various different anatomic lesions just listed, all in a short period of time. Unfortunately, all of the known animal models meet only some of the above criteria, and even another human would probably not meet all of them, because, as noted above, there is considerable human to human variation in the pattern of COPD.

A variety of different approaches have been used to attempt to model COPD in animals, but there are some more general principles that will affect any COPD model, namely, 1) considerable differences among species in lung development and maturation; 2) differences among species in lung anatomy; and 3) susceptibility to injurious agents among species and strains within a species (discussed in Ref. 16).

Comparative Anatomy

Development and maturation. The timing of pre- and postnatal development and maturation of the lung varies widely between species, and this problem needs to be taken into account when planning the initial exposure to any injurious agents.

The rat and mouse have no true alveoli at birth and develop all of their alveoli postnatally, with the majority of alveogenesis taking place between days 4 and 14 (90), whereas guinea pigs are well alveolated at birth and have only a small increase in numbers of alveoli as they age. Air space size normally increases with age, but this also varies with species. For example, guinea pigs (155) and rats (56, 96) have a progressive increase in size until 12 and 18 mo [although there appears to be some differences between strains (56)], and...
hamsters attain maximum size by 6 wk (reviewed in Ref. 61). Air space size change also varies among mouse strains, with BALB/c mice demonstrating increasing size up to 19 mo (61), whereas strain 129 has constant size after 4 wk (97).

The importance of recognizing lung maturation and growth patterns cannot be overstated. Administration of an injurious agent in the immediate postnatal period can interfere with alveolarization, and the enlarged air spaces may be falsely interpreted as emphysema. This is also true of genetically modified animals where the modification itself alters lung development in such a way that the animal has larger than normal air spaces (see Genetic and Genetically Modified Models of COPD).

Anatomic structure: bronchial tree. The various animal species commonly used to model COPD differ significantly from each other and from humans in anatomy of the bronchial tree. Mice and rats have less extensive airway branching than do humans (93). Rodents do not normally have respiratory bronchioles; rather, a respiratory bronchiole-like type of metaplasia is induced by reaction to injury (112). This is considerably different from humans, where there are several generations of membranous and respiratory bronchioles. In humans, the membranous, and to a lesser extent, the respiratory bronchioles, are the targets of smoke-induced small airway remodeling, whereas the respiratory bronchioles are the initial site of injury in centrilobular emphysema.

Anatomic structure: airway epithelium. In humans, the tracheal epithelium is pseudostratified and changes to a simple epithelial type in the subsegmental bronchi (55). The majority of the cells are ciliated and are present throughout the bronchial tree, gradually decreasing in proportion, but extending into the respiratory bronchioles (99). Goblet cells are plentiful in the large airways and progressively decrease until they are rare in the terminal bronchioles (99). This pattern is recapitulated in the guinea pig (Fig. 1) (55).

Rats and mice tend to have ~10-fold greater numbers of nonciliated bronchiolar cells in the small airways compared with humans (113, 166), and the cells vary ultrastructurally among species (113, 114). The tracheal epithelium of the mouse is a single columnar layer, whereas in the rat, the transition between pseudostratified and simple epithelium occurs at the hilum of the lung. In the mouse, goblet cells are rare in the trachea and almost totally absent in the membranous bronchioles; the majority of the tracheal epithelial cells are nonciliated Clara-like cells (105), although ciliated cells occur in large patches and account for ~37% of the total cells (104). In the rat, the proportion of ciliated cells to nonciliated cells increases from upper trachea to bronchioles where they form ~65% of total cells (55). Only a few goblet cells are present in the trachea and large airways and are generally not seen in the small airways.

Anatomic structure: bronchial glands. Interestingly, this appears to be an area of controversy, and this issue is important, since human chronic bronchitis is characterized by hypertrophied and hyperplasic bronchial glands and excess mucus secretion. Although most authors agree that the size of the bronchial glands in laboratory animals is smaller than in humans, there is disagreement as to the number and positioning

Fig. 1. Comparison of tracheal epithelium from humans (A), guinea pigs (B), rats (C), and mice (D). In the human there are large numbers of ciliated cells and goblet cells, a pattern that is also found in the guinea pig. The rat has scant goblet cells, and the mouse has none. Ciliated cells are common in the rat but are present only in clusters (arrows) in the mouse. Bar = 100 μm; hematoxylin and eosin (H&E) stain.
of glands in the various species (32, 54, 152) (Fig. 2). Despite these differences of opinion, it seems clear that bronchial glands are absent in the mouse and rabbit, almost absent in the hamster, present in the rat but concentrated in the upper portion of the trachea, and irregularly distributed in the guinea pig. Even when present, the number of glands in small animals is markedly less than large mammals, thus making small animals a very poor model for diseases such as chronic bronchitis.

Anatomic structure: pulmonary arteries and veins. In humans, the arterial and venous vasculature have definite courses and are separate from each other: the arteries follow the path of the airways, whereas the veins are present in the interlobular septa (Fig. 3). The main pulmonary artery and vessels larger than 1,000 \( \mu \text{m} \) in diameter are elastic in nature with short, branching, elastic fibrils arranged in laminae. Vessels between 500 and 100 \( \mu \text{m} \) are muscularized, with a tunica media present between two elastic laminae. Muscle becomes less apparent with decreasing size as the spiral of muscle becomes increasingly separated until it disappears at the level of the distal arterioles. Pulmonary venules have a single elastic lamina and gradually acquire a muscular layer, the borders of which are generally ill defined as it contains irregular elastic fibrils. At the level of the atrium, there may be a few cardiac muscle fibers.

In the rat, guinea pig, and mouse, interlobular septa are absent (94). The pulmonary artery branches follow the airways, but the pulmonary vein is independent and follows a separate course from the periphery of the lung to the hilum. In rodents, the walls of the small arteries have intermittent areas of increased muscular thickness. In rodents, there is a wide variation in the point at which the arterial branches change from muscular to nonmuscular, with \( \sim 50\% \) of vessels adjacent to the terminal bronchioles and \( 80\% \) of vessels adjacent to the alveolar ducts being nonmuscular (45). The arteries have a dense mesh of \( \alpha \)-smooth muscle actin immunopositive cells which is organized in a spiral until the most distal vessels (106). Unlike humans, rodent pulmonary veins have a large amount of cardiac muscle at the hilum of the lung (106), and this is accompanied by a loose mesh of \( \alpha \)-smooth muscle actin immunopositive cells, which decreases as the veins become smaller.

Anatomic structure: bronchial arteries. The bronchial arterial supply is known to be important in the overall lung reaction to injury, and thus anatomy and distribution are important in any animal model. There is considerable person-to-person variation in the human bronchial artery supply (99, 135). These arteries invest the bronchi and extend throughout the length of the bronchial tree, with communication between the bronchial and pulmonary circulations in the terminal respiratory unit. In mice (116), a bronchial artery supply exists, and there are interconnections between the two vascular systems at the level of the capillaries. Rats have a relatively similar system to humans, but with slightly different vascular derivations (135).

Lung Mechanics

A careful quantification of air space size utilizing morphometry should be mandatory to define “emphysema” as a first step to assess the response to the exposure or manipulation used in the intended animal model. However, as noted by Henson and Vandivier (44), it is remarkably easy to induce alveolar enlargement with a very wide variety of manipulations in the mouse, the most widely used animal as a model for emphysema, and this raises the question of how specific such findings might be, particularly since many such manipulations appear to be interfering with lung growth or producing abnormal lung.
Another approach is to use pulmonary function to define emphysema on the basis that, while pulmonary function tests are less sensitive than morphometry and thus may detect only more severe degrees of airways remodeling or parenchymal destruction, they demonstrate meaningful physiological changes. Although mild emphysema may have normal lung mechanics, more marked emphysematous lung destruction ought to have abnormal lung mechanics. The most specific function test representative of emphysema is loss of elastic recoil, determined from pressure-volume (P-V) curves (increased compliance or decreased elastance).

This issue is nicely illustrated in the following papers. Foronjy et al. (25) found that long-term cigarette smoke exposure in two strains of mice (CBA/JXJ67BL/Gi) and A/J resulted in a 17% and 32% increase in mean linear intercept (Lm), but no change in lung compliance. Furthermore, neither strain had a decrease in lung elastin by immunostaining or by elastin assay methods. In contrast, Cavarra et al. (14) and Takubo et al. (137) found that the pallid mouse, a strain with low levels of $\alpha_1$-antitrypsin, developed significant air space enlargement, had a 19% decrease in lung elastin content, and significant increases in compliance (Fig. 4), thus more closely resembling human emphysema. Similar results have been found by us in the AKRJ mouse strain (36) and in the guinea pig (18, 155).

Which of these approaches is “correct”? There is no simple answer to this question; at the present time, there are no definitive studies that illustrate a direct correlation of air space enlargement and compliance or a level of air space enlargement beyond which alterations of compliance would be expected, and it is unclear whether more marked emphysema (as defined in this way) is a better approximation to human disease mechanisms than less marked emphysema. Furthermore, the marked laboratory-to-laboratory variations in response in even the same (notional) mouse strains make comparisons almost impossible.

As a general rule, the greater the severity of emphysema, the greater the chance that a protective intervention is really significant, rather than just a minor experiment-to-experiment variation, but this idea must be tempered by the realization that none of these models translates to human disease in any direct fashion, and an intervention that ameliorates fairly mild emphysema might turn out to be mechanistically important. For example, there is increasing evidence for the importance of immunological reactions in the genesis of emphysema (1). In their investigations relating to the role of CD4 and CD8 T-lymphocytes in cigarette smoke-induced emphysema, Maeno et al. (81) found a 19–21% increase in air space size in wild-type C57/Bl/6J mice with a respective increase of 19% and 0.4% in CD4-/-- and CD8-/-/ cell-deficient mice. One must ask, therefore, whether the lack of lung mechanics invalidates the importance of this mechanistic data.

Normally lung mechanics in mice and other rodents are performed at the end of the experiment with the animal alive, anesthetized, paralyzed, and with the chest opened or closed. Mechanics measurements done in dead animals are not as accurate or reproducible, and measurements deteriorate as times from death increase. Lung mechanics and other pulmonary function tests can be easily measured in guinea pigs (155) or other large rodents utilizing a body plethysmograph, a tracheal canula, and an esophageal tube to obtain P-V curves, FRC, and other lung volumes. Flows can also be measured by rapidly deflating the lungs from TLC applying an airway pressure of -50 cmH2O. Measurement of lung mechanics can also be performed in mice, although it is technically more difficult, and we and others have used a small animal ventilator, Flexivent Scireq (Montreal, Canada), equipped with the necessary hardware and software to accurately measure elastance, resistance, and tissue resistance along with inspiratory and expiratory P-V curves.

Lung mechanics can also be measured in live rodents, including mice, with the expectation of recovery after the measurements (nonterminal event). After anesthesia, the animals can be (relatively) easily intubated and subsequent measurements obtained (11, 140). The obvious advantage of this technique is that it allows the documentation of functional changes over time in the same animal (161).

Vendors

There are large numbers of animal suppliers available in North America, Europe, and Asia; regular strains, transgenics, genetic models, etc., are available through these suppliers. The web site www.rodentia.com is a useful and informative starting...
pulmonary function is technically difficult, although possible,
effects (see, for example, Ref. 132).
smoked too heavily, develop nonspecific particle overload
investigators’ hands, they develop minimal disease, or, if
cross react with guinea pig proteins.

genes are cost, the lack of tools for performing molecular
extensive pulmonary function evaluations along with cardiac
metaplasia in the larger airways. As well, it is easy to perform
metaplasia in the smaller airways, although rats do demonstrate

animal models of COPD (16); here we will consider only the
advantages and limitations of such models.
Methods of smoke exposure. There are a variety of com-
cerfly and home-built smoking machines that have been used
with animal models; some systems use nose-only exposures
and some whole body exposures. There is a theoretic negative
to whole body exposure in that the animals may ingest nicotine
or tar substances when cleaning their fur. However, Mauderly
et al. (92) showed that although this was a factor, there was less
carboxyhemoglobin in the animals given whole body exposure,
and these animals had less weight loss than did animals given
a nose-only continuous or intermittent types of exposure.

When establishing any smoke exposure model, measure-
ment of either serum cotinine (a nicotine metabolite) or blood
carboxyhemoglobin (COHb) is useful as a method of confirm-
ing the relative amount of smoke exposure. In our guinea pig
nose-only exposure model, we find an acute post-smoke car-
boxyhemoglobin of 15–20% and a chronic blood carboxyhe-
moglobin level of ~5%, the latter comparable to that seen in
many human smokers. In humans, the dissociation of carboxy-
hemoglobin when breathing air results in a half-life of ~4–6
h; animal data are not available, although COHb levels rapidly
decline after smoke cessation (43). A serum cotinine level of
200–250 ng/ml is equivalent to a mild to moderate human
cigarette smoker (31, 43, 103, 127).

Choice and limitation of species. All modern studies of
cigarette smoke-induced COPD have used small laboratory
animals including mice, rats, and guinea pigs. Guinea pigs
offer numerous advantages, including easily recognizable
emphysema, more marked small airway remodeling than mice,
and the development of considerable goblet cell metaplasia
in the airways. Mice and rats develop only minimal goblet cell
metaplasia in the smaller airways, although rats do demonstrate
metaplasia in the larger airways. As well, it is easy to perform
extensive pulmonary function evaluations along with cardiac
catherization in guinea pigs (155, 162). Their main disadvan-
tages are cost, the lack of tools for performing molecular
studies, and the limited number of commercial antibodies that
cross react with guinea pig proteins.

In our experience, rats are a poor model for COPD. In most
investigators’ hands, they develop minimal disease, or, if
smoked too heavily, develop nonspecific particle overload
effects (see, for example, Ref. 132).

Most recent models of COPD have used mice. Detailed
pulmonary function is technically difficult, although possible,
in mice (36, 84, 137), but P-V curves and compliance changes
can be measured fairly easily. Mice offer the advantages of low
cost, extensive gene/protein sequence/antibody availability,
and, most important, the availability of numerous naturally
occurring mouse strains with different reactions to smoke and
the ability to produce animals with genetic modifications that
shed light on specific processes within COPD.

One point that confounds all of the literature on cigarette
smoke-induced models is laboratory-to-laboratory variations in
response. Even when the same mouse strain is used, different
laboratories get quite different degrees of disease. For example,
Churg et al. found increases in mean air space size (Lm) of
37% (20) and 38% (19) after 6 mo of exposure using C57Bl/6
mice, but Cavarra et al. (14) and Guerassimov et al. (36)
reported increases of only 14% and 13% in the same strain.
In part, this problem is caused by differences in the strains from
different providers, technical differences in morphometric
techniques, the use of different smoking systems (nose only vs.
whole body, smoke concentration, smoke time, and frequency),
and by differences in the types of cigarettes smoked. It is our
impression that currently available cigarettes (such as Ken-
tucky 2R4F or 2R5F) cause considerably less emphysema than
older ones (such as Kentucky 1R1 or 2R1), and this is a serious
problem, both in terms of producing disease and particularly in
terms of examining interventions, since one never knows
whether interventions that ameliorate small increases in air
space size would really be effective with more severe disease.

Cigarette smoke-induced models of emphysema. Emphy-
sema is the anatomic lesion that, historically, has attracted the
most attention in patients with COPD, and, similarly, has been
the focus of most animal models (Figs. 5, 6). Except for
α1-antitrypsin-deficient pallid mice (14, 137), models of
smoke-induced emphysema typically produce dilated alveolar
ducts; these changes progress with increasing amount of smok-
ing and are, anatomically, similar to a mild form of the
centrilobular emphysema commonly found in human cigarette
smokers, but the overt tissue destruction seen in humans is
much harder to demonstrate in animals (14). The parenchyma
between the dilated alveolar ducts is abnormal, with increases
in size and number of the pores of Kohn that connect adjacent
alveoli (154), a phenomenon also found in the lungs of human
cigarette smokers (21, 65, 101). There is evidence that women
are more susceptible to the effects of smoke than men (29, 70,
85, 148) and female A/J mice develop emphysema earlier than
male A/J mice (84).

The lesions produced in laboratory animals can be subtle,
even on microscopic examination; thus morphometric analysis
is required to assess the degree of damage. Measurements of
air space size (Lm) or surface-to-volume ratio (Sv) (reviewed in
Ref. 141) and sometimes destructive index (14) are used for
this purpose. Arguably, it would be better to measure only
alveolar ducts, since that is the major site of the abnormality,
and such measurements do provide more marked differences
between smoke-exposed and control animals (see Ref. 19, for
example). However, measurements of alveolar duct air require
greater morphometric effort (137) and are not widely used.

Smoke-exposed guinea pigs and mice show physiological
changes that mimic mild COPD in humans (18, 36, 84, 137,
155). The residual volume increases as does functional residual
capacity and total lung capacity. The P-V curve is shifted
upwards and to the left, compliance increases, and the flow
volume curve demonstrates diminished flow at the lower lung volumes. Ratios of FEV/FVC also decrease. These abnormalities progress with increasing length of smoke exposure (155).

Cigarette smoke-induced small airway remodeling. Small airway remodeling (increased matrix components, inflammatory cells, and goblet cell metaplasia in the airway wall with luminal narrowing, distortion, and obstruction by mucus) is now accepted as an important cause of airflow obstruction in human smokers (46, 47, 98, 107, 156). These changes increase with increasing Global Initiative on Chronic Obstructive Lung Disease (GOLD) stage (47). Excess mucus secretion is also thought to be important in the pathogenesis of acute exacerbations of COPD (reviewed in Ref. 6).

In animals, early smoke inhalation studies demonstrated increases in the numbers of goblet cells in the proximal trachea in a variety of species (7, 42, 57, 69, 108, 117), but there is a considerable species effect and a large degree of variation in the degree of metaplastic change. In the guinea pig model, chronic smoke exposure induces secretory cell metaplasia in the small airways (158), and similar to humans, smoking cessation reduces the degree of metaplasia (160).

Little attention has been paid to small airway wall remodeling in animal models, probably because these changes are too subtle to be reliably picked up by casual microscopic observation, and some deny their existence (84). Nonetheless, morphometric analysis confirms that the small airway walls are thickened in both mice and guinea pigs after long-term smoke exposure (10, 17, 18, 159). Most of the increased wall area is composed of collagen and fibronectin, and smooth muscle is not increased (10, 17, 95, 159). There is also increased collagen fiber alignment and more dense packing of the fibers, findings that imply increased mechanical stiffness in the airway wall (159). In guinea pigs, the severity of changes in collagen structure correlates negatively with PEF and FEV₀₁/FVC, and positively with airway resistance. Physiological lung volumes correlate with air space size, but RV and TLC also are related to airway wall thickness (159).

Small numbers of lymphocytes are found around the bronchioles in mice and guinea pigs and are analogous to the lymphoid aggregates seen in humans. Smoke causes quite marked increases in the number and size of these aggregates (9, 10, 146).

Cigarette smoke-induced vascular remodeling and pulmonary hypertension. In subjects with established pulmonary hypertension related to COPD, there is intimal thickening and muscular hyperplasia in the normally muscularized vessels adjacent to the bronchioles and increased muscularization of the usually partially muscularized arteries adjacent to the alveolar ducts (reviewed in Refs. 3, 157).

In Hartley strain guinea pigs, chronic cigarette smoke exposure produces about a 25% increase in pulmonary arterial pressure (160, 162), which, interestingly, appears after 1 mo of smoke exposure and does not progress. Similar to human disease, PHT in guinea pigs is associated with muscularization of the small, normally poorly muscularized pulmonary arteries adjacent to the alveolar ducts (162). Increased pulmonary artery pressure (∼30%) has also been reported in one study of smoke-exposed rats (72) but thus far has not been reported in mice. Vascular remodeling, however, has been reported in mice, and there are apparent strain differences in the severity of remodeling (100).

Conclusions regarding smoke-induced models. Cigarette smoke exposure to animals appears to be the best approxima-

![Fig. 5. Example of centriacinar emphysema in a human. Note that the respiratory bronchiole and alveolar duct complex is distorted and destroyed. Aggregates of pigmented alveolar macrophages can be found (arrow). Bar = 1,000 μm; H&E stain.](http://ajplung.physiology.org/)

![Fig. 6. Example of a guinea pig emphysema model induced by exposure to cigarette smoke for 6 mo. A shows a representative control lung, whereas B shows a representative smoke-exposed lung with an overall increase in air space size of ∼43%. Bar = 200 μm; H&E stain.](http://ajplung.physiology.org/)
Table 1. Pros and cons of cigarette smoke-induced model of COPD

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
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<tr>
<td>Induced by same insult as in humans</td>
<td>Does not produce the severe disabling disease seen in humans</td>
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<tr>
<td>Produces emphysema, airway remodeling, and vascular remodeling/pulmonary hypertension in selected species</td>
<td>Requires several months of exposure</td>
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<td>Produces physiological alterations similar to humans</td>
<td>Lesions do not appear to progress after cessation of smoke exposure</td>
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Apoptosis as a Model of COPD

There are several excellent recent reviews of this subject (144, 145, 151, 163). The idea that emphysema occurs as a failure of the lung maintenance and repair system follows from studies that have induced apoptosis by interference with vascular endothelial growth factor (VEGF) (60) or its receptor (VEGF-R) (139) or the VEGF-VEGFR complex (88). Apoptosis generally involves activation of the caspase cascade and can be induced by intratracheal instillation of activated caspase-3 as well as other agents (2). Activation of ceramide, an upstream regulator, (111) will also induce apoptosis.

These models induce air space enlargement in a very short period of time (Fig. 7) but do not appear to affect the airways. Inhibition of VEGF receptors sufficient to induce air space enlargement also causes pruning of the vascular bed and pulmonary hypertension (60, 138, 143, 150), with increased muscularization of the small pulmonary arteries.

Conclusions. Although inhibition of the VEGF system appears to result in relatively permanent air space enlargement, instillation of a single dose of activated caspase-3 produced initial air space enlargement followed by diminution of air space size within 15 days (2). Thus far, none of these models has been shown to be associated with matrix breakdown, an event that is believed to be central to the pathogenesis of emphysema (reviewed in Ref. 16), nor have they been shown to reproduce the physiological changes of emphysema. Finally, it is important to recognize that apoptosis has only been found to be increased in human lungs with severe emphysema, and analysis of the smoke-exposed mouse or guinea model has shown inconsistent results, with no significant increases in the number of apoptotic cells in some studies (Churg A and Wright JL, unpublished data) (25), whereas one study found focal areas of apoptosis, but only in DBA/2 and not in C57Bl/6 mice (5) (Table 2).

Elastase Emphysema Model

The current generally accepted hypothesis of cigarette smoke-induced emphysema is the protease-antiprotease hypothesis. This hypothesis developed out of two key observations: first, Laurell and Eriksson (71) reported that patients who were deficient in α1-antitrypsin developed early emphysema. Second, in 1965, Gross et al. (34) described the induction of emphysema in rats by instillation of the plant protease, papain. These observations led to the idea that emphysema develops as a result of a cigarette smoke-mediated influx of inflammatory cells into the lower respiratory tract with resulting release of proteases that destroy the parenchymal matrix.

The protease-antiprotease hypothesis was in large measure formulated from experiments in which emphysema was caused by administration of elastolytic enzymes, either by intratracheal instillation or aerosol inhalation; these models are currently referred to by the generic term “elastase emphysema”.

Fig. 7. Example of a mouse apoptosis emphysema model induced by installation of active caspase-3. A shows a representative control lung. B shows a representative image from lungs 48 h after installation of active caspase-3, with an overall increase in air space size of ~4%. The arrowheads indicate enlarged air spaces. Bar = 50 μm; H&E stain. [From Petrache et al. (110).]
Table 2. Pros and cons of apoptosis model of COPD

<table>
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<th>Pros:</th>
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<td>Short-term model</td>
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<td>Induces enlarged air spaces (&quot;emphysema&quot;) and vascular remodeling</td>
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<td>“Emphysema” not permanent</td>
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<tr>
<td>Apoptosis not consistently present in animal models of cigarette smoke-induced emphysema</td>
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<td>Airway remodeling not present</td>
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(Fig. 8). A variety of enzymes have been used for this purpose (52, 53, 74, 75), and one of the key findings in this regard was that only enzymes that could degrade intact elastin-produced emphysema; collagenases by themselves were ineffective (75, 136). These findings led to the current belief that destruction of the elastin framework of the lung is the fundamental pathological abnormality in emphysema.

While papain was used in initial studies, its elastase activity depends on its purity and source (75). Almost all recent studies have used either porcine pancreatic elastase (PPE) or human neutrophil elastase (HNE). PPE offers the advantages of being inexpensive and easy to obtain. Consistent quality human neutrophil elastase is now available but considerably more expensive. Experiments using crude preparations of human neutrophils first and then purified HNE were in fact instrumental in arriving at the notion that smoke-evoked inflammatory cells release elastolytic proteases that can destroy the lung (52, 53, 75). From a practical point of view, it is important to note that PPE and HNE do not have the same proteolytic spectrum; they have different primary endogenous inhibitors [α₁-antitrypsin for HNE, but α₂-macroglobulin for PPE, although α₁-antitrypsin is also effective (133)], and, even more important, a particular exogenous inhibitor may not inhibit both of these enzymes (for example, see Refs. 67 and 68 and see below). This is a crucial point to bear in mind if one is attempting to show efficacy of a novel inhibitor.

A wide variety of animals have been used in the elastase emphysema model (52, 75). Much of the early work, and some recent work, was done in hamsters, based initially on the empiric observation that hamsters appeared to develop more severe disease. The reason for this turns out to be that hamsters normally have relatively low levels of α₁-antitrypsin (52, 75). Conversely, several investigators showed that administration of α₁-antitrypsin or normal serum but not serum from persons deficient in α₁-antitrypsin prevented or ameliorated elastase emphysema (58, 86); this observation again was important in formulating the protease-antiprotease hypothesis.

Table 3 lists pros and cons of the elastase emphysema model. The major advantages are that emphysema can be rapidly induced by a single treatment with an inexpensive reagent, which makes it vastly cheaper and easy to run than a 6-mo smoke exposure. Disease severity can be controlled by selection of enzyme dose. As opposed to smoke-induced emphysema, it is relatively easy to produce severe emphysema with elastase instillation, and this facilitates detection of pulmonary function abnormalities, particularly when evaluating the effects of an inhibitor or intervention such as lung volume reduction surgery or abnormalities in connective tissue elasticity (15, 51). Biochemical and in situ hybridization studies have shown that over time there is increased synthesis of elastin and to a lesser extent collagen (77), making the model a candidate for studies on alveolar repair and regeneration [however, studies using all trans retinoic acid and/or bone marrow cell instillation or various colony-stimulating factors to attempt to drive repair are contradictory (49, 50, 76, 102, 164)]. But probably the most important current reason to use the elastase emphysema model is the situation where an intervention requires scarce/expensive resources that would not be feasible for a 6-mo smoke exposure. For example, Houghton et al. (48) recently showed that antibodies against elastin fragments ameliorated the inflammatory response and emphysema in mice given PPE.

The major disadvantages of the elastase emphysema model center around two related problems: 1) although both elastase and cigarette smoke produce emphysema through proteolytic attack on the lung matrix, the detailed mechanisms of this process are probably very different; and 2) the mechanisms behind elastase emphysema are not clear. Both light microscopic and electron microscopic studies (52, 66, 165) show rapid loss of elastin after enzyme instillation, but the disease progresses (depending somewhat on animal and dose) long after elastase activity can no longer be detected. In fact, the measured half-life of HNE or PPE in this model is ~45–50 min (133). It has been suggested that progression is caused by enzyme bound to the matrix and not detectable with conventional assays, but a late intervention (day 20) with an elastase...
Table 3. Pros and cons of elastase model of COPD

<table>
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<th>Pros:</th>
<th>Cons:</th>
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<tr>
<td>Single treatment (as opposed to 6 mo of smoke)/economic and easy</td>
<td>In case of any adverse effects, the model is not recoverable.</td>
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<td>Rapid onset</td>
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<td>Can produce severe disease depending on dose</td>
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<td>Progressive in some hands</td>
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<td>Easy to measure functional changes</td>
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<td>Good choice when dealing with scarce resources where impossible or</td>
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<td>too expensive to run a 6-mo smoke</td>
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<tr>
<td>Possibly relevant to certain aspects of regeneration of elastin</td>
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Relevance of therapies/interventions even less clear than with animal models of smoke exposure.

Inhibitor did not prevent progression (79), which the authors interpreted to mean that other processes are involved. Single dose elastase inhibitors appear to work when given immediately before or immediately after elastase administration, but are not effective if given 4 or 8 h later (35, 63, 134). However, two synthetic serine elastase inhibitors [ICI 200,800 and ICI 200,855 (153)] prevented both PPE and HNE driven emphysema when started 24 h after enzyme dosing and continued on a daily basis. Other novel elastase inhibitors/elastin protectors (13, 67, 80) also provide some degree of protection.

In fact, the model is certainly more complicated than pure elastase attack. Elastase emphysema itself is subtly different from human emphysema, with pure alveolar enlargement predominating in elastase emphysema and increased alveolar wall destruction (measured by the destructive index) in human (23) and to some extent in murine smoke-induced emphysema (84). Soon after enzyme administration, there is both hemorrhage and an inflammatory exudate which includes neutrophils and macrophages in the lower respiratory tract, and there is up-regulation of a variety of proinflammatory mediators such as TNFα, IL-1β, IL-6, and IL-8 (28). It has been claimed that there are increased numbers of apoptotic parenchymal cells (126, 164), but these studies have only used TUNEL staining and hence are inconclusive. Inflammatory processes clearly play an important role since mice lacking both TNFα and IL-1 receptors are ~80% protected against PPE-driven emphysema and inflammation (78), as are mice overexpressing CuZnSOD (26) and mice given the anti-inflammatory agent HO-1 by adenoviral transfer (129). Elgin-C, which inhibits rat NE but not PPE, was protective against PPE-driven emphysema, supporting a role for endogenous elastases (68). On the other hand, a broad spectrum MMP inhibitor decreased emphysema but not inflammation (8).

One further practical complication is that animal age appears to be important, but the literature is contradictory, with claims that either younger or older animals are more resistant to PPE (33, 59, 87).

Conclusions regard the elastase emphysema model. Overall, these findings indicate that although elastase emphysema is simple to produce, the model is not mechanistically simple at all. At best, elastase emphysema is a screening technique for mechanisms and/or interventions that could apply to human smokers; however, this approach is fairly far removed from smoke exposure and in our opinion is generally inferior to animal models using smoke exposure.

Starvation-Induced Model of COPD

In humans, chronic malnourishment or actual starvation appears to be associated with emphysema-like alterations of the lung parenchyma (reviewed in Ref. 22). Starvation models in rats have varied in the amount of reduced feeding and also have varying degrees of alteration in lung structure and physiological changes (119, 121–125). Alterations of lung mechanics were generally characterized by a decrease in lung volume related to transpulmonary pressures, and in one study (125), the shift in the saline P-V curve was accompanied by significant reductions in elastic recoil, and chord compliance (slope of the straight line between 80 and 40% maximal lung volume) was increased by 47%, with a 53% increase in air space size. Mechanical changes reverted to normal with refeeding (123), and although bulk lung hydroxyproline also reverted to normal, elastin remained decreased (122). Cycles of starvation and refeeding resulted in overall findings similar to those above, but there was also a decreased alveolar surface area interpreted as a decreased number of alveoli (120). Calorie depletion accompanied by protein depletion showed the unexpected finding of less severe emphysema than in those animals with similar caloric intake that included protein. All of the calorie-restricted groups had smaller lung volumes and fewer alveoli than did the control group indicating that the effect of starvation is a lack of lung growth.

A comparison between starvation (45% loss of body wt) and elastase-induced emphysema showed considerable differences between the two models (41). While elastase-induced emphysema mimicked human emphysema, with increased lung volumes and subdivisions of lung volume, a shift to the right of the P-V curve, and reduced expiratory flow rates, the P-V curve in the starvation group was shifted to the right, the lung volumes were not increased, and there was no decrease in airflow. Both groups had enlarged air spaces suggesting that the two models may represent lung destruction in the case of elastase emphysema and alterations of lung growth in the case of starvation emphysema. But recent studies in mice (89, 91) have questioned this explanation since alveoli appear to be lost early in calorie restriction and gained after refeeding. Starvation appears to stimulate caspase gene expression and Fas

Table 4. Pros and cons of starvation model of COPD

<table>
<thead>
<tr>
<th>Pros:</th>
<th>Cons:</th>
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<tbody>
<tr>
<td>Short-term model with relatively consistent results</td>
<td></td>
</tr>
<tr>
<td>Does not match human physiological changes</td>
<td></td>
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<tr>
<td>May represent a model of abnormal lung maintenance/repair/abnormal lung growth</td>
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<tr>
<td>Ethical considerations of induction of 45% weight loss by starvation</td>
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death receptor expression, with depression of cell replication, while refeeding increases cell replication, DNA synthesis, and increased RNA expression for a large number of cell replication-related genes.

Conclusions regarding starvation models. Starvation models are relatively short term in duration and produce enlarged air spaces, but they do not produce the physiological changes of emphysema and more likely represent models of abnormal lung growth or abnormal repair (Table 4). No airway or vascular alterations have been described.

LPS-Induced Models of COPD

LPS is a glycolipid component of gram-negative bacterial cell walls and is used because of its proinflammatory effects. It is present as a contaminant in cigarette smoke, air pollution, and organic dusts. In humans, chronic exposure to LPS-laden dusts results in decreased lung function (reviewed in Ref. 147). In the acute model, LPS induces a mixed inflammatory reaction with increases in neutrophils (8) and increases in BAL TNFα and IL-1β, and increases in MMP-9 and MMP-12 (73, 147) (Fig. 9A). In mice (147), repeated intratracheal LPS installations induces marked inflammation with predominately CD4+ T cell lymphoid aggregates around the airways and large vessels, and an increase in intra-epithelial bronchiolar epithelial cell lymphocytes. This was associated with large numbers of intra-alveolar macrophages. Cytokine mRNA profiles showed increases in TNFα, IFNγ, and IL-18, a finding that persisted after exposures ceased.

With chronic administration over several weeks, LPS instillation produces enlarged air spaces (Fig. 9B) and remodeled airways with thickened walls and increased goblet cells in the larger airways in mice, guinea pigs, and rats (40, 142, 147). In the guinea pig model (40), progressive exposures resulted in a decline in specific airway conductance and an increased duration of bronchoconstriction after exposure. These changes resemble human emphysema and small airway remodeling (albeit again relatively mild) and may be driven by mechanisms that are somewhat similar to human disease; however, these mechanisms are poorly defined.

Conclusions regarding LPS models. LPS instillation is a short term model which produces some anatomic features of human COPD, but the inflammatory infiltrate differs from that in smoke models, suggesting different mechanisms (Table 5). Probably the most important use of LPS is to mimic acute exacerbations, either given alone or given to animals also receiving cigarette smoke. The topic of models of acute exacerbation has been recently reviewed in Refs. 30, 62, 83.

Genetic and Genetically Modified Models of COPD

Much of the recent literature on COPD models has used either naturally occurring mouse strains or laboratory-produced animals that either overexpress or lack particular genes. Several excellent reviews of such models are available (12, 82, 128, 144, 149), and we will only comment briefly here on their advantages and disadvantages.

Different naturally occurring mouse strains show markedly different responses to smoke which depend in part on naturally occurring levels of antioxidant and antiprotease protection and in part on genetic factors that control the innate and acquired inflammatory response to smoke (14, 81). Different strains have a variety of genetic differences, and thus one usually cannot attribute different responses to an insult such as smoke.

Table 5. Pros and cons of LPS model of COPD

<table>
<thead>
<tr>
<th>Pros:</th>
<th>Short-term model with airway and parenchymal changes, and inflammatory cytokine upregulation</th>
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<tbody>
<tr>
<td></td>
<td>May be helpful in investigations of exacerbation</td>
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<tr>
<td>Cons:</td>
<td>Inflammatory infiltrate not analogous to that found with cigarette smoke</td>
</tr>
<tr>
<td></td>
<td>Unclear whether mechanisms involved recapitulate those of cigarette smoke-induced lesions</td>
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Fig. 9. A: example of a mouse subacute LPS model induced by exposure to LPS for 1 h per day for 5 days. Note the inflammatory reaction in the air spaces and interstitium. H&E stain. Magnification, ×10, courtesy of Dr. M. K. Lee, Battelle Science and Technology, Richland, WA. B: example of a hamster emphysema model induced by installation of LPS twice per week for 4 wk and death after 28 days after the final installation. Emphysematous areas are surrounded by relatively normal areas with an overall increase in air space size of ~30%. The arrows point to iron depositions. [From Rudolphus et al. (118)].
to a single gene change, as is possible with man-made genetically targeted animals (there are exceptions to this rule, however, such as pallid mice that are α1-antitrypsin deficient) (14, 137).

Genetically altered animals allow investigation of the effects of a specific gene/protein on all of the different anatomic lesions of COPD and potentially also are useful in designing therapeutic agents. Most attention has been directed to models of emphysema, with much less information available about small airway remodeling (64). All such models need to be interpreted very cautiously, because constitutive overexpression or knockout of a particular gene can interfere with lung development and lung growth, resulting in enlarged air spaces that superficially appear to represent emphysema but are really developmental abnormalities. Conversely, constitutive expression of a gene might also lead to accelerated aging, and accelerated senescence mice are known to develop emphysema (130).

Several different approaches have been taken to try and get around the issue of developmental abnormalities. Some constitutive transgenic models of emphysema have concentrated on selection of strains in which transgene expression is present at a very low level and disease only appears to manifest late in adulthood (27). Such models provide support for the importance of the gene in question but by definition cannot rule out subtle developmental or aging abnormalities.

Inducible models generally use constructs in which feeding of doxycycline drives the gene in question with a lung-specific promoter. This approach in theory prevents problems with developmental or premature aging effects. However, there may be small amounts of “leakage” of the transgene even in the absence of doxycycline, and it has been reported that the reverse tetracycline-transactivator gene that is used to create inducible constructs can itself can produce enlarged air spaces (130). As well, doxycycline is a matrix metalloproteinase inhibitor that inhibits MMPs such as MMP-2 and MMP-9 that are thought to play a role in COPD (37). Thus careful controls are essential in interpreting such experiments. Last, it is important to remember that overexpression of a particular gene may lead to production of huge amounts of protein, and there is no guarantee that “pharmacological” doses of a particular protein act in a similar fashion to physiological doses.

Conclusions regarding models using genetically altered animals and different naturally occurring strains. These problems notwithstanding, genetically modified animals and naturally occurring mouse strains have produced a wealth of data, either by themselves or when such animals are exposed to cigarette smoke. We have recently reviewed the topic of smoke-induced models and the mechanisms behind such models in this journal (16). How closely these models mimic the pathogenesis of human disease is still open to question (4).

Conclusions

A variety of quite widely differing types of animal models has been created to attempt to reproduce human COPD. None of them succeeds perfectly in this attempt, and all have problems. Nonetheless, cigarette smoke-induced models of either naturally occurring or man-made animal strains appear to be the best choice for most purposes if one has the resources and patience to carry out the very long exposures required. Elastase emphysema has a limited role, particularly when resources are scarce. Models that examine failure of lung maintenance or failure of repair will probably be important in understanding the pathogenesis of emphysema, but as yet don’t clearly approximate events in the human lung.

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