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## ***In Vivo* Models of Lung Disease**

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### **1. Introduction**

Development of new therapeutics for lung diseases requires good modeling systems in which to test hypotheses. Often, how lung diseases are modeled *in vivo*, are not at all initiated by the same events that cause the disease in humans. The models for interstitial pulmonary fibrosis or chronic obstructive lung disease for example, require the use of toxic reagents and models for asthma do not use the same antigenic stimuli. What this means is what is used to initiate disease *in vivo* using animal models is not necessarily totally responsible for the same disease in humans. Even in situations of generating genetic models focusing on identified genes associated with specific disease entities modeled *in vivo*, the disease in the animal model is still not the same as the disease in humans even if the gene is most certainly involved. The focus of this chapter is to describe a variety of the animal models that have been developed to study specific lung disease entities including understanding the strength and the weaknesses of the *in vivo* modeling systems. The main goal of animal modeling is to provide an *in vivo* complex scenario which allows for the pursuit of defining the underlying mechanisms of diseases or importantly to provide a format for studying new interventional therapeutics. The focus of the chapter will start with basic anatomy, physiological differences and immunological responses which either enhance the selection of the model or are used to study specific components of the disease process.

**Anatomy and Lung Models:** For an *in vivo* model to provide the appropriate conditions, modeling the anatomy and the physiology of the lung model must first be considered. Whether dealing with small rodents such as mice, rats, and ferrets or larger animal such as pigs, sheep or monkeys, a detailed understanding of the model's anatomy and physiology must be considered for the correlation to human diseases (1,2). The issues to consider include the anatomical patterns of the alveolar spaces, the bronchial tree, milieu differences including the changes in the surfactant proteins, phospholipids, and physiological differences including the respiratory rate and airway clearance mechanisms (3,4). Some of the issues of correlating with human disease have to do with how the lung structure is different with the human lung and how this relates to differences in lung structure and function. This also relates to size, oxygenation and gaseous exchange. Another important issue is how the lung structure relates to the physiology and whether the mechanisms for

homeostasis maintenance the same? This complicates things further since in most instances a direct cross-over between animal models and human disease is not complete. The relevant comparative anatomy of the lung would include all of the variables outlined in Figure 1.

## Comparative Lung Anatomy, Structure and Function

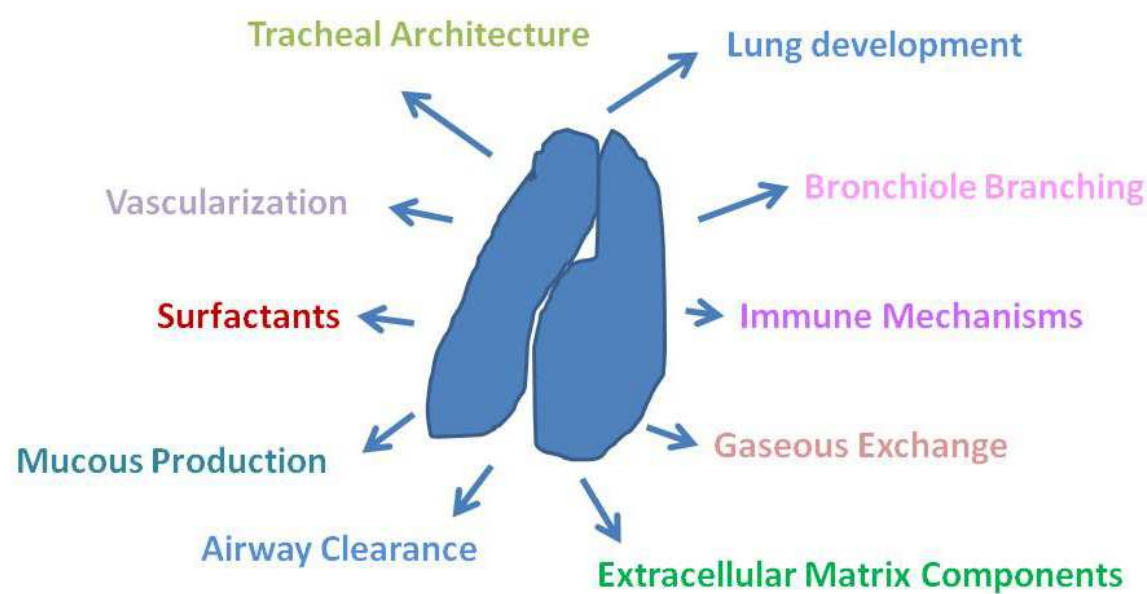


Fig. 1. Contributions to *In vivo* Lung Models. Here we show the lung and the variables associated with model selection regardless of the lung disease to be studied.

**Function and Lung Model:** The selection of an *in vivo* model must take into consideration not just the similarities and differences between the model and the human disease but also the question being asked in the disease application. The more common comparisons are listed in Table 1, when evaluating murine and rat models. Certainly, some animal models provide good *in vivo* correlates to the clinical situation; other are not so realistic. Choosing the model has to do with the question being answered and the reasoning behind selecting the model. For examples, cats and horses have been shown to develop spontaneous airway hyper-responsiveness, which would be consistent with human asthma (5,6). However, given the size of the animals, the inability to generate congenic species makes these models economically unrealistic. The opposite perspective is the ability to use mice for diseases such as asthma and cystic fibrosis (CF). Although the specific disease can be mimicked, the spectrum of the pathophysiology is different. For example, the mouse model for CF does not develop spontaneous lung disease (4,7). The model does provide an invaluable tool to study infection induced inflammation and in some case cell specific contribution of disease (8). In the murine asthma model, a variety of antigens can be used to induce disease, but it has been shown that many of the pathways associated with disease in humans are not played out in the murine model of the disease (9,10). It is a balance between the clinical or

mechanistic question and the goal of the study for the selection of the appropriate model. Animal models afford the opportunity for investigators to experimentally manipulate a number of controlled variables such as strain of animal, and environment to investigate the molecular interactions involved in the pathogenesis of many lung diseases. The selection is based upon the basic pathophysiology, anatomy and the ability to induce the disease in a time sensitive fashion for comparative and economic consistency.

Mechanism of Study	Mouse	Rat
Lung Remodeling and Repair	Offers the ability to study specific genes associated with repair and remodeling. Depends on species susceptibility to either the Th <sub>1</sub> or Th <sub>2</sub> process.	Enhanced susceptibility to develop Th <sub>2</sub> driven cellular immune responses. Larger airways, easier to measure breathing dynamics.
Inflammation	Mechanisms involve similar but often different proteins. Careful experimental design for a focused approach on the similarities but remembering the differences.	Functionally similar but several components and events which are different. Understanding the similarities and differences are important in the context of disease.
Response to Infection	Similar processes and players with proteins being both the same and different. This all depends on protein homology or even presence.	Similar processes with players of proteins being often times similar. The issue is the availability of reagents for studying the mechanisms of interests.
Response to Injury	Injury response is relative to the total surface area at the air-liquid interface. Mouse models use chemicals, which are not the initiators in human disease.	Injury associated with chemicals of mechanical contributions can be used due to the size difference from the murine counterparts. Chemicals used are always associated with real human disease.

Table 1. In Vivo Models and Studies of Lung Disease Pathophysiology

**Acute Lung Injury:** Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) results from severe injury to the lung parenchyma (11). Animal modeling experiments of ALI and ARDS have been very useful in providing some directions into the mechanisms related to disease pathogenesis and providing opportunities to explore new and innovative therapeutic targets. As with most lung disease modeling systems, the design of the model and its manipulation is predominately dictated by the hypothesis and the focal point of pathology. The pathology associated with ALI and ARDS includes inflammatory cell recruitment, exudation with edema in the small airways potentially resulting in alveolar collapse (12). The recruitment of inflammatory cells, the changes in tonicity at the tissue interface are all pathologies which contribute to the injurious process. This occurs through

enhancing the production of inflammatory proteins, proteases and reactive oxygen radicals at the tissue interface. This gets to the mechanisms associated with the development of ALI and ARDS including the processes involving the injury and down-stream response to the injury which also contributes to tissue damage (13,14). There are three different models that are used for inducing different aspects of ALI and ARDS. These include the surfactant washout (LAV) model, oleic acid intravenous injection (OAI) model and the lipopolysaccharide (LPS) model (15). The LAV model utilizes a series of bronchoalveolar lavages which requires larger animals like rats and ferrets. In the surfactant washout models, the focus is removing the protective anti-inflammatory molecules such as surfactant protein A (SP-A) potentially altering the air-liquid interface surface tension, resulting in changes in oxygenation efficiency. The change in surface milieu signals the production of pro-inflammatory cytokines with results in recruitment of inflammatory cells which ultimately contributes to interstitial tissue damage (15). The OAI model uses an infusion of oleic acid into the central vein or the right atrium (16,17), necessitating the requirement of larger animals. There is considerable diversity in terms of the dosing and the timing of the administration of OAI, making the model highly variable and not well accepted. The precise mechanisms by which the oleic acid induces lung edema, and the mechanisms associated with the recruitment of inflammatory cells and injury are not completely understood. The response of the animal to the oleic acid, results in a series of inflammatory events that create ALI/ARDS which has been theroretically associated with enhanced pro-inflammatory cytokine production.

The production of the pro-inflammatory cytokines in the LPS model is the common process involved with ALI development, as discussed in reference to the OAI and LAV models. The LPS model uses the product of gram negative bacteria (LPS) to induce cytokines and the down-stream events which result in inefficiency in the ability to resolve infection (15,18). In a sense it is a process that confuses the immune system so that it is unable to perform efficiently. The LPS is usually extracted from *Escherichia coli*, but could be from other gram negatives such as *Pseudomonas aeruginosa*, a common pathogen associated with community acquired pneumonia and ventilator associated pneumonia (19,20). The development of stable lung injury is dependent on dosage, time, route of administration and size of the model selected. In the murine models of the ALI/ARDS the LPS is administered intra-tracheally. The process of infection induced ALI and/or ARDS may include sepsis in the animal model but also in human disease. In fact, about 50% of sepsis cases ultimately account for ALI and ARDS ventilator support (21). The development of sepsis, results from a sustained and uncontrolled inflammatory response to the infectious insult contributing to dysfunction of at least one organ system. The sequences of events which lead to sepsis are unknown as well as the events that result in pulmonary failure (22,23). In the models of sepsis induced ALI/ARDS, LPS is administered intra-tracheally or induced by surgically clipping the gastrointestinal tract (11,15).

**In Vivo Models, Clinical Relevance and Limitations.** Histologically, human ALI/ARDS can be sub-divided into an exudative and fibroproliferative phase (24). The exudative phase is characterized by the accumulation of inflammatory proteins containing neutrophils (25), followed by the accumulation of macrophages initiating the fibroproliferative phase of the disease (26,27). In some patients the process and side-effects of the acute inflammatory response completely resolves whereas others progress with chronic inflammation, fibrosis and neovascularization (28). Each of the different models used to develop ALI/ARDS have



both valid and controversial contributions to studying these diseases *in vivo*. How these models compare and provide insight into ALI/ARDS is outlined in Figure 2. In the surfactant washout model, it is a useful tool in studying the importance of surfactant maintenance in airway-interface surface tension and pathophysiology of ALI. The issue is that most clinical conditions do not result in clinically significant surfactant abnormalities in the adult population (29). In the OAI model, the ability to induce the pathophysiology of ALI/ARDS is dependent on using injectable oleic acid, which is obviously not similar to the *in vivo* clinical development of the disease. However, it is still a useful model for studying the pathology of ARDS especially with a focus on membrane injury (16,17). Since infection has been closely associated with the development of ALI/ ARDS, the LPS model seems to be the most translatable. However the other two models probably represent up-stream events in the exposure, specificity and sensitivity of the development of infection based ALI/ ARDS (30-32). As with most *in vivo* animal models it does not appear that the model is consistent with all of the components of the human disease. The LPS model does not appear to develop the fibroproliferative phase of ALI/ ARDS; which limits the use of these models for studying the secondary issues associated with ALI/ ARDS chronic inflammation and fibrosis (15,30).

Acute Lung Injury In vivo Models  
Strengths and Weaknesses

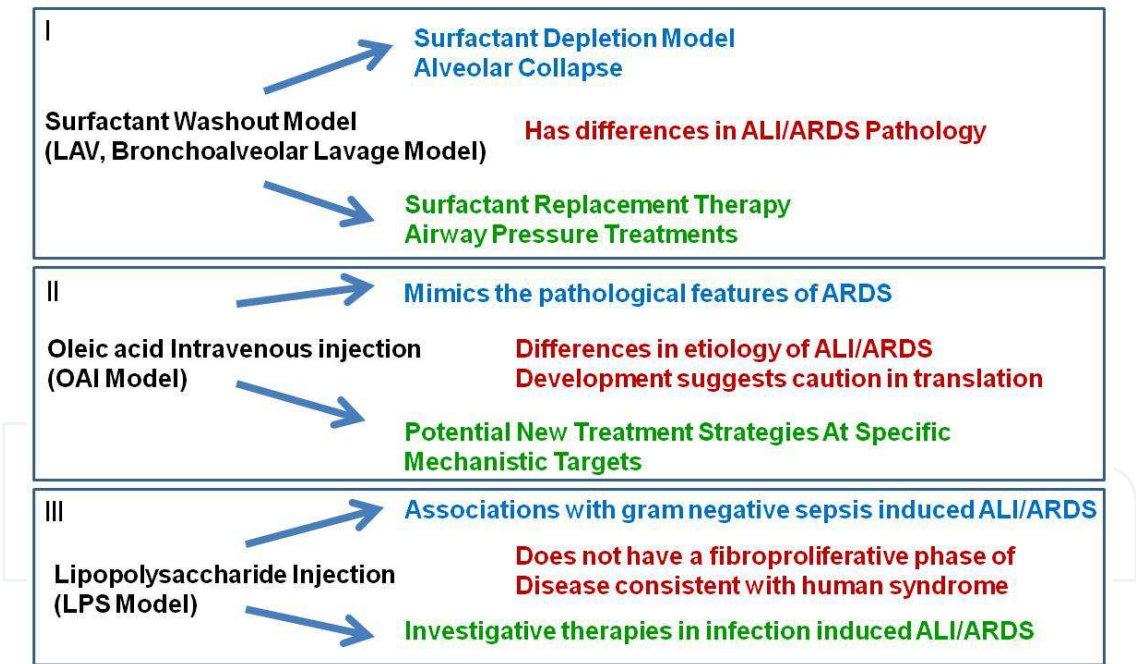


Fig. 2. In Vivo Models of Acute Lung Injury. Three principal models exist for studying ALI/ARDS. In each case the model has important contribution to the pathophysiology shown in blue, and potential therapies shown in green. The important caveats and limitations are shown in red.

**Chronic Obstructive Pulmonary Disease (COPD) and Emphysema:** COPD is the fifth leading cause of death worldwide and is associated with pollution and smoking history (33). COPD is a very complex disease with four described traits: emphysema, small airway

remodeling, chronic bronchitis and pulmonary hypertension (34). The underlying pathophysiology of COPD is dependent on the structure and function of the lung along with the immunological processes that occur post-insult. Although patients present with variations and combinations of these pathologies, all patients progress into severe pulmonary failure. The kinetics of disease progression is dependent on the patient, patient compliance to therapeutic intervention and the ability to respond to current therapeutics. In this case the animal model of choice should require a close attention to lung anatomy and physiology since these play very important roles in the overall development of COPD and emphysema (35), especially as it relates to the overall development of new therapeutics. Besides the basics of understanding the similarities and differences between lung anatomy of the animal model and that of the human disease some consideration must also be given to the overall lung mechanics.

The *in vivo* model most commonly used to study COPD includes cigarette smoke (36). The issue lies with the ability to deliver a homogenous dosing of cigarette smoke over a defined time range, and that these models do not completely recapitulate the human disease. Further, since there are genomic differences which increase susceptibility to COPD, the translatable ability is always in the background. Additionally, the pulmonary pathology produced in the context of the cigarette model produces subtle pathologies which may also introduce subjective interpretation in quantifying the histopathology (37). Better computerized-microscopic programs need to be developed that can better quantify and minimize subjective interpretation of the studies (26,38).

Non-specific inflammation is another indicator of COPD, with a predominance of neutrophils and the inflammation approach to studying COPD focuses on apoptosis and elastase (39,40). The apoptosis model focuses on the failure of the COPD lung to repair itself post-injury focusing on dysregulated normal lung tissue turnover. The mechanism associated with apoptosis induced COPD has been linked to the production of vascular endothelial growth factor (VEGF) and/or the VEGF receptor (40). It is not clear whether this VEGF/VEGF receptor dysfunction is by itself critical for inducing endothelial cell apoptosis and the processes that result in decreased vascularization in the lung or whether it is in the context of a variety of other factors which ultimately contribute to COPD.

The elastase model uses a product of the inflammatory response to initiate and perpetuate the inflammatory response seen in COPD. The original hypothesis for the importance of elastase came patients  $\alpha_1$ -anti-trypsin deficiency (41,42). Individuals with this disease develop emphysema and COPD. These patients are treated with exogenous  $\alpha_1$ -anti-trypsin, the endogenous inhibitor of elastase. In COPD/emphysema, the recruitment of inflammatory cells and the disproportionate production of proteases without the appropriate anti-protease counter-part ultimately results in extracellular matrix degradation, inflammatory cell recruitment, matrix metalloprotease activity, cellular activation which all contribute to the lung damage similar to the mechanisms in  $\alpha_1$ -anti-trypsin deficiency (43,44). The disadvantage of the elastase model, is that the function of elastase and cigarette smoke in COPD emphysema are potentially mediated through very different pathophysiological mechanisms which again brings up the issue of clinical translation. It is efficient to have a very specific inducer of emphysema for investigating specific mechanisms and therapeutic development. However, results obtained from specific products need to be taken into consideration as compared to the complex *in vivo*

environment post complex insult (45,46). Some investigators have used LPS to induce airway and parenchymal changes, although the pathophysiology is more reminiscent of ALI/ARDS than COPD (33,47). Table 2 lists the pros and cons of each of the COPD models.

Model	Pathology	Positives of Model	Negatives of Model
Cigarette Induced COPD	Dilated alveolar ducts, abnormal parenchyma and increased numbers of goblet cells. Pulmonary function tests show decrease in effectiveness.	The most similar to the human disease in terms of inducing agent that produces emphysema.	It is not debilitating in animals. Lesions do not progress beyond a certain point to mimic the human disease.
Apoptosis Induced COPD	Induction of air space enlargement. Matrix breakdown.	Induces enlarged airspaces in short period of time.	Pathophysiological mechanisms are not permanent.
Elastase Induced COPD	Increased numbers of neutrophils, elevated elastase.	Rapid and easy onset, easy to measure functional changes and possibly relevant to the repair and remodeling issues in COPD.	Mechanism of disease induction is secondary to the initiators of clinical COPD.
Starvation-Induced COPD	Decreased lung volume, changes in lung structure and function.	Limited variability and short term impact on disease development.	Compassionate care of animals. The pathology may be due to decreased repair mechanisms.
LPS Induced COPD	Produces enlarged airways in chronic scenarios. Matrix metalloproteinase production	Short-term model with parenchymal changes.	Inflammatory differential is not the same as pollutant induced insult which may reflect different mechanisms of pathophysiology.

Table 2. Models of COPD

**Bronchopulmonary Dysplasia:** Bronchopulmonary dysplasia (BPD) remains the leading cause of respiratory morbidity and mortality in severely pre-term infants (48,49). The



treatment of prematurity itself induces BPD, which complicates matters including ventilator induced surfactant deficiency and inflammation (44,50). Intrinsic BPD is characterized by immaturity, decreased growth, and immature vascularization (51). The main model for BPD is hyperoxic exposure in animal models such as rats and mice (49). Hyperoxia inhibits the normal budding and branching of the bronchi (52) leading to arrest in lung development resembling pre-term infant BPD (53). In these studies, it appears that both the airways and capillary vessels are affected requiring ventilation which can also contribute to inflammation and dysplasia (48,49,51). To understand the mechanisms and outcomes in BPD, animal models must contain elements of the normal fetal lung and the mechanisms associated with development and function. For the pulmonary mechanics studies, the *in vivo* models consist of larger animal models including lamb, rabbits and guinea pigs (49). The change in lung mechanics and the accumulation of fluid, changes the airway surface tension contributing to robust inflammatory cytokine production contributing further to the histopathology. Using these models, studies have provided avenues for understanding the role of surfactant therapy, decreased tidal volumes, improved control of oxygenation on BPD development and translation clinically (49,54).

**Alveolar Proteinosis:** The lung faces physical and environmental challenges, due to changing in lung volumes as well as exposure to foreign pathogens. The pulmonary surfactant system is integral in protecting the lung from these challenges via two different and distinct groups of surfactant proteins (55). Surfactant protein (SP)-B and SP-C are small molecular weight hydrophobic surfactant proteins that regulate air liquid interface surface tension. SP-A and SP-D are the larger hydrophilic surfactant proteins which aide in surface tension but which also have microbicidal function. Additionally, there are other non-surfactant proteins called defensins which also aid in inflammation and host defense (56). Pulmonary alveolar proteinosis (PAP) is a process by which there is a surfactant accumulation in the lungs potentially due to the inability to catabolize surfactant (57). There are three forms of the disease: genetic, exposure induced and idiopathic (58). The genetic disease specifically impacts children, and is associated with mutations in some of the surfactant protein genes (59-61). Exposure induced PAP is found in scenarios of particulate inhalation including silica and titanium (62-65). The idiopathic form is associated with circulating auto-antibodies against the macrophage growth and differentiation factor granulocyte-macrophage colony stimulating factor (GM-CSF) (66,67). Clinical studies have correlated the presence of the neutralizing antibody to PAP (66,68,69). Clinical trials of GM-CSF, plasmapheresis and whole lung lavage have shown limited successes with some sustainable relief, but none of the treatments are curative (70). In terms of animal models, most have been done with mice since the defects are most often associated with the absence of surfactant or GM-CSF proteins and murine GM-CSF knockout development of alveolar proteinosis (71-73). The surfactant protein knockouts develop diseases very reminiscent of pediatric interstitial proteinosis (50). The GM-CSF knockout mouse has many pathophysiological outcomes which are reminiscent of the human PAP adult disease (74). The nice part of these models is that they do not have to be induced, so there is relatively little variability between animal to animal. There have been some attempts to develop an autoimmune model of idiopathic PAP using monkeys and mice (75,76). These efforts have provided important insight into the potential mechanisms of development in early and late stages of PAP due to autoimmunity against GM-CSF.

Agent utilized	Pathology	Advantages	Disadvantages
Bleomycin	Induced lung damage and repair.	Ease of administration.	Requires specific dosing regimen. Toxic to investigators.
Silica	Chronic inflammatory response and repair mechanisms associated with fibrosis.	Sustained inflammation since silica is not resolved by macrophages.	Not a natural inducer of fibrosis. The mechanisms may not be translatable.
FITC	Inflammatory response and repair mechanisms. Natural hapten induced inflammatory mechanisms.	Visualize areas of repair and fibrosis.	Some characteristics of the lung disease are absent. There is significant variability depending on the FITC batch.
Irradiation	Induces direct cell death via DNA damage with a subsequent influx of inflammatory cells. The radiation may also directly induce the fibrotic processes.	Ease of study, no chemical requirement. Mimics human process in terms of initiation and progress.	Long time for the fibrosis to develop, limited to modeling radiation induced pneumonitis.
Viral Induced Transgenes	Viruses used to up-regulate mediators of fibrosis.	Specific <i>in vivo</i> molecules associated with IPF formation such as TNF or TGFB.	Deal with potential mechanisms but is not realistic to defining disease process.

Table 2. In vivo Models for Interstitial Pulmonary Fibrosis

**Interstitial Pulmonary Fibrosis:** Fibrosis is an important cause of morbidity and mortality in a variety of lung diseases, but it has a very prominent role in idiopathic pulmonary fibrosis (IPF)–(77). IPF presents with a homogenous phenotype with both definable physiologic and radiographic presentation but without identifiable etiology (78) although, the literature suggests that alveolar type II cell injury is an important early feature in the pathogenesis of pulmonary fibrosis (79). The source of injury is unknown. Different approaches to modeling pulmonary fibrosis have been used by investigating exposure to bleomycin, silica, fluorescein isothiocyanate (FITC) and irradiation (77). At the genetic level, some models of IPF have included over-expression of ‘hypothesized” genes in the pathogenesis of IPF or utilization of transgenics for cell specific contribution to IPF. Bleomycin is the most frequently used agent in modeling IPF (80,81). The advantage of bleomycin is the ease with which it can be administered and the consistency of the IPF

pathophysiology. Bleomycin is a chemotherapeutic agent which induces lung damage through direct DNA strand breakage and the generation of free radicals. The response to the injury is healing and fibrosis. Silica aerosolized into the lung induces pulmonary fibrosis through inducing chronic inflammation and frustrated phagocytosis by macrophages (77,82). Post-ingestion, the macrophages constitutively produce pro-fibrotic cytokines (83). The greatest advantage of the silica based system is that the silica particles are not easily cleared from the lungs creating a persistent stimulus and a non-reversible fibrotic process. Regardless of the model fibrosis is dependent on the strain of animals, suggesting immune dependent contribution to the overall susceptibility of IPF development. FITC is another chemical used to induce pulmonary fibrosis (77,84). Fluorescein, delivered directly into the airway acts as a hapten attaching to lung proteins providing a depot for continuous lung exposure to antigen. The advantage of the FITC model is the ability to actually image the processes as they occur in the lung.

**Asthma:** Asthma is a very complex and heterogeneous disease affecting 300 million people worldwide especially in Westernized countries (85). Why developing countries seem to be somewhat protective has been the foundation for the hygiene hypothesis (86). Asthma is a complex trait caused by multiple environmental factors with the main characteristics being airway inflammation and airway hyper-reactivity (AHR) (87). The pathogenesis of asthma is associated with many environmental factors, many cell types and several molecular and cellular pathways. Some specific presentations of asthma are outlined in Figure 3 (88,89). The majority of the induced asthmas are due to exposure to an irritant such as air pollution, allergen or viral exposure. Even aspirin and drug induced asthma can be associated with changes in the pulmonary milieu upon dosing. Interestingly, some asthma phenotypes are not associated with identifiable exposures, such as exercise induced and metabolic syndrome associated asthma.

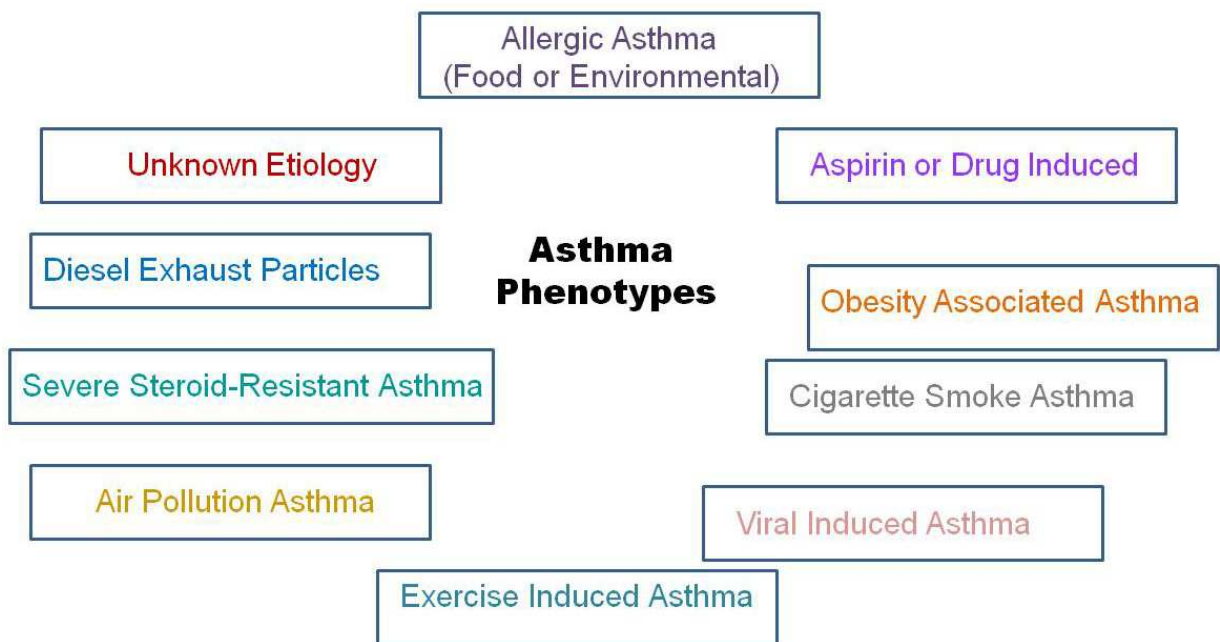


Fig. 3. Asthma Phenotypes. Asthma is a heterogeneous disease with multiple factors associated with the development and response to therapy. Given these issues, designing experiments and translating into clinical significance become a challenge.

These different pathways and phenotypes probably suggest mechanisms that are co-existent but also synergistic depending on the patient, environment, compliance and documentation.

Animal models of asthma have helped to clarify some of the underlying pathophysiological mechanisms contributing to the development of asthma (5,9,10). Much of the focus of these models is on T cell driven allergic responses contributing to understanding of the heterogeneity of asthma (90). The murine model of asthma using Balb/C mice has defined the important role of allergen-specific Th<sub>2</sub> cells in recruiting eosinophils into the airway, their activation and the release of histamine associated with atopic airway reactive disease. The major caveat in the murine asthma studies is that the allergen sensitization process does not completely recapitulate the allergic response in humans complicating the ability to utilize these models for therapeutic development (91). This has been quite frustrating in asthma therapy development, even though certain biomarkers have been identified in the *in vivo* models they have ultimately not translated into efficient therapeutic care for patients with asthma (10). The inability to translate the observations from the animal models to patient care were very disappointing and increased the lack of the appreciation of the animal models toward mechanisms and pathogenesis of asthma. The fortunate component of the murine asthma model is the ability to sensitize the animals to a variety of foreign proteins and to use transgenic animals for studying mechanisms and response to different exposures. In most scenarios, the challenge results in a Th<sub>2</sub> polarization and enhanced allergen-specific IgE production (92). Pathologically the lungs have eosinophilia, mucus secretion and goblet cell hyperplasia, airway hyper-responsiveness and remodeling with fibrosis (5,88). These asthmatic phenomena have suggested that cytokines and cells other than T-cells, such as IFN $\gamma$ , IL-17 and/or neutrophils, may also play a significant role in the lung pathology (93,94). Further, Th<sub>2</sub> targeted therapies have not been as effective as hoped in many clinical trials of asthma, suggesting alternative pathways to the lung inflammation and remodeling. These have resulted in several distinct alternatives to the traditional allergen challenge model. Table 3 outlines the different animal models currently available to study various aspects of the pathophysiology associated with asthma.

**Lung Cancer:** Several *in vivo* models exist which provide the opportunity to study cancer (95). The complications in these models are their inability to completely correlate with histologic patterns of the malignancies, natural strain susceptibility and time frames for cancer induction in humans. One important difference between the animal models and the human disease is that these animals have higher basal metabolic rates changing metastatic potential (33). Failure to develop specific tumor types is probably due to the variability of the transgene expression early in lung development. The most common compound utilized for the development of tumors in animal models is urethane (96). Mouse models have been used to study the roll of mutant oncogenes in the genesis of lung adenocarcinomas (97-99). These models have also proven useful for studying potential therapeutics. The development of a tyrosine kinase inhibitor which blocks epidermal growth factor receptor (EGFR) was found to benefit some patients after testing in mouse models. The deletion of other genes associated with human small-cell lung cancers could also be mimicked in a murine lung model aiding in therapeutic development of inhibitors (33,95).

Malignant mesothelioma is a cancer associated with environmental exposure to asbestos (95). The disease has a poor prognosis, with little to offer patients in terms of therapy. Mouse models of pleural mesothelioma have been produced by exposing mice to asbestos

Model	Advantage	Disadvantage	Pathology Association	Translation into the Clinic
Allergen Challenge	Similar to atopic induced asthmatic disease. Can be defined by the route, dosage and duration of the sensitization and challenge regime.	The model does not recapitulate all of the components of the allergic disease.	Airway hyper-reactivity, systemic IgE, mucous production, goblet cell hyperplasia.	Variability in species, sensitization/challenge regimes, and duration of studies. Has introduced some clinical failures.
Viral Respiratory Infection	Independent of Th <sub>2</sub> mechanism, Induced using sendai virus (parainfluenza) or respiratory syncytial virus (RSV).	Maybe considered a contributor to the human disease but may precede the start of asthma symptoms in humans.	Airway hyper-reactivity, alternatively activated macrophages and natural killer cells.	Evidence for viruses has been found in patients with severe asthma and in children with asthma with pre-exposure to RSV.
Air Pollution	Ozone is a common inducer. This appears to be concentration, route and dosage dependent.	Difficult to obtain consistency due to inhalation variability.	Severe airway hyper-reactivity associated with neutrophils. Also requires the presence of IL-17.	May lead to some understanding of the down-stream event in severe chronic asthma.
Intrinsic AHR	Requires strain specific manipulation and is associated with specific proteases.	Is strain specific and appears to associate with the asthma susceptibility gene.	Appears to regulate the control of airway hyper-reactivity through smooth muscle cell activation and bronchospasm.	The association of the proteases to human asthma.

Table 3. In Vivo Models for the Versatility of the Asthma Phenotypes

fibers. A wide range of natural and synthetic fibers, chemicals and metals have also been shown to induce pleural and peritoneal mesotheliomas (100,101). Recently the technology of xenographic transplantation of human malignant mesotheliomas into rats or mice has been



used to study new chemotherapeutic agents including immunotherapy, gene therapy and multimodality therapy (95). Asbestos-induced malignant mesotheliomas produced in rodents resemble the human disease with respect to latency and growth of the tumor cells. Even with these similarities, mice are not perfect models for humans.

**Cystic Fibrosis:** Cystic fibrosis (CF) is the result of defects in the gene encoding the cystic fibrosis transmembrane regulator (CFTR) and is the most common genetic disease among Caucasians (102).. Even though new therapeutics including correctors and activators like VX-770 has shown great promise in new phases of CF therapy, the cure has been elusive (103). The development of the *in vivo* models has focused on four major points of pathophysiology: anatomy, physiology, airway clearance and intrinsic and/or induced inflammation. The mouse model been the main model in CF research for several years, however, the model does not develop spontaneous lung disease requiring the introduction of bacteria to initiate the pathophysiological events associated with CF infection and inflammation (104). There are several different models of CFTR deficiency ranging from the complete absence of CFTR (null) to the partial expression and/or function (4). There have also been murine models developed in which the lung mutation remains but the gastrointestinal phenotype is corrected or it is cell specific (105). The reason for these later series of animals is that the murine CFTR null mutant consistently has gastrointestinal blockage once the mice have been weaned, increasing mortality and expense of the animals. To prevent obstruction, the animals are put on a laxative. Investigators have a choice whether to use laxative treated animals or gut corrected animals. In either case, it is likely that gastrointestinal obstruction is important in the overall immunity and host response to infection in CF. Therefore, observations in the gut corrected mouse may ultimately have to be verified in the null mouse depending on the focus of the studies. Even with the differences in the gastrointestinal constitution, the severity of the different murine models is defined by CFTR protein function related to the mRNA expressed, protein synthesis or folding of the complete CFTR protein (4). In addition to the gastrointestinal obstruction, most of the models display inflammation (106), failure to thrive (107), decreased survival (108,109) and hyper-responsiveness to stimulation (110). To improve the ability to look at CF globally, larger models of CFTR deficiency have been developed to better investigate the airway pathogenesis and progression of lung disease. Further, unique models have been developed using transgenic technology to induce CFTR deficiency in specific cell types, allowing for the sequential investigation of all of the contributing cellular abnormalities and how they contribute to the CF pathophysiology (105,107,111).

The pig has become an exciting new direction for CF model development. The pig lungs and human lungs have similar comparative anatomy (112,113) and have been used to study a variety of aspects of lung pathophysiology including surfactant homeostasis, airway hyper-responsiveness and lung injury (114). The first studies have shown that there were no differences in the newborn pig birth weight or appearance (4). Deficient CFTR in the pigs did not appear to alter normal birth weight, appearance and/or lung anatomy or function. The absence of CFTR however, did result in defective nasal transepithelial cell potential and all piglets developed severe gastrointestinal obstruction. Further, with piglet aging there appears to changes in lung physiology and function resembling that of infant with CF. However, the development of lung disease is still being investigated as to whether it is an intrinsic phenomena due to the absence of CFTR, or the result of environmental exposure (112). The pig is a great model for studies in CF lung pathophysiology, however husbandry

and cost and reproductive cycle play a major part in being able to conduct several studies with reasonable numbers of animals.

The ferret has been shown to also be a good animal model for studying CFTR lung biology (7,115). The ferret lung shows CFTR expression in the airway epithelium and submucosal glands, identical to that in humans (4,7). Like the pig model, the majority of the CFTR deficient ferrets also developed gastrointestinal obstruction with “failure to thrive” (115,116).

To study CF, the availability of three established *in vivo* model systems provides ample ability to study various components of CF pathophysiology. Besides CFTR deficient mice, pigs and ferrets, other models have also been developed or observed (117) including the sheep (118) and monkey models (119). These have been less studied for a variety of reasons. Although these models have provided invaluable insight into the development of new CF therapeutics, new model systems should be considered to get even closer to the overall mechanisms associated with CF.

**Summary:** Human lung disease is a major cause of morbidity and mortality in the world. The pulmonary dysfunction may be primary or secondary to a variety of events. The pathophysiological mechanisms associated with the disease processes are different depending upon whether the insult is external as in the case of infection or injury or internal as in the case of genetic anomalies associated with important pulmonary or secretion functions. Studying lung disease requires models that attempt to recapitulate the human phenomena. There are no perfect models, and the selection for studies must be based upon the criteria of study and the ability of the model to meet the needs of the study. In this chapter we have highlighted a variety of pulmonary diseases and syndromes with a focus on the models used to study the various pathophysiological mechanisms associated with that specific disease entity. In the end, model development and usage will continue as a conduit with which to test mechanisms and to explore the development of new and innovative therapeutics.

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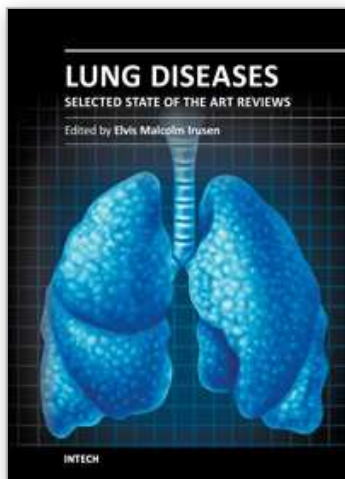
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## **Lung Diseases - Selected State of the Art Reviews**

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The developments in molecular medicine are transforming respiratory medicine. Leading clinicians and scientists in the world have brought their knowledge and experience in their contributions to this book. Clinicians and researchers will learn about the most recent advances in a variety of lung diseases that will better enable them to understand respiratory disorders. This treatise presents state of the art essays on airways disease, neoplastic diseases, and pediatric respiratory conditions. Additionally, aspects of immune regulation, respiratory infections, acute lung injury/ARDS, pulmonary edema, functional evaluation in respiratory disorders, and a variety of other conditions are also discussed. The book will be invaluable to clinicians who keep up with the current concepts, improve their diagnostic skills, and understand potential new therapeutic applications in lung diseases, while scientists can contemplate a plethora of new research avenues for exploration.

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