Research Opportunities and Advances in Lung Disease

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The Problem of Pulmonary Diseases
Lung diseases cause major morbidity, mortality, and economic burden. The most common forms include asthma, chronic obstructive pulmonary disease (COPD; including bronchitis and emphysema), pneumonia, interstitial (fibrotic) and inhalation disorders, and pulmonary embolism. In 1998, lung diseases accounted for 251,000 deaths (10%) in the United States. For 2000, the projected direct economic cost for lung disease is $91.6 billion, representing 7.3% of health costs in the United States. Ranking fourth in causes of death, COPD was responsible for 109,000 deaths in 1998. Pneumonia is the fifth leading cause of death. Asthma affects about 15 million individuals in the United States, and results in more than 1.5 million emergency department visits, 500,000 hospitalizations, and more than 3500 deaths each year, many of which are in children. It is estimated that asthma is responsible for $11.3 billion of direct and indirect costs per year. Acute respiratory distress syndrome (ARDS) (lung failure requiring mechanical ventilation and costly intensive care) has an estimated incidence of 75 persons per 100,000 per year in the United States.

Progress in the fight against lung disease has been mixed. From 1988-1998, death rates for asthma and COPD declined in men, but increased substantially in women. Death rates for most infant lung disorders declined substantially, including a 59% decline in mortality from respiratory distress syndrome of the newborn, but lung disease is still responsible for 41% of all deaths in infants younger than 1 year.

Major Advances
Remarkable achievements have been made in the past 25 years in the characterization, etiology, pathogenesis, and treatment of pulmonary disorders. Efforts from 1950 through 1975 were directed toward understanding the physiology of gas exchange, whereas research over the past 25 years has focused on the lung as a biological organ, applying biochemistry, cell biology, molecular biology, and genetics to understand lung function in health and disease. These efforts, together with epidemiologic methods, and improvements in diagnosis have led to remarkable advances in the understanding, prevention, and treatment of lung disease.

One of the great triumphs was the recognition that respiratory distress syndrome of newborns (hyaline membrane disease) is caused by a deficiency of surfactant, the protein/lipid complex that reduces surface tension at the air-liquid interface in alveoli. In premature births, if type II alveolar epithelial cells have not differentiated sufficiently to provide enough surfactant to enable enough alveoli to open, gas exchange is hindered.

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change following birth is markedly impaired. In these neonates, intratracheal administration of surfactant, either in the form of the lipid or lipid/protein complex, reduces surface tension, and enhances gas exchange until the lung epithelium can provide a normal complement of endogenous surfactant to the alveoli. An estimated 2000 neonates are saved annually in the United States by surfactant replacement.

Genetic medicine has made a major impact in the diagnosis, understanding, and treatment of lung disease. The major clinical manifestations of 2 of the most common lethal hereditary disorders in whites, cystic fibrosis (CF) and \( \alpha_1 \)-antitrypsin (AAT) deficiency, are in the lung. These disorders have been characterized at the genetic level, and the strategy used to identify the CF transmembrane conductance regulator (CFTR) gene became the paradigm to identify disease-causing genes that code for unknown proteins. The function of the AAT and CFTR proteins and the general pathogenesis of both disorders are now well understood. For AAT deficiency, augmentation therapy with purified AAT has been available for more than 10 years. Several genetic abnormalities have been linked to increased susceptibility for hypercoagulable states and pulmonary embolism. Recognition that asthma is associated with mutations of specific genes has led to major efforts to define the function of these genes and to develop drugs relevant to these targets.

Epidemiologic studies have played an important role in reducing lung disease. Myriad causes of interstitial (fibrotic) lung disease have been identified, and establishing the relationship of the extent of exposure and the risk for developing interstitial occupational disorders has led to reduction in the allowable levels of airborne asbestos, silica, and coal in the workplace, and in turn, reduction in the numbers of cases of lung disease from these agents. Although much rarer, the incidence of acute and chronic interstitial lung disease (ILD) also has declined secondary to controls in the workplace resulting from careful epidemiologic studies. The linking of asthma to specific allergens in the urban environment, such as from cockroaches, has resulted in strategies to avoid such exposures.

Remarkable technology has been developed to diagnose lung disease and to treat respiratory failure. Fiberoptic bronchoscopy makes it possible to evaluate the airways and alveoli more precisely so that infectious, inflammatory, and malignant disorders can now be diagnosed more easily and safely. Laser methods, endobronchial radiation (brachytherapy), and development of airway stents offer palliation for airway obstruction in advanced lung malignancy. The technique of bronchoalveolar lavage via the fiberoptic bronchoscope has revolutionized clinical investigation of inflammatory lung disorders, permitting safe, repetitive sampling of inflammatory cells on the respiratory epithelial surface, and the adaptation of molecular biology to the study of human lung disease. Studies of lower respiratory tract disorders (ILD, emphysema) and airway diseases (asthma, bronchitis) have established that these disorders are dysfunctions in host inflammatory/immune responses. Advances in monitoring and in the techniques of mechanical ventilation have reduced mortality from respiratory failure due to respiratory distress syndrome, congestive heart failure, and asthma. The monitoring strategies of pulmonary medicine and neurology have led to the definition of a

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variety of sleep disorders associated with dysfunction in gas exchange.30

Computed tomography of the chest, including thin section and spiral computed tomography, enables sophisticated, noninvasive, and rapid diagnosis of many lung disorders, including early diagnosis of lung cancer in high-risk individuals, direct anatomic assessment of the extent of emphysema, and the diagnosis of pulmonary embolus.27-29 Nuclear medicine methods have been developed to diagnose pulmonary embolism (ventilation/perfusion scans) and to assess lung inflammation (gallium scans).30 Video-assisted thorascopic surgery has markedly reduced the morbidity associated with lung biopsy and limited pneumonectomy.31

Current Scientific Foundation

Two general concepts are the cornerstones of thinking about pulmonary disease: (1) susceptibility for lung disease is the result of a complex interaction among environment, genetics, and host responses; and (2) pathogenesis of most nonmalignant lung disorders is associated with inappropriate inflammatory processes that injure or modify the lung.

Environment, Genetics, and Host Responses. A typical individual exchanges 8000 L of gas per day, constantly exposing the respiratory epithelium to the environment, with its burden of infectious agents, allergens, and particulates. However, despite similar airborne challenges, only a minority of individuals develop lung disease, and the extent of disease resulting from the inhalation of these agents also varies.

Interaction among the environment, genetics, and host defenses are illustrated by the 2 common autosomal-recessive pulmonary disorders—CF and AAT deficiency. In CF, mutations in both parental CFTR genes result in deficiency in CFTR levels, or less commonly impairment of CFTR function, in airway epithelial cells.13 The CFTR protein mediates chloride and sodium traffic on the epithelial apical surface in the airway epithelium.13,14 However, dysfunction of electrolyte transport does not have a major direct effect per se. Rather, chronic airway infection with Pseudomonas and other organisms, and the consequent inflammation of the airway epithelial surface are responsible for the clinical manifestations of CF.13,33 In this context, it is unlikely that the genetic abnormalities of the CFTR mutations would have significant clinical pulmonary consequences without the environmental challenge of Pseudomonas and other organisms. Further, while the inability of the host defense system to protect against the inhalation of infectious organisms allows the organisms to colonize the lung in CF, the host defense system also mediates the damage to the airways. Although there is no cure for CF, mortality has been reduced by aggressive treatment of lung infection and by strategies to clear secretions from airways.13

Deficiency of AAT is characterized by marked deficiencies in the levels of AAT in the blood and lung.7,13,32 However, the host-defense system and the environment also contribute to lung disease. The AAT is an antiprotease that protects the alveoli from destruction caused by neutrophil elastase (a neutrophil protease that normally destroys infectious organisms and clears protein debris from the lung).34 When levels of AAT are deficient, the release of neutrophil elastase into the alveoli is uninhibited, and destroys the alveoli, eventually resulting in emphysema.35 Emphysema occurs much earlier in AAT deficiency if the individual smokes cigarettes (ie, without the environmental effects of cigarette smoke, individuals with AAT deficiency may develop mild disease, occurring 10-15 years later than in individuals with AAT deficiency who smoke cigarettes).7,9

Another example of the interactions of genetics, environment, and host responses is the increased susceptibility to pulmonary embolic disease of individuals with mutations in the genes coding for coagulation factors (such as factor V Leiden, prothrombin, and antithrombin).15,16 These mutations tilt the balance of the coagulation system toward procoagulation. Mutations in these genes frequently are asymptomatic probably because the combination of environment (eg, dehydration, trauma to the calf, surgery with prolonged bedrest, long trips in vehicles without exercise, or oral contraceptives) and inadequate host responses (eg, compensation by the anticoagulant arm of the coagulation system) are needed to cause clinically relevant pulmonary emboli.

Long-term exposure (>15 to 20 years) to high concentrations of airborne asbestos causes asbestosis, a fibrotic disorder of the alveoli.39,36,37 Fibrosis is not caused by the asbestos per se, but by the host response of chronic inflammation in the presence of the asbestos. However, only a minority of exposed individuals develop clinical asbestosis, most likely because of variability in host defenses that function to prevent inhaled asbestos from reaching the alveoli, remove particulates from the lung, and protect against inflammation.36,37

Allergens in the environment also can incite lung disease, and individual susceptibility plays a critical role in determining who will develop clinical disease. For example, during the epidemic of asthma attacks in Barcelona, Spain, caused by airborne soybean allergens that resulted from dust generated from unloading soybeans at the city docks,38 emergency department visits for asthma attacks increased and several deaths occurred, but only a small proportion of the population was affected. Another example is the response to inhalation of pigeon allergens by pigeon breeders. Most pigeon breeders have circulating antibodies against pigeon proteins, but only a small proportion develop hypersensitivity pneumonitis, a potentially fatal ILD.39 Likewise, many more workers who are exposed to beryllium are sensitized to the metal compared with the small numbers who develop berylliosis.40

These examples illustrate the general principle that genetic variations determine individual susceptibility to pulmonary disease, and that the manifestations of lung disease require a complex interaction between a challenge
from the environment, and host responses in the lung.

**Stimulus/Inflammation.** Most nonmalignant lung disorders are associated with the accumulation of inflammatory cells, usually in response to an inciting agent or event that attracts and activates inflammatory cells.

Adult respiratory distress syndrome is characterized by acute failure of the lung function gas exchange. Although the cause is not always clear, ARDS is associated with acute inflammation of the alveoli incited by a specific agent (eg, viral infection), event (eg, overwhelming trauma), or both. Because it is usually not possible to prevent the inciting event, ARDS research has focused primarily on the inflammatory processes that cause alveolar dysfunction.

The pathogenesis of chronic ILD is similar to that of ARDS in that inflammation induces alveolar damage, leading to pulmonary fibrosis and loss of lung function. For some ILDs, known agents (eg, asbestos, drugs, organic dusts) are responsible for initiating the inflammatory processes in the alveoli. Although the etiology is unknown for other ILDs (eg, idiopathic pulmonary fibrosis, sarcoidosis, ILD associated with collagen vascular disorders), all ILDs involve chronic inflammation.

Asthma is a reversible limitation of airflow secondary to acute constriction of the bronchial wall. In the last 10 to 15 years, it has become apparent that asthma is a chronic inflammatory disorder of the airways, with the extent of the inflammation determined by environmental factors, endogenous factors, or both. Emphysema in cigarette smokers with normal AAT levels results from chronic inflammation in the lower respiratory tract. Cigarette smoke attracts and activates inflammatory cells in the alveoli and impairs the anti-inflammatory defenses of the lung by virtue of oxidants in cigarette smoke that inactive AAT.

These examples illustrate another general principle that the pathogenesis of most lung disorders is linked to the local accumulation and activation of inflammatory cells in response to specific stimuli, and the inability of local defenses to protect the lung against inflammatory mediators.

**Key Issues, Questions, and Challenges**

The key issues, questions, and challenges in pulmonary disease for the future involve defining the genetic basis of susceptibility to lung disease, identifying and characterizing the genes controlling the mechanisms of injury to lung tissue and host defenses against lung injury under study, the derangement of control of blood and air flow, and defining the normal processes of lung repair and how these mechanisms go awry in pulmonary disease. While research in the past decade has made major advances in all of these areas, many parallel paths contribute to these biologic processes and a major challenge is to prioritize the biologic processes and understand the hierarchy of gene expression that modulates intracellular and intercellular biologic networks that control growth, repair, and function of the lung.

**Cutting-Edge Research**

Once thought to be a relatively inactivator that simply enabled gas exchange, the lung is known to be composed of many cell types, each expressing a multitude of genes, the products of which regulate individual cells and also the integrated function of the organ. Cutting-edge research in lung disease is centered on understanding the lung as a genetically determined, complex, biological organ that mediates gas exchange and defends against a hostile environment.

Pulmonary research in the first quarter of the 21st century will focus on five major areas: (1) characterizing the expression of genes in the lung in health and in disease, starting with specific cell types, and then integrating this information into the overall functioning of the lung; (2) identifying sequence variations in genes associated with specific lung disorders, and elucidating the role of these genetic variants in disease; (3) understanding the role of the environment in modulating lung function, particularly for disorders linked to environmental factors; (4) characterizing the genetic variations in infectious organisms relevant to lung disease, and the interactions of host and pathogen genes relevant to susceptibility to infectious disorders; and (5) capitalizing on the advances in genetic medicine, including gene therapy, to develop treatments for lung disease.

**Gene Expression.** The lung is composed of at least 10 major cell types, more than 20 cell types that are less common but play an important role in lung function, and blood-derived inflammatory cells involved in host defense. Technology has been developed to isolate and culture most of these cell types, and the stage is set to assess gene expression in lung cells in health and disease. The technology of functional genomics using gene arrays is being adapted to assess lung gene expression. It should be possible to define which genes are up- and down-regulated in the normal lung, and how expression of specific genes and clusters of genes are modulated in disease, using animal models and cells from the lung in specific human disorders. A promising research area will be assessment of gene expression in the developing lung, with the eventual goal of applying modulation of gene expression to regenerate pulmonary tissue. Another key challenge in assessing lung gene expression is to integrate this information into understanding lung physiology and pathophysiology.

**Genetic Variation and Susceptibility to Disease.** With the identification and characterization of the AAT and CF genes and other single gene disorders in families with asthma and pulmonary hypertension, the easy applications of genes on lung function have been defined. While individual susceptibility also plays a role in pulmonary infections, COPD, and ILD, this involves multiple genes. Identification of genes involved in susceptibility to lung disease will require the combination of new paradigms of
epidemiologic investigation together with the development of methods to identify the respective roles of the relevant genes and sequence variants that modulate susceptibility to disease and how such disease is manifest in a given individual.

**Environment-Gene Interactions.** Genetic susceptibility to disease, even for autosomal recessive disorders (i.e., AAT deficiency and CF), is often relevant only in the context of an environmental stress that is pertinent to that disease. Identification of specific genes linked to the susceptibility of asthma will make it possible to define the specific environmental stimuli that induce the disease in individuals. For COPD and occupational lung disorders, the situation is reversed—the environmental stimulus is known (e.g., cigarette smoking and specific particulates, such as asbestos or silica, in occupational disorders), but the genes involved in governing individual susceptibility have not been identified. By identifying the genes involved in host defenses and in the pathogenesis of lung disease associated with environmental factors, it should be possible to reduce the incidence of these disorders by having individuals avoid contact with the relevant environmental agents to which they are susceptible.

**Host-Pathogen Genetic Interactions.** Now that the genomes of several human pathogens have been sequenced, and others are soon to be characterized, the stage is set to define variations of the pathogen genome over time in the same susceptible individual, and from individual to individual (including effects of age, ethnicity, and geographic location). Equally important as the pathogen genome are the host genes that defend against each pathogen, and determine how the pathogen and host interact during different phases of infection. This approach should make it possible to identify therapeutic targets of opportunity, both pathogen genes for targeting by new generations of antibiotics, and host defense genes that could be bolstered with appropriate therapy.

**Genetic Medicine and the Lung.** Genetic medicine encompasses the identification of disease-susceptibility genes and modulation of gene expression for therapeutic purposes. Gene therapy is one relevant technology in which the coding sequences of therapeutic genes are used ex vivo or in vivo to treat or prevent a specific disease. In humans, it is possible to transiently correct the deficiency in CFTR gene expression in the airway epithelium in individuals with CF. In animals, it is feasible to use gene therapy to augment blood and lung levels of AAT. Genetic vaccines, using naked plasmids or genetic modification of dendritic cells, show promise directed against pulmonary pathogens in experimental animal studies. Recombinant DNA technology to “humanize” murine monoclonal antibodies is being used to develop strategies to treat asthma by interacting with the cells and mediators involved in asthma attacks. Since the epithelial surface of the adult lower respiratory tract is approximately the size of a tennis court, and since proteins can diffuse across the pulmonary epithelium and endothelium, aerosol technology is being used to deliver therapeutic human proteins to the alveoli, where they can diffuse to the blood, thus providing a new, noninvasive route for the systemic administration of therapeutic proteins, and for local delivery of therapeutic proteins such as AAT to the lung.

**Critical Elements for Advancement**

**Short-term Goals.** The technical tools are available to achieve the goals of defining the genes controlling lung growth, and of characterizing gene expression in health and disease, including culture of primary lung cells of different types, and use of gene array methods to assess gene expression. The same is true for defining sequence variations in specific genes responsible for various lung disorders, for understanding the interaction of the environment with gene expression in the normal and diseased lung, and for defining pathogen and host defense genes pertinent to pulmonary infections.

**Long-term Goals.** While defining expression of large numbers of genes in health and disease in the lung will be achieved in the near future, there is no technology available now, or on the horizon, to modulate these genes for therapeutic purposes. The challenge is daunting—the lung is complex, and its functions depend on the integrated function of many components. To approach the therapy of multigene disorders, the technology will have to be developed to modulate the expression of different genes at different times and locations. Alternatively, it will be necessary to identify “master genes” that control large components of these pathologic processes. It also will be important to understand the control of genes involved in lung development and in lung function. While efforts have been made in this area, it will take many years to...
understand how the multitude of critical genes are regulated in the normal lung and in lung disease.

**Forecast of Major Research Advances**

Future major advances in understanding, preventing, and treating lung disease will most likely evolve from focusing on gene expression in the lung in the context of the host and the environment. What does this portend for the major lung disorders?

Genes involved in susceptibility to asthma will be defined and characterized. In the context that environmental stimuli play a significant role in triggering asthmatic attacks in susceptible individuals, asthma will be a prototype disease in which a DNA fingerprint will define susceptibility, and alert the individual to avoid interaction with the stimuli that are specific for their asthma. Identification of asthma susceptibility genes will also provide new therapeutic targets to prevent and treat this disorder.

The 2 major genetic disorders of the lung, CF and AAT deficiency, most likely will be cured by gene therapy. It is feasible to correct the genetic deficiency of CFTR function in the airway epithelium and to correct the deficiency in lung levels of AAT. The major challenge for curing CF is to develop gene transfer vectors that express the CFTR coding sequence in the airway epithelium and to correct the deficiency in lung levels of AAT. The major challenge for curing CF is to develop gene transfer vectors that express the CFTR coding sequence in the airway epithelium long term. For AAT deficiency, the major challenge is to develop vectors that can maintain sufficient levels of AAT to protect the lung. Genetic factors undoubtedly contribute to other lung disorders and it will be a challenge to define the genes that influence each disorder.

The most effective method to prevent emphysema and bronchitis is to stop the sale of tobacco products, or to convince people not to smoke. Barraging such major sociologic change, progress in defining steps in the pathogenesis of these disorders that may be vulnerable to therapeutic intervention, and in developing therapies specifically targeted to these steps will be slow. One possibility is to develop antiproteases and antioxidants to protect vulnerable lung tissues from inflammatory mediators.

Progress should occur in the development of new generations of antibiotics, including “designer” peptide antibiotics. Administration of anti-infectives by the aerosol route will gain popularity and vaccine development will become a major aspect of research in this area, particularly in gene-based vaccines. Understanding lung-host defenses, and the ability to genetically modify cells involved in host defenses will lead to development of novel vaccines against major lung pathogens. Elucidation of the genome of the pathogens and the genes involved in host defenses will lead to insights into the defense of the lung against acute infection and the eradication of chronic infection. Identification of the specific virulence genes in infections such as Mycobacterium tuberculosis and Pseudomonas aeruginosa should facilitate development of "designer" antibiotic regimens, and possibly vaccines, for the specific strain of pathogens that infect a given individual.

Progress in identification of major genes involved in the pathogenesis of ILDs should lead to identification of targets for new therapies to protect the alveoli from the effects of chronic inflammation. Pulmonary hypertension is challenging because the choice of therapies that specifically target this vascular bed is limited. Identification of genes that control susceptibility to and development of pulmonary hypertension will lead to new therapeutic targets. For both interstitial disease and pulmonary hypertension, humanized monoclonal antibodies against relevant receptors may provide a new therapeutic opportunity. Identification of susceptibility genes for development of venous thrombosis should make it possible to develop therapies to prevent pulmonary embolism.

While lung transplantation is useful for progressive respiratory failure, particularly with the development of new drug regimens to circumvent rejection, the availability of suitable organs for transplantation remains the limiting factor, and it is doubtful that availability will be solved in the next 25 years by xenotransplantation. The ultimate goal for replacement of diseased lung tissue is to regrow a new functional lung. With the technology available to identify the genes that control lung growth, major advances in this area should occur in the next few decades. It is only a matter of time before lung stem cells are identified, both within the lung and circulating. While it is not clear which direction this research will take in regard to an organ as cellular and anatomically complex as the lung, the ability to reprogram lung growth is a goal worth pursuing.

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