Chapter 7
Pulmonary Diseases

Introduction

The respiratory system extends from the nose and upper airway to the alveolar surface of the lungs, where gas exchange occurs. Inhaled tobacco smoke moves from the mouth through the upper airway, ultimately reaching the alveoli. As the smoke moves more deeply into the respiratory tract, more soluble gases are adsorbed and particles are deposited in the airways and alveoli. The substantial doses of carcinogens and toxins delivered to these sites place smokers at risk for malignant and nonmalignant diseases involving all components of the respiratory tract including the mouth.

Consider, for example, the lungs of a 60-year-old person with a 40-pack-year[1] smoking history starting at age 20 years. By age 60 years, this person will have inhaled the smoke from approximately 290,000 cigarettes and will bear a substantial risk for chronic obstructive pulmonary disease (COPD) and lung cancer. The dose of inhaled toxic particles and gases received from each of these cigarettes varies depending on the nature of the tobacco, the volume and number of puffs of smoke drawn from the cigarette, the amount of air drawn in through ventilation holes as the smoke is inhaled, and local characteristics within the lung that determine the diffusion of toxic gases and the deposition of particles. Because of this repetitive and sustained injurious stimulus, the repair and remodel process that heals the damaged lung tissue takes place at the same time the lung's defenses continue to deal with this unrelenting inhalation injury.

This chapter addresses the mechanisms by which tobacco smoke causes diseases other than cancer in the lower respiratory tract: the trachea, bronchi, and lungs. Beginning with the first Surgeon General's report in 1964 (U.S. Department of Health, Education, and Welfare [USDHEW] 1964), cigarette smoking has been causally linked to multiple diseases and to other adverse effects on the respiratory system (Table 7.1). In addition to causing lung cancer and COPD, smoking increases the risk of death from pneumonia and causes chronic bronchitis (U.S. Department of Health and Human Services [USDHHS] 2004). Typically, the lungs of smokers show evidence of diffuse changes affecting the lining of the airways, the epithelium, and the structure of the bronchioles, which are the smaller air-conducting tubes.

Previous reports of the Surgeon General have also addressed the effects of smoking on the respiratory tract. In discussing the plausibility of associations of cigarette smoke with chronic bronchitis and emphysema, the 1964 report gave full consideration to the nature of tobacco smoke and its effects on the respiratory tract (USDHEW 1964). That report concluded that cigarette smoking "… is the most important of the causes of chronic bronchitis in the United States…" (p. 302) and that "a relationship exists between pulmonary emphysema and cigarette smoking, but it has not been established that the relationship is causal" (p. 302). The 1984 report, which focused on COPD, covered mechanisms by which smoking affects the lung's structure and function and the deposition and toxicity of cigarette smoke in the lung (USDHHS 1984). The report concluded that "cigarette smoking is the major cause of chronic obstructive lung disease in the United States…" (p. vii). The mechanisms of lung injury were considered further in the 1990, 2004, and 2006 reports (USDHHS 1990, 2004, 2006).

The principal nonmalignant respiratory diseases caused by cigarette smoking—COPD, emphysema, chronic bronchitis, and asthma—are defined in Table 7.2. The definitions indicate that chronic bronchitis is a specific set of symptoms, whereas emphysema refers to a particular pattern of lung damage. COPD comprises a clinical syndrome characterized by limitation in airflow; persons with COPD often have chronic bronchitis as well, and their lungs typically display emphysema. Other nonmalignant respiratory diseases that have been linked to smoking include asthma and idiopathic pulmonary fibrosis (USDHHS 2004), but the evidence has not reached a level of certainty sufficient to warrant a conclusion of cause and effect.

The nonmalignant respiratory diseases caused by smoking contribute substantially to the burden of morbidity and mortality attributable to smoking in the United States (Table 7.1). In 2005, the Centers for Disease Control and Prevention (CDC) estimated that an average of 123,836 deaths per year could be attributed to lung cancer caused by smoking for the period 1997–2001 (CDC 2005). CDC estimated an additional 90,582 deaths from COPD and 10,872 from pneumonia and influenza annually.

Great advances have been made in our understanding of how smoking causes these diseases. Research has been facilitated by methods that directly assess changes in the lungs. Methods for obtaining biologic material from human lungs include bronchoalveolar lavage (BAL), a technique that allows recovery of cellular and

[1]Pack-years = the number of years of smoking multiplied by the number of packs of cigarettes smoked per day.

Table 7.1 Causal conclusions on smoking and diseases of the respiratory tract other than lung cancer: the 2004 and 2006 reports of the Surgeon General

Active Smoking

The evidence is sufficient to infer a **causal conclusion** between smoking and

- Acute respiratory illnesses, including pneumonia, in persons without underlying smoking-related chronic obstructive lung disease
- Impaired lung growth during childhood and adolescence
- Early onset of decline in lung function (during late adolescence and early adulthood)
- A premature onset of and an accelerated age-related decline in respiratory symptoms related to lung function in children and adolescents, including coughing, phlegm, wheezing, and dyspnea
- Asthma-related symptoms (i.e., wheezing) in childhood and adolescence
- All major respiratory symptoms among adults, including coughing, phlegm, wheezing, and dyspnea
- Poor asthma control
- Chronic obstructive pulmonary disease morbidity and mortality
- A reduction of lung function in infants of mothers who smoked during pregnancy

Involuntary Exposure to Tobacco Smoke

The evidence is sufficient to infer a **causal conclusion** between secondhand smoke exposure

From parental smoking and

- Lower respiratory illnesses in infants and children
- Middle ear disease in children, including acute and recurrent otitis media and chronic middle ear effusion
- Cough, phlegm, wheeze, and breathlessness among children of school age
- Ever having asthma among children of school age
- Onset of wheeze illnesses in early childhood

From maternal smoking during pregnancy and

- Persistent adverse effects on lung function across childhood

After birth and

- Lower level of lung function during childhood

And

- Odor annoyance
- Nasal irritation

Source: U.S. Department of Health and Human Services 2004, 2006.

noncellular components of the epithelial surface of the lower respiratory tract (Cantrell et al. 1973; Hunninghake et al. 1979; Reynolds 1987). BAL is of value in the study of immune and inflammatory mechanisms in the lower airways, because most of the cells recovered are believed to be derived from both air spaces and lung interstitium. Lung tissue obtained by biopsy or autopsy procedures can be used for cellular, protein, and nucleic acid assays. Exhaled breath condensate provides information about the composition of epithelial lining fluid (ELF) that can be used to detect inflammation and redox disturbance (Paredi et al. 2002). Blood samples may be used to assess systemic inflammatory responses, and blood cells serve as a source of nucleic acids.

Characteristics of Tobacco Smoke

Tobacco smoke, which comprises an aerosol (a mixture of solid and liquid particles) and gases, has thousands of chemical components, including many well-characterized toxins and carcinogens (International Agency for Research on Cancer [IARC] 2004). Many of these components are in the gas phase, and others are components of the particles. Nicotine, for example, is bound to particles in mainstream smoke. The chemical components in tobacco smoke were covered comprehensively in IARC Monograph 83 (IARC 2004) and described in previous reports of the Surgeon General. Numerous components of the smoke have the potential to injure the airways and alveoli.

Table 7.2 Definitions for principal nonmalignant respiratory diseases caused by cigarette smoking

Chronic obstructive pulmonary disease (COPD)	A preventable and treatable disease characterized by airflow limitation that is not fully reversible. The limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking. Although COPD affects the lungs, it also produces significant systemic consequences.
Emphysema	Permanent enlargement of the airspaces distal to the terminal bronchioles, accompanied by destruction of their walls and without obvious fibrosis. In patients with COPD, either condition may be present. However, the relative contribution of each to the disease process is often difficult to discern.
Chronic bronchitis	Chronic productive cough for 3 months in each of 2 successive years in a patient in whom other causes of productive chronic cough have been excluded.
Asthma	A chronic inflammatory disease of the airways in which many cell types play a role—in particular, mast cells, eosinophils, and T lymphocytes. In susceptible persons, the inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough, particularly at night and/or in the early morning. These symptoms are usually associated with widespread and variable airflow obstruction that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli.

Source: American Thoracic Society 2000 and American Thoracic Society/European Respiratory Society Task Force 2005.

Components of tobacco smoke with the potential to injure the lungs through a variety of mechanisms are listed in Table 7.3. Some components adversely affect host defenses; others act through specific or nonspecific mechanisms. Notably, cigarette smoking has very strong oxidant potential in that both the gas and tar phases contain high concentrations of free radicals (Repine et al. 1997). Many of the components of cigarette smoke are the targets of regulations because of their toxic effects: these include nitrogen dioxide, carbon monoxide, and various metals. For information on the toxic effects of components, see reports of the U.S. Environmental Protection Agency (EPA) and other agencies (USEPA 1993, 2000; USDHHS 2000) and standard resources in toxicology (Gardner et al. 2000; Klaassen 2001).

Assessment of toxic effects of cigarette smoke in the respiratory tract requires consideration of the complexity of the mixture inhaled and the possibility of synergistic interactions among its many components. Although it is little studied, the possibility of numerous interactions has great plausibility because of the myriad components of cigarette smoke and the interlocking pathways of lung injury.

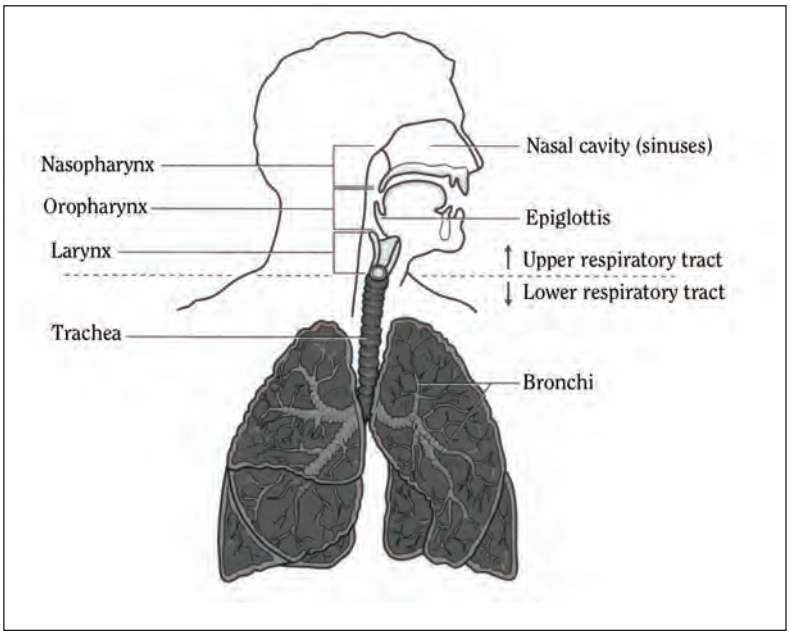
Table 7.3 Selected components of cigarette smoke and potential mechanisms of injury

Component	Mechanism
Acrolein	Cilia toxic; impairs lung defenses
Formaldehyde	Cilia toxic; irritant
Nitrogen oxides	Oxidant activity
Cadmium	Oxidative injury; promotion of emphysema
Hydrogen cyanide	Oxidative metabolism of cells affected

Dosimetry of Tobacco Smoke in the Respiratory System

To protect the lungs from injury, the respiratory tract has an elegant set of mechanisms for handling the particles and gases in inhaled air (Figure 7.1). These defenses include physical barriers, reflexes and the cough response, the sorptive capacity of the epithelial lining, the mucociliary apparatus, alveolar macrophages, and immune responses of the lung (Schulz et al. 2000). These defenses are critical because of the substantial volume of air inhaled daily: about 10,000 liters per day are inhaled by an adult. Even harmful substances present at low concentrations may eventually achieve a toxic dose after sustained exposure. In addition, high-level exposures, particularly when sustained, may overwhelm the lung's defenses, and some agents have the potential to reduce the efficacy of these defenses. Cigarette smoke, for example,

Figure 7.1 Lung defenses



Host Defenses

Nasal hair

Convoluted passages of sinuses

Coughing, sneezing, swallowing

Mucociliary cells

Normal flora

Inflammatory cells

Alveolar macrophages

Source: Cook 2000. Reprinted with permission from Elsevier Health, © 2000.

contains components that impair mucociliary clearance (Table 7.3).

The size of particles in the smoke inhaled directly from a cigarette (mainstream smoke) has been studied in a variety of systems. These studies indicate that the mass median aerodynamic diameter of particles is 0.3 to 0.4 micrometers (μm) (Martonen 1992; Bernstein 2004). Particles of this size penetrate to and are deposited in the deep lung.

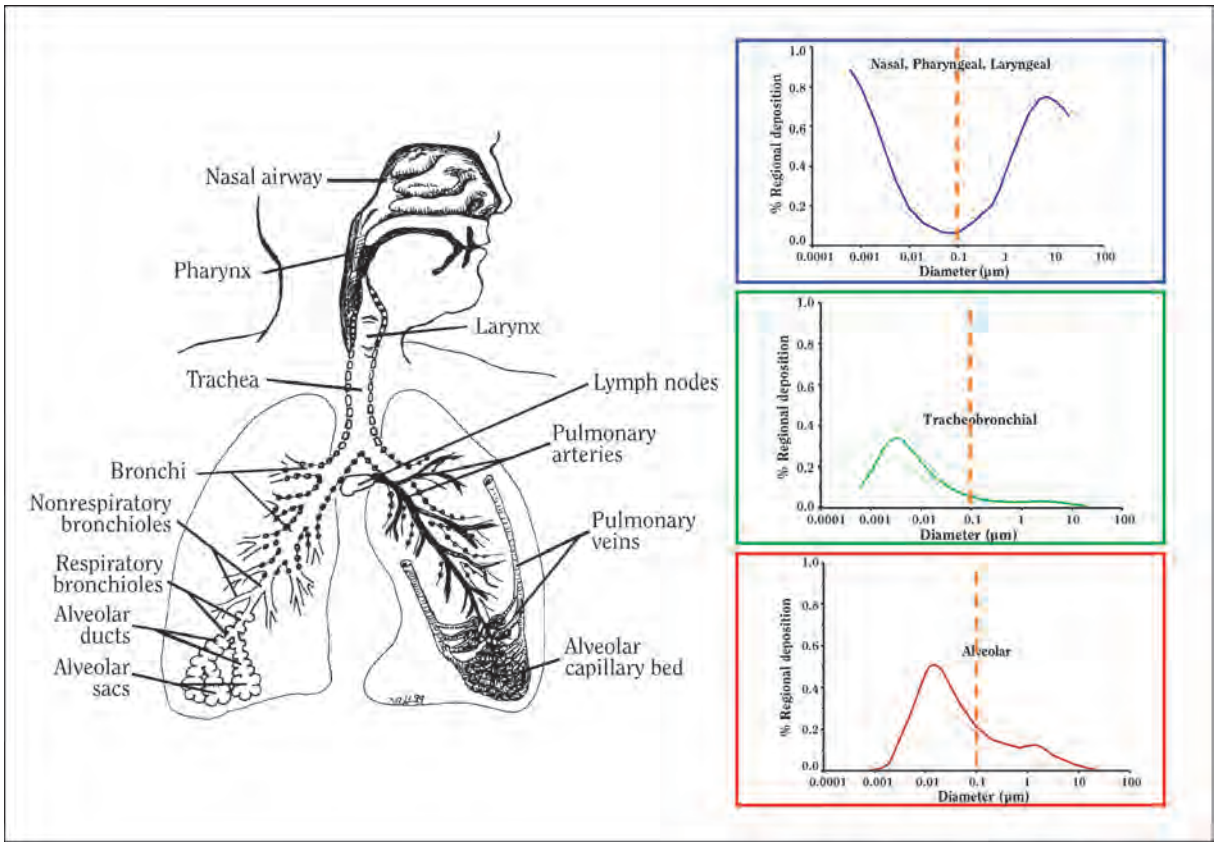
The handling of particles by the lung's defense mechanisms depends on their size (Figure 7.2). Large particles (e.g., many pollens and road dust) are removed in the upper airway, largely by impaction (USDHHS 1984). Small particles, with a mean aerodynamic diameter less than about 2.5 μm, reach the lungs, where they deposit in airways and alveoli by impaction, sedimentation, or diffusion. About 60 percent of the particles inhaled in mainstream smoke are deposited. Although these particles are subject to handling by the mucociliary apparatus and alveolar macrophages, removal is not complete because of their very high numbers in the lungs of long-term smokers, which show evidence of a substantial burden of retained particles. Similarly, evidence shows that smokers clear these particles at a reduced rate (Cohen et al. 1979; USDHHS 1984; Kreyling and Scheuch 2000).

The removal of gases in the respiratory tract is accomplished through sorption by the liquid that lines the epithelial layer (Kreyling and Scheuch 2000). Both the site and the efficacy of removal of gases depend on the solubility of the gas. Highly soluble gases are removed high in the respiratory tract, but insoluble gases (e.g., carbon monoxide) may reach the alveoli and diffuse across the alveolar-capillary membrane. These dosimetric considerations indicate a high potential for lung injury in active smokers, who inhale a rich mixture of gases and particles that penetrates throughout the lungs, with deposit of particles and sorption of gases in the two anatomic sites most critical to respiration, the airways and alveoli.

Major Pulmonary Diseases Caused by Smoking

This section provides a brief overview of the principal diseases of the lung that are caused by smoking. A brief description of pathophysiology and pathogenesis is provided as background for the more comprehensive discussions of mechanisms. These topics are covered in great detail elsewhere (Mason et al. 2005) and were addressed in the 1984 and 2004 Surgeon General's reports (USDHHS 1984, 2004).

Figure 7.2 Fractional deposition of inhaled particles in the human respiratory tract



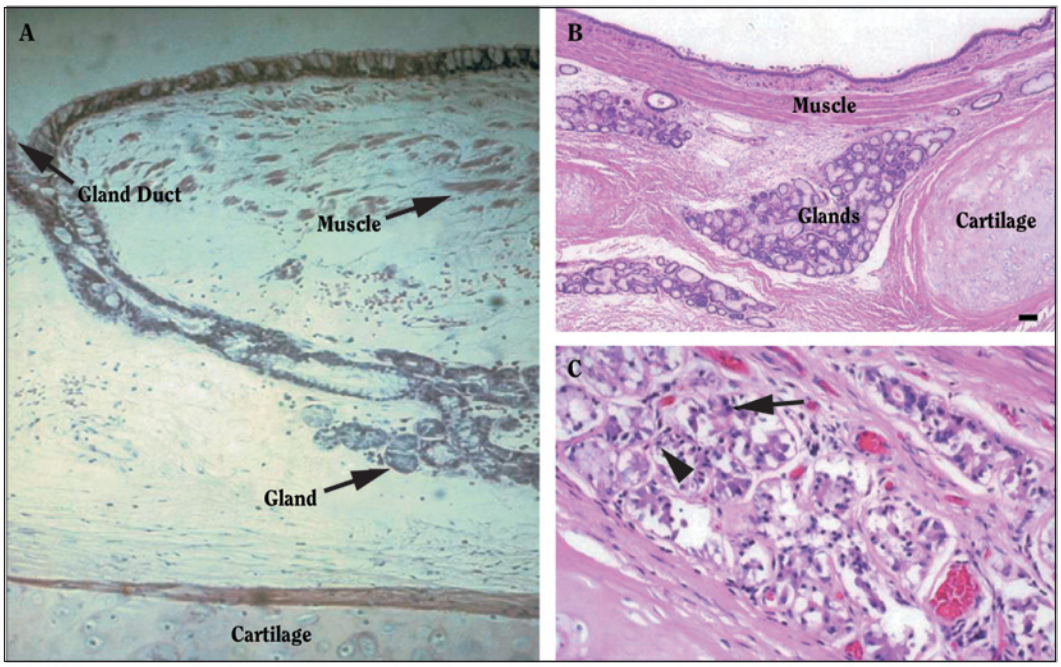
Source: Oberdörster et al. 2005. Reprinted with permission from *Environmental Health Perspectives,* © 2005. Figure based on data from the International Commission on Radiological Protection 1994. Drawing courtesy of Dr. Jack R. Harkema, Michigan State University.
Note: **µm** = micrometers.

Chronic Bronchitis

The symptom complex of chronic bronchitis has been investigated for decades. In the 1950s, the British Medical Research Council suggested that a diagnosis of chronic bronchitis was warranted when the symptoms of chronic cough and production of sputum were present on most days of the month for at least three months in two consecutive years without any other explanation (*BMJ* 1965). This proposal is reflected in the current definition of chronic bronchitis (Table 7.2). Earlier, Reid (1960) had used the size of the mucous gland layer as a predictor for the postmortem diagnosis of this condition but did not implicate the inflammatory process in the pathogenesis of either enlargement of the gland or the production of excess mucus. Subsequent studies of lung tissue surgically removed from cancer patients (Figure 7.3) have shown that the symptoms of chronic bronchitis are associated with an inflammatory response involving the mucosal surface, submucosal glands, and gland ducts, particularly in the small bronchi that are 2 to 4 millimeters (mm) in diameter (Mullen et al. 1985; Saetta et al. 1997). In addition, longitudinal studies of chronic bronchitis in persons with normal lung function have clarified that its presence does not predict future progression to more severe obstructive lung disease (Fletcher et al. 1976; Saetta et al. 1997). Presence of chronic bronchitis in persons who already have limited airflow, however, is predictive of a more rapid decline in lung function and a higher risk of hospitalization than are seen with a similar limitation of airflow but no chronic bronchitis (Saetta et al. 1997).

The inflammatory immune cells that infiltrate the epithelium, subepithelium, and glandular tissue in chronic bronchitis include the polymorphonuclear neutrophils (PMNs), macrophages, CD8-positive (CD8+) and

Figure 7.3 Comparison of normal bronchial gland (A) with enlarged bronchial glands (B and C) from a patient with chronic bronchitis



Source: Hogg 2004. Reprinted with permission from Elsevier, © 2004.
Note: (A) Histology of bronchus with epithelial lining that extends from lumen into gland duct and gland. (B) Enlarged glands from a patient with chronic bronchitis. (C) One of these glands at higher magnification showing inflammatory cells (arrow and arrowhead). Several studies of human lungs showed that the symptoms of chronic bronchitis were associated with an inflammatory response involving the mucosal surface, submucosal glands, and gland ducts, particularly in the smaller bronchi 2–4 millimeters in diameter.

CD4-positive (CD4+) T lymphocytes, and B cells that are part of the adaptive inflammatory immune process (Di Stefano et al. 1996; O'Shaughnessy et al. 1997; Saetta et al. 1997). This chronic inflammation, consisting of enlargement of the mucous glands and remodeling of the walls of both large and small bronchi reflects a deregulated healing process in tissue persistently damaged by the inhalation of tobacco smoke (Hogg 2004). The consequences of this process include both the development of a chronic cough and the accumulation of excess mucus in the airway's lumen. However, this inflammatory process has little influence on airflow limitation unless it extends to the small conducting airways that account for much of the increase in airway resistance in COPD.

Studies reported from the laboratory of Snider and associates in Israel (Breuer et al. 1993) were the first to show that elastase from PMNs was an important agent for the secretion of mucus by epithelial goblet cells. Later, Nadel (2001) and other investigators (Takeyama et al. 1999, 2000, 2001a,b; Burgel et al. 2000; Lee et al. 2000; Kohri et al. 2002) extended these observations by linking the PMN-induced production of mucin to stimulation of EGFR. They showed that PMN elastase triggered the cleavage of membrane-tethered transforming growth factor alpha (TGFα), allowing it to attach to the external binding site of EGFR. This step is followed by phosphorylation of the intracellular component of this receptor and stimulation of downstream signaling pathways that activate the expression of the *MUC5AC* gene and lead to the production of mucus (Takeyama et al. 1999). This type of experiment established that EGFR and its ligands provide a regulatory axis for the production of mucin that involves several membrane-bound ligands of EGFR, such as TGFα and heparin-binding EGF. Nadel (2001) has also shown that reactive oxygen species (ROS) can bypass the extracellular sphere of influence of this regulatory axis. Other studies have shown that ROS can directly activate EGFR's intracellular domain (Burgel et al. 2000; Takeyama et al. 2000; Kohri et al. 2002).

More recent work in transgenic mice has found that overexpression of epithelial sodium ion channels resulted in excess reabsorption of epithelial sodium and volume depletion of periciliary fluid (Mall et al. 2004). The depletion of the periciliary fluid layer interferes with the frequency of ciliary beats and results in decreased clearance and adherence of mucus to the airway surface. Results of this study showed that depletion of the periciliary fluid in animals is associated with the accumulation of mucus in the lumen of both large and small airways, leading to greater susceptibility to infection of the lower respiratory tract and early death.

Chronic Obstructive Pulmonary Disease

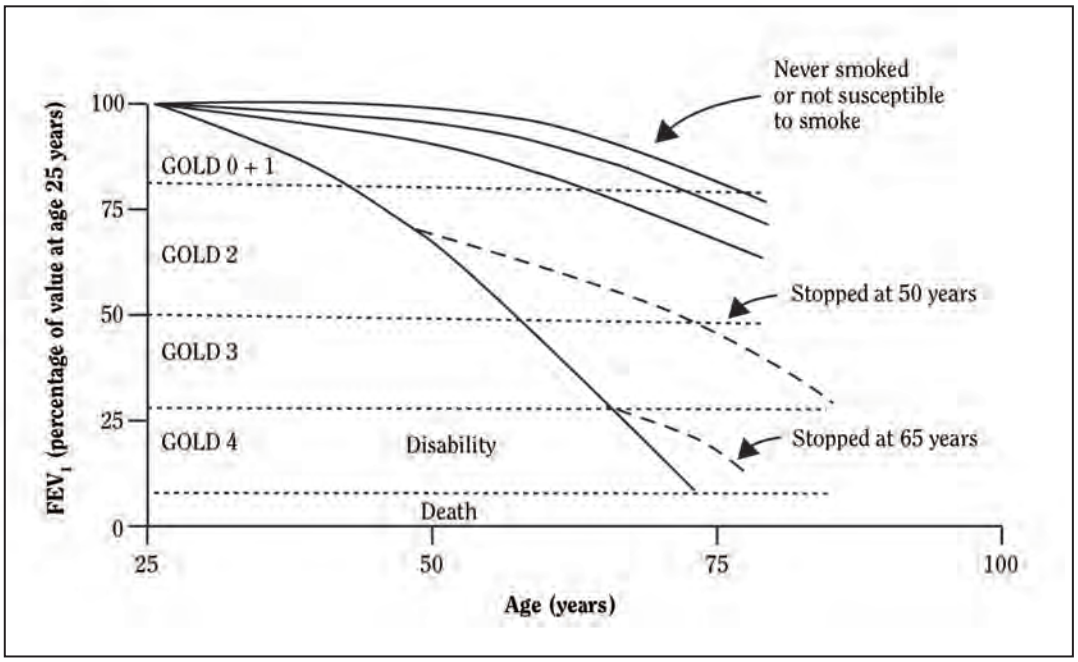
The hallmark of COPD is chronic airflow obstruction demonstrated with spirometry and the accompanying dyspnea and limitation of activity. Maximum expiratory flow is determined by the product of the resistance to flow in the small conducting airways (centimeters of water [H_2O] per liter per second) and the elastic recoil of the lung parenchyma that drives expiratory flow (liters per centimeter of H_2O). The product of these two variables, the time constant, characterizes the rapidity with which the lung fills and empties during respiration. Surprisingly, the time constant of the lung remains stable over a wide range of breathing frequencies in healthy lungs, but if disease increases either the compliance as in emphysema or the resistance as in obstruction of small airways, the time required to empty the lung is prolonged (Otis et al. 1956). The presence of a fixed limitation in airflow can be diagnosed by using a spirometer to measure the volume of air that can be forcibly expired from the lungs in one second (forced expiratory volume [FEV_1]) and then determining its ratio to forced vital capacity (FEV_1/FVC) after the administration of a bronchodilator.

The classic cohort study of the natural history of chronic bronchitis and emphysema performed by Fletcher and colleagues (*Lancet* 1965; Fletcher 1976) used this type of measurement to test the hypothesis of a sequence beginning with tobacco smoking and then moving to symptoms of chronic bronchitis or recurrent chest infections and, finally, chronic limitation of airflow. The natural history of the decline in FEV developed by Fletcher and colleagues (1976) to summarize findings of a six-year longitudinal study of men working in West London is illustrated in Figure 7.4. Subsequent studies have confirmed these findings (USDHHS 1984). The horizontal lines added to the Fletcher diagram indicate the boundaries of the five-stage classification of the severity of COPD by the Global Initiative for Chronic Obstructive Lung Disease (GOLD). The measurements used were FEV_1 and FEV_1/FVC (Pauwels et al. 2001; GOLD 2006). According to this classification, GOLD stage 0 defines persons with a normal FEV_1 and FEV_1/FVC who have symptoms attributable to significant exposure to tobacco smoke as being at risk for developing COPD. Those with mild, moderate, severe, or very severe COPD are placed in GOLD stages 1 through 4, respectively (Takeyama 2001b).

Fletcher and colleagues (1976) observed that only 15 to 25 percent of the smokers in the study developed airflow limitation, and they showed that smoking cessation slowed the rate of decline in FEV_1 in those who stopped smoking permanently. In subsequent studies of various populations, only a minority of smokers developed COPD. This repeated finding indicates a role for genetic factors that may determine susceptibility to cigarette smoke. These investigators rejected the hypothesis of a pathogenetic continuum from smoking to obstructive bronchitis. Most persons who developed airflow limitation during the study had no evidence of chronic bronchitis, a finding that was not consistent with the hypothesis of a continuum from smoking to bronchitis to obstruction. Subsequent studies have confirmed that the presence of chronic bronchitis in persons with normal lung function (GOLD stage 0) does not predict progression of disease (Vestbo and Lange 2002). Using data from the Copenhagen City Heart Study, however, Vestbo and colleagues (1996) found that the symptoms of chronic bronchitis were associated with an accelerated decline in FEV_1.

Acute exacerbations, a concern in treatment of COPD, are attributed to viral infections (Monto et al. 1975; Smith et al. 1980; Seemungal et al. 2001), bacterial infections, and occupational and environmental air pollution; an important residual of cases had no obvious cause (Pauwels et al. 2001; Rabe et al. 2007). Some unexplained exacerbations of COPD might be attributable to latent viral infection, because such infections can deregulate the expression of adhesion proteins that might initiate this response (Gonzáles et al. 1996; Keicho et al. 1997). Although Fletcher and colleagues (1976) found that these exacerbations had no effect on the rate of decline of FEV_1 in the working men in West London, the U.S. Lung Health Study showed that such exacerbations were associated with a more rapid decline in persons with mild disease who continued to smoke (Kanner et al. 2001). Subsequently, other investigators found that frequent exacerbations in patients with more severe COPD, especially those resulting from a higher bacterial load, were associated with more accelerated decline in FEV_1 (Donaldson et al. 2002; Wilkinson et al. 2003). Collectively, these data suggest that when lung defenses become compromised in the later stages of COPD, chronic infection might play a role in the pathogenesis of the airflow limitation.

Figure 7.4 Natural history of decline in forced expiratory volume with aging measured in a group of working men in West London over about six years

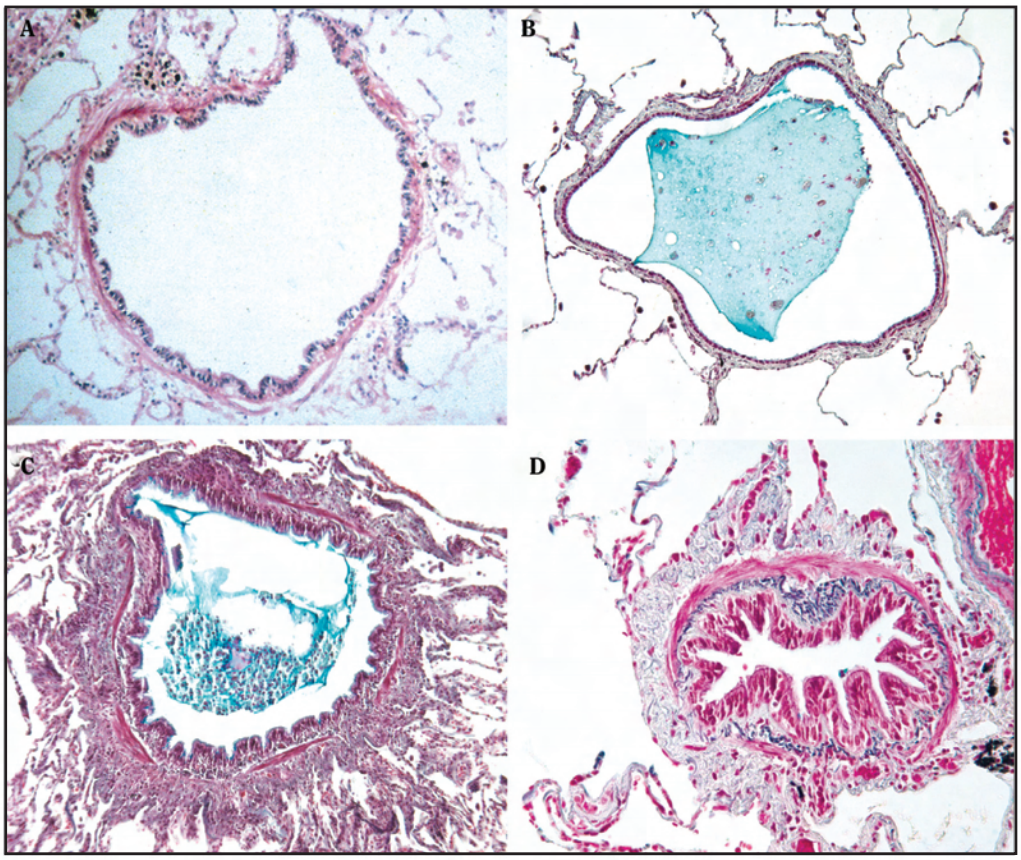


Source: Hogg 2004. Reprinted with permission from Elsevier, © 2004.
Note: Adapted from Fletcher et al. 1976. Horizontal lines have been added to their diagram to indicate the boundaries of the classification of severity of chronic obstructive pulmonary disease by the Global Initiative for Chronic Obstructive Lung Disease (GOLD).

Obstruction of Small Airways

Although spirometric measurement of FEV_1 and the FEV_1/FVC provides a reliable method for diagnosing airflow limitation and classifying its severity, spirometry cannot distinguish the contributions of either the obstruction of small airways or emphysematous destruction to the airflow limitation in COPD. Direct measurements of pressures and flows within the lung have shown that the small bronchi and bronchioles (<2 mm in diameter) are the major sites of airway obstruction in COPD (Hogg et al. 1968; van Brabandt et al. 1983; Yanai et al. 1992). This obstruction is related to an inflammatory process that thickens the airway wall, fills the lumen with exudates containing mucus, and narrows the airway by depositing connective tissue in the airway wall (Figure 7.5). McLean (1956) and Leopold and Gough (1957) recognized that an inflammatory process was present in the small bronchi and bronchioles of lungs affected by centrilobular emphysema. Leopold and Gough (1957) hypothesized that centrilobular emphysema resulted from an extension of this process from the small conducting airways into the respiratory bronchioles. Later, Matsuba and Thurlbeck (1972) demonstrated an excess deposition of connective tissue in the adventitia of the small conducting airways in advanced emphysema and suggested that peribronchiolar fibrosis narrowed the airway lumen. In addition, cross-sectional studies of the pathology of COPD have shown that the peripheral inflammatory immune process found in the lungs of all smokers is amplified in severe (GOLD stage 3) and very severe (GOLD stage 4) COPD (Fletcher et al. 1976; Hogg et al. 2004). More recent evidence indicates that at these levels of disease severity, these changes are associated with an increase in the adaptive immune response. These findings may reflect the response to an antigenic stimulus from a limited number of antigens that might be microbial or possibly from autoantigens that develop within the damaged lung tissue (Agustí et al. 2003; Voelkel and Taraseviciene-Stewart 2005).

Figure 7.5 Nature of an obstruction in the small conducting airways (<2 millimeters in diameter)



Source: Hogg 2004. Reprinted with permission from Elsevier, © 2004.
Note: A normal airway (A) is compared with another airway (B) in which the lumen is partially filled with a bland mucous plug containing a few epithelial cells. (C) An airway in which the wall contains an active inflammatory process that partially fills the lumen with inflammatory exudates containing mucus. (D) Airway narrowed by collagen deposition in the peribronchiolar space.

Emphysema

Emphysema was first described by René Laënnec in 1834 on the basis of observations made on the cut surface of postmortem human lungs that had been air-dried in inflation (Laënnec 1834), but the concept that emphysematous destruction produced airflow limitation by decreasing the elastic recoil forces required to drive air out of the lung was not fully developed until 1967 (Mead et al. 1967). The earliest concept regarding the pathogenesis of emphysema postulated that overinflation compressed the lung capillaries, leading to atrophy of lung tissue; this concept was mentioned in major textbooks of pathology as late as 1940 (McCallum 1940). As mentioned previously (see "Obstruction of Small Airways" earlier in this chapter), McLean (1956) and Leopold and Gough (1957) were the first to implicate the inflammatory response in the pathogenesis of alveolar destruction in their early descriptions of centrilobular emphysema, but skepticism about this association persisted because of the possibility that preterminal bronchopneumonia may have been responsible for the inflammation observed in the postmortem studies. The subsequent demonstration that emphysema could be produced experimentally by depositing the enzyme papain in the lung (Gross et al. 1964), combined with observational studies showing the association between emphysema and deficiency of α1-antitrypsin (AAT) (Laurell and Eriksson 1963), led naturally to the hypothesis that the pathogenesis of emphysema was based on a functional proteolytic imbalance within the

inflammatory response induced by tobacco smoke (Gadek et al. 1979).

Currently, emphysema is defined as "abnormal, permanent enlargement of air spaces distal to the terminal bronchiole, accompanied by the destruction of their walls, and without obvious fibrosis" (Snider et al. 1985, p. 183). The condition can now be diagnosed and quantified during life by several techniques. Postmortem examinations have provided indirect information on the prevalence of emphysema (Thurlbeck 1963; Ryder et al. 1971).

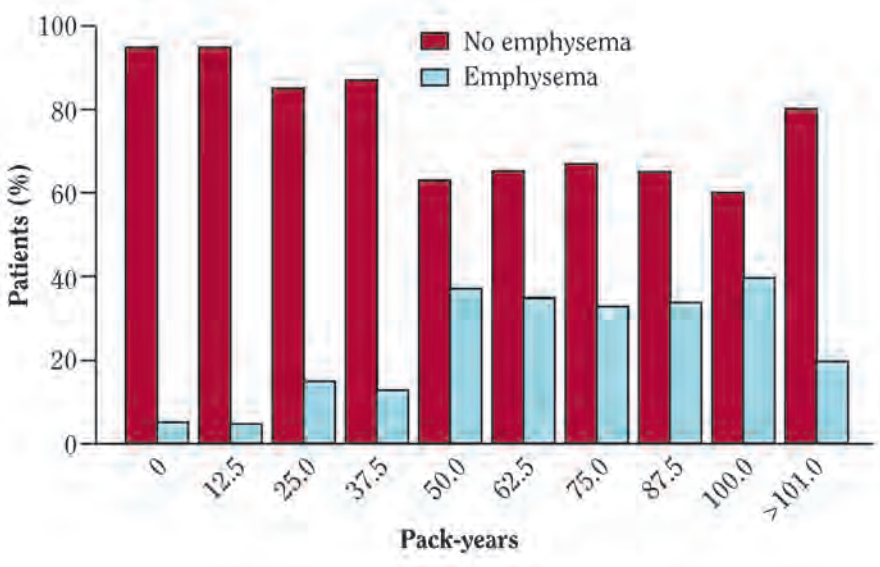
An important study from the United Kingdom (Ryder et al. 1971) found emphysema in 62 percent (219) of 353 consecutive postmortem examinations. On average, when present, the condition occupied 12.6 percent (range, 0.5 to 95 percent) of total lung volume. Smoking history was established in 179 of the 353 patients, and emphysema was present in 75 percent (80) of the 106 smokers. The mean proportion of total lung volume occupied by emphysema in smokers was 10.8 percent (range, 0 to 90 percent). Emphysema was also present in 28 percent (21) of 73 nonsmokers, but the mean proportion of the lung taken up by emphysema in nonsmokers was only 1.7 percent (range, 0 to 40 percent). In addition, the nonsmokers lived longer than the smokers (aged 64.8 versus 60.2 years; $p < 0.05$) and emphysema appeared at a later age (Ryder et al. 1971).

A laboratory study of more than 400 lungs removed from patients being treated for lung cancer (Hogg 2004) confirmed that a small proportion of smokers had emphysema and that the proportion with emphysema increases with the number of pack-years of smoking. However, the dose-response relationship plateaus at 50 to 100 pack-years, and about 40 percent of smokers are affected (Figure 7.6). Although imaging by computed tomography (CT) has now confirmed that emphysema can be found in persons with a normal FEV_1, population-based studies of its prevalence, as detected by CT, have not been attempted.

Centrilobular and Panacinar Forms of Emphysema

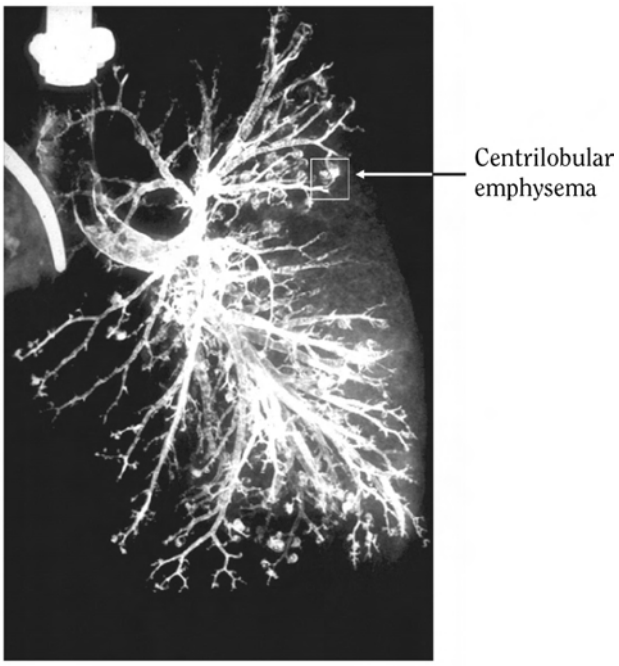
Pathologically, emphysema is characterized by its location as centrilobular or panlobular; the radiographic correlates are centriacinar emphysema and panacinar emphysema, respectively (Friedlander et al. 2007). Centrilobular emphysema is characteristic of smokers, whereas panacinar emphysema is found with AAT deficiency. In general, persons with a predominance of centrilobular emphysema have physiological abnormalities consistent with abnormal function of small airways, whereas panlobular emphysema is associated with high lung compliance. A substantial portion of people with emphysema have both types.

Figure 7.6 Dose-response relationship between level of smoking and the percentage of 408 patients in the St. Paul's Lung Study with morphologic evidence of significant emphysema in their lungs[a]



Source: Hogg 2004. Reprinted with permission from Elsevier, © 2004.
[a]The relationship plateaued with about 40 percent of the heavy smokers having emphysema.
[b]Pack-years = the number of years of smoking multiplied by the number of packs of cigarettes smoked per day.

Figure 7.7 Postmortem bronchogram performed on the lungs of a person with centrilobular emphysema



Source: Hogg 2007. Reprinted with permission from Informa Healthcare, © 2007.
Note: The lesions hang from the distal airways like "Christmas tree balls" (arrow).

A postmortem bronchogram from a patient with lesions of centrilobular emphysema visible at a microscopic low power is shown in Figure 7.7. The nature of these lesions is shown to better advantage in Figure 7.8. Several normal terminal bronchioles within a secondary lung lobule (A) and the histology of a normal acinus beyond a single terminal bronchiole (B) can be compared with a line drawing from Leopold and Gough's (1957) original description of centrilobular emphysema (C) and a postmortem radiograph showing the destruction of the respiratory bronchioles (D). These centrilobular lesions affect the upper regions of the lung more commonly than the lower regions (Figure 7.9) and are also larger and more numerous in the upper lung (Gadek et al. 1979). Heppleston and Leopold (1961) used the term "focal emphysema" to describe a less severe form of centrilobular emphysema, but Dunnill (1982) argued that this distinction was not helpful and that the two conditions probably had a similar origin, with focal emphysema being more widely distributed and less severe than the classic centrilobular form. Dunnill also preferred the term "centriacinar" to "centrilobular." "Centriacinar" seems more suitable in that each secondary lobule contains several acini (Figure 7.8A) and not all are involved in emphysematous destruction.

Wyatt and colleagues (1962) provided the first detailed account of the panacinar form of emphysema, in which more uniform destruction of the entire acinus takes place. Thurlbeck (1963) showed that it can be difficult to distinguish normal lung from lung with mild forms of panacinar emphysema, unless fully inflated specimens are carefully examined under a dissecting microscope. In contrast to centrilobular emphysema, the panacinar form tends to be more severe in the lower lobes than in the upper lobes (Figure 7.9), but this difference is substantial only in severe disease (Thurlbeck 1963). Panacinar emphysema is commonly associated with AAT deficiency but is also found in cases with no identified genetic abnormality (Thurlbeck 1963).

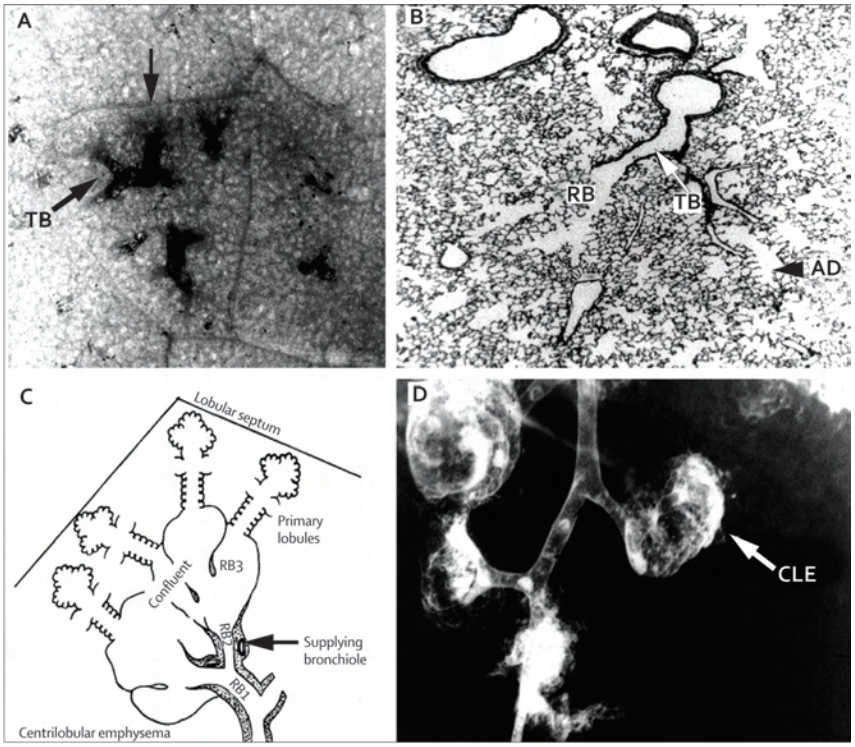
Other Forms of Emphysema

"Distal acinar," "mantle," and "paraseptal" emphysema describe lesions in the periphery of the lobule. These types of lesions are found along the lobular septa, particularly in the subpleural region (Hogg and Senior 2002). They also occur in isolation and have been associated with spontaneous pneumothorax in young adults (Ohata and Suzuki 1980) and bullous lung disease in older adults, whose lung function improved after the removal of large cysts (Morgan 1995). Less frequent forms of emphysema include the unilateral form (McLeod syndrome) that occurs as a complication of severe childhood infection by rubella or adenovirus; the congenital lobar form, a developmental abnormality in newborns; and paracicatricial emphysema, which forms around scars and lacks any special distribution within the acinus or lobule (Thurlbeck 1963; Dunnill 1982).

Pulmonary Hypertension

The invasive nature of right-heart catheterization in older adults with comorbid disease has made it difficult to study the prevalence of pulmonary hypertension in patients with COPD. In one six-year study of 131 patients (Kessler et al. 2001), COPD ranged from moderate (GOLD stage 2) to very severe (GOLD stage 4). At baseline, none of the patients had pulmonary hypertension, but after six years, it was present in 25 percent of the patients at rest and in more than 50 percent during exercise. These data suggest that the prevalence of pulmonary hypertension increases steadily with progression of COPD, appearing first during exercise and later at rest.

Figure 7.8 Details of centrilobular emphysema lesions

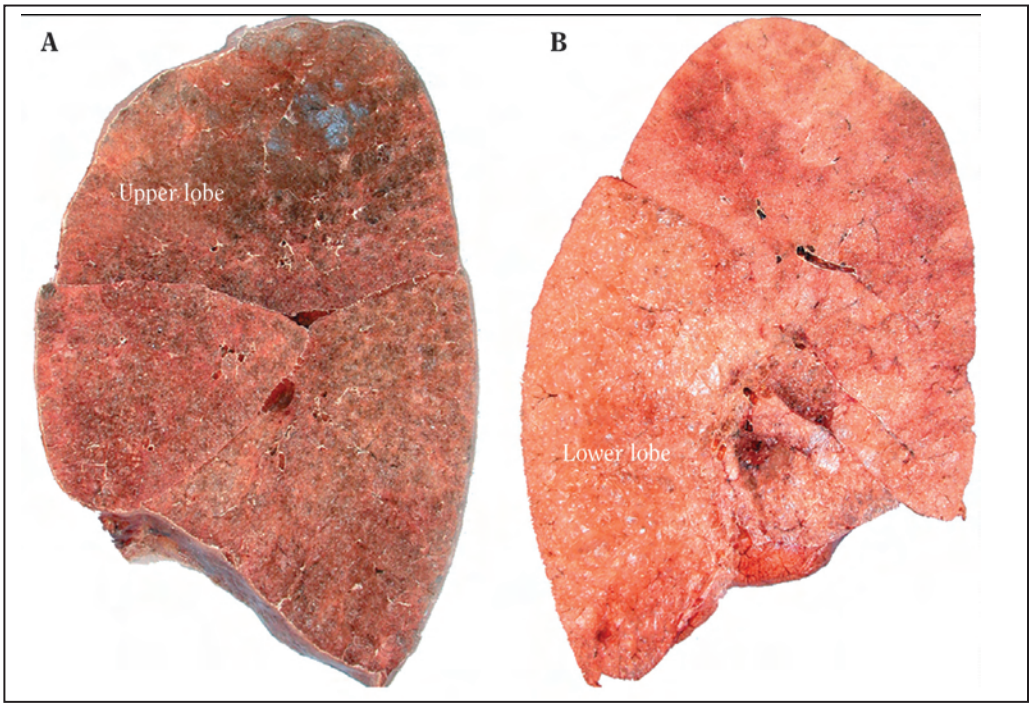


Source: Hogg 2004. Reprinted with permission from Elsevier, © 2004.
Note: (A) Several normal terminal bronchioles within a secondary lung lobule defined by its surrounding connective tissue septa (solid arrow) are shown for comparison with (B) the histology of a normal acinus beyond a single terminal bronchiole. (C) Line drawing from Leopold and Gough's original (1957) description of centrilobular emphysema showing the destruction of the respiratory bronchioles, and (D) a postmortem radiograph of the dilatation and destruction of the respiratory bronchioles. **AD** = alveolar duct; **CLE** = centrilobular emphysema; **RB** = respiratory bronchioles; **TB** = terminal bronchioles.

When pulmonary hypertension is absent at rest, but present during exercise, some of the increase in pulmonary vascular pressures can be attributed to the mechanical events associated with dynamic hyperinflation of the lung in persons with airflow limitation (Horsfield et al. 1968; Jezek et al. 1973; Weitzenblum et al. 1981; Wright et al. 1983a). When the time required to exhale becomes longer than the time between breaths, lung volume tends to increase, first as the breathing rate increases during exercise and later as it does so at rest. This increase in lung volume increases intrathoracic pressure, an increase that is transmitted to all the vessels within the thorax. As a result, both pulmonary artery and left atrial pressures are higher than atmospheric pressure but not higher than intrathoracic pressure. Treatment with oxygen at this stage of the disease lowers both pulmonary artery and left atrial pressure by slowing the breathing rate, thereby relieving the dynamic hyperinflation and lowering intrathoracic pressure. However, when lung emptying is more severely prolonged and alveolar pressure rises above intrathoracic pressure, there is a true increase in pulmonary artery pressure (Weitzenblum et al. 1981). Hypoxic vasoconstriction of the muscular pulmonary arteries and emphysematous destruction of the pulmonary vascular bed are more likely to contribute to pulmonary hypertension in severe (GOLD stage 3) and very severe (GOLD stage 4) COPD. At these more advanced stages, affected persons commonly experience chronic hypoxia and extensive destruction of the pulmonary capillary bed.

Studies of the microvessels of the lung in mild (GOLD stage 1) and moderate (GOLD stage 2) COPD show consistent changes in the intima. In more severe (GOLD

Figure 7.9 Cut surface of lungs removed from two patients with different forms of emphysema before receiving a lung transplant



Source: From Dr. Joel Cooper in Hogg 2004. Reprinted with permission from Elsevier, © 2004.
*Note: (*A) The lung on the left is affected by centrilobular emphysema, which affected the upper lobe more severely than the lower lobe. (B) The lung on the right is from a patient who had α1-antitrypsin deficiency, which involved the lower lobe to a greater degree than the upper lobe.

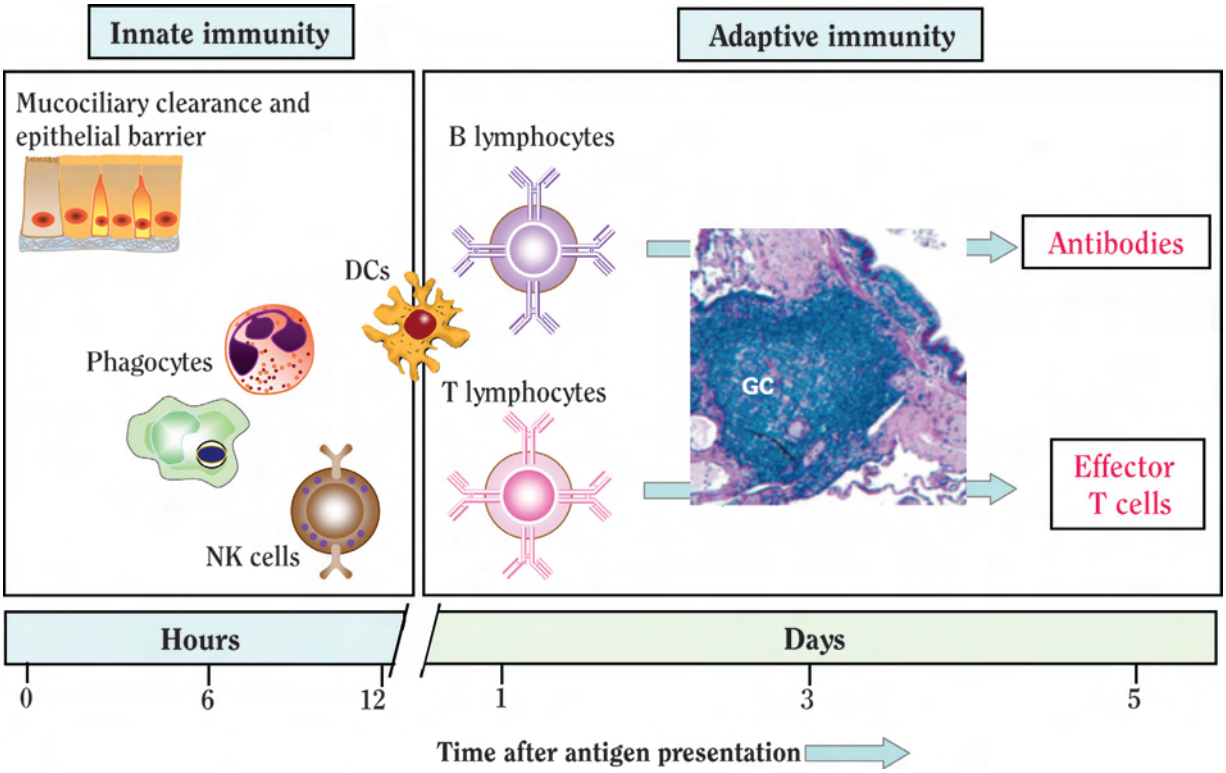
stage 3) and very severe (GOLD stage 4) COPD, the vessel wall is commonly altered by fibroelastic thickening—the proliferation of smooth muscle and extension of the muscle into small vessels that do not normally contain muscle. However, the contribution of smooth muscle to thickness of the vessel wall is also reported to be greater in smokers with normal lung function than in nonsmokers and still greater in smokers whose lung function is impaired (Horsfield et al. 1968). In patients who have very severe emphysema, the overall wall thicknesses of vessels with external diameters of 100 to 200 μm correlate with both the rise in pulmonary arterial pressure during exercise and the difference between the pulmonary artery pressures measured during rest and during exercise (Hale et al. 1984; Kubo et al. 2000). The increase in muscle in the pulmonary arteries, which is variable, probably depends on the severity of the COPD. A greater amount of muscle has been observed in the pulmonary vessels of smokers than in those of nonsmokers (Horsfield et al. 1968), but in mild COPD, little if any increase in muscle has been observed (Weitzenblum et al. 1981; Haniuda et al. 2003). Muscular medial thickening (Barberà et al. 2003), as opposed to overall wall thickening (Hale et al. 1984; Kubo et al. 2000), does not appear to be related to the severity of the pulmonary hypertension or the vascular response to oxygen in patients with COPD (Wright et al. 1992).

Reports from Barberà and associates (2003) from Spain indicate that this vascular remodeling process is also associated with, and possibly preceded by, an inflammatory process in which the vessels become infiltrated with a population of cells similar to those found around the small airways. The precise meaning of this finding and its role in the pathogenesis of the peripheral lung lesions observed in COPD is under investigation.

Smoking and Respiratory Defense Mechanisms

The innate defense system of the lung includes the apparatus for producing and clearing mucus, the epithelial cell barrier, and infiltrating inflammatory immune cells (Figure 7.10) (Abbas et al. 2000c; Knowles and Boucher 2002). The pulmonary epithelium plays a critical role in the host defense by recognizing insults and initiating innate responses (Greene and McElvaney 2005; Martin and Frevert 2005; Mayer and Dalpke 2007; Sabroe et al. 2007; Torrelles et al. 2008). The inhalation of tobacco smoke interferes with these defenses, resulting in both increased production of mucus and decreased effectiveness of the clearance process in the airway's lumen (Hogg 2008). Impairment of these defenses increases the potential for infection (Knowles and Boucher 2002; Drannik et al. 2004). Tobacco smoke also disrupts the tight junctions that form the epithelial barrier (Jones et al. 1980; Hulbert et al. 1981) and initiates the infiltration of the damaged tissue by a variety of inflammatory

Figure 7.10 Innate and adaptive immune system of the lung, including the mucous production and clearance apparatus, the epithelial barrier, and the inflammatory immune response



Source: Hogg 2007. Reprinted with permission from Informa Healthcare, © 2007.
Note: Chronic stimulation of this system by the tobacco-smoking habit results in both increased production and decreased clearance of mucus from the airway's lumen, disruption of the tight junctions that form the epithelial barrier and infiltration of the damaged tissue by polymorphonuclear and mononuclear phagocytes as well as natural killer cells and CD4+ and CD8+ T cells, and B-cell lymphocytes. The adaptive immune response requires antigen presentation primarily by dendritic cells and the organization of the lymphocytes into follicles with germinal centers. This type of response is rarely found in healthy nonsmokers but has been documented in about 5 percent of peripheral lung units of smokers with normal lung function, increasing to about 20 to 30 percent of airways in the later stages of chronic obstructive pulmonary disease. The source of antigen that drives this sharp increase in the adaptive immune response is unknown and may be related to either the colonization or infection of the lower airways by a variety of microbes in the later disease stages or to autoantigens that develop in the damaged tissue. **DCs** = dendritic cells; **GC** = germinal center; **NK** = natural killer.

immune cells, including polymorphonuclear and mononuclear phagocytes, natural killer cells, CD4+ and CD8+ T cells, and B lymphocytes (Nagaishi 1972; Niewoehner et al. 1974; Bosken et al. 1992; Richmond et al. 1993; Di Stefano et al. 1996; O'Shaughnessy et al. 1997; Ekberg-Jansson et al. 2001; Retamales et al. 2001; Cosio et al. 2002; Aoshiba et al. 2004; Hogg et al. 2004; Buzatu et al. 2005). The lymphocytes become organized into lymphoid follicles with germinal centers to mount an effective adaptive immune response. Lymphoid collections with these characteristics have been demonstrated in about 5 percent of the smaller airways of smokers (Hogg et al. 2004), and their frequency increases to about 20 to 30 percent of airways in the later stages of COPD (Nagaishi 1972; Richmond et al. 1993; Hogg et al. 2004). The source of antigen that drives this sharp increase in the adaptive immune response is unknown and may be related to either the colonization or infection of the lower airways by a variety of microbes in the later stages of the disease or to autoantigens that develop in the damaged tissue. This inflammatory immune process persists after cessation of smoking (Wright et al. 1983b; Rutgers et al. 2000). Smoking cessation slows the rate of decline in lung function and delays death (Fletcher et al. 1976; Anthonisen et al. 2005).

This section of the chapter briefly reviews both the inflammatory immune process in relation to the repair and remodeling of the tissue damaged by tobacco smoke and discusses the roles of these processes in the pathogenesis of the lesions that define COPD. This section of the chapter briefly reviews the inflammatory immune process in relation to the repair and remodeling of the tissue damaged by tobacco smoke and discusses its contribution to the pathogenesis of the lesions that define COPD. Both of these aspects of the pathogenesis of COPD are the focus of substantial research at present.

Infiltration of Innate Inflammatory Immune Cells

The epithelial cells covering the lung surface and the alveolar macrophages protecting that surface are key in defending the lung against inhaled gases and particles. Both of these cell types produce a broad array of proinflammatory chemokines and cytokines. When these signaling molecules are stimulated by tobacco smoke, they can be measured in induced sputum (Traves et al. 2002), in BAL fluid from patients with COPD (Morrison et al. 1998b), and in supernates of cultured cells exposed to particles and gases under controlled in vitro conditions (Becker et al.1996; Quay et al. 1998; Mukae et al. 2000; Fujii et al. 2001, 2002; van Eeden et al. 2001). More than 50 types of chemokine ligands (L) in four families were identified by the position of the cysteine residue; they were designated as CC, CXC, C, and CX_3C (Proudfoot 2002; Lukacs et al. 2005). These ligands interact with more than 20 chemokine receptors (R) to direct leukocyte traffic in the inflammatory immune response. Many chemokines, such as interleukin-8 (IL-8, or CXCL8), interact with more than one receptor (CXCR1 and CXCR2) to control the infiltration of PMN into damaged lung tissue (Keatings et al. 1996; Yamamoto et al. 1997). IL-8 is markedly increased in the sputum of patients with COPD (Keatings et al. 1996; Yamamoto et al. 1997) and can readily be measured in the supernates of cultured human bronchial epithelial cells (HBECs) as they take up toxic particles (Fujii et al. 2001, 2002). CXCL1 is also secreted by airway epithelial cells and alveolar macrophages and activates PMNs, monocytes, basophils, and T lymphocytes through CXCR2 (Proudfoot 2002; Lukacs et al. 2005). The migration of T lymphocytes is controlled by the chemokine receptor CXCR3 that is expressed in human peripheral airways (Saetta et al. 2002) and interacts with other chemokines, including CXCL9, CXCL10, and CXCL11 (Clark-Lewis et al. 2003). Increasingly, evidence indicates that safe and effective inhibitors of proinflammatory chemokines and cytokines may benefit persons who have COPD (Proudfoot 2002; Lukacs et al. 2005).

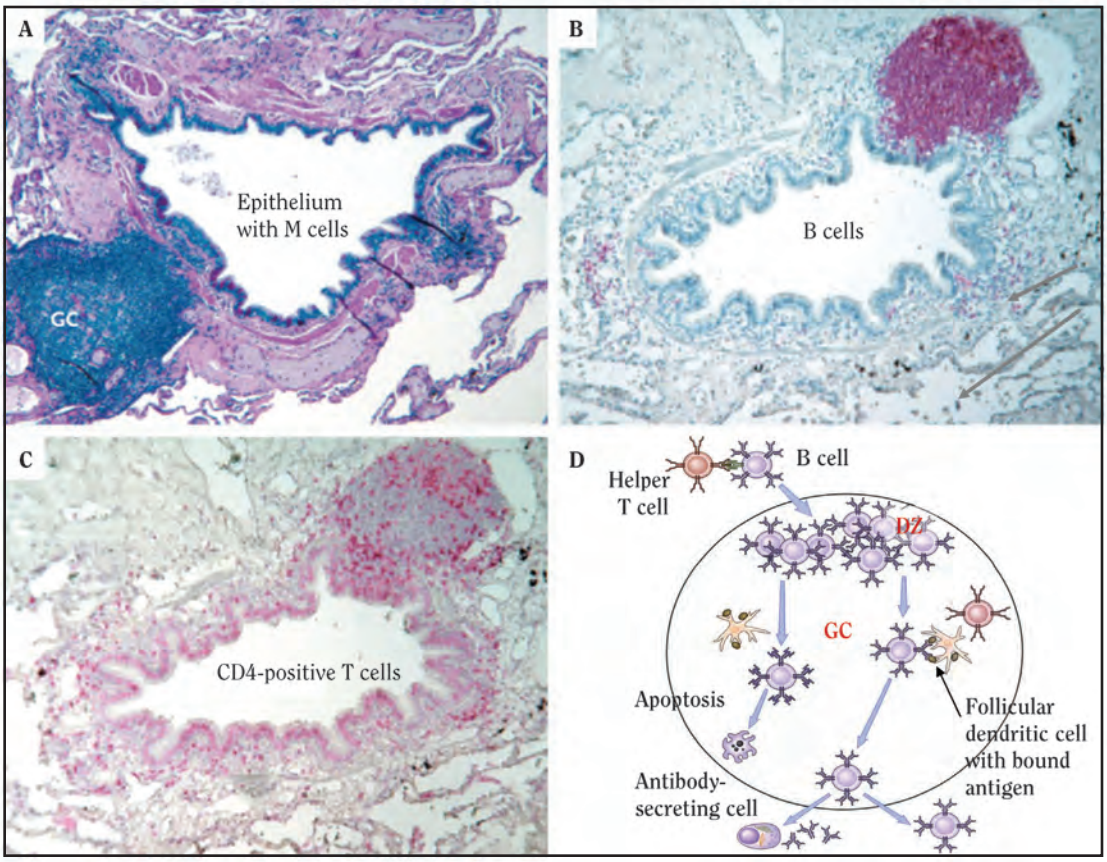
In studies involving the coculture of alveolar macrophages and HBECs, paracrine stimulation between these cell types enhances their production of chemokines and cytokines capable of controlling the recruitment and activation of leukocytes (TNFα, IL-1β, IL-8, and macrophage inflammatory protein 1α), enhancing phagocytosis (interferon-gamma), stimulating natural killer cell and T-cell function (IL-12), and initiating the repair process (granulocyte-macrophage colony-stimulating factor) (Mukae et al. 2001; Goto et al. 2004). Furthermore, instillation of the supernatants from alveolar macrophages and/or HBECs challenged with particles in vitro produces a systemic response similar to that achieved by instilling the same number of particles directly into the lungs of animals (Goto et al. 2004). The magnitude of the systemic response correlates with the number of particles phagocytosed by the macrophages (Mukae et al. 2001). More limited studies of living persons indicate that cytokines produced in the lungs (TNFα, IL-1β, and IL-6) enter the blood after smoke inhalation during natural forest fires and stimulate the liver to produce acute phase proteins and the bone marrow to increase production of leukocytes and release them into the circulation (Tan et al. 2000; van Eeden and Hogg 2000). Other studies in humans have shown a relationship among the count of circulating leukocytes, decline in lung function, and risk for early death from COPD (Chan-Yeung et al. 1988; Weiss et al. 1995).

These and other reports indicate that the inhalation of toxic particles and gases causes a local innate inflammatory immune response in the lung, initiating an adaptive immune response.

Adaptive Immune Response

The transition from the innate response to the more sophisticated adaptive immune response takes place in lymphoid follicles with germinal centers (Figure 7.10), found either in regional lymph nodes or in lymphoid collections within lung tissue (Figure 7.11). Those in the lung tissue are similar to those in the lymphoid collections observed in tonsils and adenoids in the nasopharynx and to Peyer's patches in the small bowel and the appendix of the large bowel (Nagaishi 1972; Pabst and Gehrke 1990; Richmond et al. 1993; Hogg et al. 2004). All of these structures are part of the mucosal immune system and differ from true lymph nodes in having no capsule and not receiving afferent lymphatic vessels (Nagaishi 1972; Hogg et al. 2004). The epithelium that covers the follicles in lung tissue contains specialized M cells (Figure 7.11) that transport antigens from the lumen to the lamina propria but do not function as antigen-presenting cells. Dendritic cells located in the epithelium and lamina propria pick up the antigen, which either penetrates the epithelial barrier or is transported by the M cells, and

Figure 7.11 Lymphoid collections within lung tissue



Source: Adapted from Hogg et al. 2007 with permission from Massachusetts Medical Society, © 2007.
Note: (A) Collection of bronchial lymphoid tissue with a lymphoid follicle containing a germinal center (GC) surrounded by a rim of darker-staining lymphocytes extending to the epithelium of both the small airway and alveolar surface (Movat's stain, x6). (B) Follicle in which GC stains strongly for B cells (x6). (C) Serial section of the same airway stained for CD4-positive T cells, which are scattered around the edge of the follicle and in the airway wall (x6.5). (D) Diagram of immune process triggered by antigens.

carry it to either the mucosal lymphatic collections or the regional lymph nodes (Buzatu et al. 2005). Lymphocytes enter these collections from the blood by attaching to specialized high endothelial cells lining the microvessels that supply mucosal lymphoid follicles (Abbas et al. 2000a,b).

The B cells concentrate in germinal centers rich in B lymphocytes, and the CD4+ and CD8+ T cells concentrate at the edge of the follicles and in the spaces between them (Hogg et al. 2004). This separation and concentration of B and T lymphocytes (Figure 7.11) greatly increases the opportunity for the migrating dendritic cells to present antigen to immature T and B lymphocytes as they make their way through the lymphoid collections to the efferent lymph. The T and B cells activated by the presented antigen migrate to the outer edge (dark zone) surrounding the germinal center (Figure 7.11) and enrich this zone with CD4+ helper T cells and B and T lymphocytes that have recognized similar antigens. This aggregation greatly increases the opportunity for CD4+ helper T cells, B cells, and T cells that have recognized the same antigen to interact to initiate an adaptive response.

The primary stimulus for production of antibodies is provided by the interaction between CD4+ helper T-cell receptors and the major histocompatibility complex class II antigen complex on the B cell (Abbas et al. 2000b). Secondary costimulatory signals delivered by interactions between B-7 on the dendritic cell and its ligand CD28 on the B cell and between CD40 on CD4+ helper T cells and its ligand on B cells stimulate clonal proliferation of the B cell and the production of antibodies (Abbas et al. 2000a,b).

The rich diversity of antigen receptors expressed on mature T and B lymphocytes is made possible by a somatic recombination of a limited number of gene segments encoded in spatially segregated regions of the germ line. The specificity of the antibodies produced is further enhanced by an affinity maturation process that depends on the presentation of the antigen to maturing B cells by a network of follicular dendritic cells in the germinal center (Abbas et al. 2000b). The B cells expressing high-affinity antibody to the antigen presented to them bind tightly to it and receive signals that allow them to survive and develop into either memory cells or antibody-producing plasma cells (Figure 7.11). Those B cells producing low-affinity antibody fail to make this tight connection and are removed through apoptosis (Abbas et al. 2000b).

The antigens that drive the production of antibodies in the lungs of cigarette smokers in either the early or late stages of COPD are poorly understood. The marked increase in the adaptive immune response that occurs in the later stages of the disease has been attributed to antigens introduced by colonization and infection of the lung with microorganisms (Sethi et al. 2002; Hogg et al. 2004; Murphy et al. 2005) and to autoantigens arising from within damaged lung tissue (Agustí et al. 2003; Voelkel and Taraseviciene-Stewart 2005).

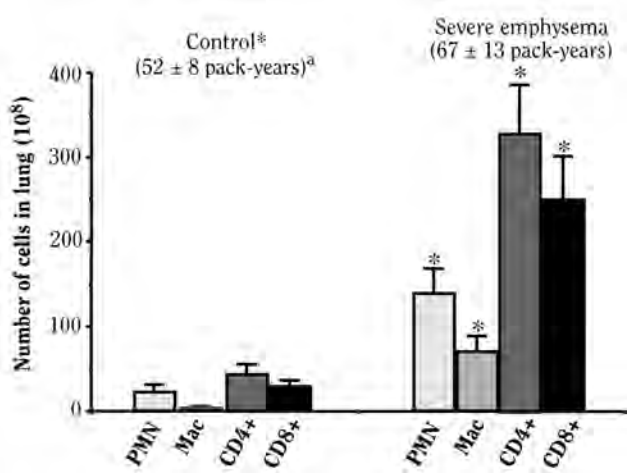
The persistent innate and adaptive immune inflammatory response described here is present in the lungs of all long-term smokers and appears to be amplified in those smokers who develop severe COPD (Figure 7.12) (Keatings et al.1996; Retamales et al. 2001; Hogg et al. 2004). Hogg and colleagues (2004), who examined predictors of FEV_1 obtained from quantitative analysis of lung specimens, measured inflammation, as well as the amount of tissue remodeling of airway walls. The various tissue indicators were compared across strata of GOLD stages for COPD. The extent of the immune response increased from the least to the most severe stage, although the total accumulated volume of cells increased only for B cells and CD8+ cells. In a multivariate analysis, these investigators found that the index of the remodeling of the wall tissue of small airways had the strongest association with the level of FEV_1, greater than the association with infiltration of the tissue by inflammatory cells (Hogg et al. 2004). Ongoing research should provide greater insight into the roles of innate and adaptive immune responses (Curtis et al. 2007).

Tissue Remodeling

Tissue remodeling in general is an intrinsic property of the wound-healing process most carefully studied in tissue damaged by an isolated injury (Clark 1996; Kumar et al. 2005). Observations of changes in small airways in lungs that represent the full range of COPD severity indicate the importance of a repair or remodeling process that thickens small airway walls (Hogg 2004). This type of injury initiates an acute inflammatory response lasting about three days (Figure 7.13). The increase in microvascular permeability that is part of this inflammatory process allows large molecules, such as fibrinogen, to leak from the vessels and initiate the formation of primitive granulation tissue. This tissue is subsequently organized by the processes of angiogenesis and fibrogenesis, which lead to the formation of a mature scar (Kumar et al. 2005). Studies of the details of these processes in the lungs of smokers have been reported (Hogg 2004).

Angiogenesis is the formation of new blood vessels within the granulation tissue by both budding from existing vessels at the edge of the wound and deposition of angioblasts derived from bone marrow, such as endothelial progenitor cells (EPCs) in the provisional matrix (Rafii et al. 2002; Reyes et al. 2002; Hill et al. 2003; Kubo and Alitalo 2003). Studies by Conway and associates (2001) and

Figure 7.12 Persistent innate and adaptive immune inflammatory response in alveolar tissue

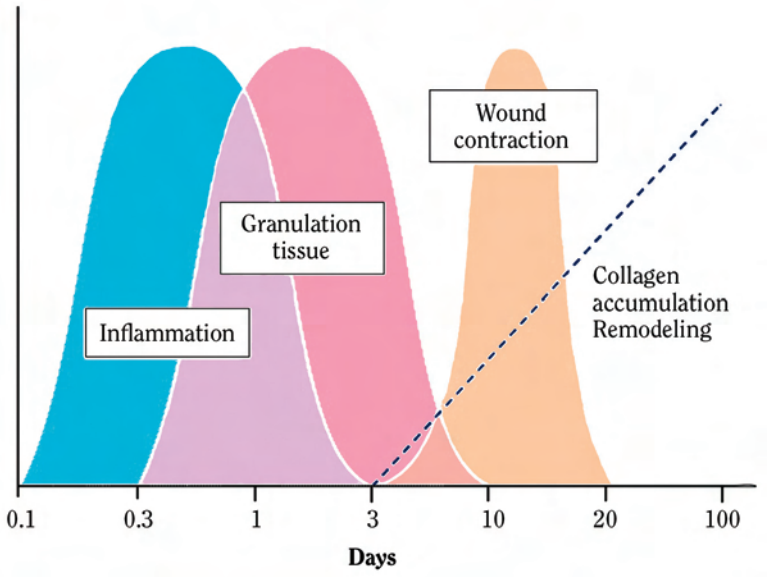


Source: Data from Table 3 in Retamales et al. 2001.
Note: Adaptive immune inflammatory response is present in the lungs of long-term smokers with normal lung function, is amplified in smokers who develop severe chronic obstructive pulmonary disease, and persists many years after smoking cessation. **CD4+** = CD4-positive T cells; **CD8+** = CD8-positive T cells; **Mac** = macrophages; **PMN** = polymorphonuclear neutrophils.
[a]Pack-years = the number of years of smoking multiplied by the number of packs of cigarettes smoked per day.
*$p < 0.05$.

Kumar and colleagues (2005) laid a foundation for understanding the process of angiogenesis. Vascular endothelial growth factor (VEGF) and one of its receptors (VEGFR-2) enhance vascular permeability, encourage the proliferation of EPCs in the bone marrow and at the injury site, and control differentiation of EPCs in the granulation tissue as they form the fragile endothelial tubes. These early vascular structures are stabilized by the interaction of angiopoietin 1 with tyrosine kinase receptors on endothelial cells. Platelet-derived growth factor and TGFβ control the recruitment of smooth muscle to their outer surface and enhance production of the extracellular matrix that stabilizes these newly formed vessels. The migration of endothelial cells formed from EPCs is controlled by integrins, especially $\alpha_v\beta_3$ and matricellular proteins. These integrins, which participate in angiogenesis, include tenascin-C and thrombospondin, a secreted acidic protein rich in cysteine (Conway et al. 2001; Kumar et al. 2005).

The fibrogenic process is initiated by the activation of resting interstitial fibroblasts that migrate into the primitive granulation tissue (Kumar et al. 2005). These resting fibroblasts have a stellate shape with octopus-like projections that form a network connecting the epithelial to the endothelial boundaries of the interstitial compartment (Walker et al. 1995; Behzad et al. 1996; Burns et al. 2003). The fibroblasts' projections send small, short extensions through tiny preformed holes in both the endothelial and epithelial basement membranes. The investigators also used three-dimensional reconstructions of serial electron micrographs of the interstitial space of the alveolar wall to demonstrate that the migrating inflammatory immune cells use both the preformed holes in the basement membrane and the surface of the fibroblast to navigate through the interstitial space. They found (Figure 7.14) that by seeking corners where three endothelial cells meet, the migrating inflammatory cells exit the microvessels in the alveolar wall without disrupting the tight junctions. After exiting, the migrating cells come into contact with the endothelial basement membrane and follow its surface until they contact one of the preformed holes that normally accommodate a fibroblast extension; the cells then crawl through the holes to enter the interstitial space. There, they contact the surface of a fibroblast that guides their movement through the interstitial compartment to bring them to the preformed holes in the epithelial basement membrane, where they exit. The cells then seek the junctions between alveolar type 1 and 2 epithelial cells to reach the alveolar surface (Walker et al. 1995; Burns et al. 2003). Pathways that are similar but not as well studied are used by migrating inflammatory cells to move

Figure 7.13 Remodeling process after a single clean surgical wound



Source: Kumar et al. 2005. Adapted from Clark 1996 with permission from Springer Science and Business Media, © 1996.
Note: A clean surgical wound initiates an acute inflammatory response that lasts for about 3 days and is associated with an increase in microvascular permeability that allows large molecules such as fibrinogen to leak from the vessels and participate in the formation of primitive granulation tissue. This tissue is subsequently organized by the processes of angiogenesis and fibrogenesis leading to the formation of a mature scar. A major difference between this process and that observed in the lung tissue of tobacco smokers is persistent stimulation of the tissue by tobacco smoke as it heals, resulting in a persistent inflammatory immune response.

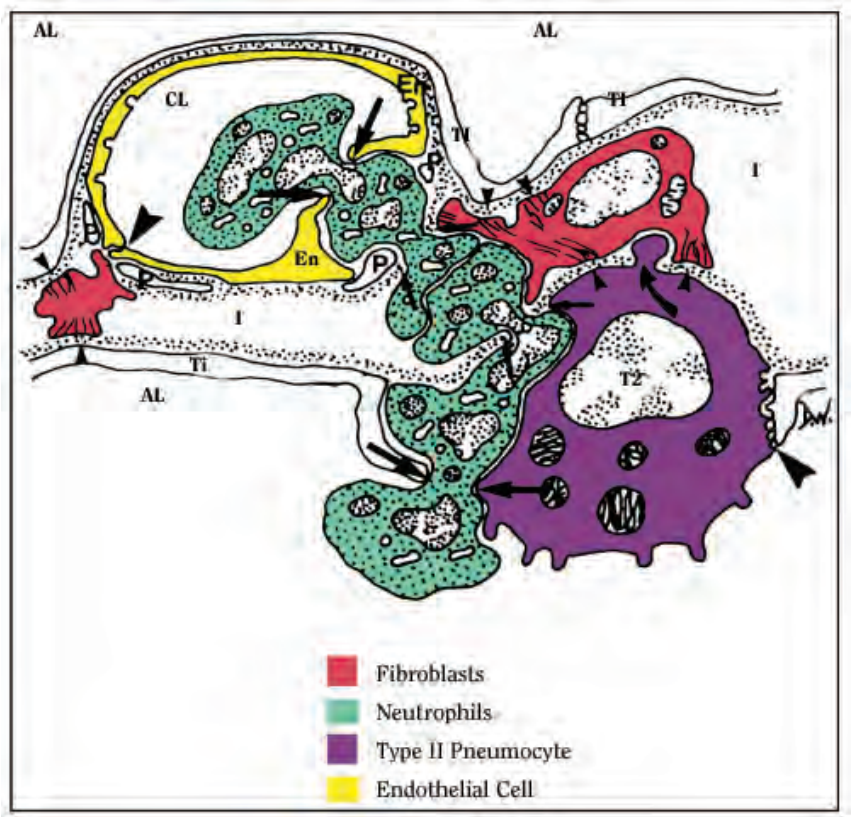
from the bronchial microvasculature to the conducting airways' surface.

Activation of the resting fibroblasts at the edge of a wound starts their migration into the primitive granulation tissue to initiate fibrogenesis (Cross and Mustoe 2003; Werner and Grose 2003). This process begins with differentiation of the fibroblasts into proto-myofibroblasts that contain bundles of microfilaments termed stress fibers and these proto-myofibroblasts then mature into myofibroblasts containing both stress fibers and α-SM actin (Kumar et al. 2005). The myofibroblasts generate contractile force within the granulation tissue in response to agonists such as endothelin; the increase in force they generate correlates with the level of expression of α-SM actin (Tomasek et al. 2002; Cross and Mustoe 2003; Kumar et al. 2005). Together with the reorganization of the extracellular matrix secreted by these cells, the forces generated by the myofibroblast reduce the size of the damaged tissue. Other reports indicate presence in the lung of myofibroblast precursors with a mesenchymal stem cell phenotype that has potential for differentiation along different pathways and for direction of specific types of tissue repair (Sabatini et al. 2005).

The inflammatory immune cells infiltrating the damaged tissue disappear within a few days of an uncomplicated single wound (Figure 7.13) but persist in the face of the relentless tissue damage caused by sustained smoking (Kumar et al. 2005). This persistent infiltration of inflammatory cells is associated with deregulation of the process of repair and remodeling that leads to the formation of a healthy scar. In a healthy scar, the balance between cellular and matrix synthesis and degradation controls the deposition of collagen that forms the scar (fibrosis). Synthesis is regulated by a wide variety of cytokines and growth factors, and degradation is controlled by the secretion and activation of proteolytic enzymes, including both matrix metalloproteinases (MMPs) and serine proteases. These processes are deregulated by the persistent injury that occurs in numerous chronic diseases, including diseases of the joint tissues such as rheumatoid arthritis, of the liver (hepatic cirrhosis), and of the lungs (pulmonary fibrosis). Deregulated healing may also underlie the pathogenesis of the lesions that develop in the lungs of smokers with COPD.

The more specific aspects of lung remodeling are a focus of research. Lung remodeling is central in the

Figure 7.14 Diagram based on three-dimensional reconstructions of serial electron micrographs illustrating how inflammatory immune cells navigate through interstitial space of alveolar wall



Source: Walker et al. 2005. Reprinted with permission from Elsevier, © 2005.
Note: Migrating inflammatory cells exit the microvessels in the alveolar wall without disrupting the tight junctions by seeking corners where 3 endothelial cells meet (arrows). They contact the endothelial basement membrane and follow it to preformed holes that allow them to enter the interstitial space and contact the surface of a fibroblast. They use the fibroblast surface to guide their movement across the interstitial compartment to the epithelial basement membrane where they find similar preformed holes and enter the epithelial compartment. They exit this compartment between the type 1 and type 2 alveolar epithelial cells. **AL** = alveolar airspace; **CL** = capillary lumen; **En** = endothelium; **F** = fibroblast; **I** = interstitial space; **P** = pericytes; **T1** = type 1; **T2** = type 2.

process that leads to airway fibrosis and narrowing in small airway obstruction, emphysema, and pulmonary hypertension (Postma and Timens 2006). Both the innate and adaptive immune responses are involved in these processes (Hogg 2008). Inflammation caused by smoking is central in driving these processes, but the heterogeneity of phenotypes among persons with COPD remains unexplained (Kim et al. 2008).

Summary

The healing of wounds inflicted by stimuli that persist as the healing takes place provides a model to study the pathogenesis of a wide variety of chronic inflammatory lesions. This model provides useful insights into the pathogenesis of the lesions found in the lungs of long-term smokers because the damage to lung tissue induced by the smoking habit must heal in the presence of a chronic stimulus. As a result, the normal tissue remodeling process essential to repair lung tissue damaged by inhaled smoke takes place in the presence of a chronic immune inflammatory process. Evidence is growing that this chronic process deregulates the normal healing process in which the deposition of collagen to form a mature scar is determined by a combination of both deposition and degradation of collagen. Deregulation of the chemokines, cytokines, and growth factors that determine collagen

deposition and the MMPs and serine proteases that control its degradation could account for both the thickening of the airway walls and the emphysematous destruction of the peripheral lung in COPD. An important feature of this hypothesis is that the application of what is known about the healing of chronic wounds to the pathogenesis of the pulmonary problems associated with smoking tobacco might lead to a better understanding of pathogenesis and new and better insights into targets for the development of new treatments for COPD.

Oxidative Stress

Rahman and MacNee (1998) elucidated the mechanisms by which oxidative stress is considered to play a central role in the lung injury caused by inhaling tobacco smoke. The lungs are directly exposed to the oxygen in inhaled air, and because the respiratory tract has direct contact with the environment through the large volume of inhaled air, it is subject to oxidative injury from inhaled oxidants generated exogenously. These exogenous oxidants come from cigarette smoke, ozone, nitrogen oxides, sulfur oxides, and other airborne pollutants. Endogenous oxidants are also generated from phagocytes and other lung cells. Consequently, the lungs have evolved an efficient antioxidant system to protect the airways and alveoli against both exogenous and endogenous oxidants. The lungs are protected against oxidative challenges by well-developed enzymatic and nonenzymatic antioxidant systems. If the balance between oxidant and antioxidant shifts unfavorably because of either an excess of oxidants or a depletion of antioxidants, oxidative stress occurs. Oxidative stress not only produces direct injurious effects in the lungs but also activates molecular mechanisms that initiate lung inflammation.

Generation of Reactive Oxygen Species

Oxygen, which constitutes 21 percent of the air inhaled, is a key element in the oxidation of organic compounds, the process by which mammalian cells produce the energy needed to sustain life (Davies 1995). One-ninth of all inhaled oxygen undergoes tetravalent reduction to produce H_2O in a reaction catalyzed by cytochrome oxidase in the mitochondrial electron-transport chain. Oxygen is also reduced in a nonenzymatic pathway in four reductions of single electrons:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \text{ (Davies 1995)} \qquad (1)$$

The terminal electron acceptor in the respiratory chain is cytochrome oxidase, which must donate its reducing equivalents to oxygen to sustain electron transport for the production of adenosine triphosphate. The sequential tetravalent reduction of oxygen by the mitochondrial electron-transport chain is the process of aerobic energy production, but it can lead to the production of ROS (Davies 1995).

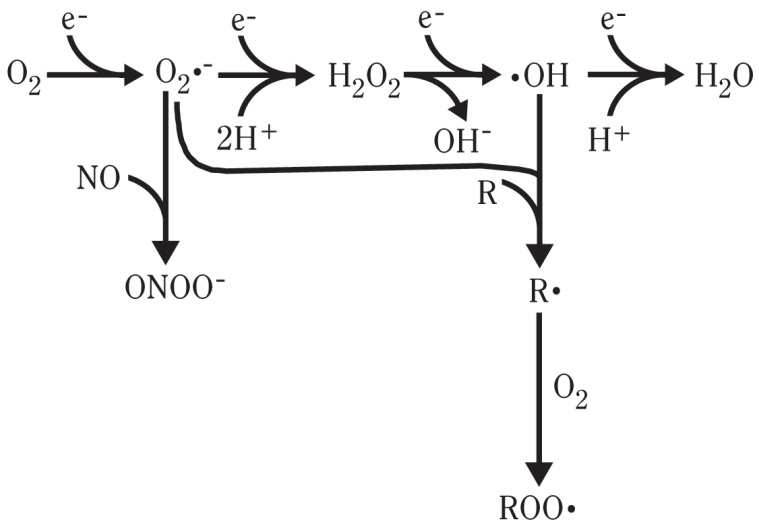
Free radicals are molecules with at least one unpaired electron (Davies 1995). The superoxide anion ($O_2\bullet^-$), the hydroxyl radical ($\bullet OH$), and nitric oxide (NO) are examples of free radicals, whereas hydrogen peroxide (H_2O_2) is not a free radical, because all of its electrons are paired. Together, the free radicals are termed ROS. The addition of one electron to oxygen produces $O_2\bullet^-$; adding a second electron leads to formation of H_2O_2, and a third electron results in the formation of $\bullet OH$ (Figure 7.15). Addition of a fourth electron to oxygen results in its full reduction to H_2O.

The mitochondria are a major intracellular locus for the generation of $O_2\bullet^-$ (Davies 1995). A further source for generating $O_2\bullet^-$ is the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymatic system (Davies 1995; Conner and Grisham 1996). $O_2\bullet^-$ is also generated by other mechanisms, including xanthine, sulfite, and aldehyde oxidases and metabolism of arachidonic acid. This anion, which is relatively unstable, has a half-life of milliseconds. Because of its charge, $O_2\bullet^-$ does not cross cell membranes easily, but it will react with proteins that contain transition metal groups, such as heme moieties or clusters of iron sulfur. These reactions may result in damage to amino acids or loss of protein or enzyme function. The majority of $O_2\bullet^-$ generated in vivo undergoes reactions that are nonenzymatic or are catalyzed by superoxide dismutase (SOD) and produce H_2O_2.

H_2O_2 is also produced directly by several oxidase enzymes, including xanthine oxidase, monoamine oxidase, and amino acid oxidase (Davies 1995):

$$O_2\bullet^- + O_2\bullet^- + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (2)$$

Figure 7.15 Formation of reactive oxygen species



Source: Bowler et al. 2004. Reprinted with permission from Taylor & Francis Group, © 2004. http://www.informaworld.com.
Note: Sequential reduction of oxygen by the addition of electrons (e^-) results in the formation of reactive oxygen species: superoxide anion ($O_2•^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH). $O_2•^-$ can combine with nitric oxide (NO) to form peroxynitrite ($ONOO^-$). $O_2•^-$ and the hydroxide radical OH^- can initiate lipid peroxidation to form lipid peroxides (ROO•) that propagate free radical chain reactions. R and R represent lipid substituent groups. H^+ is a cationic hydrogen, and $2H^+$ represents 2 cationic hydrogens.

H_2O_2 can undergo oxidation by eosinophil-specific peroxidase (EPO) and neutrophil-specific myeloperoxidase (MPO), a reaction that uses halides (X^-) as a cosubstrate to form hypohalous acids (HOX), which are potent oxidants, and other reactive halogenating species:

$$H_2O_2 + X^- + H^+ \rightarrow HOX + H_2O \quad (3)$$

where X = bromide and chloride (Davies 1995).

In a series of reactions catalyzed by transition metal irons, $O_2•^-$ and H_2O_2 react in vivo to produce •OH (Halliwell and Gutteridge 1990). One such reaction is the iron-catalyzed Haber-Weiss reaction in which the ferric ion (Fe^{3+}) is reduced to the ferrous ion (Fe^{2+}). The Fenton reaction follows, as Fe^{2+} catalyzes the transformation of H_2O_2 into •OH:

$$O_2•^- + Fe^{3+} \rightarrow Fe^{2+} + O_2$$
$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + •OH \quad (4)$$

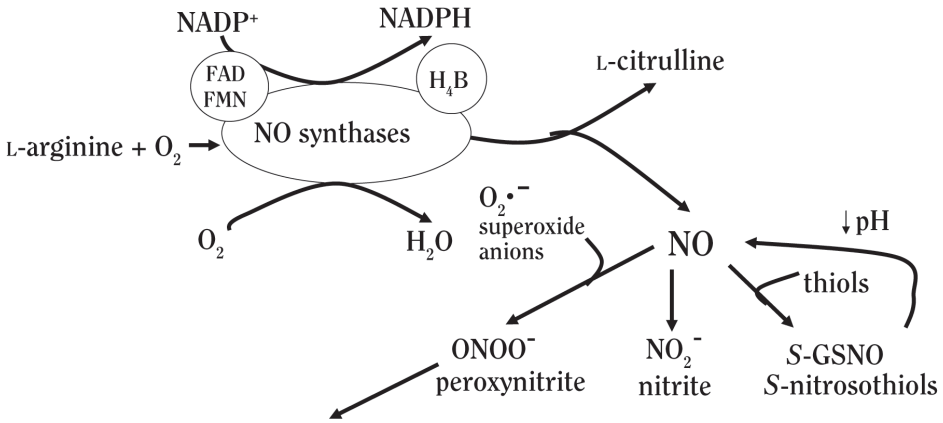
•OH can also be formed in vivo by reactions involving MPO and EPO (Halliwell and Gutteridge 1990). In conditions with physiological concentrations of halides, MPO produces hypochlorous acid and EPO produces hypobromous acid. Hypochlorous acid can generate •OH after reacting with $O_2•^-$:

$$O_2•^- + HOX \rightarrow •OH + X^+ + O_2 \quad (5)$$

•OH is the most reactive of all the radicals produced, reacting immediately with organic molecules at its site of production (Halliwell and Gutteridge 1995).

NO, which is produced endogenously throughout the human body, has a variety of roles. NO is produced from its amino acid substrate L-arginine by the reaction of NO synthases (NOSs) (Figure 7.16). Several forms of NOS have been characterized (Lowenstein and Snyder 1992) and are classified as either constitutive or inducible (Nathan and Xie 1994; Wink et al. 1996). The constitutive forms (NOS I and III) are cytosolic and were originally described and cloned from neuronal and endothelial cells, respectively (Nathan and Xie 1994). They are dependent on calcium and calmodulin and release relatively small amounts of NO for short periods in response to receptor and physical stimulation. The inducible form of NOS (NOS II) is independent of the calcium ion, and it generates NO in large amounts for long periods (Wink et al. 1996). NO contains an odd number of electrons and is therefore a radical and highly reactive in nature.

Figure 7.16 Synthesis of nitric oxide (NO) and related products



Note: **FAD** = flavin adenine dinucleotide; **FMN** = flavin mononucleotide; **H_4B** = tetrahydrobiopterin; **H_2O** = water; **NADP+** = glutamate dehydrogenase; **NADPH** = reduced nicotinamide adenine dinucleotide phosphate; **O_2** = oxygen; ***S*-GSNO** = *S*-nitrocysteine.

The reaction of NO with O_2 results in the formation of nitrite (NO_2^-) (Beckman and Koppenol 1996). Physiological concentrations of NO and O_2 may be too low for this reaction, but this result may have little importance in vivo. NO_2^- is also a substrate for MPO and EPO, which catalyze peroxidase-mediated oxidation and chlorination of biologic targets (Weiss et al. 1986). Peroxidase-catalyzed oxidation of NO_2^- results in the formation of a nitrogen dioxide radical (NO_2•). NO_2^- is a major end product of NO that does not accumulate in vivo, because it is rapidly oxidized to nitrate (NO_3^-) (Wink et al. 1996). NO also reacts rapidly with free radicals to form reactive nitrogen species (RNS) (Parks et al. 1981; Singh and Evans 1997). One such reaction is that of NO with O_2•$^-$ to form the potent oxidant peroxynitrite ($ONOO^-$). $ONOO^-$ is relatively stable, but it can be protonated to yield peroxynitrous acid (ONOOH), which then rapidly decomposes to NO_3^- (Conner and Grisham 1996). ONOOH is highly reactive, unstable, and capable of both oxidizing and nitrating reactions. The amino acid tyrosine is particularly susceptible to nitration with the formation of free or protein-associated 3-nitrotyrosine, which has been used as a marker for the generation of RNS in vivo (Ramezanian et al. 1996; van der Vliet et al. 1999).

NO also reacts with compounds containing thiol groups, resulting in the formation of *S*-nitrosothiols (SNOs). This reaction is considered to be the mechanism by which NO groups are transported and targeted to specific effector sites acting as signaling molecules (Patel et al. 1999). SNOs such as *S*-nitroso-L-glutathione may inhibit enzymes that respond to oxidative stress, such as glutathione peroxidase (GPX), glutathione reductase (GRX), glutathione-*S*-transferase (GST), and glutamate cysteine ligase (GCL) (Clark and Debnam 1988; Becker et al. 1995; Han et al. 1996).

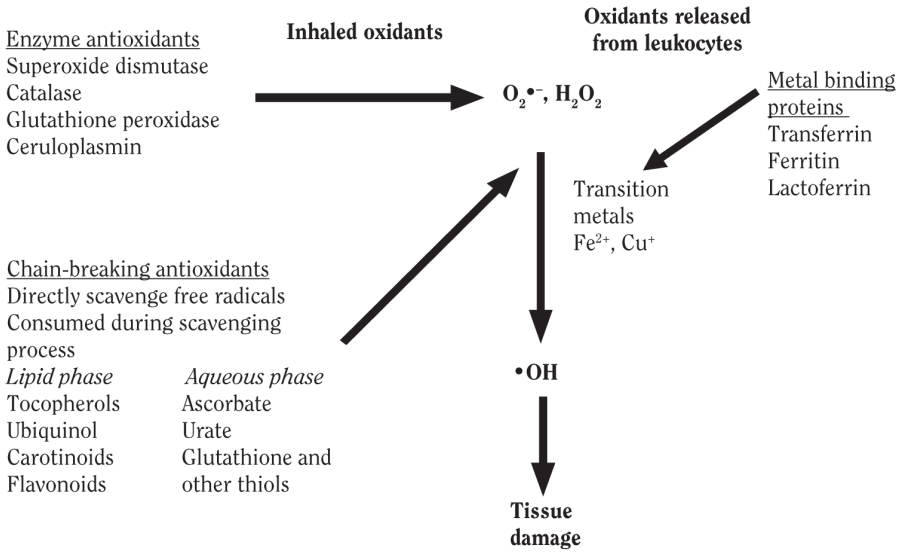
Antioxidants in Lungs

Although ROS and RNS have physiological functions, they also have the potential to cause tissue injury. The balance between these physiological functions and the potential to cause injury or damage is determined by their relative rates of formation and removal (Gutteridge 1994). Normally, ROS and RNS are removed rapidly, before they produce cellular dysfunction and eventually cell death. An antioxidant is defined as a substrate that, when present at lower concentrations than those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate. Antioxidants can be classified as either enzymatic or nonenzymatic (Figure 7.17). The enzyme antioxidants include SOD and catalase (Kinnula and Crapo 2003), the glutathione (GSH) redox system (Rahman and MacNee 2000), and the thioredoxin system (Arnér and Holmgren 2000).

Enzyme Antioxidants

The SOD family of enzymes is made up of ubiquitous antioxidant enzymes that catalyze the dismutation of O_2•$^-$ into H_2O_2 and oxygen. Three SOD enzymes have been identified in mammals: manganese SOD (MNSOD),

Figure 7.17 Oxidant and antioxidant systems in the lungs



Note: **Cu^+** = copper ion; **Fe^{2+}** = iron ion; **H_2O_2** = hydrogen peroxide; **$O_2\bullet^-$** = superoxide anion; **•OH** = hydroxyl radical.

copper zinc SOD (CUZNSOD), and extracellular SOD (ECSOD) (McCord and Fridovich 1970; Marklund 1984; Oury et al. 1996). All three enzymes are expressed widely in human lungs (Kinnula and Crapo 2003). CUZNSOD, the major intracellular SOD, is present in both the cytosol and the nucleus of lysosomes (Slot et al. 1986) of human lungs. It is highly expressed in bronchial epithelium and is the most abundant SOD in the lungs (Lakari et al. 1998). MNSOD, which is localized in the mitochondria, is highly expressed in alveolar macrophages and type II alveolar epithelial cells in human lungs (Lakari et al. 1998, 2000).

ECSOD, the major extracellular SOD, is localized to the extracellular matrix and is particularly abundant in the blood vessels of the lungs, but it has also been found in bronchial and alveolar epithelium and in alveolar macrophages (Kinnula and Crapo 2003). CUZNSOD and MNSOD are generally considered to be the major scavengers of $O_2\bullet^-$. ECSOD is present at relatively high concentrations in the lungs, and its localization to the extracellular matrix suggests that it may provide an important protective mechanism in the lung matrix. Marklund (1984) showed that concentrations of ECSOD in the lungs were 2 to 10 times higher than those in other solid organs. SODs can also be induced by cytokines and oxidants (Kinnula and Crapo 2003).

Catalase is a tetrameric hemoprotein that undergoes oxidation and reduction at its active site in the presence of H_2O_2 (Chance et al. 1979). Accordingly, it has reductive activity for small molecules, such as H_2O_2 and methyl or ethyl hydroperoxide (Pietarinen et al. 1995; Carter et al. 2004). Catalase does not metabolize the peroxides with larger molecules, such as hydroperoxide products of lipid peroxidation. It is expressed intracellularly, mainly in alveolar macrophages and neutrophils.

The most important and abundant intracellular thiol antioxidant, GSH, has a critical function in maintaining the redox status within cells, and it is involved in the detoxification of compounds by conjugation reactions through GST (Meister and Anderson 1983). The enzymes associated with reduced GSH metabolism include GPXs, GCL, and GSH synthase.

The GSH peroxidases are a selenium-containing family of enzymes that play a central role in reducing H_2O_2, but they can also reduce lipid peroxides. There are five *GPX* gene products, one of which (GPX3) can be detected in the ELF of the human lung (Comhair et al. 2001). GPX requires GSH to serve as an electron donor. The oxidized GSH that results from this reaction (oxidized glutathione [GSSG]) is subsequently reduced back to GSH by GRX, a reaction generated by NADPH from the hexose monophosphate shunt as an electron donor (Meister and Anderson 1983; Deneke and Fanburg 1989).

In healthy nonstressed cells, the intracellular ratio of GSH to GSSG is high, which ensures the availability

of GSH and thereby promotes active reduction of H_2O_2 through the GSH system (Doelman and Bast 1990; Bast et al. 1991). GSH can also function as a water-soluble antioxidant interacting directly with reactive oxygen intermediates in nonenzymatic catalyzed reactions. Scavenging of $O_2\bullet^-$ by GSH leads to the formation of thiol radicals (GS•) and H_2O_2. Thus, a substance that is generally thought of as an antioxidant may possess pro-oxidant activity under certain conditions.

GSH is synthesized by GCL and GS (Slot et al. 1986; Soini et al. 2001). The rate-limiting enzyme in GSH synthesis is GCL, which therefore plays a fundamental role in the regulation of GSH homeostasis in the lungs. GCL is a heterodimer with two subunits: a catalytic active heavy subunit and a light subunit that regulates the affinity of the heavy subunit for substrates and inhibitors. Both subunits of GCL are localized in the cytosol of cells and are particularly expressed in human bronchial epithelium and to a lesser extent in alveolar macrophages.

The fluid lining the alveolar epithelium has particularly high GSH levels. Cantin and colleagues (1987) estimated GSH levels in ELF specimens obtained by BAL. Total GSH levels in ELF, including reduced GSH and oxidized GSSG, were 140 times higher than those in plasma. Most of the GSH was present in the reduced form. GSH levels were higher for smokers than for nonsmokers.

The thioredoxin (TRX) system consists of the thioredoxin proteins TRX-1 and TRX-2; thioredoxin-like proteins (e.g., TLX-1 and 2; SPTRX-1 and 2); thioredoxin reductases (e.g., TRXR-1 and 2); peroxiredoxins (PRXs, thioredoxin peroxidases); and glutaredoxins (Rhee et al. 1999; Holmgren 2000; Powis et al. 2000; Gromer et al. 2004). These enzymes are important in reducing protein disulfides and may have additional antioxidant properties. They protect cells against high oxygen tensions and participate in the proliferation and survival of cells. TRX and TRXR are expressed in bronchial and alveolar epithelium and macrophages (Tiitto et al. 2003). Human lung expresses PRXs in bronchial epithelium, alveolar epithelium, and macrophages (Kinnula et al. 2002).

Nonenzymatic Antioxidants

Nonenzymatic antioxidant compounds may act directly with oxidizing agents and are therefore said to be "scavengers." Vitamin E (α-tocopherol) is a membrane-bound antioxidant that terminates the chain reaction of lipid peroxidases by scavenging lipid peroxyl radicals (LOO•) (Bast et al. 1991; van Acker et al. 1993; Davies 1995), thus producing the vitamin E radical, which is much less reactive than LOO•. At high concentrations, however, the radical form of vitamin E may be pro-oxidant (Bast et al. 1991). Vitamin C can also directly scavenge $O_2\bullet^-$ and •OH to form a semidehydroascorbate free radical subsequently reduced by GSH (McCay 1985). Vitamin C is not considered a major antioxidant because it also has peroxide properties. Whether the pro-oxidant or antioxidant properties of vitamin C predominate in a particular tissue is determined by the available iron stores; iron overload favors excess generation of oxidants (Rowley and Halliwell 1983; Bast et al. 1991).

Other nonenzymatic antioxidants include beta-carotene, which scavenges $O_2\bullet^-$ and peroxyl radicals, and uric acid, which scavenges •OH, $O_2\bullet^-$, and peroxyl radicals. In addition, glucose can scavenge •OH, bilirubin scavenges LOO•, taurine quenches hypochlorous acid, albumin binds transition metals, and cysteine and cysteamine donate sulfhydryl groups (Bast et al. 1991).

Mucin is a glycoprotein with a core rich in serine and threonine to which sulfhydryls are attached. The antioxidant properties of mucus are derived from the abundance of sulfhydryl moieties in its structure (Gum 1992), which actively scavenge oxidants such as •OH (Cross et al. 1984, 1997). Alveolar ELF contains high concentrations of GSH (100-fold higher than in plasma), 90 percent of which is in the reduced form (Cantin et al. 1987). ELF also contains catalase, SOD, and GPX (Cantin and Crystal 1985). Other antioxidants in ELF include ceruloplasmin, transferrin, ascorbate, vitamin E, ferritin, albumin, and small molecules such as bilirubin (Heffner and Repine 1989).

The GSTs and multidrug-resistance proteins (MRPs) are a group of detoxifying enzymes that require intracellular GSH for catalytic activity. Researchers have identified three mammalian GST families (cytosolic, mitochondrial, and microsomal) (Hayes et al. 2005) and nine related MRPs (Kruh and Belinsky 2003). Both of these classes of detoxification enzymes are expressed in healthy lungs, predominantly in the airways (Anttila et al. 1993). They function to protect cells against oxidant-generating compounds, drugs, and other end products of oxidative metabolism.

γ-glutamyltranspeptidase (γGT), an enzyme in plasma membrane is expressed in lung epithelial cells and induced by oxidative stress (Kugelman et al. 1994). GSH is not freely diffusible into cells because it must first be broken down into its amino acids. γGT breaks down the γ-glutamyl bond of GSH. Heme oxygenase-1 is a stress response protein with important functions in cell protection and homeostasis; this enzyme can also be induced by oxidants and cytokines (Choi and Alam 1996).

Oxidants and Cigarette Smoke

Cigarette smoke is a complex mixture of more than 4,700 chemical compounds, including free radicals and other oxidants at high concentrations (Church and Pryor 1985; Pryor and Stone 1993). Among the reported consequences of oxidants in cigarette smoke are direct damage to lipids, nucleic acids, and proteins; depletion of antioxidants; and enhancement of the respiratory burst in phagocytic cells (Bowler et al. 2004; MacNee 2005a). Inactivation of proteases and enhancement of molecular mechanisms involved in the expression of proinflammatory mediator genes are other oxidant-induced effects.

Cigarette smoke is often separated into two phases (tar and gas), which both contain free radicals. The gas phase is less stable and contains approximately 10^{15} radicals per puff; and the more stable tar phase has been estimated to contain more than 10^{17} free radicals per gram (Zang et al. 1995). Short-lived oxidants, such as $O_2\bullet^-$ and NO, are predominantly found in the gas phase (Pryor and Stone 1993). NO and $O_2\bullet^-$ immediately react to form the highly reactive $ONOO^-$ molecule. NO is present in cigarette smoke at concentrations of 500 to 1,000 parts per million. Free radicals in the tar phase of cigarette smoke, such as the long-lived semiquinone radical ($Q\bullet^-$), are organic and can react with $O_2\bullet^-$ to form •OH and H_2O_2 (Nakayama et al. 1989). $Q\bullet^-$ is an example of a radical in the tar phase of cigarette smoke that can reduce oxygen to produce superoxide, the •OH, and H_2O_2. The aqueous phase of cigarette smoke condensate may undergo redox recycling for a considerable time in ELF of smokers (Nakayama et al. 1989; Zang et al. 1995). The tar phase of cigarette smoke is also an effective metal chelator and can bind iron to produce tar-semiquinone + tar-Fe^{2+}, which can generate H_2O_2 continuously.

Quinone (Q), hydroquinone (QH_2), and $Q\bullet^-$ in the tar phase are present in equilibrium (Pryor and Stone 1993):

$$Q + QH_2 \rightarrow 2H^+ + Q\bullet^- \quad (6)$$

Aqueous extracts of cigarette tar contain $Q\bullet^-$. This radical can reduce oxygen to form $O_2\bullet^-$, which may dismutate to form H_2O_2:

$$Q\bullet^- + O_2 \rightarrow Q + O_2\bullet^- \quad (7)$$

$$2\ O_2\bullet^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad (8)$$

In addition, cigarette tar and lung ELF contain metal ions such as iron. In these circumstances, the Fenton reaction results in the production of •OH. Cigarette smokers deposit up to 20 mg of tar per day (≤1 gram per day) in their lungs per cigarette smoked.

Cell-Derived Oxidants

In smokers, inflammation is a characteristic feature of the lungs and other organs (Saetta et al. 2002; Bowler et al. 2004; Di Stefano et al. 2004). This inflammation generates additional oxidants that contribute to oxidative stress. Alveolar macrophages obtained by BAL from the lungs of smokers are more activated than those obtained from the lungs of nonsmokers (Schaberg et al. 1992). One consequence of this activation is the release of higher levels of ROS, such as $O_2\bullet^-$ and H_2O_2, thereby further increasing the oxidative burden produced directly by inhaling cigarette smoke. Exposure to cigarette smoke in vitro has also been shown to increase the oxidative metabolism of alveolar macrophages (Hoidal et al. 1981). Subpopulations of higher-density alveolar macrophages, which are more common in the lungs of smokers, may be responsible for the increased production of $O_2\bullet^-$ that occurs in the macrophages of smokers (Schaberg et al. 1995).

Lung epithelial cells are another source of ROS. Type II alveolar epithelial cells have been shown to release both H_2O_2 and $O_2\bullet^-$ in quantities similar to the amounts released by alveolar macrophages (Rochelle et al. 1998). ROS released from type II cells are able, in the presence of MPO, to inactivate AAT in vitro (Wallaert et al. 1993).

ROS can also be generated intracellularly from several sources, such as mitochondrial respiration, which is the largest source of free radicals. In the mitochondria, electrons leak from the electron-transport chain onto oxygen to form $O_2\bullet^-$ (Halliwell and Gutteridge 1990). A further significant cytosolic source of superoxide is the enzyme xanthine dehydrogenase, which has been shown to be present at higher levels in cell-free BAL fluid in patients with COPD than in that of healthy persons, in association with increased production of superoxide and uric acid (Pinamonti et al. 1996). A substantial amount of superoxide is also produced by membrane oxidases, such as cytochrome P-450 and the NADPH oxidase system. In addition, NO is generated from arginine by the action of NOS. Depending on the relative amounts of ROS and RNS, particularly superoxide and NO, which are almost always produced simultaneously at sites of inflammation, these species can react together to produce the powerful oxidant $ONOO^-$ (Beckman and Koppenol 1996). The generation of $ONOO^-$ is thought to prolong the action of NO and to be responsible for most of the adverse effects of excess generation of NO.

Assessment of Oxidative Stress

Oxidative stress can be measured by direct measurements of the oxidative burden, indirectly as the responses to oxidative stress, and by examining the effects of oxidative stress on target molecules (Table 7.4). Assessments of the oxidative burden in the air spaces can be derived by measuring H_2O_2 in BAL fluid or in exhaled breath condensate (Dekhuijzen et al. 1996; Nowak et al. 1998). Air space leukocytes obtained by BAL can be assessed ex vivo for the ability to produce ROS. Spin trapping, a technique in which a radical reacts with a more stable molecule, can be used to measure oxidants in biologic systems; spin trapping has shown increased ROS in the BAL fluid from patients with COPD (Pinamonti et al. 1998). NO is produced in the lungs by the catalytic activity of NOS as a marker of inflammation and indirectly as a marker of oxidative stress, and it can be measured in exhaled breath. Among the indirect measures for assessing oxidative stress is an examination of the increased activity of the hemoxygenase system, which is reflected in the carbon monoxide levels in exhaled breath. Assessment of the effects of oxidative stress on target molecules may include measuring the reaction of ROS with lipids, proteins, or nucleic acids to form markers of oxidative stress. For example, ROS attack proteins to form protein carbonyls, $ONOO^-$ reacts with tyrosine to form nitrotyrosine, and ROS react with lipids to liberate ethane and isoprostane and with DNA to form base-paired adducts (e.g., 7-hydroxy-8-oxo-2′-deoxyguanosine) or with GSH to produce oxidized GSH. These markers can be measured in blood, breath condensate, BAL fluid, and lung tissue as an indicator of the effects of free radicals on target molecules.

Table 7.4 Measurements of oxidative stress

Direct measurements of oxidative burden
Hydrogen peroxide in breath condensate or BAL fluid
Reactive oxygen species in BAL fluid and peripheral blood leukocytes
Nitric oxide in exhaled breath
Responses to oxidative stress
Carbon monoxide in breath, reflecting hemoxygenase activity
Antioxidants, antioxidant enzymes in blood, sputum, BAL fluid, and lung tissue
Effects of oxidative stress on target molecules
Oxidized proteins (e.g., carbonyl residues and oxidized and nitrated proteins)
Lipid peroxidation products (e.g., F_2-isoprostanes and 4-hydroxy-2-nonenal)
DNA oxidation products (e.g., 8-hydroxy-2′-deoxyguanosine)
Hydrocarbons in breath condensate, sputum, BAL fluid, blood, urine, and lung tissue

Note: **BAL** = bronchoalveolar lavage.

Evidence of Smoking-Induced Oxidative Stress

In Vitro Studies

Studies have examined the consequences of acute (short-term) exposure to cigarette smoke for a wide range of cells (Table 7.5). Many studies have focused on oxidative stress and have shown an increase in markers of such stress after exposure to whole cigarette smoke or condensate of cigarette smoke. The cell types studied have included alveolar macrophages, type II alveolar epithelial cell lines consisting largely of A549 cells, and neutrophils. For exposure to cigarette smoke, most of the studies have used cigarette smoke extract (CSE) as the exposure agent; a smaller number have used cigarette smoke. The concentrations of CSE and the duration of exposure have differed among studies, and concentrations of CSE from that produced by one cigarette per milliliter to that produced by four cigarettes per milliliter. The exposure times have varied between 1 second and 24 hours. All of the studies have shown that acute exposure to cigarette smoke causes increased oxidative stress.

Exposure of plasma to cigarette smoke in vitro depletes antioxidants, including vitamin C, ubiniquol-10, α-tocopherol, cryptoxanthin, retinol, and beta-carotene and leads to lipid peroxidation (Eiserich et al. 1995; Handelman et al. 1996; Scott et al. 2005). In vivo, smokers are well documented to have lower serum levels of vitamin C and beta-carotene and perhaps α-tocopherol than do nonsmokers (Tribble et al. 1993; Faruque et al. 1995; Adams et al. 1997; Lykkesfeldt et al. 1997; Motoyama et al. 1997; Munro et al. 1997; Alberg 2002; Northrop-Clewes and Thurnham 2007). This relationship between smoking status and reduced vitamin C levels may be dose related (Tribble et al. 1993; Faruque et al. 1995; Marangon et al. 1998). The hypothesis was that reduced levels of vitamin C in smokers are due to the activation of leukocytes and subsequent generation of ROS (Winklhofer-Roob et al. 1997).

Table 7.5 Studies of oxidative stress in smokers

Study	Number of participants by smoking status	Abstinence time	Cigarettes smoked	Time between inhalation and measurement	Effect of smoking
Kharitonov et al. 1995	17 smokers	8 hours	1	5 and 15 minutes	Exhaled air: ENO decreased after 5 minutes (65 ± 6.3 to 44 ± 6.4 ppb); returned to control values within 15 minutes (53 ± 6.3 ppb)
Morrow et al. 1995	10 smokers 10 nonsmokers	10 hours	3	30 minutes	Blood: F_2-isoprostane showed no significant change
Rahman et al. 1996a	12 smokers 14 nonsmokers	Unknown	1	60 minutes	Blood: TEAC decreased; TBARS increased
Chambers et al. 1998	24 smokers	1 hour	1	1 and 10 minutes	Exhaled air: ENO increased
Morrison et al. 1999	14 smokers 7 nonsmokers	12 hours	2	60 minutes	BAL fluid: leukocyte superoxide anions increased; TBARS showed no significant change; TEAC increased; glutathione showed no significant change; oxidized glutathione showed no significant change; neutrophils increased Blood: TEAC decreased; TBARS increased Epithelial permeability increased
Guatura et al. 2000	12 smokers 10 nonsmokers	10 hours	1	30 minutes	Breath condensate: hydrogen peroxide increased
Montuschi et al. 2000	12 smokers	12 hours	2	15 hours	Breath condensate: 8-isoprostane increased after 15 minutes; no significant change after 5 hours
Balint et al. 2001	15 smokers 15 nonsmokers	4 hours	2	30 and 90 minutes	Breath condensate: nitrate + nitrite increased after 30 minutes; no significant change after 90 minutes; nitrite, peroxynitrite, and ENO showed no significant change
Tsuchiya et al. 2002	20 smokers	6 hours	1	5 and 60 minutes	Blood: nitrate, nitrite, ascorbic acid, cysteine, methionine, and uric acid decreased after 5 minutes; no significant change after 30 minutes

Source: Adapted from van der Vaart et al. 2004 with permission from BMJ Publishing Group Ltd., © 2004.
Note: **BAL** = bronchoalveolar lavage; **ENO** = exhaled nitric oxide; **ppb** = parts per billion; **TBARS** = thiobarbituric acid reactive substances; **TEAC** = trolox equivalent antioxidant capacity.

GSSG, the oxidized form of GSH, is released from endothelial cells after 30 minutes of exposure to cigarette smoke (Noronha-Dutra et al. 1993). Although intracellular GSH decreased within 3 hours of exposure to cigarette smoke (Bridgeman et al. 1991; Li et al. 1994; Carnevali et al. 2003), GSH and GCL increased 24 hours after exposure. This finding suggests a protective cellular mechanism against the oxidative stress induced by cigarette smoke (Rahman et al. 1996b). Immediately after exposure to six puffs of cigarette smoke, H_2O_2 and superoxide molecules were detected in the membranes of epithelial cells in the tracheal explant model (Hobson et al. 1991), but this consequence of exposure was prevented by antioxidants. Twenty-four hours after exposure to CSE, NO was released from endothelial cells (Tuder et al. 2000). In contrast, inducible NOS (INOS) expression and release of nitrate from epithelial cells exposed to CSE were decreased (Hoyt et al. 2003). Exposure to cigarette smoke was also

shown to activate the pentose phosphate pathway, which is a source of NADPH for the enzyme GRX in endothelial cells (Noronha-Dutra et al. 1993). The activities of the main enzymes in the GSH redox cycle have been shown to be decreased by the acute exposure of alveolar epithelial cells to cigarette smoke (Rahman et al. 1996a). Cigarette smoke causes depletion of intracellular GSH in cultured airway epithelial cells and transient decreases in GPX and glucose-6-phosphate activities (Rahman et al. 1998). Exposure to cigarette smoke also causes an increase in the expression of GPX (Rahman and MacNee 1999). In vitro studies also suggest that after initial GSH depletion, GSH levels increased, apparently due to GCL induction (Rahman et al. 1996a). Exposure of neutrophils and alveolar macrophages to cigarette smoke produces morphologic changes in the cells that result in cell blebbing, which indicates oxidant-induced damage (Lannan et al. 1994).

Animal Studies

Several studies have assessed the short-term effects of the inhalation of cigarette smoke on markers of oxidative stress in lung tissue, BAL fluid, and blood in animals (Table 7.6). These studies have found increased levels of oxidative stress after such exposure.

GSH, the major thiol antioxidant in the lungs, rapidly and immediately decreases in the lung tissue of rats and other laboratory animals after exposure to cigarette smoke (Cotgreave et al. 1987; Bilimoria and Ecobichon 1992; Ishizaki et al. 1996; Li et al. 1996). GSH levels may return to normal by two to six hours after exposure to smoke (Cotgreave et al. 1987; Bilimoria and Ecobichon 1992) or may remain at levels higher than baseline (Ishizaki et al. 1996). GSSG levels increased 1 hour after exposure to smoke in animal models and decreased at 6 hours after acute exposure, returning to normal levels after 24 hours (Li et al. 1996). Acute exposure to cigarette smoke in rats did not produce any change in the amount of cysteine in the lungs; cysteine is an essential amino acid for the synthesis of GSH (Cotgreave et al. 1987). Other markers of oxidative stress, including 4-hydroxy-2-nonenal (4-HNE) and 8-hydroxy-2′-deoxyguanosine (8-OH-dG), were elevated in lung tissue after acute exposure to cigarette smoke (Ishizaki et al. 1996; Aoshiba et al. 2003a). Furthermore, INOS messenger RNA (mRNA) and endothelial NOS are increased after acute exposure to cigarette smoke (Wright et al. 1999).

ELF is the initial target for oxidative stress, and extracellular GSH levels obtained by BAL in rats were reduced immediately (Cotgreave et al. 1987) and remained at reduced levels six hours after smoke inhalation (Li et al. 1996). Twenty-four hours after acute exposure to cigarette smoke, GSH concentrations return to baseline values (Li et al. 1996). Evidence indicates that short-term cigarette smoking depletes intracellular GSH concentrations (Cotgreave et al. 1987) and increases levels of GSSG (Cavarra et al. 2001b) and 8-OH-dG (Aoshiba et al. 2003a) that are associated with decreased antioxidant capacity in BAL fluid (Cavarra et al. 2001b). Evidence of systemic oxidative stress has been shown in animal models after acute exposure to cigarette smoke, as shown by a decrease in antioxidants (Uotila 1982; Ishizaki et al. 1996). This finding is associated with an increase in products of lipid peroxidation such as 8-epi-prostaglandin$_{2\alpha}$ in blood (Cavarra et al. 2001b).

Consequences of Smoke-Induced Oxidative Stress

Epithelial Injury

Among the first injurious effects of cigarette smoke on the lungs is an increase in epithelial permeability, which has been demonstrated in animal models (Li et al. 1994). In the lung tissue of rats, increased epithelial permeability is associated with a decrease in GSH levels and an increase in GSSG levels. Depletion of GSH in the lungs increased epithelial permeability both in vivo and in vitro with use of cultured epithelial monolayers (Li et al. 1994).

Inflammatory Responses

Oxidative stress has been shown to enhance gene expression of proinflammatory mediators through the redox-sensitive transcription factors nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1). Animal studies have shown enhanced NF-κB nuclear binding after exposure to cigarette smoke, which was associated with increased gene expression and protein release of proinflammatory cytokines (Nishikawa et al. 1999). Furthermore, the molecular mechanisms associated with enhanced inflammatory responses after exposure to cigarette smoke are thought to involve an increase in histone acetylation and decreased histone deacetylase (HDAC) activity, resulting in enhanced histone acetylation, unwinding of chromatin, and hence, enhanced gene expression (Marwick et al. 2004). These effects have been demonstrated in animal models of exposure to cigarette smoke.

Susceptible Animal Models

The role of oxidative stress in the development of lung disease induced by cigarette smoke has been demonstrated in animal models: increasing oxidative stress has led to higher frequency of emphysema induced by cigarette smoke. NRF-2 is a controlling transcription factor

Table 7.6 Studies of oxidative stress in animals exposed to smoke

Study	Animals (number)	Smoke exposure			Route of administration	Effect
		Number of cigarettes	Exposure time	Time between exposure and measurement		
Uotila 1982	Syrian hamsters (NR)	5 12	1 hours 2 hours	20 hours Measurement during exposure	Smoking chamber	Experiment 1[a]—Blood: MUG increased after 20 hours Experiment 2[b]—Blood: MUG increased during smoking
Cotgreave et al. 1987	Sprague-Dawley rats (NR)	8	1 hour	0 hours	Nose only	BAL fluid: intracellular GSH decreased; free GSH decreased; extracellular GSH decreased Blood: GSH showed no significant change; cysteine increased Lung tissue: cysteine showed no significant change
Bilimoria and Ecobichon 1992	Sprague-Dawley rats (8) Hartley guinea pigs (NR)	40, 120, and 240 puffs	NR	0, 3, and 6 hours	Nose only	Lung homogenate: GSH decreased at 0 hours in rats but only at high exposures in guinea pigs; levels returned to preexposure within 3 hours Ascorbic acid: no reduction in either species
Wright et al. 1999	Rats (NR)	7	2 hours	24 hours	Nose only	Lung homogenate CNOS mRNA and protein showed no significant change; INOS mRNA increased, protein showed no significant change; ENOS mRNA increased, protein showed no significant change
Cavarra et al. 2001b	C57BL/6J mice with smoke exposure (35), controls (70)	5	20 minutes	0, 20, and 60 minutes	Smoking chamber	BAL fluid at 0 hours: trolox equivalent antioxidant capacity decreased, levels returned to preexposure within 20 minutes; glutathione disulfide increased; ascorbic acid decreased; protein thiols decreased; neither total glutathione nor vitamin E showed any significant change; 8-epi-$PGF_{2\alpha}$ increased; prevented in a subgroup of case/controls by pretreatment with *N*-acetylcysteine Plasma: 8-epi-$PGF_{2\alpha}$ increased at 0, 20, and 60 minutes; total cell count, alveolar macrophages, polymorphonuclear neutrophils, and lymphocytes showed no significant change; human secretory leukoprotease inhibitor inactivated

Table 7.6 Continued

Study	Animals (number)	Smoke exposure: Number of cigarettes	Smoke exposure: Exposure time	Smoke exposure: Time between exposure and measurement	Route of administration	Effect
Aoshiba et al. 2003a	C57BL/6 mice (6)	10	1 hour	1, 3, 16, and 24 hours	Smoking chamber	Lung tissue: 8-oxo-dG and 4-HNE increased after 1 hour in bronchial epithelial cells and type II alveolar cells; cellularity increased after 1–16 hours BAL fluid: 8-oxo-dG levels increased after 1 hour; no significant change after 24 hours

Source: Adapted from van der Vaart et al. 2004 with permission from BMJ Publishing Group Ltd., © 2004.
Note: **4-HNE** = 4-hydroxy-2-nonenal; **8-epi-PGF$_{2\alpha}$** = 8-epi-prostaglandin F$_{2\alpha}$; **8-oxo-dG** = 7-hydroxy-8-hydroxy-2′-deoxyguanosine; **BAL** = bronchoalveolar lavage; **CNOS** = constitutive nitric oxide synthase; **ENOS** = endothelial nitric oxide synthese; **GSH** = reduced glutathione; **INOS** = inducible nitric oxide synthase; **mRNA** = messenger RNA; **MUG** = methylumbelliferyl glucuronide; **NR** = data not reported.
[a]Lungs were isolated, ventilated with cigarette smoke, and perfused with 4-MUG.
[b]Isolated lungs were simultaneously ventilated with cigarette smoke and perfused with MUG.

for the expression of antioxidant genes. In NRF-2 knockout mice, exposure to smoke produced more evidence of oxidative stress in the lungs, which was associated with an increased inflammatory response and enhanced development of emphysema compared with those in wild-type mice (Foronjy et al. 2006). Furthermore, a transgenic animal that overexpressed SOD showed diminished smoke-induced emphysema. These findings suggest a role for oxidative stress in the development of emphysema (Rangasamy et al. 2004).

Antioxidants have been shown to reduce the effects of oxidative stress after exposure to cigarette smoke. The thiol antioxidants nacystelyn and *N*-acetylcysteine have each been used to reduce the inflammatory responses after exposure to cigarette smoke and also the injurious effects, principally emphysema (Antonicelli et al. 2004; Rubio et al. 2004). After exposure to cigarette smoke, recombinant SOD has been shown to reduce the inflammatory response in several ways: by decreasing the inflammatory response in the lungs, reducing the influx of neutrophils, decreasing IL-8 gene expression and release, and decreasing NF-κB activation (Nishikawa et al. 1999).

Human Studies

Several studies have shown evidence of both local and systemic oxidative stress in humans after acute exposure to cigarette smoke (Table 7.7). Some studies were undertaken in long-term smokers with normal lung function, and some have been performed in smokers who were instructed to refrain from smoking before the acute exposure at intervals between 7 and 24 hours. Reports of other studies have not provided information on abstention, and in some studies, the participants were not instructed to refrain from smoking.

Local Oxidative Stress in Lungs

The acute effects of cigarette smoking on oxidative stress have been assessed with markers in exhaled air, BAL fluid, and blood. Most of these studies have shown an immediate increase in oxidative stress after acute exposure, but some have shown no effect (Table 7.7). Five studies have described the effects of acute exposure to cigarette smoke on markers of oxidative stress in breath condensate or exhaled air. In breath condensate, the lipid peroxidation product 8-isoprostane increased 15 minutes after acute exposure (Montuschi et al. 2000). In addition, lipid peroxides have been shown to increase in exhaled breath 30 minutes after exposure to smoke (Guatura et al. 2000). Furthermore, exhaled NO has been shown to increase 1 and 10 minutes after acute exposure to smoke (Chambers et al. 1998), but in another study it decreased 5 minutes after exposure (Kharitonov et al. 1995). The inconsistency between these studies probably relates to differences in the measurements of exhaled NO and among the groups studied. High levels of exhaled NO have not been observed at time points after exposure (15, 30, and 90 minutes) (Kharitonov et al. 1995; Balint et al. 2001). Nitrate, an end product of NO, increased 30 minutes after acute exposure,

Table 7.7 In vitro studies of oxidative stress

		Smoke exposure				
Study	**Cell types**	**Source**	**Dose (cigarettes/ mL)**	**Exposure time**	**Time between exposure and measurement**	**Effect of smoke exposure**
Powell and Green 1971	Rabbit AMs	CSE	NR	NR	NR	G3PD activity in AMs decreased, effect prevented by cysteine; G6PD and LDH activities not significantly different from controls
Bridgeman et al. 1991	Erythrocytes Neutrophils A549 cell line	CS	1, 3, and 5 puffs	NR	NR	Intracellular GSH decreased, not prevented by reducing agents
Hobson et al. 1991	Rat tracheal explants	CS	1, 3, and 6 puffs	10 minutes	40 minutes	Hydrogen peroxide and $O_2^{\bullet -}$ increased along epithelial cell membranes, prevented by SOD 3 and 6 puffs: cell separation, focal membrane blebbing, and loss of cilia, cell disintegration
Tsuchiya et al. 1992	Rat PMNs	CSE	1	20 minutes	20 minutes	Radical oxidant scavenger production from PMNs decreased, prevented by SOD; oxygen consumption from PMNs increased
Noronha-Dutra et al. 1993	HUVEC	CSE	0.5	30 minutes	30 minutes	Pentose phosphate pathway activated; GSSG release increased
Li et al. 1994	A549 cell line	CSE	1	1–6 hours	1, 4, 6, and 24 hours	Epithelial permeability increased at 1 hour, prevented by GSH, no significant change 24 hours after wash; intracellular GSH decreased, no significant change 24 hours after wash
Rahman et al. 1996b	A549 cell line	CSE	1 puff/3 mL	4, 16, or 28 hours	0 hours	Intracellular CSE GSH increased after 24 hours; GSSG no significant change; γGCS activity increased; γGCS-HS mRNA increased
Pinot et al. 1999	Human peripheral blood monocytes	CSE	0, 0.006, 0.024	Overnight	Directly after	$O_2^{\bullet -}$ production showed no significant change; HSP 70 increased; membrane pseudopodes decreased; submembrane vacuoles increased; surfactant prevented CSE effects
Tuder et al. 2000	Bovine artery endothelial cells Monocytic U937 Hep G2 A549 cell line	CSE	0.1	24 hours	24 hours	VEGF decreased protein and mRNA in all cells except A549 cell line; apoptosis increased bovine artery endothelial cells

Table 7.7 Continued

Study	Cell types	Smoke exposure: Source	Dose (cigarettes/mL)	Exposure time	Time between exposure and measurement	Effect of smoke exposure
Hoyt et al. 2003	LA-4 A549 cell line HBEC	CSE	0.0004–0.00008	4 and 24 hours	24 hours	Nitrate decreased at 4 and 24 hours in all cell types; INOS-positive LA-4 cells decreased at 24 hours; INOS mRNA decreased; ENOS and NNOS mRNA showed no change in LA-4 cells; ENOS in A549 cells showed no significant change
Kayyali et al. 2003	RPMEC	CSE	20 µg/mL	4 and 24 hours	4 and 24 hours	XO activity increased at 4 and 24 hours; mRNA XO increased at 6 hours
Wickenden et al. 2003	A549 cell line HUVEC Jurkat cells	CSE	0.05–0.1	24 hours	24 hours	Necrosis increased, no apoptosis;[a] GSH inhibits necrosis and apoptosis (Jurkat cells); GSH/GSSG decreased intracellularly; inhibition CASPASE-3 activation (Jurkat cells)

Source: Adapted from van der Vaart et al. 2004 with permission from BMJ Publishing Group Ltd., © 2004.
Note: **A549** = human lung adenocarcinoma epithelial cell line; **AMs** = alveolar macrophages; **CS** = cigarette smokers; **CSE** = cigarette smoke extract; **G3PD** = glyceraldehyde 3-phosphate dehydrogenase; **G6PD** = glucose 6-phosphate dehydrogenase; **γGCS** = gamma-glutamylcysteine synthetase; **γGCS-HS** = gamma-glutamylcysteine synthetase heavy subunit; **GSH** = reduced glutathione; **GSSG** = oxidized glutathione; **HBEC** = human bronchial epithelial cells; **Hep G2** = human hepatocellular carcinoma cell line; **HSP 70** = heat shock protein 70; **HUVEC** = human umbilical vein endothelial cells; **INOS** = inducible nitric oxide synthase; **LA-4** = mouse lung epithelial cell line; **LDH** = lactate dehydrogenase; **mL** = milliliter; **µg** = microgram; **mRNA** = messenger RNA; **NNOS** = neuronal nitric oxide synthase; **NR** = data not reported; **$O_2\bullet^-$** = superoxide anion; **PMN** = polymorphonuclear neutrophil; **RPMEC** = rat pulmonary microvascular endothelial cells; **SOD** = superoxide dismutase; **U937** = human leukemic monocyte lymphoma cell line; **VEGF** = vascular endothelial growth factor; **XO** = xanthine oxidase.
[a]Light microscopy, TUNEL (terminal dUTP nick-end labeling) assay, electron microscopy.

but nitrite and nitrotyrosine, which are also products of NO metabolism, did not increase (Balint et al. 2001). In humans, all the oxidative markers of oxidative stress increase within the first hour after acute exposure, and most markers return to normal within 90 minutes (van der Vaart et al. 2004).

Only one study has investigated the effects of smoking on markers of oxidative stress in ELF or BAL fluid. In this study, release of $O_2\bullet^-$ by air space leukocytes increased after exposure to smoke (Morrison et al. 1999). In addition, systemic antioxidant capacity decreased, as measured by the Trolox Equivalent Antioxidant Capacity (Rahman et al. 1996a; Morrison et al. 1999). Surprisingly, however, the antioxidant capacity in BAL fluid increased after exposure to smoke, possibly because all of the participants were long-term smokers and already had a high antioxidant capacity in BAL fluid. After smoking, no differences were observed in levels of reduced or oxidized GSH in leukocytes or in thiobarbituric acid reactive substances (TBARS), as evidenced by measuring lipid peroxidation in BAL fluid or in ELF.

Systemic Oxidative Stress

After just one cigarette has been smoked, nitrite, nitrate, and cysteine decrease in peripheral blood (Tsuchiya et al. 2002). In a study by Hockertz and colleagues (1994), no differences were observed in the production of reactive oxygen intermediates from circulating neutrophils after exposure to smoke, but an earlier study gave conflicting findings (Drost et al. 1992). In contrast to levels in BAL fluid, TBARS in plasma increased after exposure to smoke and antioxidant capacity was decreased when measured within one hour after smoking (Rahman et al. 1996a; Tsuchiya et al. 2002). However, in smokers, levels of the lipid peroxidation product F_2-isoprostane did not change in plasma after exposure to smoke (Morrow et al. 1995), possibly because all participants were

long-term smokers who had already developed high F_2-isoprostane levels.

Epithelial Injury

Increased epithelial permeability, which can be measured by ^{99m}Tc-DTPA lung clearance (Morrison et al. 1998a), has been shown to increase in cigarette smokers one hour after exposure to smoke (Morrison et al. 1999). Another study (Gil et al. 1995), however, showed no difference in epithelial permeability 15 minutes after exposure to cigarette smoke in long-term smokers. Epithelial permeability, measured by radiolabeled urea, decreased after acute exposure to cigarette smoke (Ward et al. 2000), but no differences could be detected when measurements were made by positron emission tomography scanning with use of radiolabeled transferrin (Kaplan et al.1992).

Inflammatory Responses

The numbers of neutrophils in the blood and BAL fluid from long-term smokers are higher than in those from nonsmokers (Hunninghake and Crystal 1983; Kuschner et al. 1996; van Eeden and Hogg 2000). Findings on the effect of short-term cigarette smoking on the number of neutrophils in BAL fluid have been inconsistent. Some studies reported an increase (Morrison et al. 1999), and others reported no change (Janoff et al. 1983b). Exposure to smoke has not been shown to change the number of monocytes or the total number of leukocytes in BAL fluid (Janoff et al. 1983b). However, counts of peripheral blood granulocytes increase after acute exposure to cigarette smoke (Winkel and Statland 1981; Abboud et al. 1986; Hockertz et al. 1994), and counts of peripheral blood eosinophils decrease after such exposure (Winkel and Statland 1981). Acute exposure to cigarette smoke has also been shown to reduce the number of B cells (Hockertz et al. 1994) and the total number of lymphocytes in peripheral blood (Winkel and Statland 1981). In contrast, the number of CDB-positive cells and the ratio of CD4+ to CD8+ cells are not affected by acute exposure to cigarette smoke (Hockertz et al. 1994). In capillary blood, the total number of basophils decreased 10 minutes after the smoking of two cigarettes (Walter and Nancy 1980), and the number of degranulated basophils increased (Walter and Walter 1982).

Neutrophil kinetics in the lungs have been examined after exposure to cigarette smoke by using an assessment of the first pass of radiolabeled neutrophils through the pulmonary circulation. Retention of neutrophils in the lungs increased after acute exposure to cigarette smoke (MacNee et al. 1989). This increased retention was not due to an alteration of pulmonary hemodynamics (Skwarski et al. 1993) but resulted from decreased deformability of leukocytes (Drost et al. 1993) and/or the increased expression of the adhesion molecule L-selectin in blood neutrophils after acute exposure to cigarette smoke (Patiar et al. 2002).

After acute exposure to cigarette smoke, changes in GSH have been studied in human, animal, and in vitro models. The ratio of GSH to GSSG, which reflects oxidative stress, has been shown to decrease after acute exposure in both animal and in vitro studies but not in a single human study (Morrison et al. 1999). This discrepancy may be explained by differences in species and dose of smoke and differences between human BAL fluid and animal lung homogenate.

Exposure to cigarette smoke has been shown to damage fatty acids in cell membranes and thereby result in increased products of lipid peroxidation both in humans, as seen in exhaled air and plasma (Rahman et al. 1996a; Montuschi et al. 2000), and in animals, as seen in BAL fluid and lung tissue (Ishizaki et al. 1996; Aoshiba et al. 2003a).

Summary

The time courses of the changes in markers of oxidative stress after exposure to smoke have been studied in humans and in animal models. In humans, all the oxidative markers of oxidative stress increase within the first hour after acute exposure, and most markers return to normal quickly. In animal models, markers of oxidative stress generally increase during the first 6 hours after exposure to cigarette smoke and return to normal by 24 hours. These findings have been demonstrated in lung tissue, BAL fluid, and blood. In studies with in vitro models, only a few time points have been examined. Initial depletion of GSH after acute exposure to cigarette smoke is followed in most cases by an increase in GSH 24 hours later. This finding suggests a protective mechanism against oxidative stress from smoke that may reflect the increase in GSH seen in long-term cigarette smokers.

Oxidative Stress in Chronic Obstructive Pulmonary Disease

There is considerable evidence, largely indirect, for increased oxidative stress in the lungs of COPD patients. As explained previously, oxidative stress can be measured in several ways, including direct measurements of oxidant burden, indirect measures using response to oxidative stress, and measurements of the effects of oxidative stress on target molecules (see "Assessment of Oxidative Stress" earlier in this chapter). Spin trapping, a technique by which a radical reacts with a more stable molecule, can be used to measure oxidants in biologic systems. The technique of spin trapping has been applied to measure BAL fluid in patients with COPD and has shown increased ROS (Pinamonti et al. 1998).

Numerous studies have shown that markers of oxidative stress are increased in the lungs of COPD patients compared not only with those in healthy persons but also with those in smokers having a similar smoking history who have not developed COPD (MacNee 2000). Patients with COPD have higher levels of H_2O_2 in exhaled breath condensate, a direct measurement of air space oxidative burden, than do former smokers with COPD or nonsmokers (Dekhuijzen et al. 1996; Nowak et al. 1998). Elevated levels of H_2O_2 in the exhaled breath of smokers are thought to derive partly from increased release of $O_2\bullet^-$ by alveolar macrophages (Hoidal et al. 1981).

NO has been used as a marker of airway inflammation and indirectly as a measure of oxidative stress. Increased NO in exhaled breath has been seen in some studies of patients with COPD, but the levels are not as high as those reported in asthma (Maziak et al. 1998; Delen et al. 2000). Other studies have found either normal or even lower-than-normal levels of exhaled NO in patients with stable COPD compared with those in healthy persons (Clini et al. 1998; Rutgers et al. 1999). Smoking directly increases exhaled NO levels, however, thereby limiting the usefulness of this marker in COPD. The rapid reaction of NO with $O_2\bullet^-$, described previously, or with thiols may alter NO levels in breath (see "Generation of Reactive Oxygen Species" earlier in this chapter). Nitrosothiol levels have been shown to be higher in breath condensate in smokers and in COPD patients than those in nonsmokers (Corradi et al. 2001). $ONOO^-$, formed by the reaction of NO with $O_2\bullet^-$, can cause nitration of tyrosine to produce nitrotyrosine (Petruzzelli et al. 1997). Nitrotyrosine levels are elevated in sputum leukocytes of patients with COPD, and they are correlated negatively with FEV_1 (Ichinose et al. 2000).

Exhaled carbon monoxide, as a measure of the response of heme oxygenase to oxidative stress, has been shown to be elevated in exhaled breath in persons with COPD compared with that in persons without COPD (Montuschi et al. 2001). Carbon monoxide is also present in cigarette smoke, however, which limits its usefulness as a marker of oxidative stress in persons who smoke.

Lipid peroxidation products such as TBARS or malondialdehyde are elevated in sputum from COPD patients, and the levels correlate negatively with FEV_1 (Nowak et al. 1999; Tsukagoshi et al. 2000; Corradi et al. 2003). Urinary levels of 8-isoprostane, another lipid peroxidation product, are also higher in persons with COPD (Praticò et al. 1998). Levels of 8-isoprostane in breath condensate are also higher in persons with COPD than in healthy persons and smokers who have not developed the disease, and they correlate with the degree of airway obstruction (Paredi et al. 2000a). Isoprostanes may also reflect systemic effects caused by ROS (Morrow et al. 1995). Plasma levels of free F_2-isoprostanes are higher in smokers than in nonsmokers and are decreased after cessation of smoking.

Lipid peroxides can interact with enzymatic or nonenzymatic antioxidants and can decompose by reacting with metal ions or iron-containing proteins, thereby forming hydrocarbon gases and unsaturated aldehydes. Hydrocarbons are thus by-products of fatty acid peroxidation (Paredi et al. 2000b). COPD patients have higher levels of exhaled ethane in breath than do persons in the control group, and these levels correlate negatively with lung function (Habib et al. 1995; Paredi et al. 2000b).

There is evidence that concentrations of these markers of oxidative stress are also increased in the lung tissue of COPD patients. The lipid peroxidation product 4-HNE reacts quickly with extracellular proteins to form adducts, which have been shown to be present at higher concentrations in airway epithelial and endothelial cells in the lungs of COPD patients than in those of smokers with a similar smoking history who have not developed the disease (Rahman et al. 2002). Other markers of oxidative stress, such as 8-OH-dG and 4-HNE, have been shown to have increased expression associated with emphysematous lesions in the lungs (Tuder et al. 2003c).

Pathogenesis of Chronic Obstructive Pulmonary Disease

Many studies have shown higher levels of biomarkers of oxidative stress in COPD patients than in healthy smokers. Furthermore, several studies show relationships

between markers of oxidative stress and the degree of airflow limitation in COPD (Repine et al. 1997; MacNee 2000). However, the presence of oxidative stress and its relationship to airflow limitation may be an epiphenomenon because oxidative stress occurs in any inflammatory response. Cohort studies have not shown that the presence of enhanced oxidative stress relates to the decline in FEV_1 or to the progression of COPD.

Protease-Antiprotease Imbalance

In COPD, the protease burden in the lungs is increased because of the influx and activation of inflammatory leukocytes that release proteases. It has been proposed that a relative "deficiency" of antiproteases such as AAT, because of their inactivation by oxidants, creates a protease-antiprotease imbalance in the lungs. This hypothesis forms the basis of the protease-antiprotease theory of the pathogenesis of emphysema (Janoff et al. 1983a; Stockley 2001). Inactivation of AAT by oxidants occurs at a critical methionine residue in its active site and can be produced by oxidants from cigarette smoke or oxidants released from inflammatory leukocytes, resulting in a marked reduction in the inhibitory capacity of AAT in vitro (Bieth 1985; Evans and Pryor 1992). In vivo study of the acute effects of cigarette smoke on the functional activity of AAT show a transient but nonsignificant fall in the antiprotease activity of BAL fluid one hour after cigarette smoking (Abboud et al. 1985). In addition, in vitro exposure of lung epithelial cells to proteases leads to increased release of ROS, suggesting that proteases increase oxidative stress (Aoshiba et al. 2001b).

Hypersecretion of Mucus

Oxidant-generating systems such as xanthine and xanthine oxidase have been shown to cause the secretion of mucus from airway epithelial cells (Adler et al. 1990; Wright et al. 1996). Oxidants are also involved in the signaling pathways for EGF, which has an important role in the production of mucus (Nadel 2001). In addition, H_2O_2 and superoxide have been shown to cause a significant impairment of ciliary function after short-term exposure at low concentrations (Feldman et al. 1994). These effects may have important implications in the pathogenesis of COPD.

Lung Inflammation

Oxidative stress is present wherever inflammation exists. It may also be a mechanism for enhancing the air space inflammation that is characteristic of COPD (Pauwels et al. 2001). Oxidative stress can result in the release of chemotactic factors, such as IL-8, from airway epithelial cells (Gilmour et al. 2003), and epithelial cells from COPD patients have been shown to release more IL-8 than those of smokers or healthy persons (Profita et al. 2003). Lipid peroxidation products such as 8-isoprostane can also act as signaling molecules and cause the release of inflammatory mediators such as IL-8 from lung cells (Scholz et al. 2003). The lipid peroxidation product 4-HNE can cause increased production of TGFβ (Leonarduzzi et al. 1997) and increased expression of the gene encoding for the antioxidant enzyme γ-glutamylcysteine synthetase (Arsalane et al. 1997).

An enhanced inflammatory response in the lungs is characteristic of COPD (Di Stefano et al. 2004; Hogg 2004). Oxidative stress may have a fundamental role in enhancing inflammation through the increased production of redox-sensitive transcription factors, such as NF-κB and AP-1, and also by activation of the extracellular signal-regulated kinase, C-JUN *N*-terminal kinase, and p38 mitogen-activated protein kinase pathways (Rahman and MacNee 1998; MacNee and Rahman 2001). Cigarette smoke has been shown to activate all of these signaling mechanisms.

Genes for many inflammatory mediators are regulated by NF-κB, which is present in the cytosol in an inactive form linked to its inhibitory protein IκB. Many stimuli, including oxidants, result in activation of IκB kinase, producing phosphorylation and cleaving of IκB from NF-κB. The release of NF-κB is a critical event in the inflammatory response and is redox sensitive (Janssen-Heininger et al. 1999; MacNee 2000). Studies both in macrophage cell lines and in alveolar and bronchial epithelial cells show that oxidants cause the release of inflammatory mediators (e.g., IL-8, IL-1, and NO) and that these events are associated with increased expression of the genes for these inflammatory mediators and with increased nuclear binding and activation of NF-κB (Jiménez et al. 2000; Parmentier et al. 2000). The linking of NF-κB to its consensus site in the nucleus leads to enhanced transcription of proinflammatory genes and hence inflammation, which induces more oxidative stress, creating a vicious circle as enhanced inflammation and increased oxidative stress perpetuate each other.

Nuclear binding of NF-κB is increased in the airway macrophages and airway epithelial cells of COPD patients (Di Stefano et al. 2002). In a guinea pig model, exposure to cigarette smoke led to influx of neutrophils into the lungs and increased IL-8 gene expression, protein release, and NF-κB activation (Nishikawa et al. 1999). These increases and the neutrophil influx were reduced by pretreatment with superoxide dismutase, suggesting a role for oxidant stress. NF-κB is activated and translocated to the nucleus to a greater extent in lung tissue in smokers and in patients with COPD than in healthy persons (Szulakowski et al. 2006), and NF-κB activation in lung tissue has been shown to correlate with FEV_1 (Crowther et al. 1999).

A study of gene expression in rat epithelium after exposure to cigarette smoke showed that smoke causes rapid induction of antioxidant stress-response genes and drug-metabolizing enzymes, such as heme oxygenase and quinone oxidoreductase, all of which had decreased expression after long-term exposure to cigarettes (Gebel et al. 2004). The protein kinase C signaling pathway is also sensitive to tobacco smoke and increases its activity by twofold to threefold when stimulated by 5-percent CSE (Wyatt et al. 1999).

A further event controlling gene transcription that may be affected by oxidative stress and may enhance lung inflammation is chromatin remodeling. Under normal circumstances, DNA is wound tightly around a core of histone residues. This configuration prevents access for transcription factors to the transcriptional machinery and also reduces access of RNA polymerase to DNA, thereby resulting in transcriptional repression and gene silencing (Rahman and MacNee 1998; MacNee 2001). Histone acetyltransferases (HATs) cause the acetylation of histone residues, resulting in a change in their charge and unwinding of DNA and allowing access for transcription factors such as NF-κB and RNA polymerase to the transcriptional machinery, thereby enhancing gene expression. This process is reversed by HDACs, enzymes that deacetylate histone residues, resulting in the rewinding of DNA and gene silencing. The exact role of oxidative stress in modifying HAT and HDAC activity is unknown, but it appears that oxidative stress can result in increased HAT activity and decreased HDAC activity (Gilmour et al. 2003), which would enhance gene transcription.

Oxidative stress results in HAT activity in epithelial cells (Tomita et al. 2003). Histone acetylation can be shown to occur after the exposure of epithelial cells to cigarette smoke and is prevented by the antioxidant therapy *N*-acetylcysteine, indicating that the process is redox sensitive (Anderson et al. 2004). Furthermore, in animal models, exposure to cigarette smoke results in increased acetylated histone in the lung and decreased HDAC activity, and both of these events would enhance gene expression (Marwick et al. 2002). In addition, HDAC activity in alveolar macrophages obtained from cigarette smokers has been shown to be decreased, which would also enhance gene expression (Ito et al. 2001). This event may be due to nitration of HDAC2 by $ONOO^-$ (Ito et al. 2001, 2004a). More recent studies have suggested that acetylate histone residues, such as H4, are present to a greater extent in lung tissue in smokers and in COPD patients who smoke. These increases in H4 are associated with a decrease in HDAC2 in COPD patients who smoke and in patients with severe COPD (Ito et al. 2005; Szulakowski et al. 2006). A correlation has also been shown between decreased HDAC activity in lung tissue and FEV_1 in patients with COPD.

Apoptosis

There are two types of cell death: apoptosis, which is organized and noninflammatory, and necrosis, which is unorganized, destructive, and proinflammatory. One hypothesis is that loss of alveolar endothelial cells by apoptosis may be an initial event in the development of emphysema (Tuder et al. 2003b). Apoptosis has been shown to occur to a greater extent in endothelial cells in emphysematous lungs than in lungs of nonsmokers (Kasahara et al. 2001).

Airway lymphocytes (Majo et al. 2001) and stimulated peripheral blood leukocytes (Hodge et al. 2003) from patients with COPD also show increased apoptosis. The process of endothelial apoptosis is thought to be under the influence of VEGFR-2 receptors. Decrease of VEGFR-2 has been shown to produce emphysema in animals, and reduced expression of VEGFR-2 is evident in emphysematous human lungs (Kasahara et al. 2001). Studies have also shown that the apoptosis and emphysema induced by VEGF inhibition in animal models is associated with increased markers of oxidative stress and is prevented by antioxidants, suggesting that oxidative stress is involved in this process (Tuder et al. 2003c).

Systemic Involvement

Although COPD predominantly affects the lungs, it has important systemic consequences, including cachexia and skeletal muscle function (Wouters et al. 2002; Langen et al. 2003). Increasing evidence suggests that similar mechanisms involving oxidative stress and inflammation in the lungs may also be responsible for many of the systemic effects of COPD (Langen et al. 2003).

Peripheral blood neutrophils from COPD patients have been shown to release more ROS than such neutrophils from unaffected persons (Rahman et al. 1996a). Products of lipid peroxidation are also increased in plasma in smokers and patients with COPD (Rahman et al. 1996a). In addition, increased levels of nitrotyrosine have been shown to occur in the plasma of COPD patients (Ichinose et al. 2000).

Patients with COPD often display weight loss, which correlates inversely with the occurrence of exacerbations and is seen as an independent indicator of outcome (Gray-Donald et al. 1996; Landbo et al. 1999). In addition, loss of fat-free mass results in peripheral muscle dysfunction, decreased exercise capacity, and reduced health status (Palange et al. 1995; Baarends et al. 1997; Engelen et al. 2000b). Several factors influence the loss of weight and fat-free mass in COPD patients, including malnutrition, imbalance in overall protein turnover and the hormones involved in this process, tissue hypoxia, and pulmonary inflammation (Jenkins and Ross 1996; Engelen et al. 2000b; Eid et al. 2001; Wouters et al. 2002).

Oxidative stress may also have a role in the cachexia and loss of fat-free mass that occurs in COPD. Skeletal muscle is exposed continuously to changes in the redox environment that occur during exercise. Several studies have shown evidence of increased oxidative stress in patients with COPD both locally and systemically, particularly during exercise (Couillard et al. 2002, 2003; Langen et al. 2003). Presence of lipid peroxidation products in the serum, accompanied by an increase in the ratio of oxidized to reduced GSH, occur during exercise in COPD patients to a greater extent than in healthy persons (Sastre et al. 1992; Viña et al. 1996; Heunks and Dekhuijzen 2000). Skeletal muscle cells adapt to oxidative stress by increasing production of antioxidant enzymes such as SOD, catalase, and GPX (Franco et al. 1999). Study findings also showed evidence of disturbed redox homeostasis in COPD associated with emphysema. GSH levels in skeletal muscle were lower in COPD patients with emphysema than in those who did not have emphysema and were associated with reduced concentrations of glutamate, an important substrate in the synthesis of glutamine and GSH (Engelen et al. 2000a). Other studies demonstrate a decrease in GPX activity, elevated GRX activity, and increased lipid peroxidation, which indicate oxidative damage in the skeletal muscle of experimental hamsters with emphysema (Mattson et al. 2002). These results suggest that GSH metabolism is impaired in COPD.

Increased ROS production in skeletal muscle during exercise may result from stimulation of the mitochondrial electron-transport chain by TNFα (Li et al. 1999), which is known to be elevated in the circulation of patients with COPD who lose weight (Di Francia et al. 1994). Leukocytes infiltrating skeletal muscles in COPD patients may be another source of ROS (Adams et al. 2002). In addition, exercise increases the activity of xanthine and xanthine oxidase, a further source of ROS (Andrade et al. 1998). ROS also contribute to oxidative stress in muscles, and inducible NO expression has been shown to increase in skeletal muscle in response to inflammatory cytokines and activation of NF-κB (Adams et al. 2002). Oxidative stress may directly compromise muscle function by decreasing contractility and by increasing the susceptibility of muscle to oxidants (Barclay and Hansel 1991; Andrade et al. 1998). ROS may also oxidize proteins in the contractile apparatus, such as sulfhydryl residues in the contractile proteins, which may impair muscle function (MacFarlane and Miller 1992). In addition to impairing muscle function, resulting in muscle fatigue, oxidative stress may induce muscle atrophy. Atrophy is the result of an imbalance in muscle protein metabolism, which has been described in studies showing that oxidative stress induced inhibition of muscle-specific protein expression (Buck and Chojkier 1996; Langen et al. 2004). Furthermore, oxidative stress may result in apoptosis of muscle cells, which has been described in skeletal muscle cells, and may contribute to muscle atrophy (Stangel et al. 1996).

Summary

Considerable evidence now exists for both local and systemic oxidative stress in COPD patients. Increasing evidence suggests that oxidative stress is involved in many of the pathogenic processes involved in COPD, as well as in systemic phenomena such as skeletal muscle dysfunction. Cigarette smoke provides an extraordinarily strong dose of free radicals to the lung, initiating processes of oxidative injury that involve multiple cell types and the entire lung. Local inflammation results and markers of inflammation are higher, both in smokers and in persons with COPD, than are those in nonsmokers. Oxidative stress unfavorably tips the protease-antiprotease balance toward protease, leading to tissue damage and COPD.

Genetics of Pulmonary Disease and Susceptibility to Tobacco Smoke

α1-Antitrypsin Deficiency

Genetic Etiology

AAT deficiency is a long-established genetic risk factor for COPD and a model for the determination of susceptibility to cigarette smoking by causing COPD through a genetic mutation. However, only a minority of patients with COPD (1 to 2 percent) inherit the severe AAT deficiency that places them at highly increased risk of COPD (Lieberman et al. 1986). Consequently, only a small proportion of COPD cases are thought to be attributable to this gene-environment interaction (Lieberman et al. 1986).

The AAT protein is encoded by the *SERPINA1* gene on chromosome 14q32.1. Approximately 100 protease inhibitor (PI) alleles have been identified, some resulting in decreased serum levels of AAT (*American Journal of Respiratory and Critical Care Medicine* 2003). The **M* allele accounts for more than 95 percent of the PI alleles in U.S. populations and is associated with normal serum levels of AAT (Brantly et al. 1988). The **S* allele, which leads to mildly reduced AAT levels, and the **Z* allele, which leads to severely reduced AAT levels, occur at frequencies above 1 percent in U.S. populations. A smaller percentage of people inherit **NULL* alleles, which lead to the absence of any AAT production through a heterogeneous set of mutational mechanisms. Persons with two **Z* alleles or one **Z* and one **NULL* allele are commonly referred to as having the *PI *Z* phenotype, because their serum samples cannot be distinguished by the isoelectric focusing technique commonly used to assess PI type (Ogushi et al. 1987). Persons with *PI *Z* alleles have approximately 15 percent of normal serum AAT levels, and this quantitative reduction in circulating AAT is the primary determinant of increased risk for emphysema. In addition, molecule by molecule, the Z protein is a slightly less effective serine PI than is the M protein.

Immunologic assay of the AAT level in serum is a common test for AAT deficiency, but confirmation of the diagnosis of AAT deficiency requires determination of PI type, which is typically performed by isoelectric focusing of serum in specialized laboratories. Molecular genotyping by polymerase chain reaction can distinguish the common PI alleles (**M*, **S*, and **Z*) with use of DNA from a variety of cellular sources (von Ahsen et al. 2000; Stockley and Campbell 2001). However, high-throughput complete sequencing tests for rare PI alleles are not yet widely available, so rare alleles that produce severe AAT deficiency (e.g., **NULL* alleles) can be misclassified as normal if comprehensive molecular tests are not used.

AAT is one of the serpin protease inhibitors (serpins), an important family of PIs. The association between inherited AAT deficiency and pulmonary emphysema was critical for the development of the protease-antiprotease hypothesis on the pathogenesis of emphysema (Janoff 1985; Niewoehner 1988; Churg and Wright 2005). AAT is the major serum PI of neutrophil elastase, which is encoded by the *ELA2* gene. Neutrophil elastase is a potent elastase considered to be involved in the elastin degradation that leads to emphysema (Travis and Salvesen 1983). Although AAT demonstrates some inhibitory activity against a range of proteases, it is an extremely effective inhibitor of neutrophil elastase (Beatty et al. 1980). The functional specificity of AAT is determined by a methionine at amino acid position 358 of the AAT protein, which is the PI residue at the active inhibitory site (Mahadeva and Lomas 1998). The **Z* allele encodes a single base substitution that replaces glutamic acid at amino acid position 342 in the M protein with lysine, thus eliminating a critical salt bridge in the AAT protein. The low serum AAT levels in *PI *Z* alleles occur because the Z protein polymerizes within the endoplasmic reticulum of hepatocytes, the primary site of AAT synthesis, preventing release of the protein.

The prevalence of AAT deficiency is particularly high in populations of Northern European descent. Molecular haplotype analysis of polymorphic loci adjacent to the *AAT *Z* allele suggests a single mutational origin for the majority of **Z* alleles in modern populations, an ancestral mutation that likely occurred in Northern Europe (Byth et al. 1994). Hutchison (1998), who reviewed the European screening studies for AAT deficiency, found that the highest frequencies of the **Z* allele were in northwestern Europe. Although screening studies have typically found low frequencies of the **Z* allele in populations of African and Asian descent (Kellermann and Walter 1970), the review of the worldwide screening literature in control cohorts, prepared by de Serres (2002), suggested that there could be significant numbers of *PI *Z* carriers in almost every region of the world. These estimates were based on calculations assuming Hardy-Weinberg equilibrium and accurate AAT typing in these control populations, but it remains to be determined how significantly these estimates were affected by PI typing errors, new mutations, or migration from populations in Northern Europe.

Natural History

Although increased risk for the development of COPD among persons with the *PI *Z* allele has been well established, the magnitude of this risk and the natural history of the entire population with the *PI *Z* allele remain unclear. This population in the United States is estimated at 80,000 to 100,000. Among persons known to have the *PI *Z* allele, early-onset COPD is often observed clinically. Classic emphysema with the greatest severity in the lower lobes has been described among adults with the *PI *Z* allele and COPD, but diffuse or upper lobe emphysema can also be observed in this population (Parr et al. 2004).

Several early studies of large numbers of persons with the *PI *Z* allele demonstrated that *PI *Z*-type persons who smoked cigarettes tended to develop more severe COPD at an earlier age than did *PI *Z*-type persons who were nonsmokers (Larsson 1978; Tobin et al. 1983; Janus et al. 1985). More recently, Seersholm and colleagues (1994) demonstrated significantly higher mortality rates in smokers with *PI *Z* than in nonsmokers with *PI *Z*. Silverman and colleagues (1992) demonstrated an interaction between PI type and cigarette smoking, by comparing the patterns of phenotypic expression in smokers by the percentage with specific predicted FEV_1 values and the patterns in participants with the *PI *M*, *PI *M*/**Z*, or *PI *Z* allele, in the St. Louis Alpha-1-Antitrypsin Study.

The St. Louis Alpha-1-Antitrypsin Study also demonstrated the importance of ascertainment bias in limiting insight into the natural history of AAT deficiency. If most persons with the *PI *Z* allele are identified because they already have COPD, it would appear that most persons with this genotype will develop COPD. Among 52 persons with this allele, Silverman and colleagues (1989) confirmed the expected result that persons with the *PI *Z* allele who were tested for AAT deficiency because they already had COPD (index persons) all had significantly reduced FEV_1 values. However, marked variability in development of airflow obstruction was demonstrated in nonindex persons with the *PI* *Z allele whose genotype was ascertained, not because of existing COPD, but by genotyping in family studies or because they had liver disease. In Denmark, Seersholm and colleagues (1995) confirmed differences between lung function in index and nonindex persons with the *PI *Z* allele that were independent of age and smoking history.

The *PI *Z* type is a major risk factor for COPD, and cigarette smoking increases the risk for COPD in persons with the *PI *Z* allele (Silverman et al. 1989). Even so, some smokers with *PI *Z* maintain normal pulmonary function into older ages, whereas some nonsmokers with *PI *Z* develop COPD at an early age (Black and Kueppers 1978). For example, among 18 lifetime nonsmokers with the *PI *Z* allele, the investigators found significant variability in lung function and respiratory symptoms, despite the absence of a history of smoking or other significant environmental exposures.

In a study of 205 nonsmokers with the *PI *Z* allele in Sweden, Piitulainen and colleagues (1998) observed that using a kerosene heater and working in agriculture were associated with lower lung function. Among 128 persons with *PI *Z*, Mayer and associates (2000) found that high exposure to mineral dust was associated with increased cough symptoms and reduced FEV_1.

Because less than 10 percent of the estimated total of persons with the *PI *Z* genotype in the United States have been identified (*American Journal of Respiratory and Critical Care Medicine* 2003), the natural history of COPD in persons with the *PI *Z* allele remains uncertain. In addition to cigarette smoking, other environmental factors and genetic modifiers likely influence the development of COPD among persons with *PI *Z*. Largely in response to the underdiagnosis of persons with *PI *Z*, the American Thoracic Society and European Respiratory Society Task Force (*American Journal of Respiratory and Critical Care Medicine* 2003) recommended testing for AAT deficiency in all adults with COPD, emphysema, or asthma with chronic airflow obstruction.

Familial Aggregation of Phenotypes Related to Chronic Obstructive Pulmonary Disease

Pulmonary Function in the General Population

Several types of studies have suggested that genetic factors influence variation in spirometric measurements in the general population. Studies of twins who were not selected for lung disease have found greater correlations in the measure of lung function between monozygotic twins, who share all of their genetic variation, than between dizygotic twins, who share approximately one-half of their genetic variation. Comparison of correlations between monozygotic and dizygotic twins allows for estimating the heritability of lung function, the percentage of total phenotypic variation in lung function that is related to genetic factors. For example, in a study of 127 monozygotic and 141 dizygotic twin pairs by the National Heart, Lung, and Blood Institute (NHLBI), the estimated heritability for FEV_1 values, after adjustment for age, height, weight, and smoking was 74 percent (Hubert et al. 1982). Redline and colleagues (1987) also observed significantly higher correlations for FEV_1 between monozygotic twins than between dizygotic twins. Tishler and associates

(2002) studied 352 adult twin pairs and found evidence suggesting a relationship between history of cigarette smoking and unidentified potential susceptibility genes.

Studies in nuclear families have also supported a role for genetic determinants of pulmonary function in the general population; both path analysis and variance component analysis have been used. Lewitter and colleagues (1984), who used path analysis in a study of 404 nuclear families, estimated that 41 to 47 percent of variation in FEV_1 values was related to genetic factors. Using variance component analysis in a study of 439 persons from 108 families, Astemborski and associates (1985) estimated that after adjustment for age, gender, race, and smoking history, 28 percent of the variation in FEV_1 and 24 percent of the variation in FEV_1/FVC were related to genetic determinants. More recently, Palmer and colleagues (2001) performed variance component modeling of spirometric phenotypes in the Busselton Health Study and estimated the heritability of FEV_1 as 39 percent. Although these studies in the general population provide compelling evidence that genetic factors influence variation in level of pulmonary function, they do not necessarily provide insight into the role of genetic factors in the development of COPD.

Airflow Obstruction

Studies assessing the role of familial aggregation of phenotypes in the occurrence of airflow obstruction in relatives of patients with COPD have supported a role for genetic factors in the development of COPD. In an early study, Larson and colleagues (1970) reported higher rates of airflow obstruction in first-degree relatives of COPD patients than in the control group. Later, Kueppers and associates (1977), who studied 114 persons with COPD, compared the spirometric values in siblings with those in a matched control group and found that the siblings had significantly lower FEV_1 values after adjustment for smoking history.

In the Boston Early-Onset COPD Study, Silverman and colleagues (1998) focused on persons with severe, early-onset COPD without AAT deficiency. Among nonsmokers who were first-degree relatives of these probands with early-onset COPD, FEV_1 and FEV_1/FVC values were similar to those in nonsmokers in the control group. Using generalized estimating equations to adjust for age and pack-years of smoking, the investigators found, however, that current or former smokers among first-degree relatives of the probands with early-onset COPD had significantly higher risk for reduced FEV_1 values than did smokers in the control group. In Great Britain, McCloskey and associates (2001) compared the rates of airflow obstruction in 173 siblings of probands who had severe COPD with those for a population-based control cohort. As was found in the Boston Early-Onset COPD Study, nonsmokers who were siblings of COPD patients had risk of airflow obstruction similar to that for nonsmokers in the control group. In contrast, current or former smokers who were siblings of probands with COPD had a significantly higher risk of airflow obstruction than did smokers from the general population. The significant familial aggregation of phenotypes for airflow obstruction in COPD families, which persists after adjustment for intensity of cigarette smoking, strongly suggests genetic influences on susceptibility to developing chronic airflow obstruction.

Chronic Bronchitis

Familial aggregation of chronic cough and production of phlegm (chronic bronchitis) has also been demonstrated. In a sample of 9,226 persons from the general population, Higgins and Keller (1975) found significantly higher rates of chronic bronchitis in offspring when at least one parent had chronic bronchitis than if neither parent had the disorder, but there was no adjustment for cigarette smoking. Speizer and colleagues (1976), using National Health Interview Survey data and adjusting for cigarette smoking, demonstrated significantly higher rates of bronchitis or emphysema among offspring when at least one parent had bronchitis or emphysema. Tager and associates (1978) also adjusted for history of cigarette smoking in their analysis and found that rates of chronic bronchitis or airflow obstruction in first-degree relatives of probands with chronic bronchitis or airflow obstruction were significantly higher than those in first-degree relatives of the control group. Finally, in the Boston Early-Onset COPD Study, Silverman and colleagues (1998) found significantly higher risk of chronic bronchitis among smokers who were first-degree relatives of probands with early-onset COPD than among control smokers. This analysis was adjusted for the intensity of cigarette smoking.

Linkage Analysis of Phenotypes Related to Chronic Obstructive Pulmonary Disease

Several studies of genetic linkage across the human genome were perfomed in families from the general population who were not selected because of the presence of particular respiratory disease (Table 7.8). The purpose was to examine the relationship between spirometric values and genetic determinants of pulmonary

Table 7.8 Genomewide linkage analysis studies in general-population samples and in families with chronic obstructive pulmonary disease (COPD)

Study	Study design	Sample size	Phenotype	Chromosomal region/ maximum limit of detection score[a]	Comments
General Population					
Joost et al. 2002	Framingham Heart Study pedigrees	1,578 persons 330 pedigrees	FEV_1	• 4p: 1.6 • 6q: 2.4	
Malhotra et al. 2003	Extended Utah Centre d'Etude du Polymorphisme Humain pedigrees	264 persons 26 pedigrees	FEV_1/FVC	• 2q: 2.0	Parametric limit of detection score without heterogeneity showed most significant linkage evidence on 2q
Wilk et al. 2003a	Family Heart Study pedigrees	2,178 persons 391 pedigrees	FEV_1 FEV_1/FVC	• 3q: 2.0 • 1p: 1.7 • 4p: 3.5 • 9p: 2.0 • 17p: 2.3	Untransformed values were reported; normalized values were also analyzed
Boston Early-Onset COPD Study					
Silverman et al. 2002a	Extended pedigrees ascertained in proband with early-onset COPD	585 persons 72 pedigrees	Moderate airflow obstruction	• 12p: 1.7 • 19q: 1.5	Some regions demonstrated increased limit of detection scores for smokers only
Palmer et al. 2003	Extended pedigrees ascertained in proband with early-onset COPD	Same population 560 persons with spirometry	FEV_1 (postbronchodilation) FEV_1/FVC (postbronchodilation)	• 1p: 2.2 • 8p: 3.3 • 8q: 2.0 • 19q: 1.9 • 1p: 2.5 • 2q: 4.4 • 17q: 2.4	
DeMeo et al. 2004	Extended pedigrees ascertained in proband with early-onset COPD	Same population	FEF25–75 (postbronchodilation) FEF25–75/FVC (postbronchodilation)	• 8p: 1.8 • 12p: 1.7 • 2q: 2.6	

Note: **FEF25–75** = forced expiratory flow between 25 and 75 percent of FVC; **FEV_1** = forced expiratory volume in 1 second; **FVC** = forced vital capacity.
[a]Limit of detection scores >1.5 are presented.

function. These genetic determinants may predispose family members to COPD, or they may only contribute to variation in pulmonary function within the normal range. Joost and colleagues (2002) analyzed linkage to quantitative spirometric measurements made before use of a bronchodilator in 1,578 persons from 330 pedigrees in the Framingham Heart Study. The largest linkage signal, which did not reach the criteria for genomewide significance, was on chromosome 6q for prebronchodilator FEV_1. The score of the logarithm of the odds (LOD) ratio, or likelihood ratio, was 2.4. In a subset of this study population, flanking short tandem repeat (STR) markers were

genotyped to increase the information available for linkage analysis, and significant linkage of FEV_1 to chromosome 6q was identified with a maximum LOD score of 5.0 (Wilk et al. 2003b).

Wilk and colleagues (2003a) performed genomewide linkage analysis with prebronchodilator spirometric phenotypes in 2,178 participants in the NHLBI Family Heart Study, a population that partially overlapped that with the pedigrees from the Framingham Heart Study used by Joost and colleagues (2002). Even so, the linkage results differed substantially from those in the Framingham Heart Study. The most impressive signals suggested linkage of FEV_1 to chromosome 3q and FEV_1/FVC to chromosome 4p.

Finally, Malhotra and associates (2003) performed genomewide linkage analysis of quantitative prebronchodilator spirometric measurements in extended pedigrees. The findings suggested linkage of FEV_1/FVC values to chromosome 2q and to chromosome 5q but no linkage for either FEV_1 or FVC.

Chronic Obstructive Pulmonary Disease in Families

The Boston Early-Onset COPD Study includes extended pedigrees obtained through persons with severe early-onset COPD but without AAT deficiency. Genomewide linkage analysis has been performed with 585 members of 72 pedigrees involving early-onset COPD (Table 7.8). Initially, qualitative phenotypes of airflow obstruction and chronic bronchitis were analyzed, and no statistically significant or even suggestive regions of linkage were identified (Silverman et al. 2002a). Although limiting the sample to smokers only and genotyping of flanking STR markers identified several linkage regions of potential interest, linkage analysis of quantitative spirometric phenotypes provided more compelling evidence for linkage, especially with use of postbronchodilator spirometric values (Silverman et al. 2002b; Palmer et al. 2003). Findings suggested linkage of the postbronchodilator values of FEV_1 to chromosomes 8p (LOD = 3.30) and 1p (LOD = 2.24). Postbronchodilator FEV_1/FVC was also linked to multiple regions, most significantly to markers on chromosomes 2q (LOD = 4.42) and 1p (LOD = 2.52).

Genotyping additional STR markers and repeating linkage analysis of quantitative spirometric phenotypes provided stable-to-increased evidence for linkage on chromosomes 2q, 12p, and 19q (Celedón et al. 2004; DeMeo et al. 2004). Stratified linkage analysis of samples only from smokers also provided stable-to-increased evidence for linkage to these genomic regions. Findings suggested that genetic determinants in those regions confer increased risk for COPD because of a relationship between history of cigarette smoking and unidentified potential susceptibility genes.

Overall, the linkage results of quantitative spirometric measurements in the persons with pedigrees from the Boston Early-Onset COPD Study (Hersh et al. 2005) and samples from the general population have demonstrated only modest concordance. The most impressive linkage signals in the study have been obtained with postbronchodilator spirometric measures, which have not been used in the linkage studies of the general population. The Boston Early-Onset COPD Study demonstrated linkage of FEV_1/FVC to chromosome 2q. The suggestive linkages of FEV_1/FVC to chromosome 1p in the NHLBI Family Heart Study and the Boston Early-Onset COPD Study indicate a region that may influence spirometric measurements in both the general population and persons with COPD. One explanation for inconsistent linkage results in studies of COPD families and studies of pedigrees in the general population is that different genetic determinants could influence normal variation in spirometry and COPD. In addition, the lack of concordance among results from linkage studies in the general population could relate to genetic heterogeneity among study populations, false-positive evidence for linkage in some regions, or inadequate power of the study to replicate linkage signals.

Genetic Association with Chronic Obstructive Pulmonary Disease

A large number of studies to determine associations have assessed genetic variants in candidate genes hypothesized to be involved in the development of COPD. These were primarily case-control studies of patients with COPD and control groups. Candidate gene loci significantly associated with COPD in at least two studies are listed in Table 7.9. In addition to the *PI *M/*Z* genotype of AAT, which has been variably associated with COPD (Hersh et al. 2004), replicated associations have been demonstrated for genes of α1-antichymotrypsin (*SERPINA3*) (Poller et al. 1993; Sandford et al. 1998; Benetazzo et al. 1999; Ishii et al. 2000a), GSTM1 (*GSTM1*) (Baranova et al. 1997; Harrison et al. 1997; Yim et al. 2000; He et al. 2004), GSTP1 (*GSTP1*) (Ishii et al. 1999; Yim et al. 2002; He et al. 2004), vitamin D binding protein (*GC*) (Kauffmann et al. 1983; Horne et al. 1990; Schellenberg et al. 1998; Ishii et al. 2001; Sandford et al. 2001; Kasuga et al. 2003; Ito et al. 2004b), TGFβ1 (*TGFβ1*) (Celedón et al. 2004; Wu et al. 2004), TNF (*TNF*) (Huang et al. 1997; Higham et al. 2000; Ishii et al. 2000b; Patuzzo et al. 2000; Sakao et al. 2001;

Table 7.9 Replicated candidate gene associations in chronic obstructive pulmonary disease (COPD)

Study	Sample size	Genetic variants studied
α1-antitrypsin *PI *M/*Z* heterozygotes		
Hersh et al. 2004	>100 studies Meta-analysis	*PI *M/*Z* heterozygotes
TGFβ1		
Celedon et al. 2004	585 persons in 72 pedigrees 304 cases vs. 441 controls	5 SNPs (including same exonic SNP)
Wu et al. 2004	165 cases vs. 146 blood donor controls 76 healthy smokers	1 exonic SNP
EPHX1		
Smith and Harrison 1997	68 COPD cases 94 emphysema cases vs. 203 blood donor controls	Exon 3 nonsynonymous SNP (slow) and Exon 4 nonsynonymous SNP (fast)
Takeyabu et al. 2000	79 emphysema cases vs. 58 smoking controls and 114 healthy controls	Exon 3 and 4 SNPs
Yim et al. 2000	83 cases vs. 76 smoking controls	Exon 3 and 4 SNPs
Yoshikawa et al. 2000	40 COPD cases and 140 controls among poison-gas workers	Exon 3 and 4 SNPs
Sandford et al. 2001	283 COPD persons with rapid FEV_1 decline vs. 308 COPD persons with slow FEV_1 decline in the U.S. Lung Health Study	Exon 3 and 4 SNPs
Hersh et al. 2005	949 persons in 127 pedigrees with early-onset COPD 304 cases vs. 441 smoking controls	8 SNPs including Exon 3 and 4 SNPs
TNFα		
Huang et al. 1997	42 cases vs. 42 smoking controls and 99 blood donor controls	1 promoter SNP at -308
Higham et al. 2000	86 cases vs. 63 smoking controls and 199 blood donor controls	-308 SNP
Ishii et al. 2000b	53 cases vs. 65 smoking controls	-308 SNP
Patuzzo et al. 2000	66 cases vs. 98 healthy controls and 45 cases of nonobstructive pulmonary disease	-308 SNP

Study	Phenotype	Results/p value for association	Comments
α1-antitrypsin *PI *M/*Z* heterozygotes			
Hersh et al. 2004	Presence or absence of COPD FEV_1 in persons with *PI *M/*Z* vs. *PI *M/*M* alleles	Typically positive Typically negative	No overall consensus on risk for lung disease in persons with *PI *M/*Z* alleles
TGFβ1			
Celedon et al. 2004	Families with early-onset COPD: qualitative and quantitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	3 significant SNPs at $p < 0.05$ (1 promoter and 2 in the 3′ genomic region) 3 significant SNPs at $p < 0.05$ (2 promoter SNPs and 1 exonic SNP)	1 promoter SNP replicated in both study populations
Wu et al. 2004	Presence/absence of COPD	$p \leq 0.01$ vs. both control groups	Exonic SNP replicated in both case-control studies but not in family-based study
EPHX1			
Smith and Harrison 1997	Presence/absence of COPD or emphysema	Significant associations of exon 3 SNP with emphysema group and of exon 4 SNP with COPD group	Several negative studies had small samples
Takeyabu et al. 2000	Presence/absence of emphysema	No significant association	
Yim et al. 2000	Presence/absence of COPD	No significant association	
Yoshikawa et al. 2000	Presence/absence of COPD	No significant association	
Sandford et al. 2001	Rapid vs. slow FEV_1 decline in persons with COPD	Significant association of exons 3 and 4 SNP haplotypes with rapid FEV_1 decline	
Hersh et al. 2005	Families with early-onset COPD: quantitative and qualitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	Significant association of exon 4 SNP only in case-control sample	
TNFα			
Huang et al. 1997	Presence/absence of COPD with chronic bronchitis	Significant association vs. both control groups ($p < 0.001$)	Several studies with significant associations, but many negative studies; many studies had small sample sizes
Higham et al. 2000	Presence/absence of COPD	No significant association	
Ishii et al. 2000b	Presence/absence of COPD	No significant association	
Patuzzo et al. 2000	Presence/absence of COPD	No significant association	

Table 7.9 Continued

Study	Sample size	Genetic variants studied
Sakao et al. 2001	106 cases vs. 110 smoking controls and 129 blood donor controls	-308 SNP
Sandford et al. 2001	283 cases with rapid vs. 304 cases with slow FEV_1 decline `U.S. Lung Health Study	-308 SNP
Kucukaycan et al. 2002	169 cases vs. 358 blood donor controls	4 SNPs including -308 and +489
Ferrarotti et al. 2003	63 cases vs. 86 smoking controls	-308 SNP
Hersh et al. 2005	949 members of 127 pedigrees with early-onset COPD and 304 cases vs. 441 smoking controls	5 SNPs including -308 and +489
GSTM1		
Baranova et al. 1997	87 cases of severe chronic bronchitis vs. 102 cases of moderate chronic bronchitis vs. 172 smoking controls	Null variant
Harrison et al. 1997	111 lung cancer patients with emphysema vs. 57 without emphysema	Null variant (homozygous null vs. all others)
Yim et al. 2000	83 cases vs. 76 smoking controls	Null variant
He et al. 2004	544 persons with COPD and low FEV_1 vs. 554 with high FEV_1 U.S. Lung Health Study	Null variant
Hersh et al. 2005	Families with early-onset COPD: 949 persons in 127 pedigrees Case/control: 304 cases vs. 441 smoking controls	Null variant
GSTP1		
Ishii et al. 1999	53 cases vs. 50 healthy controls	1 nonsynonymous SNP (*ILE105VAL*)
Yim et al. 2002	89 cases vs. 94 smoking controls	1 nonsynonymous SNP (*ILE105VAL*)
He et al. 2004	544 persons with COPD and low FEV_1 vs. 554 with high FEV_1 U.S. Lung Health Study	1 nonsynonymous SNP (*ILE105VAL*)
Hersh et al. 2005	949 members of 127 pedigrees with early-onset COPD and 304 cases vs. 441 smoking controls	2 nonsynonymous SNPs (*ILE105VAL* and *ALA114VAL*)

Study	Phenotype	Results/p value for association	Comments
Sakao et al. 2001	Presence/absence of COPD	Significant differences in allele frequencies in COPD cases vs. both control groups ($p < 0.01$)	
Sandford et al. 2001	Rapid vs. slow FEV_1 decline in persons with COPD	No significant association	
Kucukaycan et al. 2002	Presence/absence of COPD	Significant association with +489 variant only	
Ferrarotti et al. 2003	Presence/absence of COPD with reduced $D_{L,CO}$	No significant association	
Hersh et al. 2005	Families with early-onset COPD: quantitative and qualitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	Significant associations with qualitative and quantitative airflow obstruction phenotypes with SNP -308 in COPD pedigrees only	
GSTM1			
Baranova et al. 1997	Presence/absence of COPD	Higher null/null frequency in both cases of moderate and severe chronic bronchitis and severe chronic bronchitis vs. controls ($p < 0.001$)	
Harrison et al. 1997	Presence/absence of emphysema in resected lung tissue	Significantly increased frequency of homozygosity for null variant in persons with emphysema ($p < 0.05$)	Only homozygosity for null variant was typically assessed
Yim et al. 2000	Presence/absence of COPD	No significant association	
He et al. 2004	High vs. low FEV_1 in persons with COPD	No significant association	
Hersh et al. 2005	Families with early-onset COPD: qualitative and quantitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	No significant association	
GSTP1			
Ishii et al. 1999	Presence/absence of COPD	Homozygous *105ILE* more common in COPD cases	Several studies with significant associations, but different alleles, are associated at amino acid 105 SNP in different studies
Yim et al. 2002	Presence/absence of COPD	No significant association	
He et al. 2004	High vs. low FEV_1 in persons with COPD	Homozygous *105VAL* gene more common in low FEV_1 group	
Hersh et al. 2005	Families with early-onset COPD: qualitative and quantitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	Borderline higher frequency of **105VAL* allele in case-control analysis only	

Table 7.9 Continued

Study	Sample size	Genetic variants studied
SFTPB		
Guo et al. 2001	97 cases vs. 82 smoking controls	4 SNPs, 1 promoter, and 3 intragenic indels
Seifart et al. 2002	118 cases vs. 118 matched controls and 110 population-based controls	SP-B *INTRON 4* indels
Hersh et al. 2005	949 members of 127 pedigrees with early-onset COPD and 304 cases vs. 441 smoking controls	1 nonsynonymous SNP (*THR131IL3*) and 1 short tandem repeat
α1-antichymotrypsin		
Poller et al. 1993	100 COPD cases vs. 100 controls	2 nonsynonymous SNPs: *PRO229ALA* and *LEU55PRO*
Sandford et al. 1998	168 COPD cases vs. 61 controls	*PRO229ALA* and *LEU55PRO*
Benetazzo et al. 1999	66 COPD cases vs. 45 controls with nonobstructive pulmonary disease and 98 healthy volunteers	4 coding SNPs (*THR15ALA, LEU55PRO, PRO229ALA*, and *MET389VAL*) and 1 indel (*1258DELAA*)
Ishii et al. 2000a	53 COPD cases vs. 65 controls	2 coding SNPs in protein (*PRO229ALA* and *LEU55PRO*) and 1 coding SNP in signal peptide (*ALA15THR*)
Hersh et al. 2005	949 members of 127 pedigrees with early-onset COPD and 304 cases vs. 441 smoking controls	*ALA15THR, LEU55PRO*, and *PRO229ALA* polymorphisms
Vitamin D binding protein (group-specific component [*GC*])		
Kauffmann et al. 1983	43 lifetime nonsmokers with low FEV_1 vs. 45 heavy smokers with high FEV_1	*GC*1S/F* and *GC*2* alleles
Horne et al. 1990	104 COPD cases vs. 413 controls	GC phenotype by isoelectric focusing
Schellenberg et al. 1998	75 COPD cases vs. 64 smoking controls	SNPs *THR420LYS* (*GC*2*) and *ASP416GLU* (*GC*1S*)
Ishii et al. 2001	63 COPD cases vs. 82 controls	*GC*1F/*1S* and *GC*2* alleles
Sandford et al. 2001	283 persons with COPD with rapid FEV_1 decline vs. 308 persons with COPD with slow FEV_1 decline	*THR420LYS* and *ASP416GLU* polymorphisms
Kasuga et al. 2003	537 persons with COPD with high FEV_1 vs. 533 with low FEV_1 U.S. Lung Health Study	*GC** haplotypes

Study	Phenotype	Results/p value for association	Comments
SFTPB			
Guo et al. 2001	Presence/absence of COPD	Significant association with SNP at +1580 ($p < 0.05$)	
Seifart et al. 2002	Presence/absence of COPD and COPD severity	*INTRON 4* variants significantly associated ($p < 0.05$) with respiratory failure subgroup	
Hersh et al. 2005	Families with early-onset COPD: quantitative and qualitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	Significant SNP association with moderate-to-severe airflow obstruction ($p < 0.05$) Significant SNP association only with gene-x-smoking interaction term ($p < 0.01$)	
α1-antichymotrypsin			
Poller et al. 1993	Presence/absence of COPD	Significant SNP association ($p < 0.05$) with *PRO229ALA*	No studies replicated associations of the same genetic variants
Sandford et al. 1998	Presence/absence of COPD	No significant association	
Benetazzo et al. 1999	Presence/absence of COPD	No significant association	
Ishii et al. 2000a	Presence/absence of COPD	Significant SNP association with *ALA15THR* only	
Hersh et al. 2005	Families with early-onset COPD: quantitative and qualitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	No significant association	
Vitamin D binding protein (group-specific component [*GC*])			
Kauffmann et al. 1983	Presence/absence of COPD	No significant association	
Horne et al. 1990	Presence/absence of COPD	Significant association with inferred *GC** allele ($p < 0.01$)	
Schellenberg et al. 1998	Presence/absence of COPD	Significant protection against COPD with *GC*2/GC*2* genotype	
Ishii et al. 2001	Presence/absence of COPD	Significantly increased frequency of *GC*1F* allele in COPD cases	
Sandford et al. 2001	Rapid vs. slow FEV_1 decline in COPD cases	No significant association	
Kasuga et al. 2003	High vs. low FEV_1 among COPD cases	No significant association	

Table 7.9 Continued

Study	Sample size	Genetic variants studied
Ito et al. 2004b	103 COPD cases vs. 88 smoking controls	*GC*1S/*1F* and *GC*2*
Hersh et al. 2005	949 members of 127 pedigrees with early-onset COPD and 304 cases vs. 441 smoking controls	*THR420LYS* and *ASP416GLU*

Note: $D_{L,CO}$ = diffusing capacity of the lung for carbon monoxide; **FEV_1** = forced expiratory volume in 1 second; **FVC** = forced vital capacity; **indel** = DNA mutation; **PI** = protease inhibitor; **SNP** = single nucleotide polymorphism; **SP-B** = surfactant protein B.
[a]In families with early-onset COPD, quantitative spirometric phenotypes included FEV_1/FVC before and after use of a bronchodilator; qualitative phenotypes included mild-to-severe airflow obstruction (FEV_1 <80% predicted with FEV_1/FVC <90% predicted) and moderate-to-severe airflow obstruction (FEV_1 <60% predicted with FEV_1/FVC <90% predicted).

Sandford et al. 2001; Küçükaycan et al. 2002; Ferrarotti et al. 2003), surfactant protein B (*SFTPB*) (Guo et al. 2001; Seifart et al. 2002; Hersh et al. 2005), and microsomal epoxide hydrolase (*EPHX1*) (Smith and Harrison 1997; Takeyabu et al. 2000; Yim et al. 2000; Yoshikawa et al. 2000; Sandford et al. 2001). Although at least two studies support an association of a genetic variant with COPD in these candidate genes, every case also has at least one negative study.

Several factors could contribute to the inconsistent results from case-control studies of genetic association with COPD. Genetic heterogeneity in different populations could contribute to difficulty in replicating associations between studies, and false-positive or false-negative results could contribute to inconsistent replication. A potentially important factor is that case-control studies of association are susceptible to supporting associations based only on population stratification; that is, they reflect differences between populations rather than true associations (Freedman et al. 2004). Population stratification can result from incomplete matching between cases and controls, which might include failure to account for differences in ethnicity and geographic origin that may affect the results. In addition, most published studies on genetic associations of COPD have not focused on genomic regions linked to COPD-related phenotypes, regions in which association studies may be more fruitful.

As of 2008, only one study, a linkage analysis of family-based genetic association for COPD, has been reported (Celedón et al. 2004). The design of the study is typically not vulnerable to effects of population stratification. The study focused on genetic variants in *TGFβ1*, a gene that is located within the region of linkage to FEV_1 on chromosome 19q in the Boston Early-Onset COPD Study and that was associated with COPD in another case-control study of genetic association (Wu et al. 2004). Five *TGFβ1* single nucleotide polymorphisms (SNPs) were genotyped in families in the Boston Early-Onset COPD Study. Family-based association analysis showed that one SNP in the promoter region of *TGFβ1* (*RS2241712*) and two SNPs in the 3′ untranslated region of *TGFβ1* (*RS2241718* and *RS6957*) were significantly associated with FEV_1 ($p < 0.05$). Among 304 case patients with severe COPD from the National Emphysema Treatment Trial and 441 smokers in the control group from the Normative Aging Study, two SNPs in the promoter region of *TGFβ1* (*RS2241712* and *RS1800469*) and one SNP in exon 1 of *TGFβ1* (*RS1982073*) were significantly associated with COPD ($p \leq 0.02$) (Celedón et al. 2004). Additional research to replicate the genetic associations in *TGFβ1* and identify the functional variants in or near *TGFβ1* is required.

A variety of candidate genes have been examined in genetic association studies focused on COPD, but no genetic loci other than the *SERPINA1* gene for severe AAT deficiency proved to be significant risk factors for COPD.

Mouse Models of Genetics for Chronic Obstructive Pulmonary Disease

Although rodent models have provided important insights into the potential biochemical mechanisms of COPD, there has been no publication of research using quantitive trait locus mapping to identify susceptibility loci through experimental crosses of relatively susceptible and relatively nonsusceptible strains. Significant differences between murine strains in susceptibility to the development of smoking-induced COPD have been demonstrated (Guerassimov et al. 2004), and use of these strain-specific differences to perform quantitative trait locus mapping may provide unique opportunities to uncover genetic determinants of COPD (Shapiro et al. 2004).

Study	Phenotype	Results/p value for association	Comments
Ito et al. 2004b	Presence/absence of COPD	Significantly increased frequency of *GC*1F* allele in COPD cases	
Hersh et al. 2005	Families with early-onset COPD: quantitative and qualitative airflow obstruction phenotypes[a] Case/control: presence/absence of COPD	No significant association	

Summary

Severe AAT deficiency is a proven genetic risk factor for COPD. Although considerable insight into the pathogenesis of COPD has been provided by studies of AAT deficiency, fundamental questions about the natural history of this deficiency remain unanswered.

Only a small percentage of patients with COPD inherit severe AAT deficiency, and additional genetic factors likely influence the development of the disorder. Further efforts in linkage analysis, association studies, and research on animal models may lead to identification of such factors. To achieve a complete understanding of COPD pathophysiology, characterization of the interactions among genetic determinants, cigarette smoking, and possibly other environmental factors is required. Identification of genetic factors influencing the development of COPD unrelated to AAT deficiency could elucidate the biochemical mechanisms causing COPD, allow identification of more susceptible persons, and lead to new therapeutic interventions as pathways of injury are better characterized.

Pathogenesis of Emphysema

Sources of Information

The information on pathogenesis of emphysema discussed here was obtained from original research articles, most published since the early 1990s. These articles were found by consulting reviews of the literature (Pardo and Selman 1999; Mahadeva and Shapiro 2002; Barnes et al. 2003; Tuder et al. 2003a; Barnes 2004b; MacNee 2005b) and by searching the Internet with use of a variety of terms relevant to the pathogenesis of emphysema. The review includes citations through June 2005.

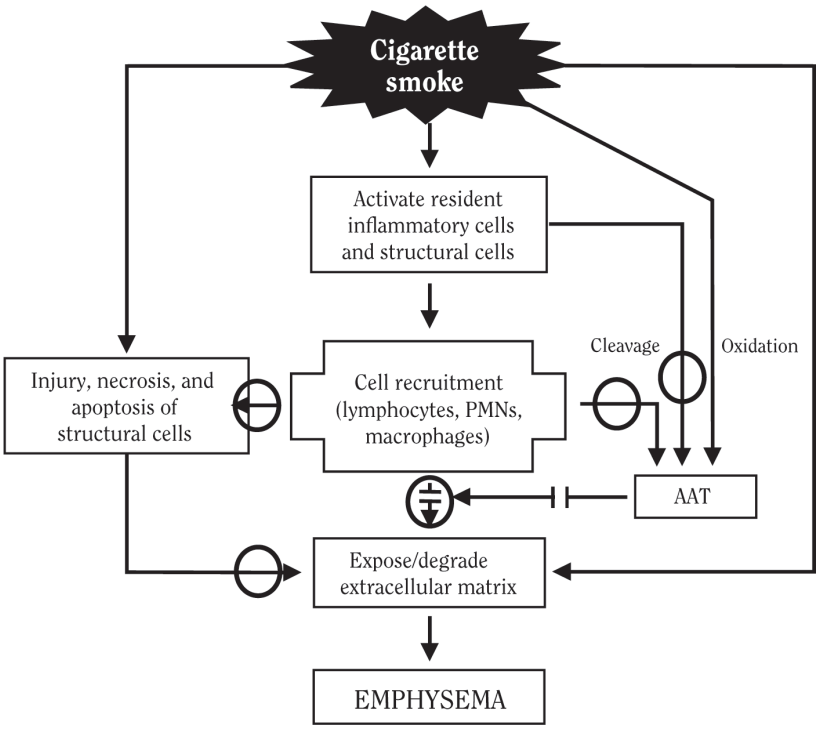
In reviewing the literature, special attention was paid to reports that distinguished between "emphysema" and the all-encompassing term "chronic obstructive pulmonary disease." In recent years this distinction has been made by using chest CT and microscopy of lung tissue.

Introduction

One long-accepted definition of emphysema is "a condition of the lung characterized by abnormal, permanent enlargement of air spaces distal to the terminal bronchiole, accompanied by destruction of their walls, and without obvious fibrosis" (Snider et al. 1985, p. 183). This definition emphasizes the loss of alveolar tissue. However, in emphysema induced by tobacco smoke, the lung tissue exhibits active synthesis of extracellular matrix (Lang et al. 1994; Wright and Churg 1995; Vlahovic et al. 1999), apoptosis, and proliferation of alveolar cells (Calabrese et al. 2005). Accordingly, emphysematous lung tissue should be viewed as undergoing remodeling rather than simply resulting from a destructive process.

The current model of the pathogenesis of emphysema, which involves diverse processes of varying importance, is summarized in Figure 7.18. In this pathogenetic

Figure 7.18 Pathogenesis of smoking-induced pulmonary emphysema



Note: Three pathways for pathogenesis are shown. The circles indicate steps in which proteases are or may be involved. (1) Smoke components recruit inflammatory cells to the lower respiratory system via factors released from alveolar macrophages and structural cells of the lungs. Recruited cells, macrophages, and structural cells release proteases and reactive oxygen species that degrade the extracellular matrix of the respiratory tissues. (2) Smoke components directly affect viability of lung cells, leading to liberation of proteases that can degrade lung matrix. (3) Smoke components may disrupt the function of protease inhibitors such as α1-antitrypsin, facilitating the activity of proteases. **AAT** = α1-antitrypsin; **PMNs** = polymorphonuclear neutrophils.

scheme, accumulation of inflammatory cells in the peripheral tissues of smokers' lungs appears to be pivotal (Finkelstein et al. 1995; Abboud et al. 1998), and proteases from inflammatory cells have multiple potential roles in causing injury.

Inflammatory cells linked to the development of emphysema include neutrophils, macrophages, and lymphocytes. How inflammatory cells are first recruited and activated in response to smoking remains incompletely understood, but the inflammatory process, once initiated, can persist for years after smoking has stopped. Thus, emphysematous tissues from transplant surgery and surgery to reduce lung volume show large numbers of inflammatory cells, even among persons who stopped smoking long before the surgery (Retamales et al. 2001; Shapiro 2001). In addition, some evidence from serial chest CT examinations indicates that emphysema also progresses after the cessation of smoking (Soejima et al. 2000). Products of neutrophils and macrophages can degrade extracellular matrix, inactivate PIs, and convert proenzymes to their active matrix-degrading form.

The role of lymphocytes in emphysema has been a topic of research over the past decade (Finkelstein et al. 1995; Cosio et al. 2002; Boschetto et al. 2003), and findings indicate that T-cell factors can induce macrophages to express proteases (Grumelli et al. 2004). ROS, both in cigarette smoke and released by inflammatory cells or epithelial cells, impinge on protease-antiprotease balance in the lungs in multiple ways, including inactivation of AAT, increased expression of chemokines such as IL-8, activation of MMPs, and induction of the transcription of NF-κB, leading to increased expression of MMPs (MacNee 2005b).

Three discoveries in the 1960s linked elastases to emphysema: (1) association between early-age onset of

emphysema and deficiency of AAT (Eriksson 1965); (2) production of emphysema in experimental animals by putting elastolytic proteases, such as papain, directly into the lung (Gross et al. 1965); and (3) demonstration that neutrophils contain and release a potent elastolytic enzyme (Janoff and Scherer 1968). Together, these discoveries led to formulation of the "elastase-antielastase" hypothesis for the pathogenesis of emphysema. This hypothesis posits development of emphysema in response to unchecked intrapulmonary activity of neutrophil elastase due to an excess of inflammatory cells, a deficiency of intrapulmonary elastase inhibition, or a combination of increased elastolytic burden and decreased elastase inhibitory capacity. This hypothesis remains tenable, but it does not incorporate more recent data indicating that (1) MMPs, including collagenases from inflammatory cells and lung structural cells (Table 7.10) (Foronjy and D'Armiento 2001), are associated with emphysema and (2) the destruction of alveolar walls in emphysema may begin with death of alveolar cells rather than with degradation of the alveolar extracellular matrix (Kasahara et al. 2001; Aoshiba et al. 2003b; Tuder et al. 2003b).

Proteases

Data from Human Studies

Several types of data link proteases to the causation of emphysema in humans: (1) assays of proteolytic

Table 7.10 Matrix metalloproteinases in emphysema

Study	Emphysema detection	Test samples	Observations	Comments
Finlay et al. 1997a	Computed tomography	AM mRNA and culture media	• Increased collagenase 1 (MMP-1) • Increased gelatinase B (MMP-9)	No increase in expression of macrophage elastase (MMP-12)
Finlay et al. 1997b	Computed tomography	BAL fluid	• Increased collagenase activity • Increased gelatinase B (MMP-9)	MMPs probably neutrophil derived
Ohnishi et al. 1998	Histology	Lung tissue from LVRS	• Increased MT-MMP-1 (MMP-14) • Increased gelatinase A (MMP-2)	No increase in expression of macrophage elastase (MMP-12)
Betsuyaku et al. 1999	Computed tomography	BAL fluid and AM culture media	• Increased collagenase 2 (MMP-8) • Increased gelatinase B (MMP-9)	MMPs in BAL fluid from inflammatory cells; no increase in gelatinase B (MMP-9) in AM culture media in emphysema
Segura-Valdez et al. 2000	Histology	Lung tissue from autopsy and LVRS; BAL fluid	• Increased collagenases 1, 2, 3 (MMP-1, -8, -13) • Increased gelatinases A, B (MMP-2, -9)	MMPs expressed by both inflammatory cells and structural cells of lung
Imai et al. 2001	Histology	Lung tissue from lung transplantation and LVRS	• Increased collagenase 1 (MMP-1)	Collagenase expressed by type II cells; no increase in expression of macrophage elastase (MMP-12)
Minematsu et al. 2001	Computed tomography	Cellular DNA	• Increased gelatinase B (MMP-9) polymorphism in smokers with emphysema	Polymorphism also associated with increased incidence of coronary atherosclerosis

Source: Adapted from Hogg and Senior 2002 with permission from BMJ Publishing Group Ltd., © 2002.
Note: **AM** = alveolar macrophage; **BAL** = bronchoalveolar lavage; **LVRS** = lung volume reduction surgery; **MMP** = matrix metalloproteinase; **mRNA** = messenger RNA; **MT** = membrane type.

activity and protease content in BAL fluid; (2) measurements of protease proteins, proteolytic activities, and protease mRNAs associated with alveolar macrophages from emphysematous lung; (3) immunostains of proteases in emphysematous tissue; and (4) determinations of protease mRNAs in lung tissue. The association between AAT deficiency and emphysema is perhaps the strongest evidence (*American Journal of Respiratory and Critical Care Medicine* 2003).

Bronchoalveolar Lavage

The BAL technique samples cells and mediators from the lower respiratory tract, but in COPD the volumes of lavage recovered are often low (Linden et al. 1993; Soler et al. 1999), and the presence of emphysema adversely influences the recovery of BAL fluid (Löfdahl et al. 2005). In one study of patients with COPD who were scored for emphysema by chest CT, patients with high scores had recoveries of fluid less than one-half the volume for those with low scores.

Numerous studies have examined proteases in BAL fluid. In studies with evaluation for emphysema, it appears that protease levels were higher in some persons with emphysema than they were in control groups (Muley et al. 1994; Yoshioka et al. 1995; Betsuyaku et al. 1995, 1996, 1999, 2002, 2003; Finlay et al. 1997a,b; Abboud et al. 1998; Takeyabu et al. 1998). The proteases included elastase, both free and in complex with AAT; MMP-1, -8, -9, -12, and -14; and cysteine proteases. However, interpretation of these data is limited by three considerations: (1) although emphysema is documented by CT, quantification of the emphysema is not reported in most studies; (2) studies do not always explicitly state whether persons with emphysema are current smokers; and (3) some control groups included nonsmokers and smokers. Regardless of these problems, the presence of emphysema is associated with no more than modest, approximately twofold, increases in proteases.

Numerous factors are known to regulate MMPs, a class of proteases increasingly implicated in emphysema, but few studies have assessed the expression of MMP regulatory factors in the context of emphysema. In one study, levels of the extracellular MMP inducer, basigin, a transmembrane protein that stimulates production of several MMPs, were much higher in BAL fluid from current and former smokers than from those who never smoked (Betsuyaku et al. 2003). Among smokers, however, levels did not differ for persons with or without emphysema.

Alveolar Macrophages

Alveolar macrophages appear to have a central role in orchestrating inflammation in COPD through production of cytokines, chemokines, and ROS, in addition to being a source of proteases and PIs (Barnes 2004a; Shapiro 2005). However, few studies have focused on macrophages from lungs with documented emphysema (Muley et al. 1994; Betsuyaku et al. 1995; Finlay et al. 1997b), and in these studies the measurements have been from macrophages that have been in culture, making the relevance of the findings to the in vivo state unclear. Despite these caveats, studies of emphysema suggest an increase in alveolar macrophage mRNA for MMP-1 (collagenase 1) and MMP-9 (gelatinase B) (Finlay et al. 1997a; Betsuyaku et al. 1999). Regardless of the presence of emphysema, however, alveolar macrophages from smokers express more MMP-9, an elastolytic protease, than do those from nonsmokers (Russell et al. 2002a). MMPs appear to account for most of the elastolytic activity released from alveolar macrophages of persons with COPD (Russell et al. 2002b). MMP-12, a protease strongly implicated in smoke-induced emphysema in mice (Hautamaki et al. 1997), was more recently found to be increased in macrophages of persons with COPD (Molet et al. 2005). The role of current smoking is not clear in this observation, however, and diagnosis of emphysema was not documented in the study.

Promising techniques are becoming available for analysis of the products of alveolar macrophages in association with smoking, COPD, and emphysema (Koike et al. 2002; Wu et al. 2005). Considering that most of the macrophages within the lungs are associated with tissue, analysis of alveolar macrophages harvested by BAL may not represent all the phenotypes of macrophages in the lung. Laser-capture microdissection of pulmonary macrophages from histological lung sections is a method for procuring macrophages within the tissue, as well as in alveolar spaces (Fuke et al. 2004), and future use of this approach is expected to provide much more data on macrophage proteases in the context of emphysema.

Studies of Lung Tissue

Immunohistochemistry. Studies of emphysematous lung tissue support the hypothesis that proteases are involved in the pathogenic process. With use of antibody to human elastin, several types of abnormalities of elastic fiber were found in the lungs of persons with emphysema, including fragmented elastic fibers in AAT deficiency and poorly formed elastic fibers and clumps of elastin in smokers with centriacinar emphysema (Fukuda et al. 1989). The clumps appear to be from synthesis of new aberrant elastin, resembling changes observed in experimental elastase-induced emphysema (Kuhn et al. 1976). In lungs confirmed to have emphysema, alveolar macrophages,

interstitial cells, and epithelial cells express immunoreactive MMP-1 and MMP-2 (gelatinase A) (Segura-Valdez et al. 2000). Structural cells in emphysematous lungs express MMP-1 (Imai et al. 2001) and MMP-14 (membrane-type 1 MMP [MT1-MMP]) (Ohnishi et al. 1998).

Gene profiling. Gene profiling of emphysematous lung tissue has found only limited changes in proteases and PIs in comparisons with control lung tissue (Golpon et al. 2004; Ning et al. 2004; Spira et al. 2004). However, these data represent small numbers of lungs, and estimates of emphysema by chest CT or morphometry of fixed lung have not been uniformly provided. Also, the tissue analyzed has not always been limited to alveolar parenchyma. Gene profiling does not quantify neutrophil proteases and circulating PIs produced at sites other than the lungs.

Direct effects of cigarette smoke. Cigarette smoke has long been thought to induce protease expression in the lungs indirectly through cytokines (Churg et al. 2002, 2004), but smoke may act directly on structural cells of the lungs to induce protease expression. In response to exposure to smoke, human small airway epithelial cells (Mercer et al. 2004) and human lung fibroblasts (Kim et al. 2004) in culture increased expression of MMP-1 without a concomitant increase in the expression of tissue inhibitor of metalloproteinase (TIMP)-1. In some studies, cytokines enhanced the direct effects of smoke (Mercer et al. 2004).

Biomarkers of Protease Involvement in Emphysema

As noted previously, elastic fibers in emphysematous lung tissue show disruptions and fenestrations of the elastin (Fukuda et al. 1989; Finlay et al. 1996) that suggest degradative events (see "Studies of Lung Tissue" earlier in this chapter). Elastin-derived peptides (desmosines) in plasma, serum, and BAL fluid are markers of elastin breakdown. A number of desmosine assays have been devised, and a highly sensitive, precise assay method (Ma et al. 2003) has been applied in studies of AAT supplementation therapy (Stolk et al. 2005). Increased urinary desmosines have been reported in smokers (Stone et al. 1995) and in persons who had rapid declines in FEV_1 (Gottlieb et al. 1996), but the presence of emphysema was not determined. Similarly, levels of elastin-derived peptides found in BAL fluid from current smokers were higher than those from former smokers and lifetime nonsmokers, regardless of associated mild COPD or emphysema (Betsuyaku et al. 1996).

Therapy to Control Proteases

The most direct antiprotease therapy for control of emphysema is supplementation with AAT for patients with severe AAT deficiency (*American Journal of Respiratory and Critical Care Medicine* 2003). Usually given intravenously once a week, this therapy appears to slow the rate of decline in FEV_1, reduce the number of lung infections, enhance survival, and reduce lung inflammation as measured by sputum markers, although the optimal dose is still under study (Stoller and Aboussouan 2004; Stolk et al. 2005). In patients with moderate-to-advanced emphysema who are not AAT deficient, oral all-*trans* retinoic acid lowered plasma levels of MMP-9 and release of MMP-9 from alveolar macrophages without affecting levels of TIMP-1, so the balance of protease activity appeared to shift toward protease inhibition (Mao et al. 2003). Whether these effects translate into a therapeutic benefit for emphysema is uncertain.

Data from Animal Models

By incorporating measurements and experimental designs that are not possible in human studies, animal models have improved understanding of the role of proteases in emphysema and are useful in evaluating agents for antiprotease therapy in patients with emphysema (Hele 2002; Voelkel 2004).

Most recent models of emphysema have used mice. These animals provide the convenience of genetic manipulation, and findings indicate that humans and mice may have shared susceptibility factors for exposure to smoke (Shapiro et al. 2004). Emphysema can be induced in normal mice with cigarette smoke (Hautamaki et al. 1997) but significant differences in susceptibility exist among strains (Cavarra et al. 2001a; Guerassimov et al. 2004). By genetic manipulation, emphysema can be induced in resistant strains. For example, mice deficient in Nrf2, a transcription factor for antioxidant and detoxifying genes, develop emphysema in response to smoke, even though their ICR strain is normally resistant to the effect of smoke (Rangasamy et al. 2004). Overexpression of certain proteins in the lung (e.g., IL-13) can lead to emphysema without exposure to an exogenous factor (Table 7.11) (Zheng et al. 2000). However, deleting the expression of certain proteins, such as surfactant protein D (Wert et al. 2000) and TIMP-3 (Leco et al. 2001), in the lungs can also lead to the development of emphysema. Inflammation is a common feature of these and other models, but inflammation does not appear to be required in all models. Emphysema induced by an *MMP-1* transgene (D'Armiento et al. 1992) or by severe caloric restriction (Massaro and Massaro 2004) occurs without overt inflammation.

Although enlarged terminal air spaces are the hallmark of emphysema, it is important to distinguish between enlargement attributable to faulty alveolar formation during development and that occurring after normal

Table 7.11 Mouse models of overexpression of a protein leading to emphysema

Study	Mediator	Promoter	Lung pathology	Mechanism
D'Armiento et al. 1992	Human interstitial collagenase (MMP-1)	Haptoglobulin	Disruption of alveolar walls and coalescence of alveolar spaces	Degradation of collagen by collagenase
Wang et al. 2000	Interferon-gamma	CC10-rtTA	Emphysema with alveolar enlargement, enhanced lung volumes, and enhanced pulmonary compliance	Increased macrophages and neutrophils in BAL fluid; decreased secretory leukocyte proteinase inhibitor; increased MMP-9 and -12 and cathepsins B, D, H, L, and S
Zheng et al. 2000	Interleukin-13	CC10-rtTA	Emphysema with enhanced lung volumes and compliance, mucus metaplasia, and inflammation	Increased MMP-2, -9, -12, -13, and -14 and cathepsins B, H, K, L, and S; treatment with MMP or cysteine proteinase antagonists significantly decreased emphysema and inflammation but not mucus

Source: Adapted from Mahadeva and Shapiro 2002 with permission from BMJ Publishing Group Ltd., © 2002.
Note: **BAL** = bronchoalveolar lavage; **CC10-rtTA** = clara cell 10-kDa protein promoter-reversible tetracycline transactivator; **MMP** = matrix metalloproteinase.

lung development. Thus, models of enlargement in which alveolar development is abnormal, such as in MT1-MMP deficiency (Atkinson et al. 2005), may not be relevant to human emphysema associated with smoking, in which the presumption is that the lung was previously normal.

Despite strong evidence implicating elastases in the pathogenesis of emphysema, research findings beginning in 1992 (D'Armiento et al. 1992) indicate that collagenolytic enzymes that do not degrade elastin may also be involved in the pathogenesis of emphysema. The key discovery was the finding of emphysema in mice engineered to harbor a transgene consisting of a haptoglobin promoter linked to the human *MMP-1* (interstitial collagenase) gene. These mice show expression of the *Mmp-1* gene in lung tissue, enlarged air spaces, bullous lesions, and reduced collagen fibers in alveolar walls and pleura. Depending on the level of transgene expression, the lung lesions can start either soon after birth or later, indicating that the emphysema is clearly postdevelopmental (Foronjy et al. 2003). Apart from demonstrating that collagenase activity could lead to emphysema, results with these mice also suggest two conclusions: (1) lung inflammation was minimal, indicating that proteases causing emphysema could come from structural cells of the lungs; and (2) the elastic fibers in the lung showed minimal inflammation, indicating that emphysema can occur without obvious disruption and resynthesis of elastic fibers.

Models of Emphysema Involving Cigarette Smoke

Model systems for exposing mice to cigarette smoke to produce emphysema vary in the cigarettes used, the manner in which cigarette smoke is delivered, and assessment of the dose of smoke actually reaching the animals. Standard research cigarettes are commonly used, and the exposure is produced by directing smoke from a single cigarette to the nose of a mouse restrained in a single-body compartment or by exposing groups of mice that are free to move in a chamber in which cigarette smoke is put into the atmosphere. The intensity of exposure to smoke, if monitored, is typically indexed by the level of carboxyhemoglobin in the blood (Wright and Churg 1995). The smoking regimens usually require nearly daily exposures for months to achieve emphysema.

As noted previously, strains of mice can exhibit extremely different susceptibility to the development of emphysema from smoke inhalation (see "Susceptible Animal Models" earlier in this chapter). These differences in susceptibility are matched by differences in the accumulation of lymphocytes and neutrophils in the lung tissue. Higher numbers of these cell types in the tissue is associated with emphysema.

For four decades, elastases have been foremost in pathogenetic schemes linking proteases to the pathogenesis of emphysema, and in recent years, the importance of elastases has been supported in studies involving mice

with deficiencies of macrophage elastase (Hautamaki et al. 1997) or neutrophil elastase (Shapiro et al. 2003). Mice with no macrophage elastase have virtually complete protection from smoke-induced emphysema; mice without neutrophil elastase have approximately 70 percent protection. The finding that a "knockout" of either of these elastolytic enzymes is protective indicates interplay between these enzymes in the pathogenesis of emphysema. The evidence suggests that neutrophil elastase is the principal culprit in matrix degradation and that macrophage elastase acts, at least partly, as a proinflammatory agent by facilitating release of TNFα (Churg et al. 2004) and as a shield for neutrophil elastase by the capacity to cleave AAT.

Although overexpression of collagenase, as described above, has been associated with emphysema, studies have performed only limited assessment of collagenase expression in response to smoke. In guinea pigs exposed to smoke, collagenase mRNA and protein were found in alveolar macrophages and structural cells of the lungs coincident with decreased lung collagen and enlarged air spaces (Selman et al. 1996).

Antiprotease Therapy for Experimental Smoke-Induced Emphysema

As noted previously, studies of gene disruption have shown that inactivating the genes for MMP-12 (Hautamaki et al. 1997) or neutrophil elastase (Shapiro et al. 2003) provides major protection against the development of emphysema from cigarette smoke (see "Alveolar Macrophages" earlier in this chapter). Similarly, PIs against MMPs or neutrophil elastase have proved effective in mice, in limiting the enlargement of air spaces associated with exposure to cigarette smoke (Table 7.12). Various routes of administration have been tried; ilomastat, a broad-spectrum MMP inhibitor, produced highly significant protection when inhaled (Pemberton et al. 2005). These studies consistently show that compounds protecting against emphysema also produce a concurrent reduction of the typical inflammatory response to exposure to smoke. Accordingly, PIs should be regarded as anti-inflammatory agents, as well as antiproteases.

Apoptosis

In contrast to findings in the lungs of nonsmokers, apoptotic epithelial cells are identifiable in the lungs of smokers (Segura-Valdez et al. 2000; Kasahara et al. 2001; Calabrese et al. 2005), and they are found in isolated alveolar macrophages subjected to cigarette smoke in vitro (Aoshiba et al. 2001a). The apoptotic cell types include alveolar macrophages and lung structural cells. Apoptosis, proteases, and emphysema were linked when emphysema developed within six hours after the protease CASPASE-3 was instilled into the lungs of mice (Aoshiba et al. 2003b). Apoptosis and emphysema have also been produced by intrapulmonary instillation of VEGF receptor blockers (Kasahara et al. 2000) and by producing a temporary reduction in lung VEGF by intratracheal administration of an adeno-associated CRE recombinase virus to mice that have a floxed VEGF (Tang et al. 2004). The mechanisms by which apoptosis leads to emphysema are still not well

Table 7.12 Effects of protease inhibitors in experimental smoke-induced emphysema

Study	Animal	Drug/route/frequency	Smoking protocol	Protection against emphysema[a]
Wright et al. 2002	Guinea pig	ZD0892 Oral Twice a day	5 cigarettes/day 5 days/week	45% (6 months)
Churg et al. 2003	Mouse	AAT Intraparenteral 48-hour intervals	2 cigarettes/day 5 days/week	63% (6 months)
Selman et al. 2003	Guinea pig	CP-471,474 Subcutaneous Once a day	Group smoke chamber 5 days/week	30% (4 months)
Pemberton et al. 2005	Mouse	Ilomastat Aerosol Daily	2 cigarettes/day 6 days/week	96% (6 months)

Note: **AAT** = α1-antitrypsin.
[a]Graded by air space enlargement in comparison of animals exposed to tobacco smoke with unexposed animals.

understood. In the CASPASE-3 study (Aoshiba et al. 2003b), elastase activity was found in BAL fluid, and induction of apoptosis of alveolar type II cells in culture resulted in liberation of elastin peptides from an elastin substrate culture medium. Accordingly, in this model, apoptosis appears to induce emphysema by causing proteolytic degradation of extracellular matrix.

Summary

Since its inception about 40 years ago, the protease-antiprotease hypothesis of emphysema pathogenesis has gained increasing support from studies in persons with emphysema and from those using animal models of emphysema. The high risk of emphysema among persons with AAT deficiency, particularly current smokers, continues to be compelling evidence for the linking of smoking and neutrophil elastase to emphysema. However, other proteases, particularly certain MMPs, appear to be involved in emphysema. Success in protecting mice from smoke-induced emphysema through genetic manipulations of proteases or by treatment with PIs has reinforced the protease-antiprotease hypothesis. For many years, the hypothesis specifically focused on elastase-antielastase imbalance and inflammatory cells infiltrating the lung as the source of proteases. These ideas have been modified in recent years to encompass discoveries that collagenolytic activity may produce emphysema and that structural cells of the lungs may contribute to the protease burden of the lung. Despite great progress, much more needs to be known about the proteolytic mechanisms involved in the pathogenesis of emphysema, such as the role of immune cells in protease regulation in emphysema and the effects of proteases on structural cells of the lungs. New techniques for analyzing biologic materials, combined with the capacity to document and quantify emphysema noninvasively, offer promise for better understanding of the role of proteases in emphysema in the years ahead.

Summary

COPD is a rising cause of morbidity and mortality in the United States and elsewhere. Smoking has long been causally linked to COPD, and decades of clinical and experimental research have provided insights into the mechanisms underlying this causal linkage. The extensive evidence reviewed in this chapter highlights the critical role of oxidative injury, driven by the high level of ROS in cigarette smoke. COPD is the only disease caused by cigarette smoking that is associated with genetic mutations leading to AAT deficiency. This genetic association led to the protease-antiprotease hypothesis on the pathogenesis of emphysema that is now well supported by work in animal models. The ROS in cigarette smoke and secondarily released by epithelial and inflammatory cells can unfavorably tip the protease-antiprotease scale in multiple ways. Although these mechanisms are now well characterized, the factors leading to COPD in the minority of affected smokers are not completely understood.

Evidence Summary

This chapter addresses mechanisms of lung injury by tobacco smoke that lead to development of COPD and considers the role of genetic factors, including specific genes, in increasing risk for COPD. The chapter acknowledges that COPD is a broad phenotypic designation with underlying damage and structural changes in the lung's airways and alveoli. This section systematically evaluates the evidence related to genetic susceptibility and to the two major mechanisms of injury considered: oxidative stress and protease-antiprotease imbalance.

Oxidative Stress

Cigarette smoke contains massive quantities of free radicals in its gas and tar phases; concentrations of free radicals are 10^{15} per puff and 10^{17} per gram, respectively. The approximately 200 or more puffs inhaled daily by a typical smoker of one pack per day would lead to a sustained high-level daily dose of free radicals. The chemical pathways by which these free radicals produce damaging ROS have been well characterized, as have the chemical

reactions by which ROS damage target molecules, including lipids, proteins, and DNA. The plausibility of oxidative stress as a mechanism of disease production is well documented and further affirmed through biomarker studies.

In various experimental systems, oxidative stress from exposure to cigarette smoke causes damage to components of the lung: the epithelium, the airways, and the alveoli. In humans, smoking is followed by a rise in markers of systemic oxidative stress and of oxidative stress affecting the lungs more specifically. The time course of the response to oxidative stress from smoking has also been characterized, showing a rise in markers after exposure to tobacco smoke. There is also substantial evidence for increased levels of oxidative stress markers in the lungs of persons with COPD. This large body of experimental and observational evidence is consistent in demonstrating that ROS can damage the lung and that evidence of oxidative stress is strongly linked with COPD. Oxidative stress is one of several mechanisms contributing to the development of COPD. However, the available evidence has not addressed whether oxidative stress is a necessary mechanism or sufficient by itself to cause COPD.

Genetic Susceptibility to Cigarette Smoke

All smokers do not develop COPD, indicating that smoking alone is not sufficient to cause COPD. A variety of lines of evidence support a role for genetic factors in determining susceptibility to cigarette smoke. Familial aggregation of phenotypes for lung function and for COPD has been repeatedly demonstrated. In addition, there is strong clinical and epidemiologic evidence on genetically inherited AAT deficiency and risk for emphysema. This chapter offers the conclusion that protease-antiprotease imbalance is involved in the development of emphysema and sufficient by itself to produce it.

The general observation that smoking alone does not lead universally to COPD, and the specific observation that genotypes associated with severe AAT deficiency lead to emphysema, imply a role for genetic factors in the pathogenesis of emphysema. To date, however, genetic loci other than *SERPINA1* have not been linked to risk for COPD.

Protease-Antiprotease Imbalance

Emphysema is a prominent and highly prevalent component of the COPD phenotype. The potential role of a protease-antiprotease balance shifted toward unchecked proteolytic activity was first identified with the finding of enhanced risk for emphysema in persons with AAT and the supporting experimental demonstration that emphysema could be produced experimentally by proteolytic enzymes. These enzymes damage the elastin in the lung, which is essential to maintaining the lung's elasticity and ventilatory function. Thus, it is directly plausible that this mechanism has a role in producing emphysema.

Three lines of evidence support a role for protease-antiprotease imbalance in causing emphysema. First, in experimental models and in smokers, a shift of protease-antiprotease balance in a destructive direction has been repeatedly demonstrated, as has corresponding injury to elastin fibers. Second, in animal models, instillation of proteolytic enzymes can produce emphysema, as does exposure to cigarette smoke. Genetically engineered mice with deficient macrophage or neutrophil elastase are protected from smoke-induced emphysema. Third, persons with homozygous AAT deficiency who smoke develop emphysema at a young age.

This substantial consistent and complementary evidence supports a causal role for protease-antiprotease imbalance in the pathogenesis of emphysema, a critical element of the COPD phenotype. This mechanism is also substantially plausible because of the importance of elastin in determining ventilator function. The evidence further indicates that protease-antiprotease imbalance is sufficient to produce emphysema in smokers. Human evidence comes from the long-described and well-documented occurrence of early-onset emphysema in smokers with low levels of AAT consequent to mutations of *SERPINA1*. Findings in animal models confirm the sufficiency of this mechanism.

Conclusions

1. Oxidative stress from exposure to tobacco smoke has a role in the pathogenetic process leading to chronic obstructive pulmonary disease.

2. Protease-antiprotease imbalance has a role in the pathogenesis of emphysema.

3. Inherited genetic variation in genes such as *SERPINA3* is involved in the pathogenesis of tobacco-caused chronic obstructive pulmonary disease.

4. Smoking cessation remains the only proven strategy for reducing the pathogenetic processes leading to chronic obstructive pulmonary disease.

Implications

Two major mechanisms underlying the causation of COPD by cigarette smoking have been identified: oxidative stress (injury) and protease-antiprotease imbalance. These mechanisms are triggered by the inhalation of combustion products directly into the lungs of smokers. Although the lung has defense mechanisms that function to check injury by inhaled agents, these defenses are overwhelmed by the sustained inhalation of cigarette smoke. Doses of inhaled smoke that could be tolerated without resulting in oxidative injury and protease-antiprotease imbalance have not been identified. Smoking cessation remains the only way to check and halt these processes.

COPD is the only disease caused by smoking that is strongly associated with a specific genetic disorder, namely, AAT deficiency. The occurrence of COPD in young smokers should trigger testing for AAT deficiency, but such screening is not recommended for the general population. Studies in progress are expected to extend understanding of the genetic basis of COPD.

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