chapter 15 Toxic Responses of the Respiratory System

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Acknowledgments

"Since the time of Hippocrates the growth of scientific medicine has in reality been based on the study of the manner in which what he called 'Nature' of the living body expresses itself in response to changes in the environment, and reasserts itself in face of disturbances and injury"

—John Scott Haldane (Haldane, 1922)

HISTORICAL PERSPECTIVE

Toxic substances can disrupt the respiratory system and distant organs after chemicals enter the body by means of inhalation. Pathological changes in the respiratory tract also can be a target of blood-borne agents. Inhalation toxicology refers to the route of exposure, whereas respiratory toxicology refers to target organ toxicity. This chapter reviews the toxic responses of the respiratory system and is an update of the previous chapter (Witschi *et al*., 2008).

Historically, respiratory toxicology is a keystone of medicine, dating back to Hippocrates. In his medical thesis *On Airs, Waters, and Places*, Hippocrates recommended that physicians evaluate local atmospheres to discover the causes of diseases (Adams, 1849). In 1661, John Evelyn appealed to the English King and Parliament for relief from the poor air quality of London that was a result of the burning of "sea-coale" (a brown coal likely enriched in sulfur that washed up on the banks of the River Thames (Evelyn, 1661). This situation continued and became worse in the 19th century when the Industrial Revolution quickened awareness of respiratory toxicology due to air pollutions (see Chap. 29).

Later, Bernardino Ramazzini proposed that clinicians evaluate the relationships between occupational atmospheres and disease pathogenesis, starting a long history of respiratory toxicology role in occupational medicine. He observed that "corruption of the

atmosphere" can be at the origin of many respiratory diseases. In his work *De Morbis Artificum Diatriba* (*Diseases of Workers*) (1713), he stated, "Miners who maintain an almost daily contact with evil powders in the earth's depths… have lungs which absorb mineral exhalations and must be the first to suffer the attack of poisonous fumes.… The mortality rate of miners is very high and, as a proof of this, we remember that their wives re-marry many times." Supporting the concept of exposure, he writes: "It is not only miners working in mines who run the risk of dying from diseases due to metals: so do many others working around mines". He also supported the concept of prevention over treatment stating "Prevention is better than cure, just as it is better, on seeing storm arrive, to get under cover than to suffer its damages" (Bisetti, 1988).

During the 19th century the relationship between dusty trades and bronchitis (Thackrah, 1832), silica dust and pneumoconiosis (Holland, 1843) became well recognized. In 1873, excessive bronchitis deaths were attributed to London fogs (smog) (British Medical Journal, 1880). In a report on London fogs (smog) in 1880 that lead to 1817 excessive deaths, Russell states "And smoke in London has continued probably for many years to shorten the lives of thousands, but only lately has the sudden, palpable rise of the death-rate in an unusually dense and prolonged fog attracted much attention to the depredations of this quiet and despised destroyer." He goes on to note: "A London fog is brown, reddish-yellow, or greenish, darkens more than a white fog, has a smoky, or sulphurous smell, is often somewhat dryer than a country fog, and produces, when thick, a choking sensation. Instead of diminishing while the sun rises higher, it often increases in density, and some of the most lowering London fogs occur about midday or late in the afternoon. Sometimes the brown masses rise and interpose a thick curtain at a considerable elevation between earth and sky. A white cloth spread out on the ground rapidly turns dirty, and particles of soot attach themselves to every exposed object." In 1884, John Aitken proposed that particles contribute to the haze and alter the color of the sunset (Aitken, 1884).

Also toward the end of the 19th century John Scott Haldane identified carbon monoxide as the lethal constituent of "afterdamp," a gas mixture created by combustion in mines, after examining many bodies of miners killed in pit explosions (Haldane, 1896). He noted skin was colored cherry-pink from carboxyhemoglobin and studied carbon monoxide's ability to displace oxygen. An experimentalist, he had investigated the effect of carbon monoxide on his own breathing in a chamber (Haldane, 1895). In the late 1800s, he supported efforts to improved mine safety by introducing gas masks for rescue workers and the use of small animals (canaries and white mice) to detect dangerous levels of carbon monoxide.

By the start of the 20th century, respiratory toxicology became even more inseparable from occupational medicine in which coal workers' pneumoconiosis (black lung), silicosis, and byssinosis were noted in specific trades, even in the absence of bacterial infection (tuberculosis) (Oliver, 1902; Hoffman, 1918; Blanc, 2005).

During World War I respiratory toxicology turned to the dark side. Efforts of Fritz Haber (Germany) (Witschi, 2000) and Victor Grignard (France) (Hodson, 1987) lead to the development and use of chlorine, phosgene, and other gases in chemical warfare. This was accompanied by toxicological studies of poison gases in laboratory animals, in which Haber noted that exposure to a low concentration of a poisonous gas for an extended time could produce the same effect (death) as exposure to a high concentration for a short time. The so-called "Haber's rule" is $C \times t = k$, where C is the gas concentration (mass/volume), t is the exposure time producing a given toxic effect, and k is a constant. Although sometimes useful, this rule has many exceptions and should be applied with caution.

In the 1920s, Yandell Henderson and Howard Haggard began testing numerous noxious chemicals and culminated a compendium of dose–response analysis and median lethal concentration (LC_{50}) of a number of noxious chemicals (Henderson and Haggard, 1943). Although a simple test of lethality clearly has limitation in understanding the mechanism of toxicity, it still has value today. The results from Henderson and Haggard's efforts are often referred to in assessments of Immediately Dangerous to Life or Health concentrations, which can be informative following accidental inhalation exposures.

From the 1920s to 1950, CN Davies (1949), Lucien Dautrebande (Dautrebande *et al*., 1948), Phillip Drinker (Drinker *et al*., 1928), Lars Friberg (Friberg, 1948), Theodore Hatch (Hatch, 1937; Hatch and Hemeon, 1948), Earl King (Robson *et al*., 1934) Frank Patty (Patty, 1949), HD Landahl (Landahl and Black, 1947), Leslie Silverman (Silverman and Lee, 1946; Silverman and Whittenberger, 1949), Henry Smyth Jr (Smyth, 1946), Herbert Stokinger (Stokinger, 1949; Stokinger *et al*., 1950), and many others advanced exposure science (ie, measure of concentrations of gas and particles in workplace and ambient atmospheres), began controlled inhalation exposure of laboratory animals, and made recommendations for human occupational exposure limits (threshold limit values [TLV]).

These investigations provided the foundation for the modern era of respiratory toxicology lead by Yves Alarie (Alarie *et al*., 1961), Roy Albert (Albert and Arnett, 1955), Mary Amdur (Amdur *et al*., 1952), David Bates (Young *et al*., 1964), Eula Bingham (Bingham *et al*., 1968), Joe Brain (Brain, 1970), Louis Casarett (Casarett, 1960), David Coffin (Coffin *et al*., 1968), John Craighead (Tegtmeyer and Craighead, 1968), Carol Cross (DeLucia *et al*., 1972), Tore Dalhamn (Dalhamn and Rodin, 1956), Robert Drew (Drew and Eisenbud, 1970), Juraj Ferin (Ferin *et al*., 1965), Robert Frank (Frank, 1970), Gustav Freeman (Freeman and Haydo, 1964), Donald Gardner (Gardner *et al*., 1969), Bernard Goldstein (Goldstein and Balchum, 1967), Elliot Goldstein (Goldstein *et al*., 1969), Gareth Green (Green and Carolin, 1967), Paul Gross (Gross *et al*., 1952), Theodore Hatch (Palm *et al*., 1956), Harold Hodge (Hodge *et al*., 1956), Sidney Laskin (Laskin *et al*., 1963), Morton Lippmann (Lippmann and Albert, 1968), Paul Morrow (Morrow *et al*., 1958), J. Brian Mudd (Mudd *et al*., 1969), Shelton Murphy (Murphy *et al*., 1963), Jay Nadel (Nadel and Comroe, 1961), Norton Nelson (Nelson, 1955), Ed Palmes (Palmes *et al*., 1959), Otto Raabe (Raabe, 1967), Verald Rowe (Rowe *et al*., 1952), Ragnar Rylander (Rylander, 1968), Irving Selikoff (Selikoff *et al*., 1965), Norman Staub (Kato and Staub, 1966), Walter Tyler (Tyler and Pearse, 1965), and Hanspeter Witschi (Witschi and Aldridge, 1968). These individuals and many others enable a better understanding of the deposition and effects of gases and particles on the respiratory tract.

RESPIRATORY TRACT STRUCTURE AND FUNCTION

Oronasal Passages

Structure The respiratory tract is divided into the upper respiratory tract (extrathoracic airway passages above the neck) and lower respiratory track (airway passages and lung parenchyma below the pharynx) (Harkema *et al*., 2006) (Fig. 15-1). The upper respiratory track reaches from the nostril or mouth to the pharynx and functions to conduct, heat, humidify, filter, and chemosense incoming air. Leaving the nasal passage, air is warmed to about 33°C and humidified to about 98% water saturation. Air is filtered in the nasal passages with highly water-soluble gases being absorbed efficiently. The nasal passages also filter particles, which may be deposited by impaction or diffusion on the nasal mucosa. Many species, particularly mice and rats, are obligate nose breathers in

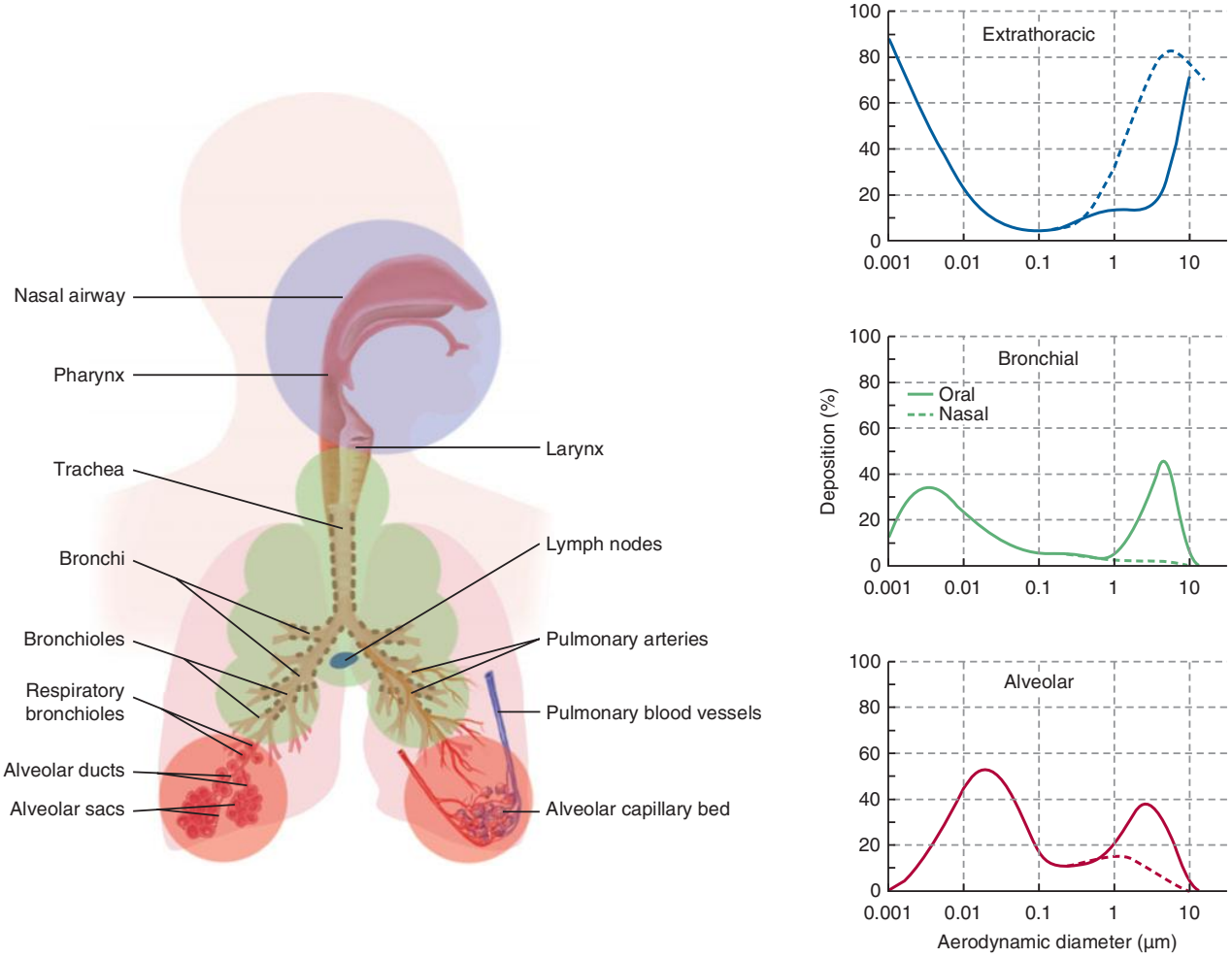

Figure 15-1. *Major regions of the respiratory tract and predicted fractional deposition of inhaled particles in the extrathoracic, bronchial, and alveolar region of the human respiratory tract during (solid line) oral or (dashed) nasal breathing.* (Adapted from Fig. 8 in Oberdörster *et al.* (2005) with drawing courtesy of J. Harkema and data from ICRP [1994].)

which air passes almost exclusively through the nasal passages. Other species, including humans, monkeys, and dogs, inhale air through both the nose and the mouth (oronasal breathers).

The surface area of oronasal region has been estimated to be 4700 cm^2. In mammals, the nasal passages are separated by a cartilaginous septum and the hard and soft palates form the base. Filtration, heating, and humidification are greatly aided by aqueous layer lining the mucosa and turbinates, which are perturbed from the lateral nasal walls. To warm the air, blood flow in the turbinates is retrograde to the inward direction of the air and can be modulated by pterygopalatine ganglion innervation of the venous plexus.

Turbinates vary in size and shape with the anterior being simple and the posterior being more complex. The airflow through the nasal passage is complex, and the narrowest region (smallest cross-sectional area) is located in the anterior aspect of the anterior turbinate. This region has the highest airflow and can be viewed as a nasal valve (ostium internum). The resistance of this region limits the amount of air that can be inhaled through the nose. In oronasal breathers, oral breathing can be initialed and will vary based on the workload, speech, and nasal congestion.

In humans, three flat turbinates are fairly simple structures. The inferior turbinates are the largest (~8 cm long) and are responsible for the majority of the control of airflow direction, humidification, heating, and filtering of air inhaled through the nose. The smaller middle turbinates (~4–6 cm) project downward over the openings of the maxillary and ethmoid sinuses, and protect the sinuses from pressurized nasal airflow. Most of the inhaled air travels between the inferior turbinate and the middle turbinate. The superior turbinates are smaller structures and serve to protect the olfactory bulb. In rodents, the anterior portion of the nasal cavity contains a dorsal nasal turbinate and a ventral maxilloturbinate, both with simple scroll structures. Posterior to these turbinates are complex multiscrolled ethmoturbinates, which contain ~50% of the surface area of the rodent nasal passage. However, the amount of airflow over the ethmoturbinates region has been estimated to be only 10% to 15% of the air passing through the nose, and thus the complexity of this structure in rodents may contribute little additional risk of contact or damage.

The nasal passages are lined with stratified squamous epithelium in the anterior vestibule, nonciliated cuboidal/columnar epithelium in the anterior chamber, and ciliated pseudostratified respiratory epithelium in the remainder of the passage including the turbinates. The cell types of the nasal respiratory epithelium are similar to the cell types of the conducting airways. The turbinates also contain airflow pressure- and temperature-sensing neural receptors linked to the trigeminal nerve.

Sensory Functions In addition to conducting, conditioning, and filtering air to the lower respiratory tract, a major function of the oronasal passage is chemosensory (Morris, 2001; Feron *et al*., 2001). Nasal epithelia can metabolize many foreign compounds by cytochrome P450 and other enzymes. Humans can distinguish between more than 5000 odors. The detection of odor can be protective and can induce avoidance behaviors. Odorant can be added to the otherwise colorless and almost odorless gas used by consumers (eg, mercaptans to methane), to assist in detecting leaks and thereby preventing fires or explosions.

Although the detection threshold concentrations can be low, a concentration only 10 to 50 times above the detection threshold value often is the maximum intensity that can be detected by humans. In contrast, the maximum intensity of sight or hearing is about 500,000 times and 1 to 10 trillion times that of the threshold intensity. For this reason, smell often identifies the presence or absence of odor rather than quantifies concentration. In addition, odor thresholds vary greatly between individuals (>1000 fold) and can be altered by allergies or nasal infections, and individuals can acclimate to odors. Some individuals cannot smell certain odors, for example, 0.1% cannot detect mercaptans in natural gas. Olfactory acuity also decreases with age (decreasing by 20%, 60%, and 70% at age of 20, 60, and 80 years). Therefore, about 30% of the elderly cannot detect mercaptans in natural gas. Lastly, odor thresholds for many compounds (eg, chlorinated solvents) are often higher than the Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PELs). Therefore, odor should not be used as a measure of safety.

Chemosensory function of the nasal passages is accomplished by a wide variety of specialized receptors in major subtypes including (1) olfactory, (2) trace amine–associated receptors (TAARs), (3) membrane guanylyl cyclase GC-D, (4) vomeronasal, and (5) formyl peptide receptors (FPRs) (Table 15-1).

The olfactory epithelium contains specialized chemosensory olfactory neurons located above superior turbinates. Airflow in this region of the nasal passage is typical low, thus sniffing can increase perception. This may enable the assessment of multiple odors and strength of a smell through intermediate sampling. Capable of regeneration, olfactory neurons form the first cranial nerve and directly lead to the olfactory bulb in the brain. These cells have surface olfactory receptor proteins in cilia that interact with odorant molecules (DeMaria and Ngai, 2010). Olfactory receptors are 7-transmembrane domain G-protein–coupled receptors that mediate transduction of odorant signals through formation of cyclic adenosine monophosphate (cAMP) (Fleischer *et al.*, 2009). The olfactory receptor gene family is one of the largest in the genome, with over 400, 850, 1100, and 1200 members in humans, dogs, mice, and rats, respectively. Olfactory receptors are also involved in developmental events, including the patterning of the olfactory sensory neuron synaptic connections in the brain.

Also in the olfactory region and originally identified because of activation by amine, TAARs detect trace amine (including 2-phenylethylamine, tyramine, tryptamine, and octopamine) and other substances (Fleischer *et al.*, 2009). Low-molecular-weight amines have a fishy or putrid odor. These odorants can be found in foods (including fish, chocolate, alcoholic beverages, cheese, soy sauce, sauerkraut, and processed meat) and can be generated during fermentation or decay. Certain trace amines are neurotransmitters and are found in the brain. Compared to olfactory receptors, the number of distinct TAAR subtypes is low (15 in mice and 6 in humans). In mouse urine, trace amine concentration varies by gender or during stress, suggesting that TAARs might be involved in the detection of "urine-borne" signals.

Another olfactory sensory neuron receptor is the membrane guanylyl cyclase GC-D receptor, which contains a cyclic guanosine monophosphate (cGMP)-dependent phosphodiesterase PDE2A and a cGMP-sensitive cyclic nucleotide-gated ion channel (Fleischer *et al.*, 2009). These receptors are localized to olfactory sensory neuron apical cilia and detect the natriuretic peptides: uroguanylin (which is also found in urine) and guanylin. In mice, GC-D receptors also can detect carbon dioxide by conversion into bicarbonate via carbonic anhydrase. In contrast to rodents, carbon dioxide is odorless to humans, and the GC-D gene is a pseudogene (ie, a gene that is present but does not yield a functional protein) in humans and other primates.

The main olfactory bulb is accompanied by the accessory olfactory bulb. Neurons from these two systems do not interconnect and the two systems function separately in the integration of specific chemicals. In rodents, the accessory olfactory bulb contains olfactory neurons that lead to the vomeronasal organ in the nose. Vomeronasal neurons can respond to olfactory stimuli that can be of higher molecular weight including nonvolatile chemicals (Touhara and Vosshall, 2009). Vomeronasal receptors exist in two protein families, VN1R and VN2R. These receptors are similar to pheromone receptors. Pheromones are chemical signals that elicit specific physiological and behavioral responses in recipients of the same species. Similar to olfactory receptor genes several vomeronasal genes exist in rodents, with few in humans, that is, 2, 8, 163, and 226 members in human, dogs, rats, and mice, respectively. Humans have not been demonstrated to generate or respond to pheromones. Expressed only during fetal gestation, vomeronasal receptors are thought to be merely vestigial in humans.

In addition, the vomeronasal organ contains FPRs that are activated by bacterial or mitochondrial formylated peptides (Fleischer *et al.*, 2009). These receptors were initially identified in leukocytes in which *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)

Table 15-1

Oronasal Sensory Receptors I

RECEPTOR PROTEIN FAMILY	SYMBOL	LIGAND	HUMAN	MOUSE
Olfactory receptor	OR	Odorants	>400	>1200
Trace amine-associated receptor	TAAR	Amines	6	15
Guanylatecyclase, type D	GUCY	Natriuretic peptides	1	2
Vomeronasal receptor	VN1R/VN2R	Pheromones	2*	226
Formyl peptide receptors	FPR	*N*-Formyl-peptides	3	8

**Only expressed in fetus and may be vestigial.*

Table 15-2

Oronasal Irritant, Thermo-, and Mechanosensory Receptors

RECEPTOR PROTEIN FAMILY	SYMBOL	LIGAND
Transient receptor potential channels	TRP	
Subfamily A (ANKTM1)	TRPA	Natural ingredients: allyl isothiocyanate (wasabi), cinamaldehyde, allicin and allyl sulfides (garlic), carvacrol, isovelleral, and polygodial
		Pain
		Cold (<17°C)
		Mechosenstory (Strech)
		Irritants (acrolein, isocyanates, tear gas, ozone, etc)
Subfamily C (Canonical)	TRPC	Mechanosensory
Subfamily M (Melastain)	TRPM2	Hydrogen peroxide
		Heat (>38°C)
	TRPM8	Menthol, eucaliptol
		Cold (<17°C)
Subfamily ML (Mucolipins)	MCOLN	Acid (low pH)
Subfamily P (Polycystic kidney disease)	PKD	Mechanosensory
		Acid (low pH)
Subfamily V (Vanilloid)	TRPV1	Capascin, allicin, and allyl sulfides (garlic)
		Moderate heat (≥43°C)
	TRPV2	High heat (>52°C)
Taste receptors	TAS	
Subfamily 1R	TAS1R	Umami (glutamate)
		Irritants (acrolein, isocyanates, tear gas, ozone, etc)
Subfamily 2R	TAS2R	Bitter
		Irritants (acrolein, isocyanates, tear gas, ozone, etc)
Subfamily 3R	TAS3R	Umami (glutamate)

mediates chemotaxis and cell activation. In the mouse vomeronasal epithelium, FPRs are activated by fMLP and other compounds (including lipoxin A4 and cathelicidin antimicrobial peptide) indicating that vomeronasal cells are likely to perform olfactory functions associated with the identification of pathogens or of pathogenic states, thereby enhancing detection of infected cells or contaminated food.

Two evolutionary hypotheses have been proposed to explain the large interspecies difference in the number of chemosensory receptor genes. One states that humans have developed full trichromatic vision and therefore do not need as many chemosensory receptor genes for finding food, mates, or supportive environments. The other is that the number of chemosensory receptor genes has expanded in the rodent lineage because rodents probably need a higher level of olfaction to survive in heterogeneous environments. However, dogs known for a good sense of smell have a smaller number of functional chemosensory receptor genes than mice or rats. This suggests that the relationship between the number of olfactory receptor genes and the sense of smell may not be straightforward. Even more complexity is suggested in that humans can detect certain odors at concentrations equal to or even below those detected by dogs or mice. One reason for this may be that olfactory perception also involves the brain. With a better memory, humans may have better olfactory ability from the small number of genes, particularly in detecting fine differences in food flavors.

Irritant, Thermosensory, and Mechanosensory Functions

In addition to the detection of odor, the detection of irritant chemicals, cold and hot temperatures, or mechanical stress can be a protective mechanism that may limit exposure. The main nerve endings that perceive irritants, the chemical nociceptors also discern temperature and mechanical stress. Two protein families, the transient receptor potential (TRP) channels and the taste (TAS) receptors, perform these functions in the upper respiratory tract (Table 15-2).

TRP channels are ion channels that are permeable to cations, including calcium, magnesium, and sodium. In mammals, 28 genes encode the TRP ion channel proteins that are divided into six subfamilies including TRPA (ANKTM1), TRPC (canonical), TRPM (melastatin), mucolipins (TRPML also known as [aka] MCOLN), polycystic kidney disease (autosomal dominant) (PCK or TRPP), and TRPV (vanilloid) families. TRPA1 and TRPV1 are the major irritant receptors in the nasal passage and are primarily within the trigeminal nerve (Bessac and Jordt, 2008). TRPA1 is responsive to a variety of natural ingredients including allyl isothiocyanate (in mustard and wasabi), cinnamaldehyde (in cinnamon), allicin and allyl sulfides (in garlic and onion), carvacrol (in oregano), isovelleral (a fungal deterrent), and polygodial (in Dorrigo pepper). TRPA1 is also responsive to pain stimuli, cold (≤17°C), stretch, and a wide range of chemical irritants. TRPV1 is responsive to capsaicin (in chili pepper) or moderate heat (≥43°C), whereas TRPV2 is responsive to higher heat (≥52°C). TRPM8 is responsive to menthol (in peppermint and cigarettes) and cold (≤28°C). Lysosomal protein, mucolipins are involved in the late endocytic pathway and in the regulation of lysosomal exocytosis. TRPC proteins are mainly located in the central nervous system and to a lesser extent in peripheral tissues. PCK1, TRPV5, and TRPV6 are calcium entry channels mainly found in the kidney and intestine.

Other chemonsensory receptors are taste receptors (TAS), which are divided into two types (Chandrashekar *et al.*, 2006). Taste buds determine salt, sour, sweet, umami (glutamates and

nucleotides), and bitter. In the mouth, salt may be perceived by sodium ion channels, but this is controversial. Sour also may be perceived by hydrogen ion channels and possibly a TRP channel (polycystic kidney disease 2-like 1). Sweet and umami are perceived by type 1 receptors, which consist of three members (TAS1R1, TAS1R2, and TAS1R3). Taste variety is achieved by formation heterodimers of these proteins, for example, umami is detected by TAS1R1 and TAS1R3 heterodimers and through metabotropic glutamate receptors 1 and 4. Bitter taste is detected by type 2 receptors (TAS2Rs), which is a larger subfamily having over 35 members. Single solitary chemosensory cells (SCCs) are present in the nasal cavity and throughout the airways. In the mouse nose, SCCs contain both TAS1R and TAS2R, which can detect irritants and foreign substances that trigger trigeminally mediated protective airway reflexes.

Conducting Airways

Structure At the beginning of the lower respiratory track is the larynx, which is responsible for speech (phonation). The conducting airways of the lower respiratory tract can be divided into proximal (trachea and bronchi) and distal regions (bronchioles). Conducting airways have a bifurcating structure, with successive airway generations containing about twice the number of bronchi progressively decreasing in internal diameter. In humans, this branching pattern is referred to as irregular dichotomous (because some branches have more or less than two daughters) and resembles the pattern of an oak tree. In laboratory animals, the branching pattern is more monopodial and resembles the pattern of a pine tree. Successive branching has two consequences—it increases total surface area of the airway epithelium, and it increases the cumulative cross-section diameter of the airways. Thus, airflow is faster in the larger diameter proximal airways, whereas airflow is slower in the smaller distal airways. The latter is somewhat counterintuitive because flow through a smaller diameter increases in many incidences (as in a weir), but flow is slower because the larger number of small airways have a much larger cumulative diameter. Thus, the bifurcations of proximal airways are flow dividers and as airway bending points they serve as sites of impaction for particles. Successively narrower diameters ultimately lead to very slow airflows and thereby favor the collection of gases and particles on airway walls by radial diffusion. Eventually a transition zone is reached where cartilaginous airways (bronchi) give way to noncartilaginous airways (bronchioles), which in turn give way to gas exchange regions, respiratory bronchioles, and alveoli. In the bronchiolar epithelium, mucus-producing cells and glands give way to bronchiolar secretoglobin cells (BSCs). The airflow is also altered by airway smooth muscle that surrounds the airways and is under autonomic innervation via the vagus nerve.

Mucociliary Clearance and Antimicrobial Functions In humans, the proximal airway and a portion of the nasal passage are covered by a pseudostratified respiratory epithelium that contains a number of specialized cells including ciliated, mucous, and basal cells (Fig. 15-2). These cells work together to form a mucous layer that traps and removes inhaled material via mucociliary clearance (Fahy and Dickey, 2010). The epithelial cells are covered by an upper mucus layer (a gel-like polymer network of high-molecular-weight mucins) and a lower periciliary liquid layer that separates the epithelial cell surface from the mucus layer. For mucociliary clearance in the airways to function optimally, regulation of ion transport, fluid, and mucus must be coordinated. To move fluid into the airway lumen, the large diameter airway epithelium can secrete chloride ion via chloride channels (Patel *et al*., 2009) and

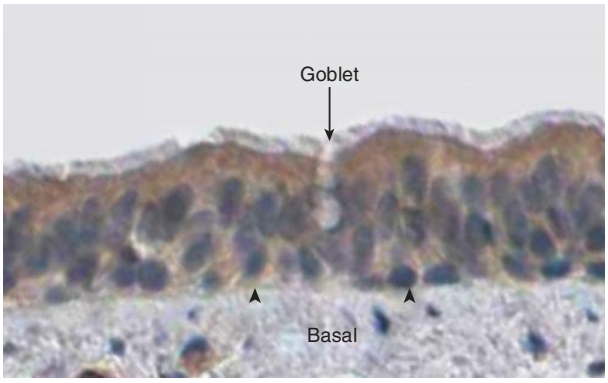

Figure 15-2. *Pseudo stratified respiratory epithelium lines the nasal cavity, trachea, and bronchi.* The surface includes mainly ciliated epithelial cells that may or may not touch the basement membrane, (arrow) surface mucous (goblet) cell, and (arrowhead) basal cells. Photomicrograph modified from the Human Protein Atlas (www.proteinatlas.org) (Uhlen *et al*., 2010).

the cystic fibrosis transmembrane regulator (Chen *et al*., 2010). To move water out of the lumen or alveolus, sodium ion is absorbed via sodium channels. These ionic gradients permit water movement that can travel pericellulary or through specialized proteins called aquaporins.

Ciliated cells have microtubule-based protrusions, cilia (Sanderson and Sleigh, 1981; Salathe, 2007). There are two general types of cilia: motile and primary. Motile cilia exert mechanical force through continuous motion to propel harmful inhaled material out of the nose and lung. Primary cilia often serve as sensory organelles. Motile cilia are ~6 to 10 μm in length with a tubulin-based axoneme motor. The axoneme of each cilia consists of nine outer doublets of microtubules and a single central pair of microtubules (9 + 2 structure) formed by heterodimers of α and β tubulin. In motile cilium, dynein heavy chains on one microtubule interact with an adjacent microtubule that enables ciliary movement through energy generated by ATPase. Primary cilium lack dynein and have 9 + 0 structure.

Ciliary beat frequency is about 12 to 15 Hz, which can change in response to cholinergic (acetylcholine) or purinergic (adenosine or ATP) stimuli that changes in the phosphorylation state of ciliary targets, in intracellular [Ca^{2+}] and in intracellular pH. In addition to controlling ciliary beat frequency, calcium is also involved in synchronizing the beat among cilia of a single cell and between cilia on different cells (Schmid and Salathe, 2011). Adenosine acts through the adenosine A2b receptor (ADORA2B) (Allen-Gipson *et al*., 2011). Motile cilia of the mammalian respiratory epithelium also exhibit both mechanosensory (via TRPV4) and chemosensory (via TAS2Rs) functions. TRPV4 channels respond to mechanical stress, heat, acidic pH, endogenous, and synthetic agonists, and activation leads to increases in intracellular calcium and ciliary beat frequency (Lorenzo *et al*., 2008). In response to bitter compounds, TAS2Rs also increase the intracellular calcium and stimulate ciliary beat frequency (Shah *et al*., 2009).

Mucus cells are full of lucid mucus granules. These granules increase in size as they move toward the apical cytoplasm, which produces a goblet shape and thus surface mucus cells are also called Goblet cells. Mucus consists mainly of water (95%) combined with salts, lipids, proteins, and mucin glycoproteins (Kesimer *et al*., 2009; Ali *et al*., 2011). Mucin glycoproteins provide the gel-like viscoelastic properties of mucus. Of the 20 identified membrane-associated or secretory mucin gene products, 16 have been identified in the airways (Leikauf, 2002b; Ali and Pearson, 2007).

Of these proteins, mucin 5, subtypes A and C (MUC5AC), and MUC5B are the predominant mucins and to a lesser extent MUC2, 7, 8, 11, 13, 19, and 20 are produced by goblet cells on the surface epithelium and mucus cells from submucosal glands (Rose and Voynow, 2006). Membrane-associated mucins in the airways include MUC1, MUC4, and MUC16. Mucus cells can secrete antimicrobial proteins including bactericidal permeablility increasing (BPI) protein, BPI fold containing family A, member 1 (BPIF1A) (aka palate, lung, and nasal epithelium associated 1 [PLUNC1]) and BPIF3 (Bingle and Bingle, 2011).

The pseudostratified epithelium also contains a basal cell with an apical membrane that does not make contact with the airway lumen (Evans *et al*., 2001). These cells have desmosomal and hemidesmosomal attachments to other columnar cells and thereby anchor the respiratory epithelium. Positioned on basal lamina, basal cells can also interact with neurons, basement membrane, underlying mesenchymal cells, lymphocytes, and dendritic cells. Moreover, they can divide and differentiate into ciliated, goblet, or BC cells (Rock and Hogan, 2011).

Serous cells contain and secrete a less viscous fluid, and are also enriched in antimicrobial proteins including lysozyme and lactotransferin. In addition to surface epithelial cells, mucus and serous cells are contained in the submucosal glands limited mainly to the cartilaginous airways. The glands contain multiple branching tubules arranged with the proximal tubules contain mucus cells and the distal ascini contain serous cells. Submucosal glands secrete MUC5B, and MUC8, with MUC5B being predominate in submucosal glands, whereas surface mucus cells secrete mainly MUC5AC. Submucosal glands are contained in the cartilaginous airways (bronchi) in humans, but are minimal in rodents (especially mice). Serous cells contain the antimicrobial protein, BPIF2 (aka SPLUNC2). Secretory leukocyte proteinase inhibitor (SLPI) is a serine proteinase inhibitor that is produced locally in the lung by cells of the submucosal bronchial glands and by nonciliated epithelial cells. The main function of SLPI is the inhibition of neutrophil elastase and other proteinases, and may also have antimicrobial functions. Neutrophil elastase (ELANE) enhances SLPI mRNA levels while decreasing SLPI protein release in airway epithelial cells. In addition, glucocorticoids (which are used to treat airway inflammation) increase both constitutive and ELANE-induced SLPI mRNA levels (Abbinante-Nissen *et al*., 1993; Sallenave, 2010). Other submucosal gland/nonciliated epithelial cell antiproteinase/antimicrobial proteins include peptidase 3, skin-derived (aka elafin), and whey acidic protein-type (WAF) 4-disulfide core domain 2.

Another airway secretory cell is the bronchiolar secretoglobin cell (BSC), previously called the Clara cell (Winkelmann and Noack, 2010). BSCs have an extensive endoplasmic reticulum and secretory granules containing secretoglobins including SCGB1A1 (aka CCSP or CC10). The roles of secretoglobins are not fully understood, but in the lung, SCGB1A1 can inhibit phospholipase A2 and limit inflammation. In humans, BSCs are found mainly in the distal airways and can act as tissue stem cells (Rock and Hogan, 2011). In mouse, BSCs are found throughout the airways and can become ciliated cells (Rawlins *et al*., 2009) or mucus-producing cells (Chen *et al*., 2009) and can express chitinases following inflammation (Homer *et al*., 2006).

Neuroendocrine cells are contained in neuroepithelial bodies or separately in the proximal airways (Van Lommel, 2001) and contact can stimulate underlying sensory nerve fibers. They synthesize, store, and release bioactive substances including 5-hydroxytryptamine (aka serotonin), calcitonin-related polypeptide α (aka calcitonin), and gastrin-releasing peptide (aka bombesin). These cells express cholinergic receptor, nicotinic, α polypeptide 7 (Chrna7) and release serotonin in response to nicotine. Serotonin can also be released following hypoxia or mechanical strain. The release of these bioactive substances can redistribute pulmonary blood flow, and alter bronchomotor tone and immune responses (Cutz *et al*., 2007). Pulmonary neuroendocrine cells and neuroepithelial bodies in the fetal and neonatal lung modulate airway development and these cells are linked to specific types of lung cancer.

Gas Exchange Region

Structure The gas exchange region consists of terminal bronchioles, respiratory bronchioles, alveolar ducts, alveoli, blood vessels, and lung interstitium (Fig. 15-3). Human lung has five lobes: the superior and inferior left lobes and the superior, middle, and inferior right lobes. In rat, mouse, and hamster, the left lung consists of a single lobe and the right lung is divided into four lobes: cranial, middle, caudal, and ancillary. A ventilatory unit is defined as an anatomical region that includes all alveolar ducts and alveoli distal to each bronchiolar–alveolar duct junction (Mercer and Crapo, 1991). Gas exchange occurs in the alveoli, which comprise ~85% of the total parenchymal lung volume. Adult human lungs contain an estimated 300 to 500 million alveoli. The ratio of total capillary

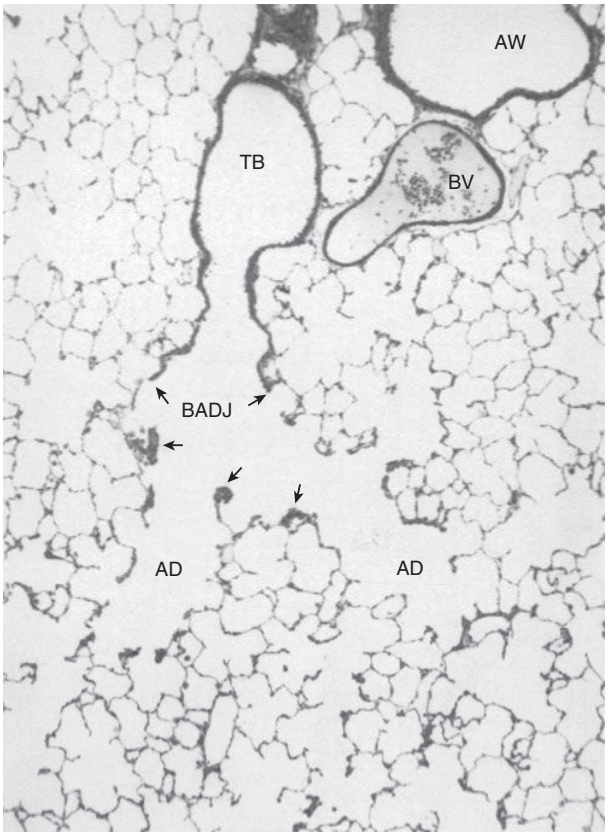

Figure 15-3. *Centriacinar region (ventilatory unit) of the lung.* An airway (AW) and a blood vessel (BV) (arteriole) are in close proximity to the terminal bronchiole (TB). The terminal bronchiole leads to the bronchiole–alveolar duct junction (BADJ) the alveolar duct (AD). A number of the (arrows) alveolar septal tips close to the BADJ are thickened after a brief (four-hour) exposure to asbestos fibers, indicating localization of fiber deposition. Other inhalants, such as ozone, produce lesions in the same locations. (Photograph courtesy of Dr Kent E. Pinkerton, University of California, Davis.)

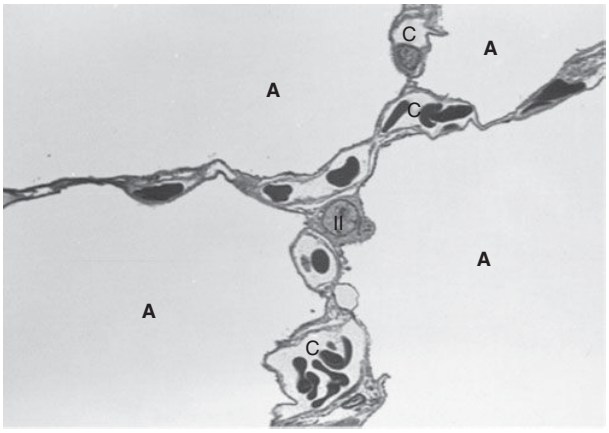

Figure 15-4. *Alveolar region of the lung.* The (A) alveolus is separated by the thin air-to-blood tissue barrier of the alveolar septal wall, which is composed of flat alveolar type I cells and occasional rounded (II) alveolar type II cells. A small interstitial space separates the epithelium and endothelium that form the (C) capillary wall. During lung injury the interstitial space enlarges and interferes with gas exchange. (Photograph courtesy of Dr Kent E. Pinkerton, University of California, Davis.)

surface to total alveolar surface is slightly less than one. Capillaries, blood plasma, and formed blood elements are separated from the air space by a thin layer of tissue formed by epithelial, interstitial, and endothelial components.

The alveolar epithelium consists of two cells, the alveolar type I and type II cell (Fig. 15-4). Alveolar type I cells cover ~95% of the alveolar surface and therefore are susceptible to damage by noxious agents that penetrate to the alveolus (Williams, 2003). Alveolar type I cells have an attenuated cytoplasm to enhance gas exchange. Alveolar type II cells are cuboidal and have abundant perinuclear cytoplasm, extensive secretory capacity, and contain secretory vesicles called lamellar bodies (Whitsett *et al*., 2010). They produce surfactant, a mixture of lipids, and four surfactant associated proteins and can undergo mitotic division and replace damaged type I cells (Rock and Hogan, 2011). Surfactant protein B and C are amphipathic and aide in spreading secreted lipids which form a monolayer that reduces surface tension. Surfactant protein A1, A2, and D are members of the subfamily of C-type lectins called collectins, which defend against pathogens. Surfactant protein A1 and A2 do not alter lipid structure but do bind lipopolysaccharides (LPS) and various microbial pathogens, enhancing their clearance from the lung. Surfactant protein D is also necessary in the suppression of pulmonary inflammation and in host defense against viral, fungal, and bacterial pathogens. Like surfactant protein B and C, surfactant protein D does influence the structural form of pulmonary surfactant. Surfactant protein D also influences alveolar surfactant pool sizes and reuptake. The shape of type I and type II cells is independent of alveolar size and is remarkably similar in different species. A typical rat alveolus (14,000 μm^2 surface area) contains two type I cells and three type II cells, whereas a human alveolus with a surface area of 300,000 μm^2 contains ~30 type I cells and ~50 type II cells (Pinkerton *et al*., 1991).

The mesenchymal interstitial cell population consists of fibroblasts and myofibroblasts that produce collagen and elastin as well as other cell matrix components and various effector molecules. Pericytes, monocytes, and lymphocytes also reside in the interstitium, as do macrophages before they enter the alveoli. Endothelial cells have a thin cytoplasm and cover about one-fourth of the area covered by type I cells.

Function

Ventilation The principal function of the lung is gas exchange, which consists of ventilation, perfusion, and diffusion. The lung is superbly equipped to handle its main task: bringing essential oxygen to the organs and tissues of the body and eliminating its most abundant waste product, CO_2 (Weibel, 1983).

During inhalation, fresh air is moved into the lung through the upper respiratory tract and conducting airways and into the terminal respiratory units when the thoracic cage enlarges and the diaphragm moves downward; the lung passively follows this expansion. The thoracic cage enlarges by the constriction of external intercostal and internal intercondral muscles, which elevate the sternum and ribs and thus increase the width of the thoracic cavity. When the parenchyma of the lung expands during inhalation, force is transferred to the airways (especially the small diameter distal airways), which increases the airway diameter and diminishes obstruction to airflow. After diffusion of oxygen into the blood and that of CO_2 from the blood into the alveolar spaces, the air (now enriched in CO_2) is expelled by exhalation. Relaxation of the chest wall and diaphragm diminishes the internal volume of the thoracic cage, the elastic fibers of the lung parenchyma recoil, and air is expelled from the alveolar zone through the airways. Any interference with the elastic properties of the lung, for example, the alteration of elastic fibers that occurs in emphysema, adversely affects ventilation, as do the decrease in the diameters of, or blockage of, the conducting airways, as in asthma.

Lung function changes with age and disease and can be measured with a spirometer (Fig. 15-5). The total lung capacity (TLC) is the total volume of air in an inflated human lung, 4 to 5 L (women) and 6 to 7 L (men) (American Thoracic Society [ATS], 1991). After a maximum expiration, the lung retains 1.1 L (women) and 1.2 L (men), which is the residual volume (RV). The functional residual capacity and residual volume cannot be measured with spirometry and are determined by several other methods including nitrogen washout, in which the concentration of nitrogen is measured in expired air following inhalation of 100% oxygen. The vital capacity is the air volume moved into and out of the lung during maximal

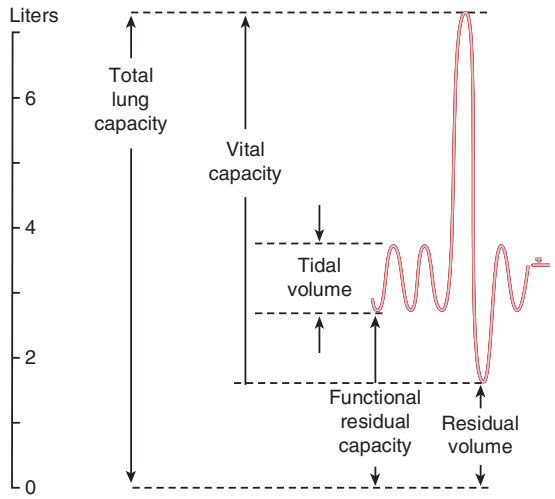

Figure 15-5. *A spirometer reading of lung volumes.* The total lung capacity is the total volume of air in an inflated human lung. After a maximum expiration, the lung retains a small volume of air, which is the residual volume. The air volume moved into and out of the lung during maximal inspiratory and expiratory movement, which is called the vital capacity. The tidal volume is typically moved into and out of the lung during each breathe. The functional residual capacity and residual volume cannot be measured with spirometer.

inspiratory and expiratory movement and typical is 3.1 L (women) and 4.8 L (men). Only a small fraction of the VC, the tidal volume (TV), is typically moved into and out of the lung during quiet breathing. In resting humans, the TV measures ~0.5 L with each breath. The respiratory frequency, or the number of breaths per minute, is 12 to 20 (thus the resting ventilation is about 6–8 L/min). During exercise, both the TV and the respiratory rate can increase markedly. The amount of air moved into and out of the human lung may increase from 12 to 15 L/min to 40 to 60 L/min with light and moderate exercise, respectively. Increased ventilation in a polluted atmosphere increases the deposition of inhaled toxic material. Thus, susceptible individuals, particularly children and the elderly, should not exercise during episodes of heavy air pollution.

Lung function changes with age and disease and can be measured by a forced expiratory maneuver with a spirometer. In this test, an individual first inhales maximally and then exhales as rapidly as possible. The volume of air expired in one second, called the forced expiratory volume 1 second (FEV1), and the total amount expired, forced vital capacity (FVC), and the ratio of FEV1/FVC, are good measures of the recoil capacity and airway obstruction of the lung. In a healthy individual the FEV1/FVC = ~80%. In chronic obstructive pulmonary disease (COPD), the parenchyma recoil is compromised, small airways close during exhalation obstructing airflow, and more air is trapped in the lung (Fletcher and Peto, 1977). Although the FVC may stay the same or may even increase slightly, narrowed small airways slow airflow at low lung volumes and thereby decrease FEV1. Thus, the FEV1/FVC is also decreased and airflow is considered obstructed when FEV1/FVC is >70% of predicted value (based on sex, height, and age). The decreased FVC is accompanied by an increase in RV. If part of the lung collapses, becomes filled with edema fluid, or is restricted due to altered lung collagen (fibrosis), FEV1, and FVC are equally reduced.

Perfusion The lung receives the entire output from the right ventricle, ~75 mL of blood per heartbeat. Blood with high CO_2 and low O_2 travels to the lung via the pulmonary artery and leaves the lung with high O_2 and low CO_2 via the pulmonary vein. The bronchi also have independent circulation with O_2-enriched blood supplied by an artery. Substantial amounts of toxic chemicals carried in the blood can be delivered to the lung. A chemical placed onto or deposited under the skin (subcutaneous injection) or introduced directly into a peripheral vein (intravenous injection) travels through the venous system to the right ventricle and then comes into contact with the pulmonary capillary bed before distribution to other organs or tissues in the body.

Diffusion Gas exchange takes place across the entire alveolar surface. Contact with an airborne toxic chemical thus occurs over a surface of ~140 m^2. This surface area is second only to the small intestine (~250 m^2) and is considerably larger than the skin (~2 m^2), two other organs that are in direct contact with the outside world. A variety of abnormal processes may severely compromise the unhindered diffusion of oxygen to the erythrocytes. Acute events may include collection of liquid in the alveolar or interstitial space and disruption of pulmonary surfactant system. Chronic toxicity can impair diffusion due to abnormal alveolar architecture or abnormal formation and deposition of extracellular substances such as collagen in the interstitium.

BIOTRANSFORMATION IN THE RESPIRATORY TRACT

Often overlooked as an organ involved in metabolism of chemicals, in favor of the liver, the lung has substantial capabilities for biotransformation (see Chap. 6). Total lung cytochrome P450 (CYP) activity is roughly one-tenth to one-third of that in the liver. However, when specific activity in a few cell types is considered, the difference is only twofold for many enzymes, and in the case of nasal mucosa, higher enzyme activity is reported per cell (Buckpitt and Cruikshank, 1997). Metabolic competence in the lung and nasal tissues is concentrated in a few cell types and these have a defined, and sometimes limited, the distribution in the respiratory tract that can vary substantially by species (Table 15-3). The balance of activation and inactivation is a critically important determinant of lung protection from injury. Protection from oxidation is another important function of enzymes in high tissue oxygen concentration that occurs in the respiratory tract. Other factors that can influence the role of phase I and phase II systems in lung toxicity include age, sex, diet, local inflammation, and the history of prior exposure (Plopper *et al*., 2001). Interestingly, many xenobiotic metabolizing enzymes have different patterns of induction (less) in the respiratory tract than in the liver, leading to the concept that regulation of these systems may be different depending on where they are located (Buckpitt and Cruikshank, 1997).

Table 15-3

Distribution of Xenobiotic Metabolizing Enzymes in the Respiratory Tract

		EPITHELIUM				
ENZYME	NASAL TISSUE	PROXIMAL AIRWAY	DISTAL AIRWAY	ALVEOLAR	MACROPHAGE	ENDOTHELIUM
Cytochrome P450 monooxygenases	+++	++	+++	++	++	++
Microsomal epoxide hydrolases	+++	++	+++	++	+	++
Flavin monooxygenases	+	++	+++	++	–	+
Prostaglandin-endoperoxide synthase 1	++	++	++	±	++	–
Prostaglandin-endoperoxide synthase 2	++	+	++	±	+++	++
Gluthathione-S-transferases	+	++	+++	+	Unknown	Unknown
Glucuronsyl transferases	+	Unknown	++	+	Unknown	Unknown
Sulfotransferases	++	+	+	±	Unknown	Unknown

Code: +++, most isoforms highly expressed; ++, some isoforms highly expressed; +, low expression; ±, low expression found in some studies but not others.

The major phase I enzyme system, the CYP monooxygenases (Nebert and Dalton, 2006), is concentrated into a few lung cells: BSCs, alveolar type II cells, macrophages, and endothelial cells. Of these cell types, BSCs have the most CYP, followed by the type II cells. The amount of total lung CYP contributed by BSCs is species-dependent, with humans having less CYP in their lungs from BSCs than mice or rats. Furthermore, the CYP isoforms present and their location in the respiratory tract also vary by species. For an extrahepatic tissue, CYPs are expressed at high levels in the nasal mucosa and this pattern of expression varies by nasal region and cell type (Thorton-Manning and Dahl, 1997; Ding and Kaminsky, 2003; Harkema *et al*., 2006). Most species have CYPs in nasal tissue and some are predominantly expressed in the olfactory mucosa (eg, CYP2G1, CYP2A3, and CYP2A13) (Ling *et al*., 2004). Metabolism by the olfactory epithelium may play a role in providing or preventing access of inhalants directly to the brain; for example, inhaled xylene may be converted into metabolites that move to the brain by axonal transport (Ghantous *et al*., 1990). The presence of the following CYP isozymes in the respiratory tract of at least one species has been reported: CYP1A1, CYP1A2, CYP1B1, CYP2A3, CYP2A6, CYP2A10/2A11, CYP2B1/4, CYP2B6, CYP2B7, CYP2E1, CYP2F1/2/4, CYP2S1, CYP2J2, CYP2G1, CYP3A4, CYP3A5, CYP3A7, and CYP4B1 (Hukkanen *et al*., 2002; Anttila *et al*., 2011).

Other phase I enzymes found in lung tissue include epoxide hydrolases, flavin monooxygenases, prostaglandin (PG)-endoperoxide synthases, carbonyl reductases, and NAD(P)H:quinone oxidoreductase 1 (NQO1). The only constant feature of the expression of these enzymes is lack of uniformity in their expression by cell type and region throughout the lung and their tendency to concentrate in epithelia. Both microsomal (EPHX1) and cytosolic (EPHX2) epoxide hydrolases are found in the lung and nasal tissues, and the activity of microsomal epoxide hydrolase can be higher in the distal airways of the lung than in the liver (Bond *et al*., 1988). Functional variants of EPHX1 have been associated with respiratory diseases including lung cancer (Kiyohara *et al*., 2006), childhood asthma (Salam *et al*., 2007), and possibly COPD (Hu *et al*., 2008; Lee *et al*., 2011). Flavin monooxygenase activity (FMO1 and FMO2) is found in rodent and human lung and nasal tissue. The isoforms present in the lung (FMO2) are different from those found in the liver (FMO1). FMO1 is the predominant isoform in the nasal mucosa (Shehin-Johnson *et al*., 1995; Henderson *et al*., 2008). The gene for FMO2 in human lung contains a premature stop codon encoding production of an inactive protein, but some ethnic groups have at least one copy of an allele that expresses the full-length protein (Whetstine *et al*., 2000). PG-endoperoxide synthases (aka cyclooxygenases) oxidize substrates at a much lower rate than CYP monooxygenases but may have a role in human pulmonary metabolism due to the relatively lower CYP activity in human lung tissue compared to rodents (Smith *et al*., 1991).

Carbonyl reductase (especially carbonyl reductase 2) enables pulmonary metabolism of endogenous carbonyl compounds, such as aliphatic aldehydes and ketones, 3-ketosteroids, fatty aldehydes, and PGs (converting PGE2 to PGF2α). NQO1 reduces quinones to hydroquinones, which then can be acted upon by NADH CYP reductase to generate semiquinone free radicals. In the lung, NQO1 activates carcinogenic heterocyclic amines found in cigarette smoke (De Flora *et al*., 1994), whereas NQO1 prevents formation of benzo[*a*]pyrene–quinone DNA adducts (Joseph and Jaiswal, 1994). Genetic polymorphisms in *NQO1* are associated with lung cancer (Kiyohara *et al*., 2005) and susceptibility to ozone (Minelli *et al*., 2011).

Phase II enzymes include glutathione-S-transferases (GSTs) (alpha, mu, and pi), glucuronsyl transferases, and sulfotransferases (SULTs). GSTs (and glutathione) and play a major role in the modulation of both acute and chronic chemical toxicity in the lung (West *et al*., 2000). A key point to keep in mind is that these enzyme systems work in concert with one another (ie, a decrease in one enzyme may result in a concomitant increase in another) and it is the combined action of all of these enzymes, and their location, that determines toxicity. The regulation of many of these enzymes is under coordinated control of the transcription factor nuclear factor, erythroid derived 2, like 2 (aka NRF2) (Slocum and Kensler, 2011).

A major determinant of the potential for detoxification may also be the cellular localization of, and ability to synthesize, glutathione in the lung. Pulmonary GST activity is 5% to 15% that of the liver in rodents and about 30% of that in human liver (Buckpitt and Cruikshank, 1997). The distribution of the isoforms of glutathione S-transferase varies by lung region with the alpha, mu, and pi isoforms (the most abundant), and the alpha and pi classes predominate in the airway epithelium of human lung. In nasal tissue, glutathione S-transferases are found in the olfactory mucosa. The mu isoform has a zonal pattern of expression increased in the lateral olfactory turbinates of the mouse (Whitby-Logan *et al.*, 2004). Polymorphisms in glutathione transferases genes have been associated with a possible increase in risk of developing lung cancer, particularly in smokers (Jourenkova-Mironova *et al.*, 1998). The GSTM1 genotype is one of the most widely analyzed genetic variants for lung cancer and an increased risk has been noted among null carriers (Shi *et al.*, 2008).

The activity of glucuronosyl transferase has been reported in both rodent and human nasal and pulmonary tissue. Glucoronosyl transferase (UGT2A1) is thought to have a role in termination of odorant signals (Lazard *et al*., 1991). The detoxification enzyme in the metabolism of polycyclic aromatic hydrocarbons (PAHs) within target tissues for tobacco carcinogens and functional polymorphisms in UGT2A1 may play a role in tobacco-related cancer risk (Bushey *et al*., 2011). Sulfotransferase activity has been demonstrated in human bronchoscopy samples (Gibby and Cohen, 1984) and SULT2B1 is immunochemically localized to the olfactory epithelium and conducting airway epithelium (He *et al*., 2005). Sulfotransferases have been localized to the sustentacular cells of the olfactory epithelium and some isoforms may be specific to the olfactory epithelium (Tamura *et al*., 1998). In lung endothelial and epithelial cells, SULT1A3 could play a role in the inactivation and/or disposal of excess chlorotyrosine (Yasuda *et al*., 2011).

GENERAL PRINCIPLES IN THE PATHOGENESIS OF LUNG DAMAGE CAUSED BY CHEMICALS

Toxic Inhalants, Gases, and Dosimetry

In inhalation toxicology, exposure is measured as a concentration (compound mass per unit of air). Typically highly toxic compounds can produce adverse effects in a concentration of mg/m^3 or μg/m^3. A m^3 is 1000 L. For gases, concentration may also be expressed as volume to volume of air, that is, parts per million (ppm) or parts per billion (ppb). This can be calculated from the mass per unit air by using the ideal gas law to determine the gas's volume. Concentration is useful because it can be measured by many air-sampling methods that rely on many chemical analytical methods. Large volumes of air can be collected so that low levels (ie, ppb) can be detected. Exposure does not equate to dose (compound mass per unit), which requires a measure of mass of the organ, cell, or subcellular target.

The sites of deposition of gases in the respiratory tract define the pattern of toxicity of those gases. Solubility, diffusivity, and metabolism/reactivity in respiratory tissues and breathing rate are the critical factors in determining how deeply a given gas penetrates into the lung (Asgharian *et al*., 2011; Gloede *et al*., 2011). Highly

soluble gases such as SO_2 or formaldehyde do not penetrate farther than the nose (during nasal breathing) unless doses are very high, and are therefore relatively nontoxic to the lung of rats (which are obligatory nasal breathers). However, formaldehyde causes cancer in the rat nasal passages (Albert *et al*., 1982). Relatively insoluble gases such as ozone and NO_2 penetrate deeply into the lung and reach the smallest airways and the alveoli (centriacinar region), where they can elicit toxic responses. Mathematical models of gas entry and deposition in the lung predict sites of lung lesions fairly accurately. These models may be useful for extrapolating findings made in laboratory animals to humans (Asgharian *et al*., 2011; Gloede *et al*., 2011). Very insoluble gases such as CO and H_2S efficiently pass through the respiratory tract and are taken up by the pulmonary blood supply to be distributed throughout the body.

Regional Particle Deposition

Because of the architecture of the airways that modulate airflow, particle size is a critical factor in determining the region of the respiratory tract in which a particle will be deposited. Deposition of particles on the airway mucosal surface is brought about by a combination of aerodynamic forces and particle characteristics (Lippmann *et al*., 1980; International Commission on Radiological Protection [ICRP], 1994; Lippmann and Leikauf, 2009). The efficiency of particle deposition in various regions of the respiratory tract depends mainly on particle size.

Aerosols are dispersed solids or liquids. Particles in air are classified by particle size (Lippmann and Leikauf, 2009). Size controls particle shape and thus influences light-scattering properties or deposition by interception. Size also controls particle mass and thus influences the probability for coagulation, dispersion, sedimentation, and impaction. Aerosols are a population of particles that can be monodispersed (essential of one size like pollens) or more typically, heterodispersed (many difference sizes). Particles generated from a single source typically have diameters that are lognormal (Poisson) distributed. This distribution will become Gaussian when plotted on a log scale and the distribution's central tendency is expressed as the mass median diameter (MMD). This measure of central tendency is accompanied by the measure of variability called the geometric standard deviation (σ_g). Monodispersed aerosols are typically defined as having a low σ_g (typically <1.2). Because the density (eg, plutonium) and the shape (eg, asbestos fibers) of aerosols can vary greatly, MMD is normalized to a unit-density sphere and is referred to as the mass median aerodynamic diameter (MMAD). In the following discussion the particle size are MMADs. Particle surface area is of special importance when toxic materials are adsorbed on particles and thus are carried into the lung.

In respiratory toxicology, aerosols (particles dispersed into air) include any of the following: (1) dusts (≥1.0 µm particles generated by mechanical division as in grinding), (2) fumes (≤0.1 µm particles generated by condensation of vapors as in heat metals or oils), (3) smoke (≤0.5 µm complex carbon particles generated condensation of products from combustion), (4) mists (2–50 µm water droplet or solutions generated by mechanical shearing of bulk liquid as in spraying), (5) fog (≤1.0 µm water droplets generated by water vapor condensation on atmospheric nuclei), or (6) smog (≥0.01–50 µm air pollution generated by stationary and mobile pollution source) (Lippmann and Leikauf, 2009). Haze has been used to describe either a dilute fog (at lower humidity) or smog. Smaller aerosols include submicrometer particles ($0.1 \le x \le 1.0$ µm), nanometer particles or nanoparticles (≤0.1 µm). All these distinguishing forms are included in the term "aerosol" or "particle."

Aerosols are typically dynamic and change by processes including dilution, dispersion, coagulation, and chemical reaction (Pandis, 2004). Atmospheric particles originate either as primary particles—by direct emission from a source—or as secondary particles—through atmospheric formation from the gas phase constituents (nucleation) (Fig. 15-6). Atmospheric particles are typically distributed into two modes and five submodes (John *et al*., 1990).

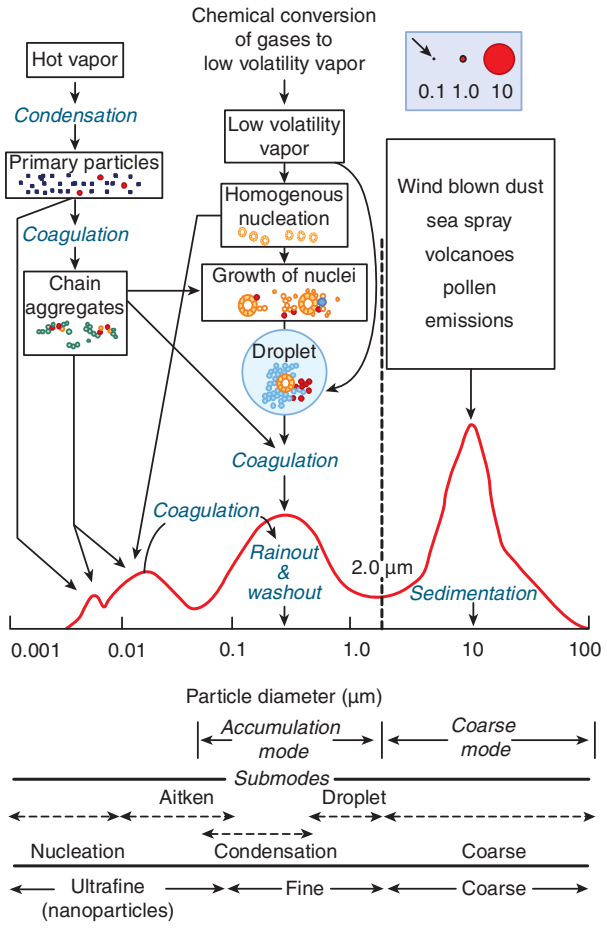

Figure 15-6. *Typically distribution of atmospheric particles consists of two major modes and five submodes.* The Accumulation and Coarse modes dominate the particle mass and Nucleation and Aitken submodes dominate the particle number. Nucleation particles are generated from gas phase emissions. Condensation can occur as plumes cool and particles and gases emitted together interact. Particles in the first two submodes typically have very short half-lives as singlet primary particles. The condensation submode is formed from the coagulation of smaller solid particles and condensation of gases including sulfates, nitrates, and organics on the particles' surface. They can be formed from chain aggregates of numerous particles of smaller diameter or also can be spherical with liquid surfaces. Particles move from the smaller submodes into the accumulation mode and these larger particles can have very long half-lives (hours to days) in the atmosphere and can travel over long distances. When the atmospheric relative humidity is very high (near 100%), particles in the accumulation mode seed rain droplets and are removed from the atmosphere. The coarse particles consist of particles >2.0 µm that are generated by mechanical processes or suspension of surface dust. Many of the particles in this range can be from natural sources (eg, wind blown desert sand). Particles larger than 50 µm readily settle and are removed from the atmosphere within minutes. Human exposure to these large particles is typically occupational (eg, dust from grinding wheels or wood sanding that is inhaled due to proximity to the source). Inset: The volume and therefore mass is to the cubed root of the radius is illustrated for difference in three orders of magnitude. This and the physical forces that maintain particles in the atmosphere are why the mass is mainly in the accumulation and coarse modes.

The two modes are the accumulation and coarse mode, which dominate the particle volume (and therefore mass) distribution.

Dominating the particle number distribution are the nucleation and Aitken submodes. The smallest nucleation submode particles are generated from gas phase emissions. This mode is dominated by a large number of nanoparticles ≤0.01 µm, but because they are so small they do not add much to the cumulative volume and therefore have little mass. For example, typical urban atmospheres can have 100,000 nucleation submode particles per cubic meter of air, but the total weight of all these particles is only about 50 ng (which is less than 0.5% of a typical urban atmosphere). The second submode is the Aitken nucleus submode, which also consists of nanoparticles ($0.01 \leq x \leq 0.1$ µm). Like the nucleation submode, particles in the Aitken nucleus submode can be formed by chemical conversion of gases from combustion processes or can be freshly generated as primary particles. This mode is named after John Aitken, an atmospheric scientist interested in cloud physics (Aitken, 1880). Most Aitken particles are primary particles that have grown due to material condensing on their surface as they move through the atmosphere. Condensation can occur as plumes cool and particles and gases emitted together interact. Particles in the first two submodes typically have very short half-lives as singlet primary particles. This is because their motion is influenced by collision with gas molecules and other particles that lead them to coagulate, especially around larger, slower moving particles.

The next largest submode is the condensation submode ($0.05 \leq x \leq 0.5$ µm). These particles are formed from the coagulation of smaller solid particles and condensation of gases including sulfates, nitrates, and organics on the particles' surface. They can be formed from chain aggregates of numerous particles of smaller diameter or also can be spherical with liquid surfaces. When these particles are hydroscopic and in a humid atmosphere, the size can increase to about 0.5 to 2.0 µm and become the droplet submode. Particles in the condensation and droplet submode contribute to the accumulation mode. These particles can have very long half-lives (hours to days) in the atmosphere and can travel over long distances from sources because they are too few in number for rapid coagulation and too small for gravitational sedimentation. Particles in this mode can travel from one state to another (eg, from the Ohio River Valley to the eastern seaboard), or even across oceans (from China to California). When the atmospheric relative humidity is very high (near 100%), particles in the accumulation mode seed rain droplets and are removed from the atmosphere. In addition, submicrometer particles have high light scattering properties that contribute to low visibility during pollution episodes. The second mode is the coarse particle and contains the coarse submode, which are particles >2.0 µm that are generated by mechanical processes or suspension of surface dust. Many of the particles in this range can be from natural sources (eg, wind blown desert sand). Particles larger than 50 µm settle readily and are removed from the atmosphere within minutes. Human exposure to these large particles is typically occupational (eg, dust from grinding wheels or wood sanding that is inhaled due to proximity to the source).

The upper respiratory tract is very efficient in removing particles that are very large (>10 µm) or very small (<0.01 µm) (Fig. 15-1). During nasal breathing, 1 to 10 µm particles are usually deposited in the upper nasopharyngeal region or the first five generations of large conducting airways. During oral breathing, deposition of these particles can increase in the tracheobronchial airways and alveolar region. Smaller particles (0.001–0.1 µm) can also be deposited in the trancheobronchial region. Particles ranging from 0.003 to 5 µm can be transported to the smaller airways and deposited in the alveolar region. Patterns of breathing can change the site of deposition of a particle of a given size. It must be kept in mind that the size of a particle may change during inspiration before deposition in the respiratory tract. Materials that are hygroscopic, such as sodium chloride, sulfuric acid, and glycerol, take on water and grow in size in the warm, saturated atmosphere of the upper and lower respiratory tract. Because adverse health effects of ambient particles have been associated with particles that were <10 µm, and subsequently <2.5 µm particles, the United States Environmental Protection Agency standard for ambient particulate matter (PM) is set at <2.5 µm particles, which is called $PM_{2.5}$ (see Chap. 29).

Deposition Mechanisms

In the respiratory tract, particles deposit by impaction, interception, sedimentation, diffusion (Brownian movement), and electrostatic deposition (for positively charged particles only) (Lippmann *et al.*, 1980; ICRP, 1994; Lippmann and Leikauf, 2009) (Fig. 15-7). Impaction occurs in the upper respiratory tract and large proximal airways where the airflow is faster than in the small distal airways because the cumulative diameter is smaller in the proximal airways. Fast airflow imparts momentum to the inhaled particle, and it is the particle's inertia that causes it to continue to travel along its original path. In airstream bends, such as an airway bifurcation, larger diameter particles deviate from the airflow and impact on the surface. In humans, most >10 µm particles are deposited in the nose or oral pharynx and cannot penetrate tissues distal to the larynx. For 2.5 to 10 µm particles, impaction continues to be the mechanism of deposition in the first generations of the tracheobronchial region.

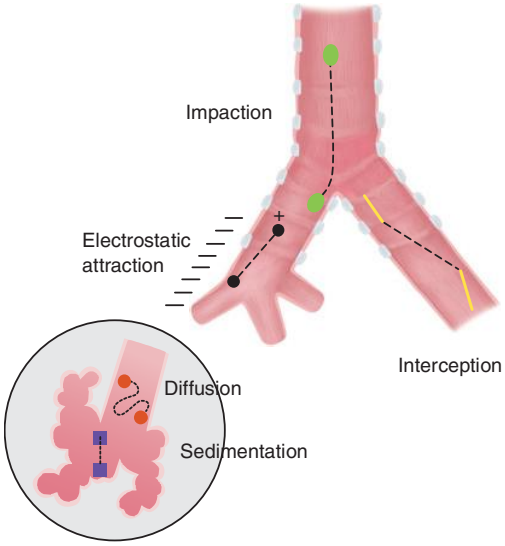

Figure 15-7. *Mechanism of particle deposition in the respiratory tract.* Impaction occurs in the upper respiratory tract and large proximal airways where fast airflow imparts momentum to the inhaled particle. The particle's inertia causes it to continue to travel along its original path and deposit on the airway surface. Interception occurs when the trajectory of a particle brings it near enough to a surface so that an edge of the particle contacts the airway surface. Sedimentation controls deposition in the smaller bronchi, the bronchioles, and the alveolar spaces, where the airways are small and the velocity of airflow is low. Sedimentation is dependent on the time a particle is in a compartment (ie, an alveolus) and can be increased by breath holding. Diffusion is an important factor in the deposition of submicrometer particles. Electrostatic deposition is a minor deposition mechanism for positively charged particles. The surface of the airways is negatively charged and attracts positively charged particles (adapted from Lippmann and Leikauf, 2009).

Interception occurs when the trajectory of a particle brings it near enough to a surface so that an edge of the particle contacts the airway surface. Interception is important for all particles but is particularly important in the deposition of fibers. Although fiber diameter determines the probability of deposition by impaction and sedimentation, interception is dependent on fiber length. Thus, a fiber with a diameter of 1 μm and a length of 200 μm will be deposited in the bronchial tree primarily by interception rather than impaction. Interception is also important for submicrometer particles in the tracheobronchial region where inertial airflow directs a disproportionately large fraction of the flow volume toward the surface of small airway bifurcations.

Sedimentation controls deposition in the smaller bronchi, the bronchioles, and the alveolar spaces, where the airways are small and the velocity of airflow is low. Indeed, in the alveoli, there is no bulk airflow. As a particle moves through air, buoyancy and the resistance of air act on the particle in an upward direction while gravitational force acts on the particle in a downward direction. Eventually, the gravitational force equilibrates with the sum of the buoyancy and the air resistance, and the particle continues to settle with a constant velocity known as the terminal settling velocity. Sedimentation is not a significant route of particle deposition when the aerodynamic diameter is ≤0.5 μm. Sedimentation is dependent on the time a particle is in a compartment (ie, an alveolus) and can be increased by breath holding.

Diffusion is an important factor in the deposition of submicrometer particles. A random motion is imparted to these particles by collisions with gas molecules. Thus, the ratio of a particle's mass to the momentum of the colliding gas molecules controls the distance the particle will travel. Larger particles are hardly moved by a gas molecule, whereas nanometer particles can be moved extensively. Diffusion is an important deposition mechanism in the nose, airways, and alveoli for particles ≤0.5 μm. Nanometer particles (0.1 μm and smaller) are also trapped relatively efficiently in the upper airways by diffusion. Particles that penetrate beyond the upper airways are available to be deposited in the bronchial region and the deep-lying airways. Therefore, the alveolar region has significant deposition efficiencies for particles smaller than 5 μm and larger than 0.01 μm.

During quiet breathing, in which the TV is only two to three times the volume of the anatomic dead space (ie, the volume of the conducting airways where gas exchange does not occur), a large proportion of the inhaled particles may be exhaled. During exercise, when larger volumes are inhaled at higher velocities, impaction in the large airways and sedimentation and diffusion in the smaller airways and alveoli increase. Breath holding also increases deposition from sedimentation and diffusion. Cigarette smoke is hydroscopic aerosol of nicotine-laden particles that grow to a median diameter of about 0.5 to 1.0 μm. Thus, a smoker's respiratory pause at the end of inhalation increases alveolar sedimentation and thereby nicotine delivery to the alveolar surface and to the blood upon absorption.

Electrostatic deposition is a minor deposition mechanism for positively charged particles. The surface of the airways is negatively charged and attracts positively charged particles. Freshly fractured mineral dust particles and laboratory-generated aerosols from evaporation of aqueous droplets can have substantial electrostatic mobilities.

Factors that modify the diameter of the conducting airways can alter particle deposition. In patients with chronic bronchitis or pneumonia, the airway lining fluid can greatly thicken and may partially block the airways in some areas. Sonic jets (eg, during wheezing and rales) formed by high air flowing through such partially occluded airways have the potential to increase the deposition of particles by impaction and diffusion in the small airways. Irritant materials that produce bronchoconstriction tend to increase the proximal tracheobronchial deposition of particles.

Nanoparticles are particles with at least one dimension of ≤100 nm. They are made from a variety of materials including carbon (eg, 60 carbon [C_{60}] fullerenes, nanotubes, or nanowires), metals (eg, gold, silver, or quantum dots), or metal oxides (eg, cerium oxide, titanium dioxide, or zinc oxide). Engineered biological nanoparticles include liposomes and viruses designed for gene or drug delivery. These particles can be generated in a wide array of dimensions and physicochemical properties. For example, carbon nanotubes can be single-walled (ie, SWCNT) or multiwalled (ie, MWCNT) and generated at various lengths (>1.0 μm). Carbon nanotubes can have many surface modifications (eg, metal coat) or can be core loaded. In addition, nanoparticles make up a portion (small by mass, but large by number and surface area) of emissions from vehicle engines, especially diesel engines, and industrial furnaces and burners. The toxicity of nanoparticles may be enhanced over large particles because of an increased surface area that can provide a biological reactive surface that can generate secondary reaction products (eg, reactive oxygen species) or may provide an inert surface that carries adsorbed copollutants (Oberdörster *et al*., 2005). Commercial nanoparticles are often rod shaped with lengths of 5 to 10 μm and thereby share aspect properties with asbestos (see below) (Donaldson *et al*., 2010). Additional concern about potential toxicity of nanoparticles is triggered by epidemiological studies that associate increased mortality in sensitive populations with increased ambient PM exposure (see Chap. 29). Lastly, nanoparticles may have increased toxicity because normal host defenses may have limited effectiveness against these particles. These particles may be too small to be recognized by macrophage and because nanoparticles can move through membranes, these particles can escape from phagosomes (see Chap. 28).

Particle Clearance

Lung defense is dependent on particle clearance (Table 15-4). Rapid removal lessens the time available to cause damage to the pulmonary tissues or permit local absorption (Lippmann *et al*., 1980). However, it is important to remember that particle clearance is not equivalent to complete protection for many reasons. An inert particle that can penetrate to the alveolar region can be a vehicle carrying adsorbed toxic gases. Once deposited in the lung, the adsorbed materials may dissolve from the surfaces of particles and enter the epithelium, endothelium, bloodstream, or lymphatics. Small particles (eg, nanoparticles) may directly penetrate cell membranes and evade clearance. Moreover, particle clearance from the respiratory tract is not equivalent to clearance from the body. The only mechanisms by which deposited particles can be removed from the body are nasal wiping and coughing.

Nasal Clearance Particles deposited in the nose are cleared depending on their site of deposition and solubility in mucus. Particles deposited in the anterior portion of the nose are removed by extrinsic actions such as wiping and blowing. Particles deposited in the posterior portion of the nose are entrapped in mucus and removed by mucociliary clearance that propels mucus toward the glottis, where the particles are swallowed. Insoluble particles are generally cleared from this region in healthy adults and swallowed within an hour of deposition. Particles that are soluble in mucus may dissolve and enter the epithelium and/or blood before they can be mechanically removed.

Tracheobronchial Clearance Particles deposited in the tracheobronchial tree are also removed by mucociliary clearance.

Table 15-4

Respiratory System Structures, Histology, Functions, and Particle Deposition and Clearance

REGION	HISTOLOGY	CELL TYPES	FUNCTIONS	NUMBER	SURFACE AREA EXPOSED TO AIR (m^2)	VOLUME OF BREATH (l)	DEPOSITION MECHANISM	CLEARANCE MECHANISM
Nares and anterior nasal passage	Anterior vestibule: Stratified squamous epithelium Anterior chamber: Nonciliated cuboidal/ columnar epithelium with nasal hair	Anterior vestibule: Squamous epithelium Anterior chamber: Cuboidal epithelium, ciliated cells, mucus cells, serous cells, brush cells, neuroendocrine cells, basal cells, subcutaneous hair follicles	Conduct, heat, humidify, and filter air	2	2×10^{-2}	0.175	Impaction and interception	Sneeze
Posterior nasal passage	Ciliated pseudostratified respiratory epithelium, submucosal glands	Ciliated cells, mucus cells, serous cells, brush cells, neuroendocrine cells, basal cells, smooth muscle cells, submucosal glands (mucus and serous cells)	Conduct, heat, humidify, and filter air	2	4.5×10^{-2}			Sneeze and mucociliary clearance
Olfactory bulb	Olfactory epithelium, Bowman glands	Olfactory cells, supporting cells, basal cells, brush cells	Smell	2	2×10^{-4}			Sneeze
Mouth	Nonkertinized stratified squamous epithelium	Stratified squamous epithelium	Conduct, heat, humidify, and filter air	1	2.2×10^{-2}			Cough, Swallowing
Teeth and gingiva	Tooth: Bone with enamel, dentin, cenetum, pulp Gingiva: Gingival, junctional, and subcular epithelium	Tooth: Odontoblasts, fibroblasts, preodontoblasts Gingiva: Nonkertinized stratified squamous epithelium	Mastication	20 (primary) 32 (permanent)	5×10^{-3}			Swallowing
Tongue	Fungiform, filiform, foliate, circumvallate papillae	Supporting cells, gustatory cells	Taste, food manipulation	1	1×10^{-2}			Cough, swallowing
Salivary glands (partoid, submaxilary, sublingual, and minor)	Lobular secretory glands with adenomeres	Serous and mucous secretory epithelial cells	Moisten food, amylase digestion, lysozyme secretion	6 major 600 minor	*			Saliva secretion

Pharynx	Nonkertinized stratified squamous epithelium, adenoid tissue, tonsils	Nonkertinized stratified squamous epithelium, adenoid and tonsils: mucosal associated lymphoid tissue	Conduct, heat, humidify, and filter air Adenoid, Immunoglobin A secretion	1	1×10^{-2}			Sneeze, cough, and mucociliary clearance
Larynx	Vocal folds (cords), 3 single (thyroid, cricoid, and epiglottic), and 3 paired (arytenoid, corniculate, and cuneiform) cartilages	Stratified squamous epithelium (true folds), respiratory epithelium (false fold), chondroblasts (cartilage)	Phonation	1	5×10^{-4}			Cough and mucociliary clearance
Trachea	Ciliated pseudostratified respiratory epithelium, submucosal glands, cartilage rings	Ciliated cells, mucus cells, serous cells, brush cells, neuroendocrine cells, basal cells, smooth muscle cells, submucosal glands (mucus and serous cells)	Conduct, heat, humidify, and filter air	1	7.5×10^{-3}			
Bronchi	Ciliated pseudostratified respiratory epithelium, cartilage plates, airway smooth muscle	Ciliated cells, mucus cells, serous cells, brush cells, neuroendocrine cells, basal cells, smooth muscle cells, submucosal glands (mucus and serous cells)	Conduct and filter air	510	0.26			
Bronchioles	Transition from ciliated pseudostratified to cuboidal ciliated respiratory epithelium (no cartilage and no submucosal glands)	Ciliated cells, clara cells, few or no mucus cells	Conduct and filter air	6.5×10^{4}	7.5			Mucocilary clearance
Respiratory bronchioles	Cuboidal nonciliated respiratory epithelium with alveoli in a portion of the wall	Cuboidal epithelium and alveolar epithelium	Conduct and filter air	4.6×10^{5}	*	0.2	Sedimentation, diffusion, and electrostatic attraction	Macrophage phagocytosis and migration
Alveolar duct and sac	Cuboidal nonciliated respiratory epithelium with alveoli in a portion of the wall	Some low cuboidal cells and mostly type I (squamous nonsecretory), type II (secretory cuboidal) alveolar epithelial cells, macropahge	Conduct and filter air, gas exchange	$3–5 \times 10^{8}$	140	4.5		
Pleural cavity and diaphragm	Mesothelium and skeletal muscle	Mesothelial cells, skeletal muscle cells, nerves	Generation of negative pleural pressure for inhalation	1	1.8	0	Translocation	Macrophage lymphatic migration

In addition to deposited particles, particle-laden macrophages are also moved upward to the oropharynx, where they are swallowed. Mucociliary clearance is relatively rapid in healthy individuals and is completed within 24 to 48 hours for particles deposited in the lower airways. Infection and other injuries can greatly impair clearance.

Alveolar Clearance Particles deposited in the alveolar region are removed by specialized cells, the alveolar macrophage. Lung defense involve both the innate and adaptive and immune systems. The innate immune system confers immediate recognition, phagocytosis, and killing of bacteria and microbes that are in the airway or alveolus. It is nonspecific and does not confer longer-term memory of the invading pathogenic stimuli. Alveolar macrophages are major effectors of innate immunity in the lung. Other innate immune cells include polymorphonuclear leukoctyes (aka neutrophils) that can augment this capacity but are typically present only when the lung is inflamed. The adaptive immune system confers long-lasting or protective immunity to the host that is specificity to a foreign microbe or material (antigen). Adaptive immunity involves dendritic cells that take up and present antigens to T lymphocytes (T cells) or antibody-producing B lymphocytes (B cells). Dendritic cells are derived from monocytes and reside in the airway epithelium. Lymphocytes reside in the hilar or mediastinal lymph nodes, lymphoid aggregates, and lymphoepithelial nodules, as well as in aggregates or as single cells throughout the airways. Ideally, the antibody generated by the B cell is recognizes a single molecular signature on the antigen and initiates other cells to evoke responses that protect the host (see Chap. 12).

Macrophage phagocytosis depends on the recognition of foreign or damage cells by a variety of macrophage surface macromolecules and receptors. Phagocytosis requires (1) particle binding to the membrane specifically via recognition molecule–receptor interactions or nonspecifically by electrostatic forces (inert materials), (2) receptor activation that initiates cell signaling, (3) actin polymerization and coordinated cytoskeletal movements that leads to extension of membranes, and (4) vesicular membrane closure closely apposed to the particle or the fiber ingested forming a phagosome shaped by the material ingested (Bowden, 1987).

Particles in the formed phagosome fuse with a lysosome to create a phagolysosome, where the ingested material is eventually degraded. Degradation can be oxygen-dependent (involving the respiratory burst) or oxygen-independent. Oxygen-independent degradation requires the fusion of granules containing proteolytic enzymes such as defensins, lysozyme, and cationic proteins. Additional antimicrobial peptides are present in these granules, including lactoferrin, which sequesters iron to provide unfavorable growth conditions for bacteria. Inhaled nanoparticles deposit along the entire respiratory tract, but are not efficiently engulfed by surface macrophages or may escape from phagosomes (Geiser, 2010).

Following alveolar deposition, macrophages rapidly engulf particles (≥50% within three hours and nearly 100% by 24 hours) (Alexis *et al*., 2006). After several days or weeks, most macrophages then move to the airways and are removed mainly by mucociliary clearance. A small portion of insoluble particles may be phagocytized by alveolar macrophages and removed via lymphatic drainage. A portion of deposited particles not cleared by macrophages can be found in epithelial and interstitial cells or may be found in the lymphatic system with clearance time of months or years. Insoluble particles, especially long narrow fibers, may be sequestered in the lung for very long periods, often in macrophages located in the interstitium.

ALVEOLAR MACROPHAGE RECEPTORS

Alveolar Macrophage Receptors and Innate Immunity

Several receptors are involved in the phagocytic uptake of micrometer-sized particles (Geiser, 2010). Among these are Fc receptor and pattern-recognition receptors (PRRs) including complement, mannose, scavenger receptors (Table 15-5), and other PRRs (Table 15-6). These receptors differ in pathogen recognition motifs and are opsonin-dependent or -independent. Opsonins are binding enhancers (eg, antibodies), which coat the negatively charged molecules, especially those on bacterial membranes. Molecules that activate the complement system also are considered opsonins.

Phagocytosis of particles is mediated by various Fc receptors, which recognize immunoglobulin-coated particles. Macrophage Fc receptors recognize Fc fragments of the antibodies immunoglobin (Ig) A, E, and G. Fc receptors are classified based on the type of antibody they recognize, for example, Fc fragment of IgA, receptor for (FCAR aka CD89) recognizes IgA. Other macrophage Fc receptors include FCER1A/G, FCER2 (aka CD23) that recognize IgE, and FCGR1A/B/C (aka CD64), FCGR2A/B/C (aka CD32), and FCGR3A/B (aka CD16) that recognize IgG. The main receptors associated with alveolar macrophage phagocytosis are the IgA (FCAR) and IgG (FCGR1, 2, 3 types) receptors. Phagocytic cells do not have a FC receptor for IgM, making IgM ineffective in assisting phagocytosis. However, IgM efficiently activates complement and is therefore considered an opsonin. Clustering and activation of the low-affinity receptor FCGR2 by uncoated quartz has been demonstrated in vitro (Haberzettl *et al*., 2008).

Once activated, FCAR modulates the respiratory burst (Ouadrhiri *et al*., 2002). The respiratory burst involves the freshly generated reactive oxygen species produced by the phosphorylation, assembly, and activation of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Iles and Forman, 2002). Assembly of the NADPH oxidase complex that includes cytochrome b-245 and β polypeptide ([CYBB] aka NADPH oxidase 2 [NOX2]) involves Ras-related C3 botulinum toxin substrate 1 (RAC1), neutrophil cytosolic factors (NCFs) 1, 2, and 4 (aka p47PHOX, p67PHOX, and p40PHOX, respectively), and CYBA (aka p22PHOX). Activation requires phosphorylation of the NCF2 subunit of the NADPH oxidase complex that activates the oxidase, which utilizes cytosolic NADPH to reduce extracellular oxygen to superoxide. Superoxide is converted to hydrogen peroxide by superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), and diatomic oxygen. H_2O_2 can be combined with chlorine by myeloperoxidase to produce hypochorite (chlorine bleach), which plays a role in destroying bacteria. There are three types of SODs: SOD1 (containing Cu/Zn) is the cytoplasmic isoform, SOD2 (containing Mn/Fe) is the mitochondrial isoform, and SOD3 (containing Cu/Zn) is the extracellular isoform (Zelko *et al*., 2002). The latter is particular abundant in the lung's extracellular matrix (ECM).

Under ideal conditions, superoxide anion is released totally into the phagosome and thereby held inside the cell. However, it can be released and react with NO. The oxidation of L-arginine produces NO endogenously by a reaction catalyzed by NO synthase. The enzyme exists in constitutive neuronal (NOS1), inducible (NOS2), and endothelial (NOS3) isoforms. In aqueous solution, freshly generated NO persists for several minutes in micromolar concentrations before it can react with O_2 to form much stronger oxidants such as NO_2. Nonetheless, in the body the half-life of NO is only seconds because NO diffuses

Table 15-5

Major Alveolar Macrophage Receptors

RECEPTOR PROTEIN FAMILY	SYMBOL	SYNONYMS	LIGAND
Fc receptors			
Subfamily A (FCAR)			
Fc fragment of IgA, receptor for	FCAR	CD89	Immunoglobin A
Subfamily E (FCER)			
Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	FCER1A		Immunoglobin E
Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	FCER1G		Immunoglobin E
Fc fragment of IgE, low affinity II, receptor for (CD23)	FCER2	CD23	
Subfamily G FCGR)			
Fc fragment of IgG, high affinity Ia, receptor (CD64)	FCGR1A	CD64a	Immunoglobin G
Fc fragment of IgG, high affinity Ib, receptor (CD64)	FCGR1B	CD64b	Immunoglobin G
Fc fragment of IgG, high affinity Ic, receptor (CD64)	FCGR1C	CD64c	Immunoglobin G
Fc fragment of IgG, low affinity IIa, receptor (CD32)	FCGR2A	CD32A	Immunoglobin G, quartz
Fc fragment of IgG, low affinity IIb, receptor (CD32)	FCGR2B	CD32B	Immunoglobin G, quartz
Fc fragment of IgG, low affinity IIc, receptor (CD32)	FCGR2C	CD32C	Immunoglobin G, quartz
Fc fragment of IgG, low affinity IIIa, receptor (CD16a)	FCGR3A	CD16a	Immunoglobin G
Fc fragment of IgG, low affinity IIIb, receptor (CD16b)	FCGR3B	CD16b	Immunoglobin G
Complement receptors			
integrin, alpha M (complement component 3 receptor 3 subunit)	ITGAM as a dimer with ITGBM	CD11b	Complement component 3 (iC3b), beta glucan, intercellular adhesion molecule I (ICAM1 aka CD54)
integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	ITGBM as a dimer with ITGAM	CD18	Complement component 3 (iC3b), beta glucan, intercellular adhesion molecule I (ICAM1 aka CD54)
Mannose receptor			
mannose receptor, C type 1	MRC1	CD206	Microbial mannose structures
Scavenger receptors			
macrophage scavenger receptor 1	MSR1	CD204	Acetylated LDL, oxidized LDL, advanced glycation end products-modified, maleylated-bovine serum albumin, lipoteichoic acid (LTA), lipid A component of LPS
macrophage receptor with collagenous structure	MARCO	SCARA2	Titanium dioxide, iron oxide and polystyrene latex particles. Acetylated LDL, oxidized LDL, advanced glycation end products-modified, maleylated-bovine serum albumin, LTA, lipid A component of LPS

(>100 μm) from the tissue and enters red blood cells and reacts with oxyhemoglobin. The direct toxicity of endogenous NO is modest but is greatly enhanced by reacting with superoxide to form peroxynitrite (ONOO–) (Beckman, 2009; Ferrer-Sueta and Radi, 2009). Most NO formed in the lung is excreted as nitrate in the urine within 48 hours or it can be exhaled (Meyer and Piiper, 1989). Other metabolites include nitrogen gas, ammonia, and urea (Yoshida and Kasama, 1987; Kosaka *et al*., 1989). Nonetheless, NO is produced in sufficiently high concentrations to outcompete SOD for superoxide. Compared to other reactive oxygen and nitrogen species, peroxynitrite reacts relatively slowly with most biological molecules, making peroxynitrite a selective oxidant. Peroxynitrite mainly modifies tyrosine in proteins to create nitrotyrosines, leaving a biological signature. Nitration of structural proteins, including neurofilaments and actin, can disrupt filament assembly with major pathological consequences. Nitrotyrosines

Table 15-6

Major Alveolar Pattern Recognition Receptors

RECEPTOR PROTEIN FAMILY	SYMBOL	SYNONYMS	LIGAND
Toll-like receptors (TLRs)			
toll-like receptor 1	TLR1		Bacterial PAMPs (lipoproteins, lipoteichoic acid [LTA], peptidoglycan), Fungal PAMPs, beta-glucan, zymosan, mannan
toll-like receptor 2	TLR2		Bacterial PAMPs [Lipoproteins, lipoteichoic acid (LTA), peptidoglycan], Fungal PAMPs, beta-glucan, zymosan, mannan
toll-like receptor 3	TLR3		Viral PAMPs (double-stranded [ds] RNA)
toll-like receptor 4	TLR4		Gram-negative bacterial lipopolysacchrides (LPS), mannan
toll-like receptor 5	TLR5		Bacterial flaggen
toll-like receptor 6	TLR6		Bacterial PAMPs (Lipoproteins, LTA, peptidoglycan) Fungal PAMPs, beta-glucan, zymosan
toll-like receptor 7	TLR7		Bacterial or viral PAMPs (single-stranded [ss] RNA)
toll-like receptor 8	TLR8		Bacterial or viral PAMPs (ssRNA)
toll-like receptor 9	TLR9		Bacterial, viral, fungal, or parasitic DNA, unmethylated CpG motifs
toll-like receptor 10	TLR10		Lipoproteins, lipoteichoic acid (LTA), peptidoglycan (possibly)
toll-like receptor 11	TLR11		Parasitic PAMPs (profilin)
C-type lectin receptors (CLRs)			
C-type lectin domain family 4, member E	CLEC4E	Mincle	Cord factor (trehalose-6,6′-dimycolate (tuberculosis)
C-type lectin domain family 7, member A	CLEC7A	Dectin-1	Bacterial PAMPs (beta-glucans, zymosan)
Nucleotide binding oligomerization domain (NOD)-like receptors (NLRs)			
Caspase activation and recruitment domain (CARD) subtype			
nucleotide-binding oligomerization domain containing 1	NOD1	CARD4	*Legionella pneumophilia, Pseudomonas aeruginosa,* and *Staphylococcus aureus,* and other gram-negative and some gram-positive bacterial peptidoglycan: muropeptides (iE-DAPs)
nucleotide-binding oligomerization domain containing 2	NOD2	CARD15	Muramyl dipeptide in all peptidoglycan types
Pyrin domain (PYD) (aka death fold domain) subtype			
NLR family, pyrin domain containing 1	NLRP1	CARD7	Inflammasome initiators
NLR family, pyrin domain containing 3	NLRP3	NALP3	Inflammasome initiators
NLR family, CARD domain containing 4	NLRC4	IPAF	Inflammasome initiators
PYD and CARD domain containing	PYCARD	ASC	Inflammasome initiators
RNA helicase retinoic acid-inducible gene I-like receptors (RLRs)			
DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	DDX58	RIG-I	Shorter dsRNA fragments, influenza A/B, respiratory syncytial virus, and sendia virus
interferon induced with helicase C domain 1	IFIH1	MDA5	Longer nucleotide fragments poly(I:C) motifs and picoaviral ssRNA
DEXH (Asp-Glu-X-His) box polypeptide 58	DHX58	LPG2	Regulator of DDX58/IFIH signaling

have been detected in acute lung injury and several other inflammatory diseases (Beckman *et al.*, 1990; Freeman *et al.*, 1995; Squadrito and Pryor, 1998).

In addition, H_2O_2 produced by the respiratory burst also functions as a second messenger and activates signaling pathways in alveolar macrophage (Iles and Forman, 2002; Gwinn and Vallyathan, 2006). A major event is the activation of nuclear factor of kappa light polypeptide gene enhancer in B cells 1 (NFKB1) by H_2O_2. Activated NFKB1 translocates into the nucleus and stimulates the expression of genes involved in a wide variety of biological functions, especially cytokines and chemokines that modulate the inflammatory response. Cytokines are intercellular mediators of inflammation that activate other inflammatory cells. Chemokines are intercellular mediators that attract other leukocytes through chemotaxis. The main cytokines generated by classically activated macrophages (aka M1 macrophages) include tumor

necrosis factor (TNF), and interleukin (IL) 1, β (IL1B), IL6, and IL12. Interferon-γ (IFNG) released from activated macrophages or T cells (especially the Th1 subtype lymphocyte) drive immature macrophages toward the classically activated phenotype. Classically activated macrophages also express increased nitric oxide synthase 2, inducible (NOS2), which generate nitric oxide that can react with superoxide to form the more stable peroxynitrite, a reactive nitrogen species (Pacher *et al*., 2007). H_2O_2 also activates the mitogen-activated protein kinase (MAPK) pathways that phosphorylate transcription factors including MAPK1/3 (aka extracellular-regulated kinase [ERK]) and the MAPK8 (aka c-Jun N-terminal kinase [JNK]). Phosphorylation of MAPK1/3 also modulates the expression of genes via phosphorylation of the transcription factor ELK1, member of ETS oncogene family (ELK1) that controls the production of the FBJ osteosarcoma oncogene (FOS) transcription factor.

Complement receptors recognize complement-coated particles (van Lookeren Campagne *et al*., 2007). Macrophage complement receptors include integrin, α M (complement component 3 receptor 3 subunit) (ITGAM aka CD11b) and integrin, β 2 (complement component 3 receptor 3 and 4 subunit) (ITGBM aka CD18). Complement component C3 is central to opsonization and its first cleavage product, C3b, forms the multisubunit enzyme, C3bBb, that proteolytically cleaves additional C3 molecules on the pathogen surface. C3b is further degraded to iC3b, C3c and C3dg. These receptors dimerize to form the ITGAM/ITGBM (aka CD11b/CD18) complex, which recognizes multiple ligands including iC3b, β-glucans, and intercellular adhesion molecule 1 (ICAM1 aka CD54). β-Glucans are glucose polymers found in the cell walls of plants, fungi, and some bacteria (Palma *et al*., 2006). Because cell walls are actively remodeled during bacterial cell growth and division, the constant release of these ligands from bacteria allows the innate immune system to survey its surroundings for the presence of active bacteria. ICAM1 is also a ligand for integrin α L (antigen CD11A (p180), lymphocyte function-associated antigen 1; α polypeptide) (aka CD11a) when complexed with ITGBM.

Macrophage mannose receptor, namely mannose receptor C type 1 (MRC1 aka CD206), is a C-type lectin receptor (CLR) that recognizes high-mannose structures on the surface of potentially pathogenic viruses, bacteria, and fungi (Gazi and Martinez-Pomares, 2009). Unlike other CLRs that elicit microbicidal effector functions (see below), MRC1 expression and its endocytic function are selectively decreased by IFNG, but increased by IL4 and IL13. IL4 and IL13 are cytokines generated by Th2 lymphocytes that increase in parasitic infections (eg, Helminthic infections) or acquired allergies (eg, allergic asthma). The induction of MRC1 is a marker of the alternatively activated macrophage (aka M2 macrophage) (Gordon and Martinez, 2010). Alternatively activated macrophages are thought to be immunesuppressive because they release IL10, do not produce IL12, and have decreased Th1-attracting chemokine, chemokine (C–C motif) ligand 3 (CCL3 aka MIP1A). However, alternatively activated macrophages express increased levels of arginase 1 (ARG1), resistin-like alpha (RETNLA), and chitinase 3-like 3 and 4 (CHI3L3 and CHI3L4). ARG1 can reduce NO production. RETNLA can modulate inflammation. CHI3L3/4 can bind chitins in insects, Helminth eggs, and Nematode pharynx. MRC1 also can interact with tumor cell mucin glycoproteins (eg, MUC16 aka CA125 and TAG-72), which induces MRC1 internalization and thereby modulate (perhaps suppress) cytokine production (Allavena *et al*., 2010).

Macrophage scavenger receptors, macrophage scavenger receptor 1 (MSR1 aka CD204), and macrophage receptor with collagenous structure (MARCO) recognize a range of modified host polyanionic molecules and apoptotic cells (Bowdish and Gordon, 2009). Ligands for MSR1 shared for the most part with other SR, include acetylated low-density lipoprotein (LDL), oxidized LDL, advanced glycation end products (AGEs)-modified, and maleylated-bovine serum albumin. Other ligands include lipoteichoic acid (LTA) and the lipid A component of LPS. Three MSR1 protein isoforms can be generated from the *MSR1* gene. The isoform type 3 does not internalize modified LDL (acetyl-LDL) despite having the domain that mediates this function in type 1 and 2 isoforms. Isoform 3 has an altered intracellular processing and is trapped within the endoplasmic reticulum, rendering the macrophage unable to complete endocytosis. The isoform type 3 can inhibit the function of isoforms type 1 and type 2 when coexpressed, indicating a dominant negative effect and suggesting a mechanism for regulation of scavenger receptor activity in macrophages. MARCO is also involved in uptake of unopsonized particles such as titanium dioxide (TiO_2), iron oxide (Fe_2O_3), and polystyrene latex (PSL) (Arredouani *et al*., 2005). These particles were about 1 mm in diameter.

Alveolar Macrophage Pattern-Recognition Receptors

PRRs have various ectodomains that recognize pathogen-associated molecular patterns (PAMPs) present on microbial surfaces. PRRs include Toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and the RNA helicase retinoic acid-inducible gene I (RIG-I) (Table 15-6).

TLR proteins contain three major domains, a leucine-rich repeat motif containing ectodomaim, a transmembrane region, and a cytosolic Toll-IL1 receptor (TIR) domain (Kawai and Akira, 2011). Expressed either on the cell surface or associated with intracellular vesicles, 10 and 13 functional TLRs have been identified in human and mouse, respectively. TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are expressed on the plasma membrane and mainly recognize microbial membrane components; TLR3, TLR7, TLR8, and TLR9 are expressed in the endosome and mainly recognize nucleic acids. Membrane-associated TLRs detect distinct PAMPs with the leading example being LPS, an endotoxin in the cell membrane of gram-negative bacteria, which is specifically recognized by TLR4. Other bacterial PAMPs include lipoproteins, LTA from gram-positive bacteria, and peptidoglycan (PGN) that are recognized by heterodimeric TLR2/TLR1 or TLR2/TLR6 and possibly TLR10, whereas bacterial flagellin is recognized by TLR5. Fungal PAMPs, β-glucan, and zymosan are recognized by TLR2 and TLR6, and mannan is recognized by TLR2 and TLR4. Parasitic PAMPs include profilin of *Toxoplasma gondii* recognized by TLR11. Viral PAMPs include nucleic acid variants of double-stranded (ds) RNA recognized by endosomal TLR3 with bacterial or viral single-stranded (ss) RNA being recognized by endosomal TLR7 and TLR8. Bacterial, viral, fungal, or parasitic DNA and unmethylated CpG motifs are recognized by endosomal TLR9. Alveolar macrophages express all the known TLRs, but most prominently TLR2, TLR3, TLR4, TLR5, TLR6 (Maris *et al*., 2006), and TLR9 (Juarez *et al*., 2010). In addition to TLRs, other membranes and cytosolic PRRs are found in the lung.

Other macrophage membrane PRRs include CLRs that recognize β-glucan and mannose structural molecules (Osorio and Reis e Sousa, 2011). For example, C-type lectin domain family 7, member A (CLEC7A aka Dectin-1) binds β-glucans and zymosan present in lung bacterial pathogens such as *Mycobacterium tuberculosis*, *Aspergillus* spp, and *Pneumocystis* spp. Another important macrophage CLR is CLEC4E, which recognizes trehalose-6,6′-dimycolate

(aka cord factor), a major cell wall glycolipid of *M tuberculosis* (Ishikawa *et al*., 2009). *M tuberculosis* produces several other molecules that are recognized via MRC1, TLR2, and TLR9.

Other cytosolic PRRs are NOD-like receptors (NLRs). NLR proteins are characterized by a shared domain architecture that includes a nucleotide-binding domain (NBD) and, like TLRs, a leucine-rich repeat (LRR) domain (Elinav *et al*., 2011). NLRs can be grouped into two subfamilies based on additional domains, which can be either a caspase activation and recruitment domain (CARD) or a pyrin domain (PYD aka death-fold domain). The major CARD NLRs are NOD containing 1 (NOD1) and NOD2. NOD1 and NOD2 are cytosolic receptors that recognize distinct PGN subunits contained in gram-positive bacteria cell walls. NOD1 recognizes muropeptides (iE-DAPs) that are found in the PGN of gram-negative bacteria and only some gram-positive bacteria, whereas NOD2 recognizes a minimal motif of muramyl dipeptide that is found in all PGNs. In the lung, NOD1 is important because it recognizes and participates in regulating inflammatory responses to *Legionella pneumophila* (Berrington *et al*., 2010), *Staphylococcus aureus* (Travassos *et al*., 2004), and *Pseudomonas aeruginosa* (Travassos *et al*., 2005).

PYD NLR proteins along with other members of the NLR protein family can form multiprotein complexes called inflammasomes (Sutterwala *et al*., 2007). Inflammasomes are composed of one of several NLR and Pyhin proteins (Schattgen and Fitzgerald, 2011), including NLR family, pyrin domain containing 1 (NLRP1), NLRP3, and NLR family, CARD domain containing 4 (NLRC4 aka IPAF) sense endogenous or exogenous PAMPs (Sutterwala *et al*., 2007). Inflammasomes are assembled through homophilic CARD–CARD and PYD–PYD interactions between NLRs, PYD, and CARD domains containing (PYCARD aka apoptosis-associated speck-like protein containing a CARD [ASC]) (Elinav *et al*., 2011). Inflammasome assembly initiates the activation of inflammatory caspases, cysteine proteases that are synthesized as inactive prozymogens. Upon activation, caspases trigger cellular programs that lead to inflammation or cell death. Caspase 1, apoptosis-related cysteine peptidase (IL1, β, convertase (CASP1), which joins the inflammasome and is regulated in a signal-dependent manner, is the most prominent member of proinflammatory caspases. Other proinflammatory capases include CASP4, CASP5, CASP11, and CASP12. CASP1 catalytic activity is tightly regulated and essential for pro-IL1B and pro-IL18 processing and secretion. Inflammasomes require two signals to accomplish their biological function. Signal I initiates transcriptional activation of inflammasome components and is often provided through TLR and NFKB1 signaling, whereas signal II is required to initiate inflammasome assembly. For example, during influenza infection, signal I requires TLR7 viral RNA recognition and signal II activation of inflammasomes requires an acidified Golgi compartment (Ichinohe *et al*., 2010).

Additional cytosolic PRRs are the RIG-I–like receptors (RLRs), a 3-protein family of DExD/H box RNA helicases that function as cytoplasmic sensors of viral PAMPs. The RLRs signal downstream transcription factor activation to drive type 1 IFN production and antiviral gene expression that elicits responses to control virus infection (Loo and Gale, 2011). The founding member of the family is DEAD (Asp–Glu–Ala–Asp) box polypeptide 58 (DDX58 aka RIG-I: retinoic acid-inducible gene I), which recognizes viral dsRNA. Lung pathogens recognized by DDX58 include influenza A, influenza B, respiratory syncytial virus, and Sendai virus. The second member is IFN induced with helicase C domain 1 (IFIH1 aka MDA5 melanoma differentiation associated factor 5). IFIH was originally named MDA5 because it increases in response to β-IFN, β1, fibroblast (IFNB), and mezerein, a protein kinase C-activating compound, which causes melanoma differentiation. These proteins contain an N-terminal region consisting of tandem CARD domains, a DExD/H box RNA helicase domain with the capacity to hydrolyze ATP and to bind and possibly unwind RNA, and a C-terminal repressor domain embedded within the C-terminal domain that in the case of RIG-I is involved in autoregulation. DDX58 preferentially associates with influenza's shorter RNA fragments (eg, 50 triphosphorylated ends-RNA sequence motifs) and some dsRNA regions (Baum *et al*., 2010). In contrast, IFIH interacts with and preferentially recognizes high-molecular-weight fragments (eg, picornaviral ssRNA or longer synthetic poly(I:C) motif). The third member is DEXH (Asp–Glu–X–His) box polypeptide 58 (DHX58 aka LGP2: laboratory of genetics and physiology 2 and a homolog of mouse D11lgp2). Although similar to DDX58 and IFIH in structure, DHX58 lacks the N-terminal CARD domains and may function as a regulator of DDX58/IFIH signaling.

Upon PAMP recognition, PRR proteins recruit a specific set of adaptor molecules that share a cytoplasmic domain that helps to initiate the cell signaling that actives antimicrobial responses and transcription of intra- and intercellular signaling molecules. For example, the TIR domains of TLR4 interact with the TIR-domain containing adaptor proteins, including the myeloid differentiation primary response gene (88) (MyD88) or TLR adaptor molecule 1 (TICAM1 aka TRIF), and initiate downstream signaling events (Kawai and Akira, 2010). The TLR4–TICAM1–MyD88 complex activates the IL1-receptor–associated kinase 1 (IRAK1) and TNF-receptor–associated factor 6 (TRAF6) complex, which in turn, activates transforming growth factor (TGF)-beta–activated kinase 1 (TAK1). This leads to the activation of NFKB1 by removal of the inhibitory protein, nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, α (NFKBIA), and other members of an inhibitory complex that prevents NFKB1 movement to the nucleus. Nuclear NFKB1 increases transcription of a wide array of cytokines and chemokines. The secretion of inflammatory the newly generated cytokines attract and activate other leukocytes and structural cells. Alternatively, when TLR4 is internalized within the phagosome, it can form a complex with RAB11A, member RAS oncogene family (RAB11A), TICAM1, and TICAM2 (aka TRAM). This initiating step can either activate TRAF6 or add to a late NFKB1 response that causes additional recruitment of neutrophils and activation of macrophages. In addition, phagosomal TLR4 can activate TRAF3 that leads to IFN regulatory factor 3 (IRF3) activation. IRF3 translocates to the nucleus and activates the transcription of type I IFN (eg, *IFNA1* and *IFNB1*) genes, as well as other IFN-induced genes. This can result in direct killing of infected pathogens. Moreover, activation of TLR signaling leads to maturation of dendritic cells, a macrophage that presents antigens to lymphocytes, contributing to the induction of adaptive immunity (see below).

ACUTE RESPONSES OF THE LUNG TO INJURY

Trigeminally Mediated Airway Reflexes

Inhaled toxic chemicals or particles can come into contact with cells lining the respiratory tract from the nostrils to the gas-exchanging region. The sites of deposition in the respiratory tract have important implications in evaluating the risks posed by inhalants. For example, rats have more nasal surface on a per body weight basis than do humans. Measurement of DNA–protein cross-links formed in nasal tissue by formaldehyde has demonstrated that rats, which

readily develop nasal tumors, have many more DNA cross-links per unit of exposure (concentration of formaldehyde × duration of exposure) than monkeys. Because the breathing pattern of humans resembles that of monkeys more than that of rats, extrapolation of tumor data from rats to humans on the basis of formaldehyde concentration may overestimate nasal doses of formaldehyde to humans. Patterns of animal activity can affect dose to the lung; nocturnally active animals such as rats receive a greater dose per unit of exposure at night than during the day, whereas humans show the opposite diurnal relationships of exposure concentration to dose.

Nasal and airway irritation represents a common response to inspired toxic compounds (Lanosa *et al.*, 2010). Nasal irritation is mediated by irritant receptors (eg, TRPA1) that trigger trigeminal nerves characterized by tickling, itching, and painful nasal sensations (Alarie, 1973; Nielsen *et al.*, 2007). TRPA1 is sensitive to several irritants including acrolein, allyl isothiocyanate, chlorine, 4-hydroxynonenal, and hydrogen peroxide (Bautista *et al.*, 2006; Bessac and Jordt, 2008; Bessac *et al.*, 2009). Nasal tissues of rodents express high levels of cytochromes P450 (CYP450) (Morris, 2000; Ding and Kaminsky, 2003), and biotransformation enzymes that can result in the formation of electrophilic metabolites from volatile organic compound vapors that activate TRPA1 (Lanosa *et al.*, 2010). For example, styrene (Morris, 2000) and naphthalene (Morris and Buckpitt, 2009) are extensively metabolized by CYP2F isozymes within nasal mucosa. When the concentration of an inhaled substance exceeds the biotransformation capacity of the nasal passages, it can penetrate on to the lower respiratory tract (Morris, 2001).

Nasal irritation has been used as a basis for occupational exposure levels and is a common component in sick building syndrome resulting from poor indoor air quality (Hall *et al.*, 1993; Hodgson, 2002). One of the consequences of nasal irritation is nasal mucus secretion that can dilute the irritant. In some cases, severe irritation that limits exposure can occur at concentrations below those that induce toxic response upon chronic exposure. These can be viewed as protective mechanisms. However, because the threshold dose of an irritant response can vary greatly among individuals (much like olfactory acuity), nasal irritation is not a reliable method for occupational safety. In mice, sensory irritation can lead to decreased breathing frequency during irritant exposure or increased pause at the onset of expiration, which can be highly quantifiable (Alarie, 1973; Vijayaraghavan *et al.*, 1993; Morris *et al.*, 2003). If continued exposure cannot be avoided, many irritants will produce cell necrosis and increase permeability of the airway epithelium.

Bronchoconstriction, Airway Hyperreactivity, and Neurogenic Inflammation

Substances that penetrate the nasal passage or are inhaled orally (with less efficient deposition) can trigger irritant receptors in the airways. Large diameter airways are surrounded by collagen and bronchial smooth muscle, which helps maintain airway tone and diameter during expansion and contraction of the lung. Bronchoconstriction can be evoked by irritants (acrolein, etc), cigarette smoke, or air pollutants, and by cholinergic drugs such as acetylcholine. Bronchoconstriction causes a decrease in airway diameter and a corresponding increase in resistance to airflow. Bronchoconstriction can also be due to an accumulation of thick mucus. Characteristics associated with signs include coughing, wheezing, and rapid shallow breathing, and those associated with symptoms include a sensation of chest tightness, substernal pain, and dyspnea (a feeling of breathlessness). Dyspnea is a normal consequence of exercise, which potentiates these problems as well as leads to more mouth breathing. Because the major component of airway resistance usually is contributed by large bronchi, inhaled chemicals that cause bronchoconstriction are generally irritant gases with moderate solubility.

Bronchial smooth muscle tone is regulated by the autonomic nervous system. Postganglionic parasympathetic fibers will release acetylcholine to the smooth muscle layer surrounding the bronchi. These smooth muscle cells have membrane cholinergic receptors, muscarinic 3 (CHRM3). The activation of these receptors by acetylcholine activates an intracellular Gq protein domain, which increases cGMP. The increase in cGMP in turn activates a phospholipase C (PLC) pathway that increases intracellular calcium concentrations $[Ca^{2+}]_i$. Increased $[Ca^{2+}]_i$ leads to contraction of the smooth muscle cells. The actions of cGMP can be antagonized by increased cAMP evoked by protein kinase A (PKA). Increased cAMP can be accomplished by agents that bind to β-adrenergic receptors on the cell surface. The latter can be stimulated by inhaled bronchodilators (β-adrenergic agonists such as albuterol) or by injected epinephrine (adrenaline).

In addition to inducing an acute bronchoconstriction, irritants can prime the autonomic response by lowering the threshold dose of acetylcholine needed to induce bronchoconstriction. A lower threshold of acetylcholine-mediated bronchoconstriction is called airway hyperreactivity (or hyperresponsiveness). This response serves as the basis for a sensitive measure of whether a toxicant can cause bronchoconstriction in animals or humans primed by a prior dose of an acetylcholine-like chemical (bronchoprovocation testing). These tests are performed by measuring airway resistance following inhalation of increasing doses of a methacholine aerosol. Methacholine is used because it is more stable than acetylcholine. Other important bronchoconstrictive substances include histamine, various eicosanoids (including PGs [mainly $PGF_2\alpha$ and PGD_2], thromboxane A_2, leukotrienes C_4 and D_4), and adenosine. The bronchial smooth muscles of individuals with asthma contract at a lower threshold dose during provocation than do those of individuals without asthma (see Asthma).

In addition to bronchoconstriction, cough, and airway hyperreactivity, irritants can stimulate TRP channels (especially TRPA1 and TRPV1) that cause neurogenic inflammation. Mediated by neuropeptides (including tachykinins) released from nociceptive nerve terminals, neurogenic inflammation also includes vasodilatation, plasma protein extravasation, and leukocyte adhesion to the vascular endothelium (Geppetti *et al.*, 2006). Tachykinins including substance P (aka neurokinin 1) activate tachykinin receptors (TACRs) on airway tissues. Tachykinins stimulate airway smooth muscle TACR2s and TACR1s to mediate bronchoconstriction, submucosal gland TACR1s to mediate mucin secretion, and cholinergic nerve TACR3s to mediate terminal stimulation. TRPV1 mediates the tussive action (cough) of capsaicin, which is widely used in cough provocation studies. TRPA1 is targeted by acrolein, 4-hydroxy-2-nonenal, and hydrogen peroxide. TRPV1 and TRPA1 antagonists may therefore represent potential antitussive and anti-inflammatory therapeutics for respiratory airway diseases.

Acute Lung Injury (Pulmonary Edema)

Initiated by numerous factors, acute lung injury (aka adult or infant respiratory distress syndrome) is marked by alveolar epithelial and endothelial cell perturbation and inflammatory cell influx that leads to surfactant disruption, pulmonary edema, and

atelectasis (Ware and Matthay, 2000). Pulmonary edema produces a thickening of the alveolar capillary barrier and thereby limits O_2 and CO_2 exchange. Matching ventilation to vascular perfusion is critical to efficient gas exchange and is disrupted during acute lung injury. Alterations in coagulation and fibrinolysis accompany lung injury. Pulmonary edema may not only induce acute compromise of lung structure and function but also cause abnormalities that remain after resolution of the edematous process. Alveolar and interstitial exudates are resolved via fibrogenesis, an outcome that may be beneficial or damaging to the lung. Accumulation and turnover of inflammatory cells and related immune responses in an edematous lung probably play a role in eliciting both mitogenic activity and fibrogenic responses. During acute lung injury, profibrotic growth factors, TGFB1 (Wesselkamper *et al.*, 2005) and platelet-derived growth factor (PDGF) (Bellomo, 1992) are activated and can initiate epithelial–mesenchymal transition (EMT) (see Pulmonary Fibrosis).

When inhaled in high concentrations, acrolein, HCl, NO_2, NH_3, or phosgene may produce immediate alveolar damage leading to a rapid death. However, these gases inhaled in lower concentrations may produce very little apparent damage in the respiratory tract. After a latency period of several hours, exposure to these compounds may compromise alveolar barrier function that leads to delayed pulmonary edema that is often fatal. In addition, acute lung injury can result from systemic effects including sepsis, transfusion, and blunt trauma to other organs. In several animal studies, neutrophil depletion can be protective; however, acute lung injury can develop in the absence of circulating neutrophils.

During acute lung injury, innate immunity is activated through activation PRRs (Xiang and Fan, 2010). In addition, a number of damage-associated molecular pattern proteins (DAMPs) are formed and include AGEs, high-mobility group protein B1 (HMGB1), amyloid β-peptide, and members of the S100 calcium binding protein (especially S100A8 [aka calgranulin A], S100A9 [aka calgranulin B], and S100A12 [aka ENRAGE]). These ligands bind to the ectodomain of AGE receptor (AGER aka RAGE) and initiate intracellular signaling leading to NFKB1-mediated induction of inflammatory cytokines and through positive feedback, AGER expression. In particular, HMGB1 is a potent mediator of acute lung injury and can induce PMN accumulation, edema formation, and production of proinflammatory mediators in the lung. HMGB1 is also a mediator of lethality during endotoxemia and sepsis in mice. In humans with severe trauma, plasma HMGB1 levels correlate positively with severity of injury and progression to acute lung injury. Activated macrophages and other inflammatory cells produce excessive reactive oxygen (superoxide anion, hydroxyl radicals, hydrogen peroxide) and nitrogen (nitric oxide, peroxynitrate) species. Because these oxidant species are potentially cytotoxic, they may mediate or promote the actions of various respiratory toxicants. Such mechanisms have been proposed for paraquat- and nitrofurantoin-induced lung injury. In this regard, the lung is well equipped with antioxidant enzymes, especially SOD3, extracellular, which converts superoxide anion to hydrogen peroxide. Hydrogen peroxide is the mediator of the extracellular cytotoxic mechanism of activated phagocytes. In addition, hydrogen peroxide is a potent intracellular signaling molecule that readily crosses cell membranes, and can thereby amplify cell damage. Phagocytic production of reactive oxygen species causes activation of proteinase enzyme and inactivation of proteinase inhibitors. Platelets (and platelet microthrombi) also have the ability to generate activated O_2 species.

Pulmonary edema is customarily quantified in experimental animals by measurement of lung water content. Lung water content can be expressed as the wet (undesiccated) weight of the whole lung or that of a single lung lobe. This value is often normalized to the weight of the animal or to the weight of the lung after complete drying in a desiccator or oven. The latter is typically expressed as lung wet weight:dry weight ratio.

CHRONIC RESPONSES OF THE LUNG TO INJURY

Chronic Obstructive Pulmonary Disease

Characterized by a progressive airflow obstruction, COPD involves an airway (bronchitis) and an alveolar (emphysema) pathology. With more than 200 million cases worldwide, COPD is the fourth leading cause of death (Pauwels and Rabe, 2004). The major risk factor for COPD is tobacco smoking and about 20% of smokers will develop COPD. Indoor air pollution from burning biomass fuels is associated with an increased risk of COPD in developing countries (Salvi and Barnes, 2009).

Chronic bronchitis is defined by the presence of sputum production and cough for at least three months in each of two consecutive years. Bronchitis in COPD involves airway inflammation with excessive mucus production from surface epithelial (goblet) cell and submucosal glands (Leikauf *et al.*, 2002a; Rogers, 2007). The number of goblet cells increases, the number of ciliated cells decreases, and the size of the submucosal glands increase markedly. The latter are the major source of mucus and thereby sputum in the disease (Reid, 1960). The associated decreased mucociliary clearance and mucus retention may obstruct the airways and contribute to COPD exacerbations and possibly mortality (Miravitlles, 2011). Acrolein can increase mucin overproduction and can be formed endogenously in the airways during COPD (Bein, 2011).

COPD is almost characterized by chronic cough. A cough starts with activation of the sensory terminals of cough receptors located in the airway mucosa that triggers a deep inhalation and a forced respiratory exhalation against a closed glottis. It ends with the opening of the glottis that produces a rapid acceleration of air from the lungs accompanied by a distinct sound (Chung and Pavord, 2008). Frequent coughing in COPD can be exacerbated by respiratory infections, and many viruses and bacteria induce cough to move from host to host. Respiratory mechanical and ligand-gated cough receptors on rapidly adapting receptors, C fibers, and slowly adapting fibers provide input to the brainstem medullary central cough generator through the intermediary relay neurons in the nucleus tractus solitarius. As noted above, cough can be evoked by thermal, osmotic, and chemical (especially capsaicin) stimuli that engage the TRP channel (especially TRPV1) protein family. In addition, respiratory ligand-gated receptors are the degenerin/epithelial sodium channel proteins that include nonvoltage-gated sodium channels (eg, SCNN1A) and amiloride-sensitive cation channels (ACCN2 aka acid-sensing ion channel 1) (Kollarik and Undem, 2006). Members of both families have been implicated in the transduction of mechanical and acidic stimuli. Cough can also be evoked by stimuli of other ligand-gated receptors including 5-hydroxytryptamine activation of 5-hydroxytryptamine (serotonin) receptor 3A (HTR3A), ATP activation of purinergic receptor P2X, ligand-gated ion channels, and nicotine activation of chloinergic receptors, nicotinic subtypes. The central cough generator then establishes and coordinates the output to the muscles that cause cough, bronchoconstriction, and mucus secretion. Cough is effective in removing mucus in the first five to eight bronchial generations but as the cumulative diameter of the airways increases the acceleration of airflow is diminished.

Emphysema is physiologically defined by airflow obstruction that leads to dyspnea (especially on excursion) (Fig. 15-8) accompanied by diminished FEV1. The diagnosis of COPD is made by decreased FEV1:FVC ratio (which is not reversible by administration of a bronchodilator) and includes mild (<80% of normal; GOLD: I), moderate (50%–57%; GOLD: II), severe (30%–49%; GOLD: III), and very severe (<30% or chronic respiratory failure; GOLD: IV) (Rabe *et al*., 2007). Emphysema is pathologically defined by an abnormal enlargement of the airspaces distal to the terminal bronchiole accompanied by destruction of the walls without obvious fibrosis (Snider *et al*., 1985). Destruction of the gas-exchanging surface area results in a distended, hyperinflated lung that no longer effectively exchanges oxygen and carbon dioxide as a result of both loss of tissue and air trapping. Pathologically, emphysema can be (a) centriacinar emphysema that begins in the respiratory bronchioles and spreads peripherally (typically starting in the apical lung and is associated with cigarette smoking), (b) panacinar emphysema that destroys the entire alveolus uniformly (typically in the lower half of the lungs), and (c) paraseptal emphysema that forms giant bullae.

The pathogenesis of emphysema involves a proteinase–antiproteinase imbalance that leads to the remodeling of the supportive connective tissue in the parenchyma and separate lesions that coalesce to destroy lung tissue (Shapiro and Ingenito, 2005). This mechanism was proposed following the associated of serpin peptidase inhibitors, clade A (α-1 antiproteinase, antitrypsin), member 1 (SERPINA1) deficiency with early-onset emphysema (Laurell and Eriksson, 1963) and the ability to induce emphysema in rodents instilled with proteinase, for example, papain (Gross *et al*., 1965) or neutrophil elastase (ELANE) (Janoff *et al*., 1977). SERPINA1 inhibits serine proteinases including trypsin and ELANE and protects against cigarette smoke–induced alveolar enlargement in mice (Churg *et al*., 2003; Churg, 2003). ELANE-deficient mice are partially protected against emphysema (Shapiro *et al*., 2003) and macrophage matrix metalloproteinase 12 (MMP12 aka macrophage elastase)–deficient mice do not develop cigarette smoke–induced alveolar enlargement (Hautamaki *et al*., 1997). In addition to MMP12, MMP1 (D'Armiento *et al*., 1992), MMP2, MMP9 (Senior *et al*., 1991), proteinase 3 (PRTN3) (Kao *et al*., 1988), and plasminogen activator tissue (PLAT) (Chapman *et al*., 1984) can mediate alveolar enlargement and MMP14, and ADAM metallopeptidase domain 17 (ADAM17) can mediate mucus overproduction. Both have been demonstrated in laboratory animals or human cells in culture. These changes can be accompanied by decreases in antiproteinases, for example, tissue inhibitor of metalloproteinase 3 (TIMP3) decreases when MMP9 increases in mucus overproduction (Deshmukh *et al*., 2008).

In all individuals, elastolytic events accumulate with time and can cause a decline in lung function that is normally associated with aging. Toxicants that activate macrophages or cause inflammatory cell influx (which increases the burden of ELANE) can accelerate this process. Macrophages also can be activated by endogenous intracellular DAMPs released by activated or necrotic cells and degraded ECM molecules that increased following tissue damage. For example, ECM elastin fragments have been implicated in cigarette smoke–induced emphysema in mice (Houghton *et al*., 2006).

In addition to macrophage and neutrophil activation, COPD involves cytotoxic T-cell lymphocytes (CTLs) that express a killer cell lectin-like receptor subfamily K, member 1 (KLRK1 aka natural killer cell group 2D) receptor, which recognizes MHC class I polypeptide-related sequence A (MICA) and MICB, and the related UL16-binding proteins (ULBP1, ULPB2, and ULBP3)

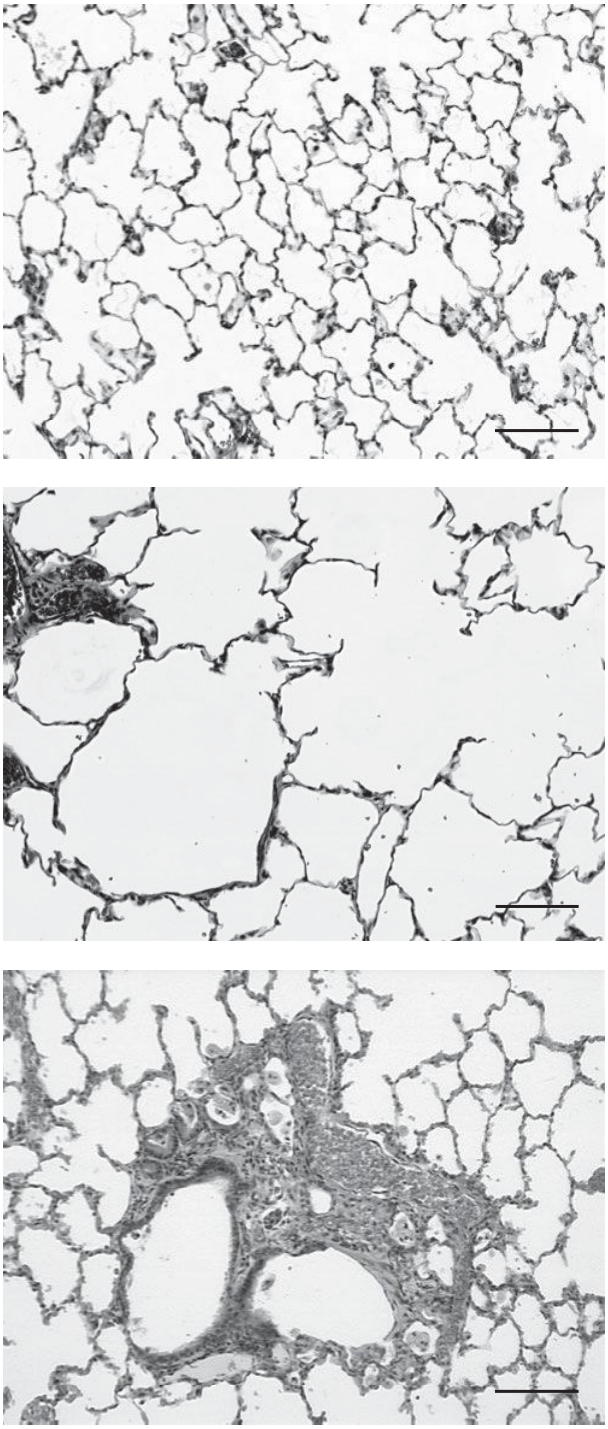

Figure 15-8. *Airspace enlargement induced by tobacco smoke and pulmonary fibrosis induced by asbestos in rat lung.* Top panel: Normal rat lung. Middle panel: Extensive distention of the alveoli (emphysema) in rat lung following inhalation of tobacco smoke (90 mg/m^3 of total suspended particulate material). Bottom panel: Lung of a rat one year after exposure to chrysotile asbestos. Note accumulation of connective tissue around blood vessel and airways (fibrosis). Bar length: 100 µm. (Photograph courtesy of Dr Kent E. Pinkerton, University of California, Davis.)

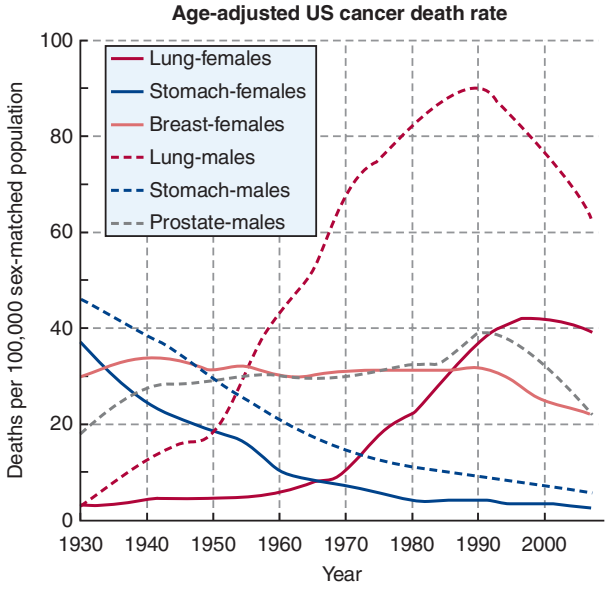

Figure 15-9. *Trends in age-adjusted United States cancer death rates for stomach, lung, breast (females), and prostate (males) cancers.* Solid line: females; dashed line: males. (From US Mortality Volumes 1930 to 1959, US Mortality Data, 1960 to 2007. National Center for Health Statistics, Centers for Disease Control and Prevention.)

(Borchers *et al*., 2009). Respiratory epithelial cells undergoing toxicant stress (eg, caused by cigarette smoke or infection) express MICA on the cell surface and emphysema results from epithelial expression of the murine homolog (retinoic acid early transcript 1, α). These receptors are recognized by KLRK1 expressing CTLs, which then selectively perform cell cytolysis on the targeted cell and thereby remove damage or infected cells.

Lung Cancer

At the beginning of the 20th century, lung cancer was a rare disease. Because of cigarette smoking, lung cancer is now the leading cause of death from cancer among men and women in the United States (Siegel *et al*., 2011) (Fig. 15-9). In addition to inducing COPD, retrospective and, more conclusively, prospective epidemiological studies unequivocally associated tobacco smoking with this epidemic in lung cancer. Currently, ~75% to 85% of lung cancer cases (and many cases of cancer of the bladder, esophagus, oral cavity, and pancreas) are caused by cigarette smoking. The main factor responsible for smoking dependence is nicotine, a ligand for the nicotinic acetylcholine receptor α subunit, which makes up 0.3% to 5% of tobacco plant and accumulates in the tobacco leaf during curing. The increased risk of developing lung cancer for average smokers compared with nonsmokers is 8- to 10-fold and for heavy smokers about 20- to 40-fold (Pope *et al*., 2011). Stopping smoking reduces the risk of developing lung cancer or COPD (International Agency for Research on Cancer [IARC], 2004). The global burden is ~1.2 million cancer deaths per year, with 85% of lung cancer cases in men and 47% of lung cancer cases in women being attributable to tobacco use.

The development of lung cancer is likely to be a result of multiple genetic and gene–environment interactions because not all smokers develop lung cancer and nonsmokers develop lung cancer (Sun *et al*., 2007). Single-nucleotide polymorphisms (SNPs) located on chromosomal regions 5p15.33 and 15q25.1 have been consistently identified by genome-wide association studies (GWAS) as significant predictors of lung cancer risk, and an SNP in 6p22.1 has been associated with survival time in small-cell lung cancer (SCLC) patients (Xun *et al*., 2011). Others have used transcription signatures to predict prognosis and therapeutic options in lung cancer (Chen *et al*., 2007; Kadara *et al*., 2011).

Many chemical exposures encountered in industrial settings also pose a lung cancer risk. Arsenic, asbestos, beryllium, cadmium, chromium, and nickel have been associated with cancer of the respiratory tract (IARC, 1993). Workers exposed to chloromethyl ether, mustard gas, or effluent gases from coke ovens have an increased lung cancer risk. First noted among radium miners and then in homes, radon gas is a known human lung carcinogen. Formaldehyde is a probable human respiratory carcinogen. Silica, human-made fibers, and welding fumes are suspected carcinogens. Smokers who inhale radon or asbestos fibers increase their risk for developing lung cancer several fold, suggesting a synergistic interaction between the carcinogens. For example, working with asbestos increases the risk about twofold and for persons who smoke moderately the risk is eightfold. However, asbestos workers who smoke moderately have an associated risk of 16-fold.

Lung cancer in never-smokers is the seventh leading cause of cancer death worldwide (Sun *et al*., 2007). In addition to radon, indoor environmental tobacco smoke increases the risk of developing lung cancer in nonsmokers (IARC, 2004). In addition, biomass cooking, high-temperature oil cooking, and coal heating especially in poor-ventilated households are associated with lung cancer, COPD, and pneumonia, together leading to over 2 million deaths per year worldwide (World Health Organization [WHO], 2006). Ambient $PM_{2.5}$ has been associated with increased lung cancer risk (Beeson *et al*., 1998; Pope *et al*., 2002).

Human lung cancers may have a latency period of 20 to 40 years, making the relationship to specific exposures difficult to establish. The two major forms of lung cancer are non–small-cell lung cancer (NSCLC; which accounts for ~85% of all lung cancer cases) and SCLC (which accounts for ~15% of all lung cancer cases) (Herbst *et al*., 2008). NSCLC can be subdivided into three major histological subtypes: squamous-cell carcinoma, adenocarcinoma, and large-cell lung cancer. Smoking causes all types of lung cancer but is most strongly linked with SCLC and squamous-cell carcinoma. Adenocarcinoma is the most common type in patients who have never smoked (Sun *et al*., 2007). Large-cell lung is really rare and makes up less than 10% of the NSCLC cases.

Compared with lung cancer, cancer in the upper respiratory tract is less common in humans but can occur frequently in experimental animals exposed to inhaled carcinogens (Harkema *et al*., 2006). Nonetheless, head and neck cancers are still a major cause of cancer deaths worldwide. Upper respiratory cancers are associated occupational exposures to chromium, nickel, mustard gas, and isopropyl alcohol. Nasal cancers also are associated with wood dust, textile dust, and possibly leather dust or formaldehyde. Buccal carcinogens are associated with tobacco smoke, smokeless tobacco, betel quid chewing, and alcohol consumption.

Epithelial lung cancers develop in a sequence of distinct morphological changes. Initially, cell numbers in the epithelium lining the airways increase (hyperplasia) and eventually display abnormal nuclei and changes in shape (dysplasia), often assuming squamous-cell characteristics (squamous metaplasia). The lesions then progress to first carcinoma in situ, an accumulation of cancerous cells in small foci and then into large tumor masses. Eventually tumor cells invade adjacent local tissues, blood vessels, and lymphatics, leading to the formation of distant metastases. The histologically visible sequential development is accompanied by multiple molecular lesions (Wistuba and Gazdar, 2003).

The potential mechanisms of lung carcinogenesis have been studied extensively by means of analysis of tumors and human bronchial cells maintained in culture. A key component is DNA damage induced by reactions with an activated carcinogen or its metabolic product, such as PAHs (eg, benzo[*a*]pyrene: BaP) or alkyldiazonium ions derived from tobacco specific *N*-nitrosamines (TSNAs). Formed from nicotine in the tobacco curing process, TNSAs include a potent carcinogen, nitrosoaminoketone (NNK) (ie, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) (Ter-Minassian *et al*., 2011). Found in unburned tobacco and tobacco smoke, NNK is metabolized to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide metabolites (NNAL-Gluc). NNK and NNAL form DNA adducts, which are found in lung tumors in mice treated with NNK. Urinary levels of total NNAL (NNAL plus NNAL-Gluc) are associated with lung cancer in a dose-dependent manner in a cigarette smoker (Yuan *et al*., 2009).

Persistence of O^6-alkyl-deoxyguanosine mutations in DNA appears to correlate with carcinogenicity (Hecht, 1999). However, tumors do not always develop when adducts are present, and adduct formation may be a necessary but not sufficient condition for carcinogenesis. DNA damage caused by reactive oxygen species is another potentially important mechanism. Ionizing radiation leads to the formation of superoxide, which is converted through the action of SOD to hydrogen peroxide. In the presence of Fe and other transition metals, hydroxyl radicals may be formed, which then cause DNA strand breaks. Cigarette smoke contains high quantities of reactive oxygen species (Pryor, 1997) and acrolein (Bein, 2011). Additional oxidative stress may be placed on the lung tissue of smokers by the release of reactive oxygen/nitrogen species formed by activated macrophages and other inflammatory leukocytes. In addition, diminished DNA repair and loss of enzymes that maintain chromosomal integrity (eg, aberrant telomere homeostasis that evades senescence) are critical events in many cancers, including lung cancer (Buch *et al*., 2011).

Genomic instability is a hallmark of lung cancer (Sato *et al*., 2007). Critical genetic and epigenetic changes include DNA mutations, loss of heterozygosity, and promoter methylation and global transcriptome changes that can include stimulation of mitogenic pathways and suppression of apoptosis pathways. Loss of heterozygosity is the loss of normal function of one allele of a gene in which the other allele was already inactivated. In the context of lung cancer, an inactivated allele of a tumor suppressor gene could be inherited from a parent's germline cell that was passed on to the offspring. The offspirng would thereby be heterozygous for that allele and subsequent exposure to a mutagen during the offspring's life could then inactivate the functional allele. Genetic and epigenetic changes can persist over years and eventually lead to aberrant cellular function to produce premalignant changes, including dysplasia and clonal patches.

Many molecular changes in earliest-stage cancer also occur in advanced disease (Herbst, 2008). Premalignant patches contain clones and subclones, which often involve mutations in tumor protein p53 (TP53 aka p53) and epidermal growth factor receptor (EGFR). Lung cancers unrelated and related to smoking have different molecular profiles, mutation in EGFR being more common in never-smokers, and v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) being more common in smokers. Mutation TP53 are found in both smokers and nonsmokers; however, TP53 are more frequent in never-smokers (Sun *et al*., 2007). In addition, the TP53 mutational signature (ie, relative amounts of specific transitions, transversions, or deletions), and spectrum (ie, specific mutation within the gene) differ between smokers and never-smokers.

In response to diverse cellular stresses, TP53 regulates target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. TP53 is a DNA-binding protein containing transcription activation, DNA-binding, and oligomerization domains. Normally, TP53 can bind to a consensus DNA-binding site and activate expression of downstream genes that inhibit growth and/or invasion, and thus it functions as a tumor suppressor. Found in both NSCLCs and SCLCs, mutant TP53 protein fails to bind these sites, and hence lacks tumor suppressor activity. Acrolein (Tang *et al*., 2011) and BaP (Denissenko *et al*., 1996) adducts of TP53 are common in lung cancers.

A receptor tyrosine kinase (RTK), EGFR is a receptor for a number of growth factor ligands (including EGF and transforming growth factor α) and subsequently can stimulate downstream signaling pathways of mitogen-activated protein kinase and phosphatidylinositol-3 kinase. Amplications of *EGFR* are more common in squamous-cell carcinoma, whereas a kinase domain mutation is more common in adenocarcinoma. The latter occurs more frequently in women, persons of Han decent, and nonsmokers who develop lung cancer. This kinase is critical because it is the target of the RTK inhibitors gefitinib and erlotinib, which have been particularly successfully in treatment of this type of lung cancer. Also a member of the EGFR family, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma-derived oncogene homolog (avian) (ERBB2 aka HER2) protein lacks a ligand-binding domain and therefore cannot bind growth factors. However, it does bind tightly to other ligand-bound EGF receptor family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signaling pathways that stimulate cell proliferation. Mutations in this gene are associated with adenocarcinoma.

KRAS is a guanine nucleotide (GDP/GTP)-binding protein that acts as a self-inactivating signal transducer. Normal KRAS can be transiently activated by signals from cell surface receptors that promote the exchange of bound GDP for GTP. The transient nature of this response is ensured by the intrinsic ability of KRAS to hydrolyze GTP, and thereby to switch itself off. Single point mutations produce KRAS proteins with amino acid substitutions that have reduced GTPase activity. Mutant KRAS proteins are thereby locked in a constitutively activated GTP-bound state. KRAS mutations are more common among or are associated with adenocarcinomas among smokers and activated NNK and BaP for KRAS bulky adducts. Mutation or amplification of *TP53*, *EGFR*, *ERBB2*, or *KRAS* thus provides a proliferative advantage to tumor cells.

Other RTKs mutated in lung cancer include Met proto-oncogene (hepatocyte growth factor receptor) (MET) and anaplastic lymphoma RTK (ALK). The ligand for MET is hepatocyte growth factor, HGF, which despite its name is a growth factor in the lung and important in repair (Ware and Matthay, 2002). ALK belongs to the insulin receptor superfamily. A chromosomal rearrangement of the *ALK* gene that leads to fusion with echinoderm microtubule-associated protein like 4 (*EML4*) can be found in about 7% of adenocarcinomas. Other fusion proteins can be generated with the ALK protein. A specific RTK inhibitor of MET and ALK, Crizotinib, has been effective against NSCLC cell lines that express oncogenic ALK fusion proteins and has been useful in an initial clinical trial for lung cancers of this type (Rodig and Shapiro, 2010).

Serine/threonine kinase 11 (STK11 aka liver kinase B1 homolog or LKB1) regulates cell polarity and other cellular functions. Normally, STK11 protein activates AMP-activated protein kinase (AMPK), which leads to growth suppression. However, mutant STK11 proteins lack the ability to phosphorylate AMPK and to interact with TP53 that diminish transcription of target genes.

Mutations in *STK11* are associated with smoking and are common in adenocarcinoma and squamous-cell carcinoma in persons of European decent, whereas it is uncommon in adenocarcinoma in persons of Hun decent. *STK11* mutations often are present in tumors with *KRAS* mutations, but are not found in tumors with *EGFR* mutations.

Other mutations common in lung cancer occur in NK2 homeobox 1 (NKX2-1 aka thyroid transcription factor 1: TITF1), and phosphoinositide-3-kinase, catalytic, α polypeptide (PIK3CA). Cancer stem cells (CSCs) are a subset of cancer cells with the ability to initiate cancer when injected into mice (Martelli *et al*., 2011). CSCs are thought to be responsible for tumor onset, self-renewal/maintenance, mutation accumulation, and metastasis. The existence of CSCs could explain the high frequency of neoplasia relapse and resistance to therapies. Critical for alveolar type II cell development, NKX2-1 may have a role in modulating stem cell functions and *NKX2-1* amplication (with increased copy number) is common in adenocarcinoma. PIK3CA is the catalytic subunit of PI-3-kinase (PI3K), which is a member of the v-akt murine thymoma viral oncogene homolog 1 (AKT1) and mechanistic target of rapamycin (serine/threonine kinase) (MTOR) survival pathway. The PI3K–AKT1–MTOR pathway is downstream of EGFR and mutation in this pathway protects against apoptosis in tumor cells. Pharmaceuticals that inhibit PI3K/AKT1/MTOR can induce apoptosis in tumors and is a new target for eliminating CSCs.

Another common alteration is increased v-myc myelocytomatosis viral oncogene homolog (avian) (MYC aka c-Myc). MYC is frequently amplified in SCLC cell lines in a higher frequency than tumors, suggesting that MYC is more related to tumor progression and a relatively late event in lung cancer development. A transcription factor, MYC may contribute to the production of multiple growth factors that can promote and inhibit the proliferation via paracrine and autocrine loops via specific receptors.

In toxicological testing, lung tumors or cancers in laboratory animals do not always mimic those in humans. Most lung tumors in mice are peripherally located adenomas and adenocarcinomas, even after tobacco or NNK exposure. Rarely do mice or rats develop bronchial squamous-cell carcinoma found in humans that smoke. Rats on occasion develop lung tumors that are characterized by an epithelium surrounding a space filled with keratin. The mass may compress the adjacent lung parenchyma and occasionally invades it. These lesions are classified by some pathologists as bona fide tumors, whereas other pathologists characterize this type of lesion as a cyst filled with keratin. Classification of such a lesion is important because these lesions often are found in long-term tests in animals that have been exposed to agents that are not considered carcinogens, such as carbon black, titanium dioxide, and certain human-made fibers (ILSI, 2000). In other animal tests, a large particle number or mass is instilled into the trachea as a single exposure, which is used to mimic a daily or a lifetime exposure burden. These instillations may not be evenly distributed in the lung and histological examination often demonstrates focal accumulations of a large number of particles almost never observed in humans even after a lifetime of exposure. A unifying hypothesis postulates that because clearance mechanisms in the deep lung depend predominantly on phagocytosis and migration of pulmonary alveolar macrophages, such large doses overwhelm clearance mechanism and elicit irresolvable inflammation (called particle overload) (Morrow, 1992). As a consequence, lung burdens of these dusts persist for months or years, and may evoke unique mechanisms of disease pathogenesis not applicable to human experience with inhaled chemicals or particles. The issue of whether particle overload is useful in defining a toxicity threshold in such experiments remains unresolved.

Asthma

Asthma is defined by sporadic bouts of airflow obstruction, leading to dyspnea. Airflow obstruction is measured as increased airway resistance (or decreased predicted FEV1). In asthma, resting levels of airway resistance can be normal or slightly increased, which typically is reversible with bronchodilators. The hallmark of asthma is a persistent or recurrent airway hyperreactivity. As noted above, airway hyperreactivity is defined by a lower threshold to acetylcholine- or methacholine-mediated increased airway resistance. Airway hyperreactivity can be specific, as in allergic asthma, and induced by exposure to a single known irritant or antigen. Knowledge of the causal agent is extremely valuable because avoidance can reduce the frequency of or even eliminate bronchospasm. Airway hyperreactivity can also be nonspecific, and bronchoconstriction can result from a wide range of triggers including irritants, cold-dry air, or exercise. Therapy for nonspecific airway hyperreactivity is therefore more difficult.

Responses to a methacholine or histamine challenge can vary with severity of the disease. The response in mild asthma is marked by a greater sensitivity (ie, the dose–response curve is shifted to the left) but may not include a greater maximal airway resistance (Woolcock *et al*., 1984). This may be caused by altered neural control, inflammation, mediator metabolism, or epithelial damage (Sterk and Bel, 1989). In more severe asthma, greater sensitivity is accompanied by a greater maximal response than observed in healthy subjects. This may be due to additional increases in airway smooth contractility, thickening of the airway wall, and decreased lung elastic load. Airway inflammation is typically marked by activation of receptor and epithelial injury that leads to an influx of eosinophils, which are rarely present in a healthy lung. During exacerbations, the inflammation involves eosinophils, mast cells, lymphocytes, and neutrophils.

The pathogenesis of asthma involves the adaptive and innate immune systems. In allergic asthma, previous exposure to an antigen typically leads to the generation of immunoglobin E (IgE) molecules that have molecular recognition sites specific to the antigen. Upon reexposure, the antigen causes cross-linking of IgE molecules and activation of lymphocytes, eosinophils, macrophages, and mast cells, with elaboration and release of an array of cytokines, chemokines, eicosanoids, histamine, tachykinins, and other mediators. Together, these mediators induce smooth muscle constriction, vascular leakage, mucus secretion, and inflammatory cell recruitment. These processes lead to airway obstruction, airway edema, formation of intraluminal mucus and plugging, and neutrophil recruitment with additional mediator release, respectively. Severe airway obstruction and ventilation-vascular perfusion mismatching lead to impaired gas exchange and hypoxemia. In irritant-induced (nonallergic) asthma, the inflammatory cascade is not initiated by an inhaled antigen but many of the same mediators and effector pathways are activated. Persistent inflammation and epithelial damage contribute to airway hyperreactivity. In laboratory animals, short-term airway hyperreactivity can be induced by many irritant stimuli but this response is likely to be reservable (lasting a few hours or days after a single exposure). It can also be induced by antigen sensitization (typically ovalbumin injections) and subsequent antigen challenges (typically an aerosol of albumin in saline). This is followed by a determination of airway resistance following increasing doses of methacholine or histamine.

Asthma has been associated with a number of occupations (Malo and Chan-Yeung, 2009). Occupational asthma can involve adaptive immunity induced by high-molecular-weight and some low-molecular-weight substances. High-molecular-weight agents

including flour-, cereals-, latex- or animal-derived proteins and enzymes cause sensitization through an IgE-mediated mechanism, such as in common atopic asthma. Occupational asthma, for example, is common among laboratory technicians and veterinarians who become sensitized to proteins excreted in rodent urine. Many low-molecular-weight agents that induce occupational asthma include acid anhydrides and platinum salts that induce asthma through an IgE mechanism, but most low-molecular-weight agents involve an uncertain mechanism of induction. Many of the low-molecular-weight agents can cross-link biological macromolecules (Jarvis *et al*., 2005). The latter low-molecular-weight agents include metals (eg, nickel, vanadium, chromium, cobalt, zinc, cadmium, and aluminum), diisocyanates (eg, toluene diisocyanate), cleaning agents, wood dusts, soldering fluxes, pesticides, pharmaceuticals, and reactive dyes. Low-molecular-weight agents may act as haptens that combine with endogenous proteins to form a complex that is recognized as an antigen by the immune system. A subtype of asthma (aka reactive airways dysfunction syndrome) or persistent cough can be a sequele to accidental high-level irritant (eg, chlorine or ammonia) exposure that can also induce acute lung injury (Brooks *et al*., 1985). It is unclear whether the acute exposure alone causes asthma or uncovers existing asthma. Nonetheless, it is clear that persons with asthma may be susceptible to lower doses of irritants (such as sulfur dioxide) (Sheppard *et al*., 1980). In addition, because ambient particle matter and other air pollutants contain many of the metals and other chemicals that are associated with asthma in the workplace, it is likely that these irritants can exacerbate asthma (Leikauf, 2002b).

Pulmonary Fibrosis

The pathological hallmark of pulmonary fibrosis is increased focal staining of collagen fibers in the alveolar interstitium. Excess lung collagen is usually observed not only in the alveolar interstitium but also throughout the centriacinar region, including the alveolar ducts and respiratory bronchioles (Fig. 15-8). The foci can coalesce to form large masses of noncompliant lung. The pleural surface of the lung may also become fibrotic and together with parenchymal stiffening prevent full lung inflation. Ultimately the lung is unable to inflate or deflate properly and thus is restricted. Restrictive lung disease can be detected by a decrease in the predicted FVC, with or without change in the FEV1.

The pathogenesis of pulmonary fibrosis involves epithelial cell injury and macrophage activation produced by a wide range of toxic insults (Wynn, 2011). For example, macrophages can be activated by phagocytosis of crystalline silica or crocidolite asbestos, which activate inflammasome receptor-mediated TNF and IL1B formation. Epithelial cells and macrophages also release chemokines that recruit and activate other inflammatory cells including neutrophils and T cells. These cells combine to produce excessive TGFB1, TNF, IL1B, IL13, and IL17. Of these, TNF and TGFB1 are major mediators in pulmonary fibrosis because fibrosis can be diminished experimentally by inhibition of TNF (Piguet *et al*., 1990) or TGFB1 (Sheppard, 2004; Gharaee-Kermani *et al*., 2009) activity. Importantly, TGFB1 and other TGFB family members (TGFB2 and TGFB3) are secreted in inactive complexes with a latency-associated peptide (LAP), a protein derived from the N-terminal of the region of the gene product. LAP is a ligand for the integrins, heterodimeric proteins consisting of α and β subunits that bind matrix molecules or cellular counterreceptors. In the lung, integrin $\alpha v \beta 6$ (through cell–cell contact) and integrin $\alpha v \beta 8$ (through a proteolytic process) can activate extracellular latent TGFB1 and TGFB3.

Repeated injury and the inability to resolve macrophage activation lead to areas of focal hypoxia. The consequence of the combined TGFB1/3, cytokine release, and hypoxia leads to epithelial-to-mesenchymal transition in which lung epithelial cells transdifferentiate into fibroblast-like cells (Chapman, 2011). These cells contribute to a larger population of myofibroblasts that arise from local mesenchymal cells, and bone marrow–derived fibrocytes. The migration, proliferation, and activation of myofibroblasts contribute to excessive excellular matrix deposition that has altered collagen cross-linking. The consequence of this process is disorderly repair and substained fibrogeneis, which leads to progressive stiffness of the fibrotic lung.

AGENTS KNOWN TO PRODUCE LUNG INJURY IN HUMANS

Currently, the American Chemical Society (ACS) lists over 50 million chemicals with a unique Chemical Abstracts Service (CAS) Registry Number and over 280,000 are listed as inventoried/regulated chemicals by CHEMLIST, but only ~7900 compounds are listed as commonly used by industry (ACS, 2011). Many of these compounds represent hazards to the respiratory tract. For example, the American Conference of Governmental Industrial Hygienists (ACGIH) lists over 700 TLVs for atmospheric exposures (ACGIH, 2011). TLVs refer to occupational airborne concentrations of substances "that nearly all workers may be repeatedly exposed day after day without adverse health effects." These values and other exposure limits have been developed because prevention of exposure is one of the most effective approaches to prevent lung injury and disease. Nonetheless, given the large morbidity and mortality associated with current acute and chronic lung disease, a great need exists to develop additional preventative and therapeutic strategies based on the knowledge of the cellular and molecular events that determine lung injury and repair. Table 15-7 lists a portion of the respiratory toxicants that can produce acute and chronic lung injury in humans. In the following sections, a few examples of our current understanding of lung injury at the mechanistic level are discussed, with emphasis on agents directly responsible for human lung disease.

Inhalation Hazards

Acrolein As an α–β-unsaturated aldehyde, acrolein (2-propenal) is volatile at room temperature and is highly irritating to upper and lower respiratory tract (Bein, 2011). Acrolein can be formed by heating cooking oils and fats above 300°C (eg, wok cooking), and thus its name refers to the pungent "acrid" (from Latin stem: acer meaning sharp or sour) smell that is produced from "oleum" (Latin meaning "oil"). Acrolein also can be formed in cigarette smoke, environmental tobacco smoke, domestic cooking with biomass fuels (WHO, 2006), smoke from fires, and automotive and diesel exhaust. Additional human exposure can result from acrolein use as a biocide or chemical feedstock.

Acrolein contains a reactive carbonyl group and an electrophilic α-carbon and thus is highly reactive (often forming cross-links) with biological macromolecules. Irritant stimuli, of which acrolein is one of the most potent, activate respiratory sensory nerve endings, especially TRPA1 (Bautista *et al*., 2006; Andre *et al*., 2008). More plentiful in cigarette smoke than PAHs, acrolein can adduct tumor suppressor p53 (TP53) DNA (Tang *et al*., 2011). Acrolein is also generated endogenously at sites of lung injury, and excessive breath levels (sufficient to activate metalloproteinases and increase mucin transcripts) (Deshmukh *et al*., 2009) have been detected in persons with COPD or asthma (Deshmukh *et al*., 2008). Because of its high reactivity, acrolein alters gene regulation, increases inflammation, decreases mucociliary transport, and

Table 15-7

Agents That Produce Lung Injury and Disease

TOXICANT	DISEASE	EXPOSURE	ACUTE EFFECT	CHRONIC EFFECT
Acrolein	Acute lung injury, chronic obstructive pulmonary disease	Biomass or hot oil cooking, fire fighters, environmental tobacco smoke, biocide water treatment	Cough, shortness of breath, extreme oronasal irritation, pulmonary edema, airway hyperreactivity	Chronic obstructive pulmonary disease, possibly asthma or lung cancer
Aluminum abrasives	Shaver disease, corundum smelter's lung, bauxite lung	Abrasives manufacturing, smelting	Alveolar edema	Interstitial fibrosis, emphysema
Aluminum dust	Aluminosis	Aluminum, firework, ceramic, paint, electrical good, and abrasive manufacturing	Cough, shortness of breath	Interstitial fibrosis
Ammonia		Farming, refrigeration operations, ammonia, fertilizer, chemical, and explosive manufacturing	Oronasal and bronchial irritation, pulmonary edema	Acute lung injury, chronic bronchitis
Arsenic		Pesticide, pigment, glass, and alloy manufacturing	Bronchitis	Laryngitis, bronchitis, and lung cancer
Asbestos	Asbestosis	Mining, construction, shipbuiliding, brake repair, vermiculite contaminant		Fibrosis, pleural calcification, lung cancer, mesothelioma
Aspergillus	Framer lung, composte lung, malt worker's lung	Working with moldy hay, compost, or barley	Bronchoconstriction, cough, chest tightness	Extrinc allergic alveolitis (hypersensitivity pneumonitis)
Avian protein	Bird fancier's lung	Bird handling and farming with exposure to bird droppings	Bronchoconstriction, cough, chest tightness	Extrinc allergic alveolitis (hypersensitivity pneumonitis)
Beryllium	Berylliosis	Mining, alloy, and ceramic manufacturing, Milling beryllium	Pulmonary edema, pneumonia	Interstitial granulomatosis, progressive dyspnea, cor pulmonarle, fibrosis, and lung cancer
Cadmium		Welding, smelting, and electrical equipment, battery, alloy, and pigment manufacturing	Cough, pneumonia	Emphysema, cor pulmonale
Carbides of tungsten, titanium, or tantalum	Hard metal disease	Metal cutting and manufacturing	Bronchial epithelial hyper- and metaplasia	Peribronchial and perivascular fibrosis
Chlorine		Paper, plastics, chlorinated product manufacturing	Cough, hemoptysis, dyspnea, bronchitis, pneumonia	
Chromium (VI)		Chromium compound, paint, pigment, chromite ore reduction manufacturing	Oronasal and bronchial irritation	Fibrosis, lung cancer
Coal dust	Coal worker's pneumoconiosis	Coal mining		Fibrosis with emphysema
Cotton dust	Byssinosis	Textile manufacturing	Chest tightness, wheezing, dyspnea	Restrictive lung disease, chronic bronchitis

Hydrogen fluoride		Chemical, photograph film, solvent and plastic manufacturing	Airway irritation, hemorrhagic pulmonary edema	
Iron oxides	Siderotic lung disease, silver finisher's lung, hematite miner's lung, arc welder's lung	Welding, steel and jewelry manufacturing, foundry work, hematite mining	Cough	Silver finisher's lung with subpleural and perivascular macrophage aggregates; Hematite miner's lung with diffuse fibrosis-like pneumoconiosis; Arc welder's lung with bronchitis
Isocyanates		Auto painting, and plastic and chemical manufacturing	Airway irritation, cough, dyspnea	Asthma
Kaolin	Kaolinosis	Pottery making		Fibrosis
Manganese	Manganese pneumonia	Chemical and metal manufacturing	Acute pneumonia (often fatal)	Recurrent pneumonia
Nickel		Nickel mining, smelting, electroplating, battery manufacturing, fossil fuel combustion	Delayed pulmonary edema, skin allergy	Acute lung injury, chronic bronchitis, non-small-cell lung cancer, nasal cancer
Nitrogen oxides	Silo-filler's diseases	Silo filling, welding, explosive manufacturing	Immediate or delayed pulmonary edema	Bronchiolitis obliterans, emphysema in experimental aniimals
Nontuberculous mycobacteria	Metalworking fluid hypersensitivity	Working with metal cutting fluid contain water and contaminated with mycobacteria	Bronchoconstriction, cough, chest tightness	Extrinc allergic alveolitis (hypersensitivity pneumonitis)
Organic (sugar cane) dust (possibly contaminated with thermophilic actinomycete)	Bagassosis	Sugar cane and molasses manufacturing (bagasse is the fibrosis residue from sugar extraction)	Bronchoconstriction, cough, chest tightness	Extrinc allergic alveolitis (hypersensitivity pneumonitis)
Ozone		Welding, photocopying, bleaching flour, water treatment, deordorizing	Substernal pain, exacerbation of asthma, bronchitis, pulmonary edema	Fibrosis (including airways)
Perchloroethylene		Dry cleaning, metal degreasing, grain fumigation	Edema	Hepatic and lung cancer
Phosgene		Plastic, pesticide, and chemical manufacturing	Severe pulmonary edema	Bronchitis and fibrosis
Silica	Silicosis, pneumoconiosis	Mining, stone cutting, sand blasting, farming, quarry mining, tunneling	Acute silicosis (inflammation)	Fibrosis, silicotuberculosis
Sulfur dioxide		Chemical manufacturing, refrigeration, bleaching, fumigation	Bronchoconstriction, cough, chest tightness	Chronic bronchitis
Talc	Talcosis	Mining, rubber manufacturing, cosmetics	Cough	Fibosis
Thermophilic actinomycete	Farmer lung, mushroom worker's lung, penguin humidifier lung	Farming (hay or grain degradation)	Bronchoconstriction, cough, chest tightness	Extrinc allergic alveolitis (hypersensitivity pneumonitis)
Tin		Mining, tin processing		Widespread mottling in chest x-ray often without clinical impairment
Vanadium		Metal cutting and manufacturing, specialty steel manufacturing	Airway irritation and mucus production	Chronic bronchitis

diminishes alveolar-capillary barrier integrity (Jang *et al*., 2011). In laboratory animals, high acrolein exposures have lead to acute lung injury and pulmonary edema (Leikauf *et al*., 2011) similar to that produced by smoke inhalation, whereas lower concentrations have produced bronchial hyperreactivity (Leikauf *et al*., 1989), excessive mucus production (Deshmukh *et al*., 2005), and alveolar enlargement (Borchers *et al*., 2007). Susceptibility to acrolein exposure is associated with differential regulation of cell surface receptor, transcription factor, and ubiquitin–proteasome genes (Fabisiak *et al*., 2011; Leikauf *et al*., 2011). Thus, acrolein contributes to the morbidly and mortality associated with acute lung injury and COPD, and possibly asthma and lung cancer. Although irritant-induced sensory responses can serve as warning signs and promote avoidance behavior or protective measures, acrolein exposure remains a global health problem because its release is closely linked to basic human needs (eg, cooking), emergencies (eg, fire fighting), or personal habits (eg, smoking).

Asbestos Asbestos refers to a group of silicate minerals in fiber form. The most commonly mined and commercially used asbestos fibers include the serpentine chrysotile asbestos and the amphiboles crocidolite, anthophyllite, amosite, actinolite, and tremolite asbestos. Exposure to asbestos fibers occurs in mining operations and in the construction and shipbuilding industries, where asbestos was at one time widely used for its insulating and fireproofing properties. Concern about asbestos in older buildings has led to the removal of asbestos-based insulating material; abatement workers may now represent an additional population at risk.

Asbestos causes three forms of lung disease: asbestosis, lung cancer, and malignant mesothelioma (Mossman *et al*., 2011). Asbestosis is a form of pulmonary fibrosis with characteristically diffuse collagen foci and the presence of asbestos fibers, either free or coated with a proteinaceous material (asbestos bodies). Alveolar macrophage clearance is critical to the prevention of asbestosis and depends on fiber length, biopersistance, and dose (Churg *et al*., 2003; Churg, 2003). Lung cancer develops in workers in the asbestos mining industry and smoking of cigarettes greatly enhances risk. Malignant mesothelioma is a rare form of cancer that develops mainly in the pleural mesothelium, the protective lining that covers the lungs, diaphragm, and interior of the chest wall. Unlike lung cancer, mesothelioma is not associated with smoking history.

Observations in humans sometimes differ from those in laboratory animals. In animal experiments, chrysotile produces mesothelioma much more readily than do the amphibole fibers. In humans, amphibole fibers are implicated more often even when the predominant exposure is to chrysotile asbestos. Chrysotile breaks down much more readily than do the amphiboles. One possibility is that in laboratory animals, the chrysotile fibers, even if broken down, are retained longer relative to the life span of the animal than they are in humans, thus explaining the higher rate of mesothelioma developed.

Health hazards associated with asbestos exposure depend on fiber shape, length, and surface properties (Lippmann, 1994; Oberdörster *et al*., 2005; Donaldson *et al*., 2010). Chrysolite asbestos are curved fibers and tend to be less toxic than crocidolite fibers that are long and thin, straight fibers. Fibers 2 µm in length may produce asbestosis; mesothelioma is associated with fibers ≥5 µm long, and lung cancer with fibers ≥10 µm. Fiber diameter is another critical feature. Fibers with diameters larger than ~3 µm do not readily penetrate into the peripheral lung. Only fibers ≥0.15 µm in diameter are likely to produce asbestosis or lung cancer. Mesothelioma, however, is more often associated with fiber diameter <0.5 µm because thinner fibers may be translocated from their site of deposition via the lymphatics to other organs, including the pleural surface (Oberdörster *et al*., 2005). The surface properties of asbestos fibers also contribute to toxicity (Upadhyay and Kamp, 2003; Mossman *et al*., 2011). Crocidolite contains more iron (including ferrous iron (Fe^{2+}) that may participate in Fenton chemistry to generate reactive oxygen species) than chrysolite. The interaction of iron on the surface of asbestos fibers with oxygen may lead to the production of hydrogen peroxide and the highly reactive hydroxyl radical—events that have been associated with asbestos toxicity (Upadhyay and Kamp, 2003). The protection afforded by SOD or free radical scavengers in asbestos-related cell injury in vitro support a role of reactive oxygen species and concomitant oxidized lipids as a mechanism of asbestos toxicity.

Once asbestos fibers have been deposited in the lung, they may become phagocytized by alveolar macrophages (Churg *et al*., 2003; Churg, 2003). Short fibers are completely ingested and subsequently removed via the mucociliary escalator. Longer fibers are incompletely ingested, and the macrophages become unable to leave the alveoli. Activated by the fibers, macrophages release mediators such as cytokine, chemokines, and growth factors, which in turn attract immunocompetent cells or stimulate collagen production. Asbestos-related lung disease thus may be mediated through the triggering of an epithelial injury and macrophage activation, and inflammatory events or through the production of changes that eventually lead to the initiation (DNA damage caused by reactive molecular species) or promotion (increased rate of cell turnover in the lung) of the carcinogenic process (Mossman *et al*., 2011).

Silica Inhaled particles of silicon dioxide (silica) cause a characteristic human lung disease—silicosis. Crystalline silica is a major component of the earth's crust; silicon is only second to oxygen as the most common element. Mineral forms of silicon exist primarily as crystalline SiO_2 with a central silicon atom forming a tetrahedron with four shared oxygen atoms. The three principal crystalline isomeric forms are quartz, tridymite, and cristobalite. The tetrahedral structure is biopersisent and linked to fibrogenic potential. Stishovite, a rare crystalline variant without the tetrahedral conformation, is biologically inert. Amorphous forms of silica (eg, kieselguhr and vitreous silica) also have low fibrogenic potential. The ubiquitous presence of silica has made it an occupational hazard ever since tools were cut from stone, and silicosis remains a significant industrial hazard throughout the world in occupations such as mining and quarrying, sandblasting, and foundry work.

In addition to its structure, particle size, concentration, and surface properties affect the pathogenicity of silica both in vivo and in vitro. In humans, the most fibrogenic particle size appears to be about 1 µm (range 0.5–3 µm). In animal experiments (rats, hamsters), the comparable values appear to be 1 to 2 µm (range 0.5–5 µm). In animals, the concentration of silica dust is directly related to the intensity and rapidity of the histological reaction in the lung. When compared with stored silica or coated silica, freshly fractured silica particles produce more free radicals from their surface, increasing the respiratory burst when phagocytized and more pulmonary inflammation (Vallyathan *et al*., 1995).

Silicosis may be acute or chronic with distinct pathological consequences. Acute silicosis occurs only in subjects exposed to a very high level silica (most often quartz or sand) small enough to be respirable (usually <5 µm) over a relatively short period, generally a few months or years. These patients have worsening dyspnea, fever, cough, and weight loss that can rapidly progress to respiratory failure, usually ending in death within two years. No known therapeutic strategy controls the relentless course of acute silicosis.

Chronic silicosis has a long latency period, usually >10 years and can be divided into simple and complicated silicosis. Even after radiographic changes, simple silicosis may be asymptomatic (ie, no dyspnea) with little change in pulmonary function. The x-ray

presents fibrotic nodules, generally in the apical portion of lung. The hilar lymph nodes have peripheral calcifications known as eggshell calcifications. Simple silicosis may progress into complicated silicosis, which is defined as the presence of conglomerate nodules larger than 1 cm in diameter. These nodules usually occur in the upper and mid-lung zones. In advanced stages, the nodules may be surrounded by emphysematous bullae. Chronic silicosis is associated with an increased incidence of tuberculosis.

The pathophysiological basis of pulmonary fibrosis in chronic silicosis is probably better understood than is the etiology of any other form of pulmonary fibrosis (Huaux, 2007). The role of pulmonary alveolar macrophages in the ingestion of silica is an initiating event. Macrophages phagocytose silica particles into phagosomes that fuse with endosomes during the internalization process (Costantini *et al*., 2011). Similar to the fate of microorganisms, macrophages experience phagosomal destabilization (Hornung *et al*., 2008) and activate pathways including the NALP3 inflammasome (Cassel *et al*., 2008; Dostert *et al*., 2008), PRRs, and antiviral pathways to release inflammatory cytokines (Giordano *et al*., 2010). In contrast to microorganisms, silica particles cannot be degraded and macrophages undergo cell death, releasing these particles that are engulfed by other macrophages, thus perpetuating the process of phagocytosis and cell death (Huaux, 2007). This leads to elevated mediator release (TGFB1, TNF, etc) (Di Giuseppe *et al*., 2009), EMT (Chapman, 2011), and initiates and maintains myofibroblast collagen (Munger and Sheppard, 2011).

Naphthalene Naphthalene occurs in cigarette smoke, tars, petroleum and is a precursor in the chemical synthesis of tanning agents, phthalic acid anhydride, carbaryl, and 2-naphthol. In laboratory animals, inhaled or parenterally administered naphthalene produces extensive necrosis in the bronchiolar epithelium of the mouse but much less necrosis in the airways of rats, hamsters, or monkeys (Buckpitt *et al.*, 2002). The primary target in the surface epithelium is the bronchiolar secretoglobin cells. These cells were thought to be very important because they contain a stem cell subpopulation. They were once thought to be capable of cellular maintenance of the epithelium in bronchi, bronchioles, and alveolar regions. However, recent evidence suggests that these cells are only involved in the long-term maintenance and repair of airways but not alveolar epithelium (Rawlins *et al*., 2009). In mice, naphthalene is metabolized to naphthalene oxide primarily through CYP2F enzymes (Shultz *et al*., 1999; Baldwin *et al*., 2005). In rats and other species, including monkeys, conversion of naphthalene is less stereospecific and the rates of formation of the epoxide are much slower than in mice (Buckpitt *et al*., 2002). Naphthalene epoxides may subsequently be conjugated with glutathione and form adducts that are eliminated as mercapturic acids. The epoxide can undergo rearrangement to 1-naphthol with subsequent metabolism to quinones, which are potentially toxic compounds. Naphthalene metabolites bind covalently to cellular proteins that are important in normal cellular homeostasis and protein folding and this may be related to the mechanism of toxicity by this chemical. Interestingly, in both mice and rhesus monkeys the total amount of adducted protein is similar (Lin *et al*., 2006).

Blood-Borne Agents That Cause Pulmonary Toxicity in Humans

A number of compounds administered systematically can enter the lung through pulmonary circulation and cause lung injury and disease. For example, intraparenteral napthalene has marked lung toxicity in the mouse, probably due to an enrichment of an activating enzyme (CYP2F) in a specific cell in the lung epithelium (Buckpitt *et al.*, 2002). Another example is that the ingestion of arsenic has been associated with lung cancer (Putila and Guo, 2011). Below are two more examples that have had toxicological significance in clinical settings.

Bleomycin Bleomycin is a cancer chemotherapeutic drug with a major complication—pulmonary fibrosis that can be fatal (Jules-Elysee and White, 1990). Bleomycin produces a sequence of injury and necrosis capillary endothelial cells, alveolar type I cell, edema formation and hemorrhage, delayed (after one–two weeks) proliferation and apoptosis of alveolar type II cells, and eventually thickening of the alveolar walls by fibrotic changes. In many tissues, the cytosolic enzyme bleomycin hydrolase inactivates bleomycin (Schwartz *et al*., 1999). In lung and skin, two target organs for bleomycin toxicity, the activity of this enzyme is low compared with that in other organs. Bleomycin stimulates the production of collagen in the lung. Before increased collagen biosynthesis, steady-state levels of mRNA coding for fibronectin and procollagens are increased, subsequent to a bleomycin-mediated release of cytokines such as TGFB1 and TNF (Ortiz *et al*., 1999). In mice, bleomycin-induced TGFB1 is critical because mice deficiency in its receptor TGFBR2 in alveolar epithelial cells have attenuated fibrosis and increased survival following bleomycin treatment (Degryse *et al*., 2011). Bleomycin also combines with Fe(II) and molecular oxygen; when it combines with DNA, single- and double-strand breaks are produced by a free radical reaction. Animal models of belomycin-induced pulmonary fibrosis have been used to study the efficacy of promising antifibrotic drugs (Giri, 2003) and stem cell therapy (Ortiz *et al*., 2003).

Cyclophosphamide and 1,3 Bis (2-Chloroethyl)-1-Nitrosourea (BCNU) A number of chemotherapeutic drugs can produce lung damage and pulmonary toxicity in patients treated with these drugs and can be a significant problem (Meadors *et al*., 2006). Cyclophosphamide is widely used as an anticancer and immunosuppressive drug. The undesirable side effects include hemorrhagic cystitis and pulmonary fibrosis (Fraiser *et al*., 1991). Cyclophosphamide is metabolized by the cytochrome P450 system to two highly reactive metabolites: acrolein and phosphoramide mustard. In the lung, cooxidation with the PGH synthase system, which has high activity in the lung, is a possibility. Although the exact mechanism of action for causing lung damage has not been established, studies with isolated lung microsomes have shown that cyclophosphamide and its metabolite acrolein initiate lipid peroxidation (Patel and Block, 1985).

Another chemotherapeutic drug that has pulmonary fibrosis as a complication is carmustine (BCNU) BCNU exerts its antitumor properties by reacting with cellular macromolecules and forms inter- and intrastrand cross-links with DNA. In humans, a dose-related pulmonary toxicity is often noticed first by a decrease in diffusion capacity, which can develop into fatal pulmonary fibrosis (Weiss *et al*., 1981). The mechanism of action is not entirely clear (Wu *et al*., 2001). It is possible that BCNU inhibits pulmonary glutathione disulfide reductase, an event that may lead to a disturbed GSH/GSSG state in pulmonary cells. Eventually, this state leaves the cell unable to cope with oxidant stress. High concentrations of oxygen in the inspired air may enhance the pulmonary toxicity of BCNU and also that of the other anticancer drugs known to affect lung tissue: cyclophosphamide and bleomycin.

EVALUATION OF TOXIC LUNG DAMAGE

Humans Studies

Although the lung is susceptible to multiple toxic injuries, it is also amenable to a number of tests that allow evaluation of proper functioning (Utell and Frampton, 2000; Rennard and Spurzen, 2006;

Huang and Ghio, 2009). Commonly used tests include measurement of FEV1, FVC, and airway resistance. Additional tests evaluate the maximal flow rates and different lung volumes, diffusion capacity, oxygen, and carbon dioxide content of the arterial and venous blood, distribution of ventilation, and lung and chest wall compliance. Many pulmonary function tests (eg, FEV1 and FVC) require active collaboration by the subject examined. This noninvasive test is easy to administer to adults (ATS, 1991) and children (Beydon *et al*., 2007), and does not require sophisticated equipment or a hospital setting. The subject is asked first to inhale deeply and then to exhale the air as quickly as possible. The test is repeated typically three times and the result of the best effort is recorded. The test is often used in epidemiological studies or controlled clinical studies designed to assess the potential adverse effects of air pollutants. A reduction in FEV1 is usually indicative of impaired ventilation such as that found in restrictive (increased lung stiffness) or obstructive (obstructed airflow) lung disease.

To accomplish proper oxygenation of venous blood and elimination of CO_2, the gases have to diffuse across the air–blood barrier (Macintyre *et al*., 2005). The structural properties that control gas exchange include the lung gas volume, the path length for diffusion in the gas phase; the thickness and area of the alveolar capillary membrane, and the volume of blood in capillaries supplying ventilated alveoli. The functional properties that control gas exchange include the uniformity of ventilation and perfusion with respect to each other, the diffusion characteristics of the alveolar membrane; the binding properties of hemoglobin (Hb) in the alveolar capillaries, and the gas tensions in blood in the pulmonary vascular bed that exchanges gas with the alveoli. Gas exchange may be hindered by the accumulation of fluids or cellular elements in the alveoli (edema, pneumonic infiltrates), thickening of the alveolar wall (fibrosis), insufficient ventilation of the alveolar region (emphysema), ventilation–perfusion mismatching, or insufficient presence of oxygen transport elements (reduced alveolar blood volume or reduced amount of Hb in the blood). Gas exchange can be evaluated by measuring the arterial partial pressure of both oxygen and CO_2. In general, blood gas analysis is a comparatively insensitive assay for disturbed ventilation because of the organisms' buffering and reserve capacities, but may be a useful tool in clinical medicine. Measurement of diffusion capacity with CO, a gas that binds with 250 times higher affinity to Hb than does oxygen, is more sensitive. The test is easy to perform in humans and laboratory animals and is widely used in clinical studies.

Proper lung function in humans can be evaluated with several additional techniques. Computed tomography provides detailed roentgenographic information of airways and lung parenchyma and has been useful in screening high-risk patients for lung cancer (National Lung Screening Trial, 2011). Increased concentrations of nitric oxide are often found in exhaled air when inflammatory processes have led to induction of iNOS (Barnes *et al*., 2010). As an experimental method, exhaled breath or induced sputum analysis may also be useful in assessing other inflammatory indicators in exhaled breath condensate such as oxidative stress markers (eg, hydrogen peroxide and isoprostanes), nitric oxide derivatives (eg, nitrate and nitrates), arachidonic acid metabolites (eg, prostanoids, leukotrienes, and epoxides), adenosine, and cytokines (Popov, 2011). Fiberoptic bronchoscopy has become one of the most valuable tools for the detection of toxic lung injury (Reynolds, 2011). The procedure allows direct visual inspection of the major lobar and segmental airways; the depth of penetration is limited by the external diameter of the bronchoscope, usually 5 mm. Bronchoscopy also allows the introduction and retrieval of saline solutions into the lung and subsequent analysis for cellular and molecular constituents (broncholveolar lavage). Excision of small tissue samples (biopsies) during bronchoscopy is an additional diagnostic tool, most helpful in the evaluation and staging of precancerous and cancerous lesions.

Animals Studies

The toxicology of inhaled materials has been and continues to be extensively studied in experimental animals (Mauderly, 1996). In such studies, selection of animals with a respiratory system similar to that of humans is particularly desirable (Phalen *et al*., 2008). The respiratory system of monkeys most closely resembles that of humans. However, the availability and cost of these animals and the necessity for special facilities for housing monkeys and performing long-term exposures, along with ethical considerations, including the confinement of primates in small exposure chambers for prolonged periods, severely limits the use of primates. Rats and mice are widely used, although fundamental differences in respiratory anatomy (eg, lack of respiratory bronchioles) and function (rats and mice are obligate nose breathers) can complicate the extrapolation of effects to humans. Experimental studies with guinea pigs and rabbits provided the first conclusive evidence that sulfuric acid and SO_2 may damage human lungs (Amdur, 1989). The following techniques are used to study the effects of inhaled toxicants in animals.

Inhalation Exposure Systems

In inhalation studies, animals are kept within a chamber that is ventilated with a defined test atmosphere (Phalen, 1976; Wong, 2007). Generation of such an atmosphere is comparatively easy for gases that are available in high purity in a compressed tank, for example, SO_2, O_2, or NO_2. Metering and dilution produce appropriate concentrations for exposure. Final concentrations within the chamber monitored continuously with direct reading detectors (eg, UV for ozone or infrared for CO_2) that are calibrated. Exposure should be within 5% of the targeted concentration. Alternatively, wet chemical analysis procedures are applied after sampled gases from the chambers are bubbled through traps. Such sampling yields a time-weighted average for the concentration. More challenging is the generation of particles or complex mixtures (eg, tobacco smoke, diesel, and gasoline exhaust or residual oil fly ash), particularly because of the possibility of interactions between individual mixture constituents and the possibility of formation of artifacts (Pauluhn, 2005).

Exposure chambers must allow for the rapid attainment of the desired concentrations of toxicants, maintenance of desired toxicant levels homogeneously throughout the chamber, adequate capacity for experimental animals, and minimal accumulation of undesired products associated with animal occupancy (usually ammonia, dander, heat, and carbon dioxide). As a general rule, the total body volume of the animals should not exceed 5% of the chamber volume. Nose-only exposure chambers avoid some of these problems (Phalen *et al*., 1984). However, nose-only exposure systems create different problems, including a great deal of stress on the animals due to confinement during exposure, and the very labor-intensive handling that is required for exposures by this route. The proper selection of exposure techniques for a given chemical is ultimately a decision that must be made on a case-by-case basis. Finally, concern for the environment and the safety of facility personnel suggest prudence in how chambers are exhausted.

Pulmonary Function Tests in Experimental Animals

Conducting pulmonary function tests in experimental animals poses distinct challenges, especially in small rodents (Reinhard *et al*., 2005). Experimental animals cannot be made to maximally inhale or exhale at the investigator's will. For example, FEV_1 can be obtained in experimental animals only under anesthesia. Expiration is forced

by applying external pressure to the thorax or negative pressure to the airways (Vinegar *et al*., 1979). Alternatively, analysis of pressure–volume curves is a comparatively easy test to perform in animals, not requiring a specialized apparatus (Alarie *et al*., 1961; Sinnett *et al*., 1981). The test provides some indication of lung compliance. Compliance (volume/pressure) is calculated as the slope of the volume–pressure curve. The volume–pressure curve involves deflating the lung and then inflating the lung with incremental volumes and recording the pressure, and can be obtained from lungs filled with air and/or physiological saline. The lung is then deflated with incremental volumes and pressure again recorded. Compliance provides an indication of the intrinsic elastic properties of the lung parenchyma and, when measured in vivo, the thoracic cage. In emphysema, compliance of the lung increases because elatic recoil is decreased. When the inflation and deflation are performed with air, the curves will not match and this hysteresis is an indication of surfactant function. The use of saline is sensitive to structural changes in lung parenchyma, as the effects of surfactant are eliminated in a saline-filled lung.

Another pulmonary function test is the analysis of airway resistance, which can be measured in laboratory animals by restrained plethysmography (Amdur and Mead, 1958), unrestrained video-assisted plethysmography (Bates *et al*., 2008), or unrestrained acoustic plethysmography (Reynolds *et al*., 2008). An increase in airway resistance is a measure of bronchoconstriction inasmuch as the airway smooth muscle contraction or airway mucosal edema narrows the airway lumen and obstructs airflow. Airway resistance also can be measured after a challenge of incremental doses of inhaled methacholine (or histamine) (Bates and Irvin, 2003) or infused acetylcholine (Swiecichowski *et al*., 1993). This challenge provides a measure of airway hyperreactivity, a distinguishing feature of asthma in humans. In asthma, resting airway resistance is typically normal but when challenged, airway resistance will increase at a lower threshold dose of methacholine. Analysis of breathing pattern can also be used and may differentiate between upper airway and lower airway irritants (Alarie *et al*., 1988; Bates *et al*. 2004). In rodents, upper airway ("sensory") irritants produce a breathing pattern of decreased respiratory frequency with increased tidal volume, whereas lower airway ("pulmonary") irritants produce a breathing pattern of increased respiratory frequency and decreased minute volume (ie, the total volume of air breathed in 1 minute).

Morphological Techniques The pathology of acute and chronic injury may be examined by gross inspection and under the microscope and should include the nasal passages, larynx, major bronchi, and the lung parenchyma.

Regional distribution of lesions in nasal passages can be assessed after fixation and decalcification. Various regions of the nasal passages can then be examined by obtaining cross sections at multiple levels. Proper fixation of the lung is done by vascular perfusion with fixative through the pulmonary artery or by instillation of fixative through the trachea. Perfusion fixation does not dislodge material (lining fluid, deposited particles) or cells in the lumen of the airways or the alveoli from their original position. Fixation by intratracheal instillation can be done under controlled pressure (usually 30 cm H_2O in rodents), which preserves alveolar architecture. This permits semiquantitative or quantitative measurements to be made. The choice of fixative depends on how the lung will be further analyzed. Formalin-based fixatives are satisfactory for routine histopathology, whereas the use of more sophisticated techniques such as electron microscopy, immunohistochemistry, and in situ hybridization require careful selection of the fixative.

Paraffin sections of respiratory tract tissue are suitable for routine histopathological analysis of gross pathological changes, for example, inflammation, fibrosis, or lung tumors. The tissue can be stained with (a) hematoxylin and eosin stain for routine assessment (Llewellyn, 2009), (b) periodic acid-Shiff's Alcian blue stain for glycoproteins (mucus cells) (Jeffery *et al*., 1982), or (c) Masson trichrome stain for collagen (Goldner, 1938). Plastic or Epon sections 1-μm thick are required for proper identification of additional cell types or recognition of cytoplasmic changes as in damaged BSCs. Other structural alterations, such as degenerative changes or necrosis of type I epithelial cells or capillary endothelial cells, usually are detected by transmission electron microscopy (TEM). TEM is essential for an unequivocal identification of cells in the alveolar interstitium and is used mainly in morphometric analysis of the lung. Scanning electron microscopy allows visualization of the surface of interior lung structures, reveals alterations in the tissue surface, and detects rearrangement of the overall cell population. Confocal microscopy, consisting of a laser microscope coupled to a computer, allows examination of thick sections and discovery of specific cell types deep within the tissue labeled with fluorescent markers; it is an ideal tool for three-dimensional reconstruction of normal and damaged lung.

Morphometry, the quantitative description of structure, refers to a quantitative analysis of tissue (Gehr *et al*., 1993). Measurements made in two dimensions on photographs taken under the microscope allow one to measure areas, the thickness of a structure, and numerical density. Using the appropriate formula, values such as the volume occupied by a specific cell population in the entire lung parenchyma can be calculated (Hyde *et al*., 2007).

Additional tools for the study of toxic lung injury include immunohistochemistry, in situ hybridization, and analysis of cell kinetics. Transcriptome (Mohr *et al*., 2002), proteomic (Miller *et al*., 2008), and metabolome (Fabisiak *et al*., 2011) profiling are additional value tools to assess the lung in health and disease. Antibodies to a variety of enzymes, mediators, and other proteins are available. It is possible to identify cell types that carry certain enzymes and their anatomic locations. In situ hybridization allows one to visualize anatomic sites where a specific gene product is expressed, for example, collagen production in a fibrotic lung. Two useful sites to obtain lung-specific expression include the Human Protein Atlas (Uhlen *et al*., 2010) and GenePaint (Visel *et al*., 2004). Ascribing a given metabolic capability to a specific cell type requires evaluation of gene expression and/or protein production in specific cells in situ. The normal adult lung is an organ for which under normal circumstances very few cells appear to die and to be replaced. When damaged by a toxic insult, the lung parenchyma is capable of repairing itself in an efficient manner. Type I cell damage is followed by proliferation of type II epithelial cells, which eventually transform into new type I cells (Adamson and Bowden, 1974; Fujino *et al*., 2011); in the airways, the BSCs proliferate and divide following injury to ciliated cells (Rock and Hogan, 2011; Delgado *et al*., 2011). Quantitative data can be obtained by either injection of the DNA precursors (tritated thymide, bromodeoxyuridine) or visualization of proliferating cell nuclear antigen (PCNA). Flow cytometry is valuable in the study of cell populations prepared from the lung.

Pulmonary Lavage and Pulmonary Edema Pulmonary edema and/or pulmonary inflammation are early events in acute and chronic lung injury. The fluid lining the pulmonary epithelium can be recovered by bronchoalveolar lavage. Analysis of the lavage fluid is a useful tool to detect respiratory tract toxicity (Henderson, 2005; Reynolds, 2011). Influx of neutrophils or other leukocytes such as lymphocytes or eosinophils into the lavage fluid is the most sensitive sign of inflammation. Measurements of lung injury

include total protein and/or albumin. Additional measurements include secretory products of macrophages and epithelial cells include fribronectin, chemokines, and other cytokines (eg, TNF or IL1B). Reduced glutathione levels may be an indicator of oxidative stress. Lactate dehydrogenase activity (and its substituent isoenzymes), *N*-acetylglucosaminidase, acid or alkaline phosphatase, other lysosomal hydrolases, and sialic acid add additional information. In addition pulmonary edema can be assessed by determining lung wet:dry ratio or injection of Evan blue dye albumin (Patterson *et al*., 1992; Li *et al*., 2010).

In Vitro Studies

Isolated Perfused Lung In vitro systems with materials originally obtained from either human tissues or experimental animals are particularly suited for the study of mechanisms that cause lung injury (Aufderheide, 2005; Bakand and Hayes, 2010). The methods include isolated perfused lung, microdissection/organotypic tissue culture systems, and cell type–specific cell culture. The isolated perfused lung method is applicable to lungs from many laboratory species (eg, mouse, rat, guinea pig, or rabbit) (Mehendale *et al*., 1981). The lung is perfused with blood or a blood substitute through the pulmonary arterial bed. At the same time, the lung is actively (through rhythmic inflation–deflation cycles with positive pressure) or passively (by creating negative pressure with an artificial thorax in which the lung is suspended) ventilated. Toxic agents can be introduced into the perfusate or the inspired air (Rhoades, 1984). Repeated sampling of the perfusate allows one to determine the rate of metabolism of drugs and the metabolic activity of the lung.

Airway Microdissection and Organotypic Tissue Culture Systems Many inhalants act in specific regions of the respiratory tract. For example, the terminal bronchioles, a region especially rich in the highly metabolically competent BSCs, is often a target of gases with low water solubility. Microdissection of the nasal passage (Fanucchi *et al*., 1999) and airways (Plopper *et al*., 1991) consists of stripping away surrounding tissue or parenchyma while maintaining the airway structure and exposing the epithelium. Microdissected airways can be studied in culture for up to one week, can be used to study site-specific gene expression, morphological changes in toxicant injury and repair, or can be used for biochemical analyses including enzyme activity measurements and determination of antioxidant concentrations (such as glutathione). This facilitates study of the metabolically active BSCs found in the airways (Plopper *et al*., 2001). Tissue culture systems have been developed in which epithelial cells maintain their polarity, differentiation, and normal function similar to what is observed in vivo. Epithelial cell surfaces are exposed to air (or a gas phase containing an airborne toxic agent), while the basal portion is bathed by a tissue culture medium (Widdicombe and Welsh, 1980).

Lung Cell Culture Many lung-specific cell types have been isolated and can be maintained as cell culture. Human (Reynolds, 2011) and animal (Kobzik *et al*., 1988) alveolar or interstitial macrophages can be obtained from lavage or lung tissue. Their function can be examined in vitro with or without exposure to appropriate toxic stimuli (Brain, 1986; Brain *et al*., 1994). Type II alveolar epithelial cells can be isolated and primary cell cultures maintained in culture for short periods (Dobbs *et al*., 1997; Wang *et al*., 2007). Direct isolation of type I epithelial cells has also been successful (Williams, 2003).

Maintenance of the epithelial cells at the air–liquid interface is important to maintain polarity and differentiation. Epithelial cells may be seeded on top of a suitable supporting material (eg, collagen or nitrocellulose membranes) with mesenchymal cells seeded on the other side to observe epithelial cell–fibroblast interactions. Systems are available for the isolation and culture of human bronchial epithelial cells (Lechner *et al*., 1982; Lechner and LaVeck, 1985) and BSCs (Belinsky *et al*., 1995). These cells can be differentiated into ciliated and mucin-producing cell by growth in air–liquid interface systems (Whitcutt *et al*., 1988; de Jong *et al*., 1994). Serous and mucus cells can be obtained from submucosal glands and maintained as primary cell culture (Finkbeiner *et al*., 2010). Lung fibroblasts are easily grown (Baglole *et al*., 2005) and have been studied in coculture with epithelial cells (Delgado *et al*., 2011). Primary cell cultures have been transformed (Gruenert *et al*., 1988) and/or immortalized (Reddel *et al*., 1988; Vaughan *et al*., 2006) and can be passaged and maintained for long periods in the laboratory. Cell lines established from lung tumors have been extensively used by investigators and have yield many novel insights into lung cancer (Gazdar *et al*., 2010).

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