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Host Defense and Metabolism in the Lung

LEARNING OBJECTIVES

Upon completion of this chapter, the student should be able to answer the following questions:

1. How do the components of the mucociliary clearance system function to remove xenobiotic substances and particulates?
2. How does particle shape and size influence their pattern of airway deposition and/or clearance?
3. What are the major mechanisms of particle deposition?
4. How are the mucosal and systemic immune responses similar? How are they different?
5. What unique features of immunoglobulin A make it well suited for immunologic protection in the mucosal environment?
6. What are the cellular components of the adaptive and innate immune cells in the respiratory system?

In addition to their primary function of gas exchange, the lungs act as a primary barrier between the outside world and the inside of the body, with host defense functions. They are also active organs in the metabolism of xenobiotic and endogenous compounds.

Host Defense

To cope with the inhalation of foreign substances, the respiratory system and, in particular, the conducting airways have developed unique structural features: the mucociliary clearance system and specialized adaptive and innate immune response mechanisms.

Mucociliary Clearance System

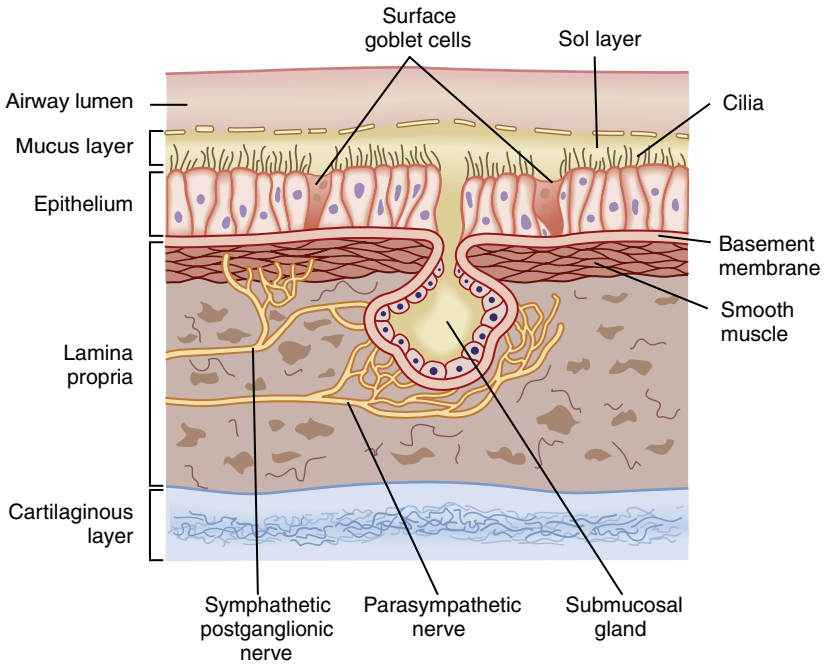
The mucociliary clearance system protects the conducting airways by trapping and removing inhaled pathogenic viruses and bacteria, in addition to nontoxic and toxic particulates (e.g., pollen, ash, mineral dust, mold spores, and organic particles), from the lungs. These particulates are inhaled with each breath and must be removed. The three major components of the mucociliary clearance system are two fluid layers, referred to as the *sol* (**periciliary fluid**) and *gel* (**mucus**) layers, and **cilia,** which are positioned on the surface of bronchial epithelial cells (Fig. 26.1). Inhaled material is trapped in the viscoelastic (sticky) mucus layer, whereas the watery periciliary fluid allows the cilia to move freely and establish an upward flow to clear particulates from the lung. Effective clearance requires both ciliary activity and the appropriate balance of periciliary fluid and mucus.

Periciliary Fluid Layer

The periciliary fluid layer is composed of nonviscous serous fluid, which is produced by the pseudostratified ciliated columnar epithelial cells that line the airways. These cells have the ability to either **secrete** fluid, a process that is mediated by activation of cystic fibrosis transmembrane regulator (CFTR) chloride (Cl^-) ion channels (**Na^+ secretion** follows passively between cells across the tight junctions), or **reabsorb** fluid, a process that is mediated by activation of epithelial sodium channels (ENaC; **Cl^- absorption** follows passively between cells across the tight junctions). NaCl secretion or reabsorption temporarily establishes an osmotic gradient across the pseudostratified epithelium, which provides the driving force for passive water movement. The balance between CFTR-mediated Cl^- secretion and ENaC-mediated Na^+ absorption is regulated by a variety of hormones and determines the volume of the periciliary fluid, which in the healthy lung is 5 to 6 μm deep, a level that is optimal for rhythmic beating of the cilia and mucociliary clearance.

Mucus Layer

The mucus layer lies on top of the periciliary fluid layer and is composed of a complex mixture of macromolecules and electrolytes. Because the mucus layer is in direct contact with air, it entraps inhaled substances, including pathogens. The mucus layer is predominantly water (95%–97%), 5 to 10 μm thick, and exists as a discontinuous blanket (i.e., islands of mucus). Mucus has low viscosity and high elasticity and is composed of glycoproteins with groups of oligosaccharides attached to a protein backbone. Healthy individuals produce approximately 100 mL of mucus each day. Four cell types contribute to the quantity and composition of the mucus layer: **goblet cells** and **Clara cells** within the tracheobronchial epithelium, and **mucous cells** and **serous cells** within



• **Fig. 26.1** Overview of the epithelial lining and innervation of the tracheobronchial tree. The cilia of the epithelial cell reside in the periciliary fluid layer, and the mucus layer is on top. Interspersed between the ciliated epithelial cells are surface secretory (goblet) cells and submucosal glands. Sympathetic and parasympathetic nerve fibers descend into the submucosal glands and smooth muscles.

IN THE CLINIC

Cystic fibrosis (CF) is the most common lethal inherited disease among white people. It is an autosomal recessive disease caused by mutations in the *CFTR* gene. It is characterized by chronic bacterial lung infection, progressive decline in lung function, and premature death at an average age of 48 years. More than 2000 mutations of the *CFTR* gene have been described, but 70% of affected individuals have a deletion of phenylalanine at codon 508 (F508del-CFTR) in at least one allele. This mutation results in a lack of Cl^- secretion and an increase in ENaC-mediated Na^+ reabsorption, which in turn results in a reduction in the volume of the periciliary fluid.

Detailed study of the different *CFTR* mutations has resulted in an understanding of various disease-related phenotypes, some of which are associated with milder disease and some with more severe disease. Since the 1980s, research findings have elucidated how many of the most common mutations in the *CFTR* gene cause CF, and this has led to the development of drugs that target specific mutations and reverse the progressive reduction in lung function. For example, in one mutation (G551D-CFTR, which affects ≈5% of patients with CF), the CFTR Cl^- channel reaches the plasma membrane of airway epithelial cells but does not secrete Cl^-. Through precision medicine, a drug, ivacaftor (Kalydeco), has been found to stimulate Cl^- secretion via the G551D-CFTR, thereby improving lung function and decreasing the rate of disease progression. When the alleles are homozygous for the F508del-CFTR mutation (which affects ≈50% of patients with CF), the CFTR Cl^- channel does not reach the plasma membrane of airway epithelial cells.

In 2015 and 2018, the U.S. Food and Drug Administration approved combination drug therapies, lumacaftor/ivacaftor (Orkambi) and tezacaftor/ivacaftor (Symdeko), that have been shown to correct the gene defect, increase the amount of F508del-CFTR in the plasma membrane, and improve Cl^- transport. More recently, the combination of elexacaftor/tezacaftor/ivacaftor (Trikafta) has been approved to treat additional mutations in the *CFTR* gene. Both Symdeko and Trikafta have been approved for use in people with two alleles of F508del-CFTR as well as people with one F508del-CFTR allele and the other allele containing a variety of other mutations in the *CFTR* gene. Clinically, these medications have been shown to improve lung function and to decrease the rate of decline in lung function.

the tracheobronchial submucosal glands. Goblet cells, also referred to as *surface secretory cells,* represent approximately 15% to 20% of the tracheobronchial epithelium, and are found in the tracheobronchial tree up to the 12th division. In many respiratory diseases, goblet cells appear further down the tracheobronchial tree; thus the smaller airways are more susceptible to obstruction by mucus plugging. Goblet cells secrete neutral and acidic glycoproteins rich in sialic acid in response to chemical stimuli. In the presence of infection or cigarette smoke or in patients with chronic bronchitis, goblet cells can increase in size and number, extend above the 12th division of the tracheobronchial tree, and secrete copious amounts of mucus. Injury and infection increase the viscosity of the mucus secreted by goblet cells, which reduces mucociliary clearance of inhaled particles and pathogens.

Submucosal tracheobronchial glands are present wherever there is cartilage in the upper regions of the conducting airways, and they secrete water, ions, and mucus into the airway lumen through a ciliated duct (Fig. 26.1). Although both mucous and serous cells secrete mucus, their cellular

TABLE 26.1 Properties of Serous and Mucous Cells in Submucosal Gland

Property	Serous Cells	Mucous Cells
Location	Most distal	Middle to distal
Granules	Small, electron-dense	Large, electron-lucent
Glycoproteins	Neutral Lysozyme, lactoferrin	Acidic
Hormones	α-Adrenergic > β-Adrenergic	β-Adrenergic > α-Adrenergic
Receptors	Muscarinic	Muscarinic
Degranulation	α-Adrenergic Cholinergic Substance P	β-Adrenergic Cholinergic

structure and mucus composition are distinctly different (Table 26.1). In several lung diseases, including chronic bronchitis, the number and size of submucosal glands are increased, which leads to increases in mucus production, alterations in chemical composition of mucus (i.e., increased viscosity and decreased elasticity), and the formation of mucus plugs that cause airway obstruction. Mucus secretion from submucosal tracheobronchial glands is stimulated by parasympathetic (cholinergic) compounds such as acetylcholine and substance P and inhibited by sympathetic (adrenergic) compounds such as norepinephrine and vasoactive intestinal polypeptide. Local inflammatory mediators such as histamine and arachidonic acid metabolites also stimulate mucus production.

Clara cells, located in the epithelium of bronchioles, also contribute to the composition of mucus through secretion of a nonmucinous material containing carbohydrates and proteins. These cells play a role in bronchial regeneration after injury.

Ciliated Cells and Cilia

As noted previously, the respiratory tract to the level of the bronchioles is lined by a pseudostratified, ciliated columnar epithelium (see Fig. 26.1). These cells maintain the level of the periciliary fluid in which cilia and the mucociliary transport system function. Mucus and inhaled particles are removed from the airways by the rhythmic beating of the cilia. There are approximately 250 cilia per airway epithelial cell, and each is 2 to 5 μm in length. Cilia are composed of nine microtubular doublets that surround two central microtubules held together by dynein arms, nexin links, and spokes. The central microtubule doublet contains an adenosine triphosphatase (ATPase) that is responsible for the contractile beat of the cilium. Cilia beat with a coordinated oscillation in a characteristic, biphasic, and wave-like rhythm called **metachronism.** They beat at approximately 1000 strokes per minute, with a power forward stroke and a slow return or recovery stroke. During their power forward stroke, the tips of the cilia extend upward into the viscous mucus layer and thereby move it and the entrapped particles. On the reverse beat, the cilia release the mucus and withdraw completely into the sol layer. Cilia in the nasopharynx beat in the direction that propels the mucus into the pharynx, whereas cilia in the trachea propel mucus upward toward the pharynx, where it is swallowed.

AT THE CELLULAR LEVEL

Sputum is expectorated mucus. However, in addition to mucus, sputum contains serum proteins, lipids, electrolytes, Ca^{++}, DNA from degenerated white blood cells (collectively known as *bronchial secretions*), and extrabronchial secretions, including nasal, oral, lingual, pharyngeal, and salivary secretions. The color of sputum is more closely correlated with the amount of time that it has been present in the lower respiratory tract than with the presence of infection. Although not precisely identifiable with disease diagnosis, the color of sputum can be informative in helping lead to a diagnosis and stage of disease. Mucus has many colors: white, yellow, green, red, pink, brown, gray, and black. The coloration is commonly due to the type of cell present in the airways (inflammatory cells, such as neutrophils or eosinophils, or red blood cells) and how long they have been there. Clear or cloudy **white** thin mucus is considered normal; however, if amounts and thickness are increased, it may represent an early sign of infection. Thick white mucus can be the only identifiable feature of gastroesophageal reflux disease caused by gastric acid reflux into the airways. **Yellow** and **green** coloration of mucus is due to the presence and breakdown of neutrophils and eosinophils in infectious and allergic diseases. Yellow is typically associated with more acute disease (infection, allergy), and green usually indicates a more chronic stage with the presence of bacteria (chronic bronchitis, bronchiectasis, cystic fibrosis, and lung abscess). **Red** mucus indicates the presence of red blood cells in the airways and is associated with pneumococcal pneumonia, lung cancer, tuberculosis, and pulmonary emboli. **Pink** mucus is typically associated with the breakdown of eosinophils in individuals with allergies. Gray, brown, and black mucus is often associated with cigarette or marijuana smoking, cocaine use, air pollution (workplace environment, such as coal mines), and old blood.

Particle Deposition and Clearance

In general, deposition of particles in the lung depends on the particle size, density, and shape; the distance over which it has to travel; airflow speed; and the relative humidity of the air. The four major mechanisms for deposition are **impaction**, **sedimentation, interception,** and **Brownian movement.** Particle characteristics and properties, which influence the mechanism of deposition, are listed in Table 26.2. In general, particles larger than 10 μm are deposited by **impaction** in the nasal passages and do not penetrate into the lower respiratory tract. Particles 2 to 10 μm in size are deposited in the lower respiratory tract predominantly by inertial impaction at points of turbulent airflow (i.e., nasopharynx, trachea, and bronchi) and at airway bifurcations because their tendency to move in a straight direction prevents them from changing directions rapidly. In more distal areas, where airflow is slower, smaller particles (0.2–2 μm) are deposited on the surface by **sedimentation** as a result of gravity. For substances with elongated shapes (i.e., asbestos, silica), the mechanism of deposition is **interception**. The elongated

TABLE 26.2 Particle Deposition Characteristics

Method of Deposition	Particle Size (μm)	Deposition Site	Airflow	Determining Factors
Impaction	>10	Nasal passages	Fast	Size, density
	2–10	Nasal pharynx Trachea Bronchi	Fast	Size, density
Sedimentation	0.2–2.0	Distal airways	Slow	Size, density, diameter
Interception	NA	NA	Slow	Shape (elongated)
Brownian movement	<0.2	Smaller airways Alveoli	Slow	Diffusion coefficient (not density)

NA, Not applicable.

particle's center of gravity is compatible with the flow of air; however, when the distal tip of the particulate comes in contact with a cell or mucus layer, deposition is facilitated. Particles smaller than 0.2 μm are deposited in the smaller airways and alveoli and are influenced mainly by their diffusion coefficient and **Brownian motion**. Unlike the deposition of larger particles in the upper airways, particle density does not influence diffusion of these smaller particles, and deposition is enhanced with decreased size. These smaller particles come in contact with the alveolar epithelium, where cilia and the mucociliary transport system do not exist; thus they are removed by the phagocytic activity of alveolar macrophages or absorption into the interstitium with subsequent clearance by lymphatic drainage. Although most alveolar macrophages are adjacent to the epithelium of the alveolus, some are located in the terminal airways and interstitial space.

In the conducting airways, the mucociliary clearance system transports deposited particles from the terminal bronchioles to the major airways, where they are coughed up and either expectorated or swallowed. Deposited particles can be removed in a matter of minutes to hours. In the trachea and main bronchi, the rate of particle clearance is 5 to 20 μm/minute, but it is slower in the bronchioles (0.5–1 μm/minute). In general, the longer an inhaled material remains in the airways, the greater is the probability that the material will cause lung damage. The region from the terminal bronchioles to the alveoli is devoid of ciliated cells and is considered the "Achilles heel" in what is otherwise a highly effective system. The relatively slow rate of particle clearance in this area, which is mediated by macrophages, renders it the most common location for many occupational lung diseases.

IN THE CLINIC

In some lung diseases—for example, those caused by inhalation of silica particles **(silicosis)** or coal dust particles **(pneumoconiosis,** the "black lung" disease of coal miners)—alveolar macrophages phagocytize the particles but are unable to destroy them, and the macrophages eventually die. Alveolar macrophages localize and concentrate the particles in the "Achilles heel" region of the lung. These particles are not removed via mucociliary clearance and eventually enter the lung interstitium. The ensuing inflammatory response leads to a granulomatous-like lesion with fibrosis, a restrictive lung disease. Silicosis and pneumoconiosis are classical examples of diseases originating through environmental workplace exposure. Increased awareness of the cause of these diseases and improved workplace environments have led to reduction in the incidence of these types of lung diseases.

Mucosal Immune System: Adaptive and Innate Immunity

Mucosal Immune System

In nonmucosal tissues (e.g., spleen, liver, kidney), the body's primary defense is the classical proinflammatory adaptive, antigen-specific immune response orchestrated in the local draining lymph nodes with afferent and efferent lymph flow. The major adaptive immune cells in the systemic immune system are T lymphocytes with **αβ T cell receptors (TCRαβ T cells)** for specific antigen recognition and plasma B cells that synthesize immunoglobulin M (IgM) and immunoglobulin G (IgG) complement–binding antibodies, which can induce inflammation. However, mucosal tissues (i.e., those of the respiratory, gastrointestinal, and urinary systems, as well as the eyes, nose, throat, and mouth) must constantly discriminate between what is harmful and what is not, and although inflammation is protective, it usually disrupts the normal physiologic processes and is not desirable unless absolutely necessary. Accordingly, mucosal tissues have developed specialized "noninflammatory" defense mechanisms, which form the basis of the **mucosal immune system** and can function independently of the systemic immune system. The mucosal immune system contains both specialized **innate lymphoid cells** (macrophages, natural killer cells, dendritic cells [DCs]) and **adaptive** T lymphocytes with **γδ T cell receptors (TCRγδ T cells)** and plasma B cells that synthesize immunoglobulin A (IgA), a nonclassical complement-binding antibody. These innate and unique adaptive responses can prevent or limit responses to foreign nonpathological agents while eliminating pathological agents/substances with little or no inflammation. In addition, if this front-line defense system fails or is bypassed,

the lungs do have a classical adaptive immune response system in which lymphatic drainage is via the mediastinal lymph node located in the upper region of the thoracic cavity adjacent to the main left-right lung bifurcation.

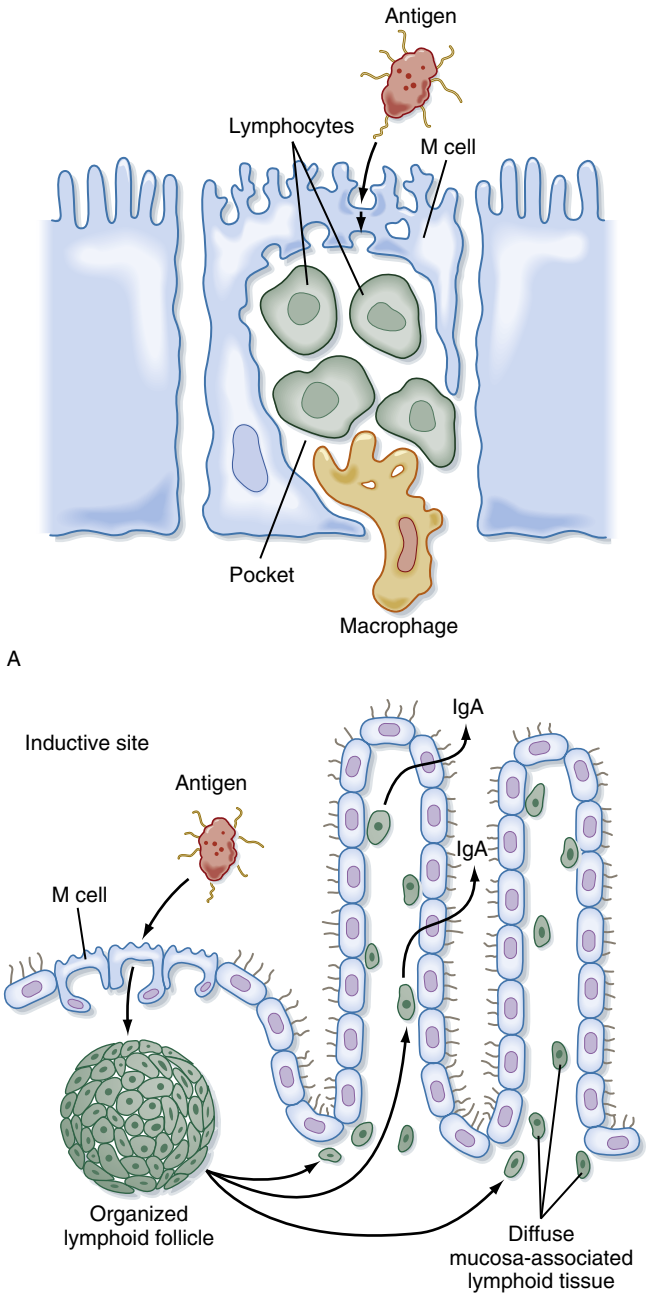
A distinctive feature of the mucosal immune system is that antigens are processed through **lymphoid aggregates** rather than through a true lymph node. Unlike a true lymph node, which has afferent and efferent lymph flow, lymphoid aggregates have only afferent drainage of material into the aggregate without efferent flow. This lymphoid network is commonly referred to as *mucosa-associated lymphoid tissue* (MALT); in the gastrointestinal tract, it is referred to as *gut-associated lymphoid tissue* (GALT); and in the lungs, it is known as **bronchus-associated lymphoid tissue** (**BALT;** Fig. 26.2). BALT is present in the conducting airways, where the epithelium is composed mainly of ciliated cells with consistent mucus flow. However, an interesting feature of BALT is that its airway epithelium is not ciliated; it is referred to as a *lymphoepithelium,* which creates a break in the mucus flow (like a drain) and allows the substances/particulates to be processed in the lymphoid aggregate (or follicle).

It appears that there is communication between mucosal tissues and that sensitization via one organ is transposed to all MALT/BALT tissues via a lymphatic-like drainage network. The systemic immune system and MALT/BALT may work independently of each other, and the fact that one is sensitized may not be true of the other. This may serve as a defense mechanism in limiting sensitization only to mucosal tissue. Lymphocytes also cluster in smaller numbers and density in what is referred to as **tertiary ectopic lymphoid tissue (TELT),** in which they can also process antigens (see Fig. 26.2*A*). Another prominent feature is a diffuse submucosal and intraepithelial network of **solitary lymphocytes** and innate lymphoid cells scattered throughout the respiratory tract. Because inhaled particles are broadly dispersed throughout the respiratory tract, each type of lymphoid cell and tissue (BALT, TELT, and solitary lymphocytes) plays important and unique roles in the overall defense of the lungs.

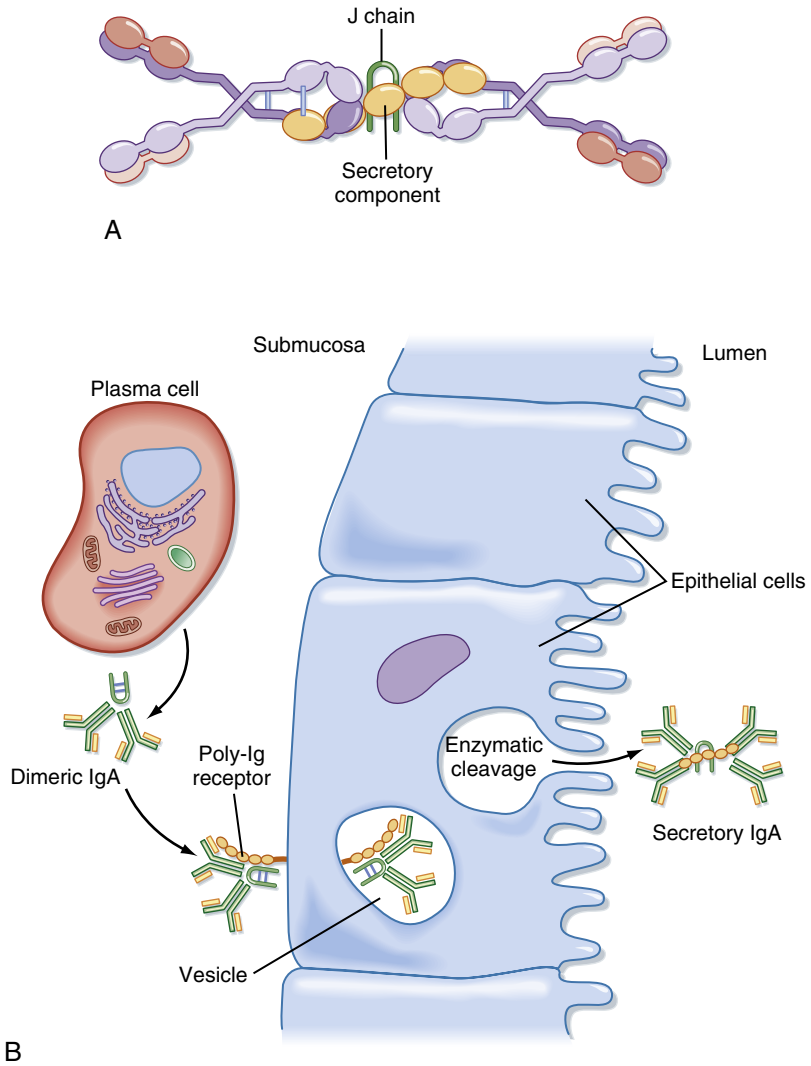
Specialized Adaptive Lymphoid Cells

Plasma Cells Producing Immunoglobulin A

One of the specialized features of MALT, GALT, and BALT is a unique antibody system in which specialized features of the IgA antibody (non–complement-fixing, J chain for transport, and dimeric structure for stability in the airway lumen) are used. In submucosal areas, plasma cells synthesize and secrete IgA, which migrates to the submucosal surface of epithelial cells, where it binds to a surface protein receptor, polymeric immunoglobulin (poly-Ig; Fig. 26.3). The poly-Ig receptor aids in the pinocytosis of IgA into the epithelial cell and its eventual secretion into the airway lumen. During exocytosis of the IgA complex, the poly-Ig is enzymatically cleaved, and a portion of it, the secretory piece, remains associated with the complex. The secretory piece stays attached to the IgA complex in the airway and helps protect it from proteolytic cleavage. The IgA-antigen immune complex does not bind complement in the same classical manner as do other immune complexes, and thus its proinflammatory properties are limited. The IgA-antibody system is very effective in binding particulates and viruses to form a large complex, which promotes its removal via the mucociliary clearance system, before they invade epithelial cells.



• **Fig. 26.2** Representation of bronchus-associated lymphoid tissue (BALT)/tertiary ectopic lymphoid tissue (TELT), M cells, and immunoglobulin A *(IgA)* synthesis. **A,** M cells located in mucosal epithelium endocytose antigen in the lumen and transport it for processing to loosely organized submucosal pockets of lymphoid cells, predominantly lymphocytes and macrophages (TELT). **B,** Diagram of a mucous membrane, showing secretion of IgA antibodies in response to antigen endocytosed by M cells. Activated B cells differentiate into IgA-producing plasma cells (lymphocytes) and migrate from the densely organized lymphoid follicle (in BALT) to the nearby submucosa, where they secrete IgA.



• **Fig. 26.3** Structure and formation of secretory immunoglobulin A *(IgA)*. **A,** Secretory IgA consists of at least two IgA molecules that are covalently linked via J chain and covalently associated with the secretory component. The secretory component contains five immunoglobulin-like domains and is linked to dimeric IgA through binding to an IgA heavy chain. **B,** Secretory IgA is formed during transport through epithelial cells. *Poly-Ig*, Polymeric immunoglobulin.

T Lymphocytes With γδ T Cell Receptors

Most classical adaptive immune T lymphocytes are $CD3^+$ cells with TCRs that are composed of α and β chains (TCRαβ T cells). These cells mature in the thymus and egress mostly to **lymph nodes and the spleen.** The classical activation of TCRαβ cells requires antigen processing/presentation, typically via the major histocompatibility complex, in a dendritic cell (DC), usually to induce an inflammatory response. $CD3^+$ T lymphocytes with TCRs expressing γ and δ chains (TCRγδ T cells) also mature in the thymus, but the majority of these cells egress to mucosal tissues (i.e., lung, intestines, and skin) and represent only a minority of T cells in the peripheral blood and systemic lymphoid tissues. TCRγδ T cells, often referred to as *intraepithelial lymphocytes,* preferentially localize to submucosal sites and epithelium and are considered a first line of defense of epithelial surfaces.

In contrast to TCRαβ T cells, the classical antigen activation of TCRγδ T cells does not require antigen processing or presentation by DCs. Of interest is that TCRγδ T cells have been shown to be capable of responding to antigen either in the manner of an innate cellular response, via pathogen-associated molecular patterns (PAMPs, described later in this chapter), or, in some circumstances, in the manner of a classical TCRαβ T cell response. Whether this variation in responsiveness is due to the presence of subpopulations of TCRγδ T cells or to plasticity (alterations) of the same cell is not clear. Because these cells express this unique duality, they are considered to be a link between adaptive and innate immune responses. Furthermore, TCRγδ cells can respond immediately to antigen and generally do not demonstrate memory. In contrast to the typical TCRαβ T cell proinflammatory response, TCRγδ T cells can provide protection without inducing inflammation. TCRγδ T cells have been shown to expand in response to viral and bacterial infections and synthesize either proinflammatory cytokines (interleukin [IL]–17, interferon [IFN]–γ) or regulatory/suppressive

cytokines (transforming growth factor β [TGFβ] and lymphocyte-activation gene 3). TCRγδ T cells also suppress the immunoglobulin E (IgE) response to inhaled antigen, further preventing allergen-induced inflammation.

Specialized Innate Lymphoid Cells

Innate lymphoid cells (ILCs) generally fall into four categories, TCRγδ T cells, dendritic cells (DCs), macrophages, natural killer (NK) cells, and subpopulations of each (Table 26.3). ILCs are distinctively different from adaptive immune cells by their lack of "antigen" specificity for the offending agents and their ability to distinguish self from nonself through recognition of PAMPs present on the pathogens. Since most inhaled substances are nonpathogenic, the body has developed a specialized recognition system to identify harmful pathogenic substances and organisms. Rather than specific antigen recognition, this system enables discrimination of self from nonself through the recognition of PAMPs on the pathogenic organisms/substances, which are then recognized by a family of receptors on host defense cells (i.e., ILCs) called *pattern recognition receptors* (PRRs). PAMPs are common distinguishing features on many pathogens and are composed of peptidoglycans, lipopolysaccharides, and unmethylated 5′-C-phosphate-G-3′ (CpG) DNA, which bind to their corresponding PRRs on ILCs. There are two major classifications for PRRs: **toll-like receptors** (TLRs), which are expressed mainly on cell surfaces, and **nucleotide-oligomerization domain** receptors, which are expressed intracellularly in the cytoplasm of ILCs. Both of these pathways activate nuclear factor Kβ (NFKβ), a transcription factor, which stimulates release of proinflammatory cytokines, as well the antiviral type I interferons (IFN-α and IFN-β). The TLRs are a family of transmembrane proteins with different specificities for various pathogens. TLR-2 is specific for lipoteichoic acids associated with Gram-positive bacteria, whereas TLR-4 is specific for lipopolysaccharide (endotoxin), a product of Gram-negative bacteria. In the lung, bronchial epithelial cells, macrophages, DCs, mast cells, eosinophils, and alveolar type II epithelial cells express both TLR-2 and TLR-4. Other TLRs are specific for viruses: TLR-3 binds to double-stranded RNA viruses, and TLR-7 and TLR-8 bind to single-stranded RNA viruses. NK cells express TLR-3, TLR-7, and TLR-8 and plasmacytoid DCs, eosinophils, and B cells express TLR-7. A variety of phagocytic cells and DCs in the lung and other mucosal tissues also express TLRs. Thus in addition to classical phagocytic cells, bronchial and alveolar epithelial cells play active roles in host defense by means of the PAMP-PRR nexus.

Perhaps the most unique and intriguing aspect of this specialized mucosal defense system is the ability of ILCs to respond to pathogens immediately, as opposed to the days or weeks it takes to mount a classical TCRαβ T cell adaptive immune response with clonal expansion and memory. The fast response, and the lack thereof for innocuous substances, are highly advantageous in the mucosal tissues, which are

TABLE 26.3 Innate and Adaptive Immune Cells in the Respiratory System

Cell Type	Location	Function
Innate Lymphoid Cells		
T lymphocytes with TCRγδ chains	Intraepithelial, submucosa	Selective antigen recognition Immunoregulation (decrease IgE)
Dendritic cells	Diffuse in the lung interstitium	
Conventional		Antigen presentation Immunoregulation (tolerance)
Plasmacytoid		Antiviral
Alveolar macrophages	Alveoli and alveolar ducts	Phagocytosis
Macrophages	Diffuse in the lung interstitium, BALT, and TELT	
M-1 cells		Phagocytosis, proinflammatory cytokines
M-2 cells		Phagocytosis, suppressive cytokines
NK cells	Diffuse in the lung interstitium	Targeted cytotoxicity Immunoregulation (tolerance)
iNKT cells	Diffuse in the lung interstitium	Immunoregulation (cytokines, IL-10, IFN-γ)
Adaptive Immune Cells		
T lymphocytes with TCRαβ chains	Submucosa, BALT, TELT	Specific adaptive immunity Proinflammatory (Th1/Th2 cytokines)
B lymphocytes	Submucosa, BALT, TELT	IgM IgG, IgA, and IgE antibody synthesis

BALT, Bronchus-associated lymphoid tissue; *IFN-γ*, interferon γ; *IgA, IgE, IgG, and IgM*, immunoglobulins A, E, G, and M; *IL-10*, interleukin 10; *iNKT*, invariant natural killer T; *NK*, natural killer; *TCR*, T cell receptor; *TELT*, tertiary ectopic lymphoid tissue; *Th1 and Th2*, T helper 1 and T helper 2.

common sites for parasitic invasion and toxic chemical exposure. In addition, many of the ILCs demonstrate plasticity, meaning that they can be induced by the environment (i.e., cytokines) to alter their functionality to either a proinflammatory or regulatory (suppressive) phenotype.

Macrophages and Dendritic Cells

The lungs contain resident alveolar macrophages, recruited macrophages, and DCs. Resident alveolar macrophages are embryonically derived and self-renewing. Both recruited macrophages and DCs originate in the bone marrow but vary somewhat in their differentiation lineage: recruited macrophages develop through the common myeloid-granulocyte/macrophage progenitor linage with other granulocytic cells (basophils, mast cells, eosinophils, neutrophils), and DCs develop through either the common lymphoid progenitor or the common myeloid progenitor lineages. Macrophages and DCs are the first nonepithelial cells to contact and respond to a foreign substance. In the lungs, there are at least two major types of recruited macrophages (M-1 [proinflammatory] and M-2 [regulatory]) and two types of DCs (conventional and plasmacytoid).

Macrophages

If the inhaled foreign material stays within the airspace in the lower respiratory system (alveolar ducts and alveoli), it will probably be phagocytized by resident alveolar macrophages. However, if the foreign material/organism penetrates and reaches submucosal areas, it will come into contact with M-1 or M-2 recruited macrophages and conventional or plasmacytoid DCs. Alveolar macrophages are found mostly in the alveolus adjacent to the epithelium and less frequently in the terminal airways and interstitial space. They migrate freely throughout the alveolar spaces and serve as a first line of defense in the terminal bronchioles and alveoli. They phagocytize foreign particles and substances, as well as surfactant and cellular debris from dead cells. For a particle/organism that is phagocytized by a macrophage, the major mechanisms of destruction include formation of O_2 radicals, enzyme activity, and halogen derivatives within lysosomes.

The phagocytic activity of the macrophage inhibits the binding of particulates to the alveolar epithelium and their subsequent penetration into the interstitium. The alveolar macrophage also transports engulfed particles to ciliated regions of the mucociliary transport system for elimination and thus provides an important link between the alveolar spaces, the post-terminal bronchiole "Achilles heel" region, and the mucociliary clearance system.

In addition, alveolar macrophages and M-2 macrophages present in the submucosa can also suppress T cell activity by direct contact or by the secretion of soluble factors such as nitric acid, prostaglandin E2, and the immunosuppressive cytokines (IL-10 and TGF-β). M-1 macrophages are also located in submucosal sites and represent the classical "proinflammatory" phagocytic cell, with similar killing capabilities to those of alveolar macrophages. In addition, the T helper 2 (Th2) proinflammatory (and proasthma) cytokines IL-4 and IL-13 have been shown to promote the differentiation of M-1 macrophages into regulatory/suppressive M-2 macrophages, which demonstrates the plasticity of these cells. The ability of these macrophage populations to demonstrate plasticity and dispose of foreign material rapidly with either a proinflammatory or regulatory response considerably enhances the lung defense system and is a unique contributor to the overall mucosal defense system.

Dendritic Cells

Conventional DCs reside in the submucosa of lung and other mucosa tissues and are considered the major antigen-presenting cells. They are usually in a resting immature state in which they function as sentinels to capture and process antigen, after which they mature and migrate to the local draining lymph node (mediastinal node for the lungs). At this node, they present antigen to T cells, which initiates an adaptive immune response, either proinflammatory or suppressive, depending on the antigen and DC. $CD103^+$ DCs have been shown to induce regulatory/suppressor T cells by means of their synthesis and secretion of TGF-β and indoleamine dioxygenase. Plasmacytoid DCs are located in similar submucosal areas but function more in an antiviral role, as opposed to antigen presentation; upon viral activation, they rapidly secrete the antiviral cytokine IFN-γ. Macrophages and DCs commonly synthesize and secrete many similar cytokines, depending on the stimulus; such cytokines include IL-1β (which activates vascular endothelium and lymphocytes), IL-6 (which activates lymphocytes and enhances antibody production), IL-12 (which activates NK cells, CD4 T cells to Th1 T cells), tumor necrosis factor α (which activates vascular endothelium and increases permeability), IL-8 (which recruits neutrophils, basophils, and T cells), and IFN-γ (which has antiviral effects).

Natural Killer and Invariant Natural Killer T Lymphoid Cells

NK cells originate in the bone marrow and differentiate through the common lymphoid progenitor lineage. Resident populations of functionally active NK and invariant natural killer T (iNKT) cells are present in mucosal sites and the lung interstitium. NK cells are a major component of the body's innate immune system of defense against invading pathogens such as herpesviruses, various bacterial infections, and tumor cells. NK cells can be activated by interferons (IFN-α and IFN-β) and other macrophage-derived cytokines (IL-12 and IL-18), which enhances their killing capabilities. The mechanism of killing is through the release of granular enzymes (granzymes, perforins, and serine esterases), which create holes or pores within the target cell membranes and thereby cause cell death. In addition, NK cells can synthesize IFN-γ, which is capable of inhibiting viral infections and stimulating CD4 and CD8 T cell responses.

NK cells have a complicated set of receptors to recognize a wide array of infectious bacteria and viruses. Individuals who lack NK cells are very prone to herpesvirus infections and often have recurrent infection. The iNKT cells belong to a category of lymphoid cells termed *innate-like lymphocytes,*

reside together with TCRγδ cells in mucosal tissues, and have antigen receptors with limited diversity, unlike the adaptive immune cells. These types of cells are thought to be links between innate and adaptive immunity. A critical aspect of the link is that these cells can respond must faster than cells of the classical adaptive immune response, limiting early damage until the adaptive response can mobilize a stronger defense. The iNKT cells have an invariant T-cell receptor α chain and, on activation, secrete the regulatory cytokines IL-10 and IFN-γ.

Epithelial Cells and Commensal Microbiota Protect the Lumen of the Airways

Epithelial Cells

Airway epithelial cells can produce and secrete both **antibacterial enzymes** and amphipathic (hydrophilic–positively charged) **antimicrobial peptides**, which generally target and disrupt cell wall components of the bacteria, which leads to cell death. Lysozyme, elastase, hydrolases, and secretory phospholipase A_2 are examples of antibacterial enzymes targeted at various pathogens. Antimicrobial peptides are typically cationic peptides synthesized as propeptides and activated through cleavage of an anionic propiece. Peptides secreted from epithelial cells and alveolar macrophages usually enter the mucosal fluid, whereas peptides secreted from submucosal macrophages typically stay within the local tissue.

There are three classes of antimicrobial peptides: defensins, cathelicidins, and histatins. Defensins, which are categorized as α and β can disrupt cell membranes within minutes and kill bacteria, fungi, and viruses. **β-Defensins** are synthesized in alveolar type II cells, are stored in lamellar bodies, and are a component of surfactant. **α-Defensins** are synthesized and stored within epithelial cells, macrophages, and neutrophils. **Cathelicidins** are stored in secondary granules and activated intracellularly by fusion of the phagosomes with the granule or also directly by elastase. In the lungs, cathelicidins are secreted from lung type II epithelial cells, as well as from neutrophils and macrophages. **Histatins** are present mainly in the oral cavity and are more specific for pathogenic fungi. Although it is not clear whether they exist in the respiratory tract, carbohydrate-binding proteins (lectins) in the gut can directly kill Gram-positive bacteria by binding to their cell walls. Furthermore, lactoferrins and lysozyme present in epithelial cells have bacteriostatic and bactericidal effects; the precise mechanism by which this occurs is not clearly understood, although it is postulated that they may work synergistically with iron and calcium.

Lung Microbiome-Commensal Microbiota

The human microbiome, especially in the gut, has received considerable attention, and exploration into the role of these commensal microorganisms in health and disease is rapidly developing. Although much is known about the gut microbiome, the lung microbiome has been difficult to explore. Once thought to be a sterile environment, the lung remains a difficult site to sample for a variety of reasons, including subject safety during sampling (invasive procedures), complexity and variety of epithelial surfaces throughout the respiratory tract, and the simple fact that far fewer bacteria are available to sample. Furthermore, the lumen ecosystem and epithelium of the respiratory tract differ considerably along the respiratory tract, from the upper airways to the alveoli. Thus it is likely that the density and types of bacteria residing in these various regions also differ. It is clear, however, that the lung does have a microbiome, and three primary phyla appear to be represented: Bacteroidetes (*Prevotella, Bacteroides*), Firmicutes (*Veillonella, Streptococcus, Staphylococcus*), and Proteobacteria (*Pseudomonas, Haemophilus, Moraxella, Neisseria, Acinetobacter*).

Alterations in the lung microbiome have been shown to be associated with several pulmonary conditions (including asthma, cystic fibrosis, and chronic obstructive pulmonary disease) and with lung transplantation. The increased rate of asthma in children has been linked to the increased rate of antibiotic use, which has a considerable effect on the microbiome. Organisms in the Proteobacteria *(Haemophilus, Moraxella)* and Firmicutes *(Streptococcus)* phyla correlate with asthma. An experimental study in mice showed an improvement in asthma when Proteobacteria organisms were replaced with Bacteroidetes, which coincided with an increase in regulatory T cells and suppression of the Th2 proinflammatory cytokines prominent in the pathogenesis of asthma.

From a therapeutic standpoint, the use of probiotics to maintain a "normal" microbiome has proved useful in treating some infectious diseases in the gut, and the use of probiotic therapy in patients with chronic lung infections (e.g., cystic fibrosis, upper respiratory tract infections) have been promising. The precise mechanism or mechanisms by which the bacteria may "protect" the lungs is still poorly understood but probably varies considerably according to the microorganism.

It has been shown that *Bifidobacterium* can activate DCs and TLRs to secrete IL-10 and retinoic acid, mediators capable of inducing regulatory T cells, which downregulate the Th2 inflammatory response. From a causative perspective, histamine, a major component of mast cell granules and secreted from *Lactobacillus* organisms, may be involved with priming DCs to stimulate Th2 inflammatory responses, as seen in individuals with asthma. In addition, ILCs play a role in influencing the microbiome through their ability to bind to receptors on epithelial cells, thus altering the interaction of the epithelium with the microorganisms and possibly promoting colonization of either pathogenic or nonpathogenic microorganisms. Additional research is required to understand fully the role of the lung microbiome in health and disease.

Clinical Manifestations Associated With Abnormalities in Mucosal Innate and Adaptive Immunity

By far the most common pathological conditions associated with mucosal tissue are allergic responses (e.g., allergic

IN THE CLINIC

Coronaviruses are a family of RNA viruses (*Coronaviridae*) enclosed in an envelope from which surface "spike proteins" project. Electron microscopy reveals these spikes give the virus a crown-like appearance. They are visually similar to the image of the solar corona. In humans, most cause symptoms of a common cold (low-grade fevers, cough, rhinitis, pharyngitis) and are self-limited. In 2002, coronavirus SARS-CoV-1 emerged and raised initial concerns of epidemic spread, although the disease remained relatively rare. The outbreak subsided by 2004. A second coronavirus, MERS-CoV, was reported in 2012 and continues to cause sporadic cases and occasional clusters. In 2019, the coronavirus SARS-CoV-2 (COVID-19) emerged and quickly sparked a global pandemic. Many infected individuals demonstrate only mild to moderate symptoms, while others develop severe disease with extensive pulmonary involvement and death. Risk factors for severe disease include age >80, diabetes, obesity, hypertension, and male gender. The virus is spread primarily by respiratory droplets or fomites produced when an infected individual coughs or sneezes, although transmission may occur by other mechanisms as well. Infected individuals can transmit the virus days before onset of their own symptoms. SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor found on the surface of alveolar type II cells. This can cause diffuse alveolar damage and progress to acute respiratory distress syndrome and death. Extrapulmonary manifestations include vascular complications (pulmonary emboli, stroke, and other blood vessel damage). Damage to the alveolar-capillary network leads to impaired gas diffusion. This in turn can produce capillary leak introducing serum proteins into the alveoli and disrupting the surfactant system. Combined, these processes decrease lung compliance and increased work of breathing. SARS-CoV-2 may also trigger an acute hyperinflammatory response known as a cytokine storm leading to elevations in IL-1, IL-2, IL-6, TNF-alpha, and interferon-gamma. Cytokine storm can worsen respiratory distress and lead to blood clotting events (including stroke and myocardial infarction), acute kidney injury, and inflammation of the vascular endothelium.

asthma, allergic rhinitis, and food and skin allergies). Some common human allergens include components of inhaled house dust mites, cockroaches, and cat dander, as well as bee stings and ingestion of peanuts. One of the most common drug allergens is penicillin, which can bind to many endogenous proteins and also alter their antigenicity.

As previously described, the predominant antibody response in MALT is IgA; however, in an allergic response, IgE is the predominant antibody. It is generated by a switchover mechanism induced by the synthesis of IL-4 from Th2-primed CD4 T cells. The IL-4 induces the antibody-producing B cells to switch over from synthesizing IgG antibodies to IgE. IgE binds to the surface of mast cells in the submucosa through its crystallizable fragment (constant) region of the antibody molecule. Upon reexposure to the inhaled antigen and its subsequent migration from the airway lumen to the submucosa, the allergen then binds to the antigen-binding fragment region of the IgE molecule, which forms an immune complex (IgE-Ag) on the surface of the mast cell.

The final step is that IgE-Ag complexes must crosslink with other IgE-Ag complexes on the surface of the mast cell, which induce intracellular signaling pathways to initiate degranulation and immediate release of preformed Th2 mediators (histamine, heparin, prostaglandins, leukotrienes, IL-4, IL-5, IL-13, proteases). These mediators induce the classical signs of asthma: smooth muscle constriction (bronchoconstriction), eosinophil recruitment and activation (inflammation), and connective tissue remodeling. Symptoms of wheezing, coughing, and shortness of breath occur within minutes, followed by a late response of eosinophilia and airway inflammation.

The inflammatory response can resolve spontaneously or as a result of therapy (bronchodilator or anti-inflammatory drugs, such as corticosteroids). Low-grade inflammation may persist and result in a process called *airway remodeling,* manifested by permanent, irreversible structural changes such as submucosal fibrosis and airway smooth muscle hypertrophy. The mechanisms responsible for airway remodeling in allergic diseases are not fully understood, but chemokines and cytokines such as TGF-β, a potent profibrotic cytokine, play important roles. Thus a very elegant, highly effective defensive system against infectious and parasitic organisms is "tricked" into responding to an innocuous substance, an allergen, as if it were harmful and initiates its defenses; the result is allergic airway disease.

Metabolic Functions of the Lung

The lungs are exposed to and metabolize a wide variety of xenobiotic substances. The endothelial cells within the lung capillary bed have a large surface area that receives very high blood flow, and lung endothelial cells have developed various mechanisms and cell surface receptors to metabolize xenobiotics. Most of the metabolic processing of inhaled or ingested xenobiotic compounds occurs enzymatically within the liver and intestinal tract with members of the cytochrome P-450 (CYP) enzyme families (e.g., CYP1, CYP2, CYP3). The lungs and other organs also selectively participate in the processing of xenobiotics and typically have lower levels of cytochrome P-450 enzymes. Prominent cytochrome P-450 enzymes in the lung include CYP1B1, CYP2B6, CYP2E1, CYP2J2, CYP3A5, and CYP1A1, the last of which is present in high levels in people who smoke cigarettes.

Drugs for the treatment of asthma and chronic obstructive pulmonary disease—such as corticosteroids, long-acting β_2 receptor agonists, leukotriene receptor antagonists, and methylxanthines—are degraded enzymatically in the lungs. In addition, a wide array of endogenous substances are metabolized by endothelial cells within the pulmonary capillary bed, including vasoactive amines, cytokines, lipid mediators, and proteins. Box 26.1 provides a list of compounds metabolized in the lung. Metabolism can occur through either intracellular or extracellular processing of endogenous substances that pass though the capillaries or by direct synthesis and secretion by endothelial cells. For example, angiotensin I is activated by angiotensin-converting enzyme, which is on the surface of endothelial cells. Serotonin, a vasoconstrictor, binds to a specific receptor

• BOX 26.1 Compounds Metabolized in the Lungs

Enzymatic Degradation in Pulmonary Circulation

- Corticosteroids
- Long-acting beta agonists
- Methylxanthines

Endothelial Cells in Pulmonary Capillary Bed

- Vasoactive amines
- Cytokines
- Lipid mediators
- Proteins

on the surface of endothelial cells and is internalized and metabolized inside cells. Approximately 80% of the serotonin entering the lungs is metabolized in a single pass through the pulmonary capillary bed.

Pulmonary vascular endothelial cells synthesize and secrete prostacyclin, endothelin, clotting factors, nitric oxide, prostaglandins, and cytokines. Vascular endothelial cells, however, lack 5-lipoxygenase and are not able to synthesize leukotrienes (smooth muscle constrictors). Compounds not metabolized by the pulmonary capillary bed include epinephrine, dopamine, histamine, isoproterenol, angiotensin II, and substance P.

AT THE CELLULAR LEVEL

Angiotensin-converting enzyme (ACE) is present in small indentations (caveolae) on the surface of pulmonary endothelial cells and catalyzes the conversion of the physiological inactive angiotensin I to the active angiotensin II, a potent vasoconstrictor. This is a major mechanism in the body's ability to supply systemic levels of angiotensin II and thus influence blood pressure. The therapeutic use of ACE inhibitors is important in the management of patients with high blood pressure.

IN THE CLINIC

The lungs play a key role in the metabolism of many prodrugs, which are inactive, into active drugs. Administration of prodrugs improves the amount of active drug delivered to their targets in the body. In many cases, inactive precursor prodrugs are delivered systemically or locally (through inhalation) to the lungs, where they are activated in situ. An example of this is beclomethasone dipropionate (Qvar, Beconase AQ), a medication inhaled by patients with asthma, which is activated by esterases within the lung to the active form 17-beclamethasone monopropionate.

Key Points

1. The respiratory system has developed unique structural (mucociliary transport system) and immunological (mucosal immune system) features to cope with the constant environmental exposure to foreign substances; these features limit or inhibit inflammation.
2. The three components of the mucociliary transport system are the sol phase (periciliary fluid), the gel phase (mucus), and cilia.
3. The depth of the periciliary fluid layer is maintained by the balance between Cl^- secretion and Na^+ absorption and is essential to normal ciliary beating.
4. Mucus is a complex macromolecule composed of glycoproteins, proteins, electrolytes, and water. It has low viscosity and high elastic mechanical properties.
5. Goblet cells, Clara cells, and the mucous and serous cells residing in the tracheobronchial glands produce mucus.
6. Particle deposition in the lung is dependent on their size, density, and shape; the distance traveled; airflow speed; and relative humidity. The major mechanisms for particle deposition are impaction (particles larger than 10 μm, in nasal passages, and particles 2 to 10 μm, in the nasopharynx, trachea, and bronchi), sedimentation (particles 0.2–2 μm in size, in distal airways), interception (particles with elongated shape, in the lower airways), and Brownian movement (particles smaller than 0.2 μm, in the alveoli).
7. The respiratory system is part of the mucosal immune system, which is composed of the intestinal (GALT), the respiratory (BALT), and urinary tract systems. These systems do not contain true lymph nodes with afferent and efferent lymph flow; they are composed mainly of nonencapsulated lymph nodules without true lymphatic drainage.
8. The nonciliated lymphoepithelium of BALT establishes a break in the mucociliary blanket that acts as a drain to facilitate the collection and immune processing of foreign particulates throughout the conducting airways.
9. TCRγδ T cells, IgA synthesizing plasma cells, NK cells, and alveolar macrophages are highly specialized innate and adaptive immune cells unique to the anti-inflammatory defense system in the lung and other mucosal tissues.