3
Lung Defenses

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We breathe to live, but the air we breathe carries many potentially harmful agents. To protect us against these constant challenges, our lungs have defenses that are remarkably effective, biologically complex, and scientifically fascinating. It is not hyperbole to say that the pathogenesis of most lung disease begins with a breach of these defenses. This chapter surveys these normal lung defense systems. Just as this text assumes familiarity with general pathology, we also assume knowledge of basic immunology. This chapter emphasizes the lung's variations on themes of innate and adaptive immunity, and discusses the special role of granulomatous inflammation in lung defenses.

To the extent possible, we will consider the evidence that a putative defense mechanism is functionally important. For example, relevant data include studies of people with genetic or acquired deficiency states, or experimental animal models with genetic or pharmacologic disruption (or enhancement) of defense components. In some cases, we must admit to a lack of formal proof and await future developments in the field. Fortunately, however, these are a minority, and our current knowledge of lung defenses will be shown to be well grounded in abundant clinical and experimental data.

Problems in the Air

Let us begin by considering broadly the potential problems for which lung defenses must be prepared: namely, agents carried into the lungs during inhalation. A first realization is that lung defense systems must cope with a staggering volume of air. The volume of air inhaled by the average person can be estimated as 10,000 L per day or 400 million liters per lifetime.[1] This volume ensures exposure to infectious and toxic agents, even if present in the air at quite low concentrations. There are three major categories of potentially injurious agents that merit attention: pathogens, particles, and toxic gases.

Pathogens

Pathogens enter the airways from two major sources: inhalation of bioaerosols in the environment and aspiration of nasopharyngeal secretions. The latter illustrates well the general capacity of lung defenses for effective clearance of small inocula of organisms, especially those of low to moderate virulence. We must recall that there normally is abundant colonization of the nasopharynx by a complex ecology of microorganisms that can include pathogens such as *Streptococcus pneumoniae* in up to 10% of normal adults.[2] Importantly, numerous studies have documented that nocturnal aspiration of small volumes of these secretions is a normal occurrence.[3] These secretions (e.g., saliva) contain an estimated 10^8 anaerobes and 10^6 aerobes per milliliter, as well as pneumococci and other pathogens (depending on colonization status). The general absence of infection despite this daily dose of bacteria is a testimonial to the efficacy of lung defenses. For inhalation exposures, quantitative culture data show that ambient air contains variable, albeit generally low, levels of bacteria and fungi.[4] It must be recognized, of course, that the systems that are so effective against low-level challenges can fail us with larger doses or more virulent organisms. Exposure to aerosols generated by expiration, sneezing, and coughing of other infected individuals can increase local concentrations of pathogens, especially in crowded conditions. Classic studies of tuberculosis provide dramatic evidence for aerosol transmission of a virulent pathogen, showing infection of guinea pigs by exposure to air from the rooms of patients with active disease (especially remarkable for calculations indicating infection after inhaling only one mycobacterium) (see Chapter 9).[5]

Particles

The broad term *particles* describes the complex collection of solid particulates found suspended in the air we breathe. They derive from both natural sources (e.g.,

crustal soil dusts, pollen, spores) and from man-made pollution sources (e.g., power plants, automobiles). Air particles vary greatly in size, pathogenicity, and concentration (e.g., rural vs. urban vs. occupational environments). A key point is that particle size (diameter) is an important determinant of pulmonary deposition and clearance. Particles larger than 10 μm in diameter are filtered in the nose and upper airway and do not enter the lungs. In contrast, "respirable" size particles (<2.5 μm in diameter) easily enter and deposit in airways and alveolar parenchyma. The pathogenicity of such inhaled particles depends on dose, the physicochemical characteristics of the particle, and the host response. Particles of different types span a spectrum of pathogenicity. At one end are inert or innocuous particles that cause minimal inflammation or injury at ambient concentrations or when introduced into the lungs experimentally (e.g., iron oxide, TiO_2, volcanic ash). In contrast, pathogenic particles like quartz cause human disease (silicosis, see Chapter 26) and robust inflammation and injury in experimental animal models.

The most commonly inhaled pathogenic particles are those found in polluted urban air, which is composed of complex combustion-derived particles. Their toxicity is illustrated dramatically by historical episodes of high mortality during extreme elevations of particulate air pollution (e.g., the London fog of 1952 where over 4000 excess deaths were recorded).[6,7] Recent epidemiologic studies find a more insidious but consistent problem. There is increased mortality and morbidity as air particle levels rise in urban atmospheres. This observation has generated considerable new interest in the pathogenesis and control of particle-mediated health effects.

The astute reader will have observed that, despite the goal of this section of quantifying the challenges faced by the lungs, no estimate of particle numbers typically inhaled has yet been offered. This reflects in part the devilish complexity of particle chemistry and health effects. Air levels of particles are typically measured in micrograms per cubic meter ($\mu g/m^3$), but it is not yet clear whether particle number, total mass, surface area, chemical composition, metal content, or (most likely) some combination of these determine pathogenicity.[8–12] The same liter of typical urban air may be described as containing merely hundreds of large "fine" size particles (respirable, 2.5 μm diameter) or millions of very small "ultrafine" size particles. Hence, this field still relies on the $\mu g/m^3$ standard used for epidemiologic studies. For some perspective, we note that typical particle levels in U.S. cities are 10 to 20 $\mu g/m^3$, that epidemiologists link detectable increases in mortality and morbidity with increases of 10-$\mu g/m^3$ increments, that many polluted cities in the world have levels fivefold or higher, and that levels during the fatal London Fog of 1952 are estimated to have been 300-fold or more higher!

The deposition and clearance of inhaled particles has been extensively studied. For larger particles that deposit on the airways, clearance is rapid and primarily via action of mucociliary clearance (see below). Smaller particles that reach the alveoli are phagocytosed by lung macrophages, and then cleared within the macrophages when they exit the lungs via mucociliary clearance or, less frequently, when they enter the tissue lymphatics and migrate to thoracic lymph nodes.

A key point that is instructive for our review of lung defenses is the observation that most air pollution particle effects are detected in people with preexisting disease (i.e., "the sick getting sicker").[13,14] This indicates that normal lungs cope with these noncatastrophic particle loads without apparent acute effects, even though higher doses or deposition in diseased lungs can cause injury. This is achieved through mechanisms detailed below.

Toxic Gases

A number of pollutant gases provide an oxidant stress against which the lungs must be prepared to defend. Major problem gases include ozone, nitrogen dioxide, and sulfur dioxide, each of which has been linked in epidemiologic and laboratory studies to myriad respiratory health problems.[10] Additional culprits include CO, semivolatile organics, and a host of other "air toxics." While substantial improvements in air pollution levels have occurred over recent decades, large numbers of people are still frequently exposed to unhealthy levels of these oxidant gases. For example, in 2003, U.S. Environmental Protection Agency (EPA) standards for ozone levels were exceeded in areas where a total of over 100 million people live.[15] The chemistry and pathophysiology for these oxidant gases have been extensively studied and reviewed for the interested reader.[16,17] This brief mention will suffice to complete our consideration of the potential problems inhaled by the lungs.

Defense Mechanisms

Anatomic

Deposition studies show that the upper respiratory tract is especially effective in filtering out large airborne particles (e.g., >10 μm in diameter), precluding their entry into the lungs. This is likely aided by the complex structure of the nasal cavity and turbinates, which promote turbulence and impaction of large particles. This constitutes the first of many defense mechanisms, as summarized in Figure 3.1. However, smaller particles remain suspended in the airstream and enter the lungs ("respirable" particles, <2.5 μm in diameter). The closure of the glottis during swallowing is another anatomic defense
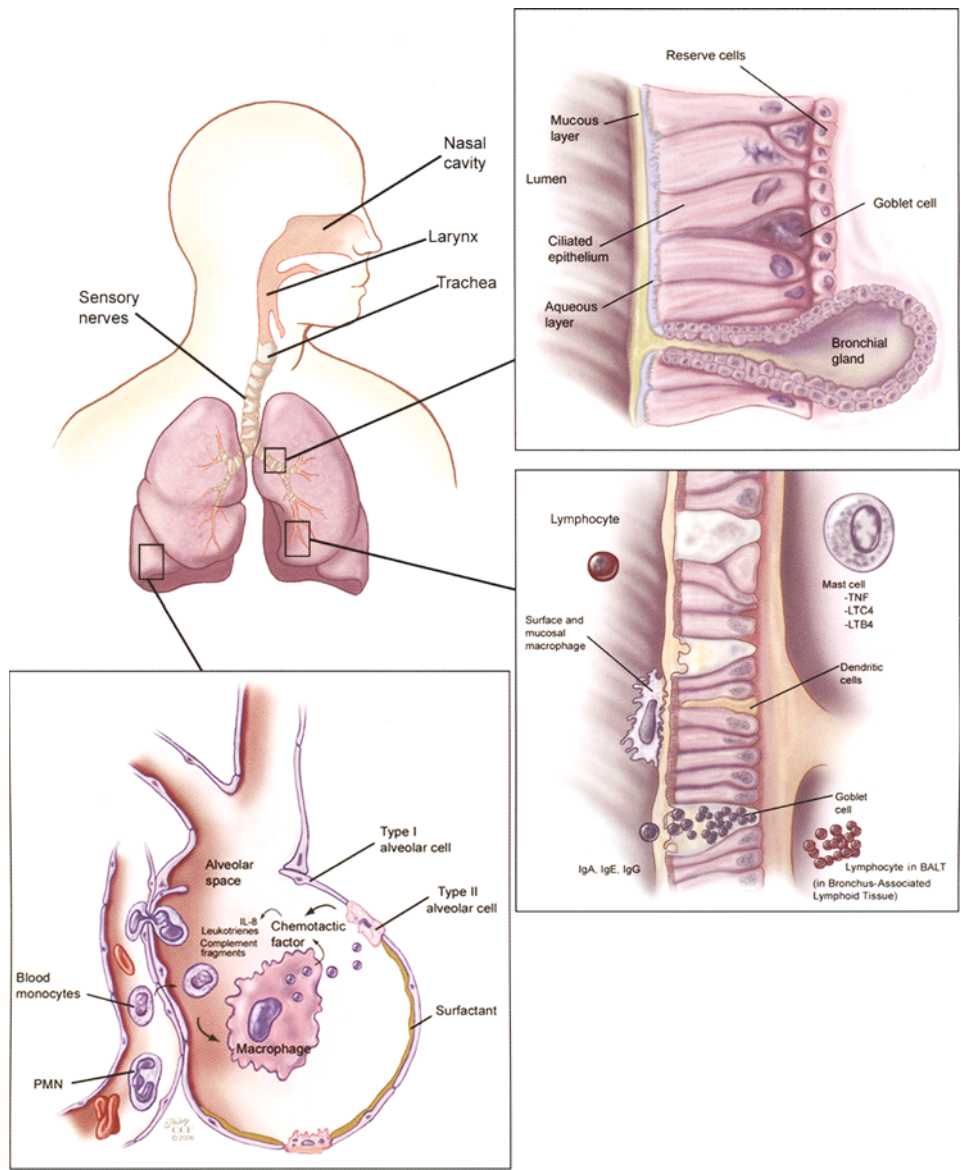
FIGURE 3.1. Summary of lung defenses. These defenses include aerodynamic filtration by the upper airway and cough reflexes initiated by sensory nerves in the trachea and bronchi. Additional airway defenses include mucociliary clearance, antibodies and other soluble mediators, and innate and adaptive cellular immunity. Alveolar defenses rely on antimicrobial properties of surfactant and initial clearance by alveolar macrophages; if needed, additional inflammatory cells are recruited by chemoattractants produced by macrophages and epithelial cells.

against entry of secretions (and food fragments) into the lungs, as illustrated by the increase in aspiration pneumonia seen in people with dysfunctional or structural disruptions of normal glottis closure.

Cough is a normal protective reflex that helps keep the airways free of obstruction and harmful substances. The stimuli that initiate the cough reflex stimulate sensory nerve fibers that have been divided broadly into three main groups: A delta fibers, C fibers, and slowly adapting stretch receptors (SARs) (see also Chapter 2).[18] An intact cough reflex is an important respiratory defense mecha-

nism, supported by findings of marked depression of the cough reflex in elderly patients with pneumonia and that the greater the derangement of the cough reflex, the greater the risk of pneumonia.[19]

Mucociliary Clearance

Ciliated respiratory epithelial cells act together with secretory cells of the airways to constitute a mucociliary transport system. Each epithelial cell has approximately 200 cilia that beat 300 to 600 times/min and continuously

propel mucus and other admixed debris up the airways (hence the common moniker of "mucociliary escalator") at 0.5 to 20 mm/min in small to large airways, respectively. Under normal conditions, between 10 and 100 mL of secretions pass up the trachea into the pharynx daily.[20] The tight junctions between the apical connections of these cells form a physical barrier to the penetration of particles or gases.

The lining fluid that facilitates mucociliary clearance consists of two phases. The sol or periciliary fluid phase adjacent to the epithelial cells provides a low viscosity environment for the beating cilia. The overlaying mucous gel phase is more viscous, and is propelled (along with any entrained inhaled particles) by the beating of the cilia. A second view of this innate airway defense system emphasizes a role for a "chemical shield" in the airway lining fluid in protecting the lung against inhaled bacteria.[21] This hypothesis emphasizes two other important functions for epithelia: the secretion of salt-sensitive antimicrobial peptides (defensins, see below), and the production of a low-salt (<50 mmol NaCl) milieu on airway surfaces that allows the defensins to be active against bacteria. It is striking that the antimicrobial substances appear to be limited to suppression of bacterial growth for a short period (about 2 to 6 hours), which conveniently matches the time normally required to clear inhaled bacteria from the airways by mucous transport.

We can again turn to experiments of nature for validation of the functional importance of this lung defense component. Congenital structural abnormalities (e.g., Kartagener's syndrome) in cilia disrupt normal mucociliary clearance. Such patients experience increased sinopulmonary infections. Similarly, acquired defects in ciliary function are caused by sundry agents associated with increased susceptibility to infection (e.g., tobacco smoke, alcohol). Additional details of ciliary structure and diseases linked to ciliary dyskinesis are presented in Chapter 5.

Surveillance by Soluble Mediators

The lining of the airways and alveoli contains numerous extracellular substances that contribute to defense. Their function is best characterized against microbes, but they may also interact with inhaled particulates.

Antimicrobial Components

Two of the most abundant antimicrobial proteins of airway secretions are lysozyme and lactoferrin, with estimated concentrations of 0.1 to 1 mg/mL.[22] Lysozyme is an enzyme that breaks the glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid residues in bacterial cell wall peptidoglycan. In addition to enzymatic lysis of bacterial cell walls, lysozyme can also kill bacteria nonenzymatically. Lysozyme is highly active against many gram-positive species, but is relatively ineffective against gram-negative bacteria unless aided by certain cofactors (e.g., lactoferrin, hydrogen peroxide, and ascorbic acid). It is likely that these cofactors damage and render the outer membrane of gram-negative bacteria permissive for lysozyme access to sensitive peptidoglycans. Lysozyme is produced by both epithelial cells and leukocytes. Since it is about tenfold more abundant in the initial "airway" aliquot than in later samples of bronchoalveolar lavage, it is likely that airway epithelium and its glands are the major sources of basal lysozyme in airway lining liquid. Elegant support for the role of lysozyme comes from studies showing that genetic deletion of lysozyme in mice increases susceptibility to pneumonia, while transgenic overexpression enhances resistance.[23]

Lactoferrin is an iron-binding protein highly abundant in the specific granules of human neutrophils and in epithelial secretions. Lactoferrin inhibits microbial respiration, and therefore growth, by sequestering essential iron. It can also be directly microbicidal, an activity found in its N-terminal cationic fragment lactoferricin. Secretory leukoprotease inhibitor (SLPI) is another protein found in airway lining fluid that has antimicrobial activity against in vitro gram-negative and gram-positive bacteria. Notably, lysozyme, lactoferrin and SLPI show marked synergy when tested together for bacterial killing in vitro.[24] Although the data for both lactoferrin and SLPI are strongly suggestive, no direct support (i.e., showing increased infection) from a human deficiency state or genetically altered mouse model exists.

There are two major families of antimicrobial peptides to consider: the defensins and the cathelicidins. Human defensins are relatively small, 3- to 5-kDa peptides with a characteristic six-cysteine/three-disulfide structure, and are members of a large family of microbicidal peptides.[25] Although genomic data indicate that the family includes more than 20 members, only a few human defensins have been characterized, and divided into α and β subgroups based on structure. The human neutrophil peptides (HNP)-1, -2, and -3, are closely related and found in the dense azurophil granules of neutrophils, and a fourth, HNP-4, is found in the same location but is much less abundant. Two other human defensins, HD-5 and -6, are located in the lysozyme-rich secretory granules of intestinal Paneth cells. Three recently characterized defensins, HBD-1, -2, -3, differ slightly structurally but are noteworthy since their messenger RNAs (mRNAs) are expressed in epithelia, including respiratory tract expression. As with the bovine tracheal antimicrobial peptide, a prototypic airway defensin, the synthesis and secretion of HBD-2 (and presumably HBD-3) are regulated by both responses to lipopolysaccharide (LPS) and other microbial stimuli and by cytokines. This conclusion is based on both in vitro data and on patient studies, showing that

the concentration of antimicrobial peptides such as β-defensins is increased in various body fluids during inflammatory or infectious diseases, such as pneumonia or cystic fibrosis. Cathelicidins are a more heterogeneous collection of antimicrobial peptides found in many animals, but the sole member found in humans is designated LL-37.[26]

Recent studies provide proof of the host defense function of antimicrobial peptides in living organisms.[27] For example, mice deficient in the antimicrobial peptide mBD-1 show delayed clearance of *Haemophilus influenzae* from the lung.[28] Overexpression of LL-37 by viral gene transfer resulted in augmentation of innate host defense in a bronchial xenograft model of cystic fibrosis[29] and in murine animal models of pneumonia and septic shock.[27]

In vitro, defensins are potent microbicidal agents at micromolar concentrations against many gram-positive and gram-negative bacteria, yeast and fungi, and certain enveloped viruses. However, this activity requires low-salt conditions, and increasing salt concentrations rapidly inhibit defensin activity. This requirement prompts the hypothesis that one function of airway epithelia is to maintain an optimally low-salt milieu within the airway lining fluid that facilitates mucociliary clearance.[21] Defensins damage microbes by permeabilizing microbial membranes rich in anionic phospholipids, with relative sparing of host membranes that are rich in cholesterol- and neutral phospholipid.

At the alveolar level we find two components of the surfactant layer with important defense properties: surfactant proteins A and D. They are collectins, which are a small family of secreted glycoproteins with C-type lectin domains and collagenous regions.[30] The collectins surfactant proteins A and D are the major protein constituents of surfactant. In vitro, these proteins bind to microorganisms via sugars on the microbial surface, and enhance adhesion and phagocytosis of microorganisms by agglutination and opsonization. In addition to facilitating pathogen uptake and killing by immune cells, SP-A and SP-D are directly antimicrobial, that is, they show direct bactericidal activity in the absence of effector cells.[31] In vivo evidence is also available and indicates a prominent host defense function. Mice genetically deficient in SP-A and SP-D show increased susceptibility to lung infections by bacteria[32,33] and viruses.[34,35] Importantly, the decreased microbial clearance can be reversed by addition of exogenous SP-A. While similar, SP-A and SP-D show different patterns of antimicrobial activity and likely contribute distinctly to overall pulmonary host defense.[36,37]

Antibodies and Complement

These potent immune system molecules are present in airway and alveolar lining fluid, as shown in extensive quantitative analyses of the proteins in bronchoalveolar lavage fluids.[20] The major immunoglobulin (Ig) classes found are IgA and IgG. IgA is predominantly found along the nasopharyngeal mucosa and in large airway samples; its relative concentration decreases progressively from larger to smaller airways. In contrast, IgG is the major antibody found in alveolar fluid samples. Similarly, complement components are detectable in fluid samples from normal lungs, albeit at much lower concentrations than found in plasma. The true in-situ concentration for both antibody and complement remains unresolved due to limitations introduced by the dilutional aspects of lavage techniques. Nevertheless, it is clear that both are present.

A more difficult question is what do the "pre-positioned" antibody and complement in lung lining fluids contribute to initial lung host defense? The importance of *systemic* antibody and complement to the ultimately successful defense against infection is eminently clear from numerous human deficiencies and forms the foundation for the field of immunology. Experimental complement depletion studies show decreased lung clearance of certain pathogens (*Streptococcus pneumoniae* and *Pseudomonas aeruginosa*) but not others (*Klebsiella pneumoniae* and *Staphylococcus aureus*).[38] Mice with genetic deletion of complement components or receptors also show increased susceptibility to lung infections.[39,40] However, in experimental analysis of bacterial clearance it is difficult (perhaps impossible) to separate the effect of lining fluid immunoglobulins and complement from the same mediators delivered from plasma after the inevitable increase in vascular permeability caused by introduction of the organisms. Vaccination against pneumococci generates protective antibody that reduces systemic disease and mortality, but does not apparently change the rate of local lung infection (pneumonia).[41] Similarly, for the special case of mucosal IgA, we learn from patients with selective IgA deficiency that most are asymptomatic, but a small minority do experience increased sinopulmonary infections,[42] supporting a perhaps modest, but definite, function for lining fluid IgA. Overall, it is intuitive and logical that these proteins contribute somehow to lung defense, but their precise role in initial defense remains difficult to characterize.

Antioxidants

The first line of defense against inhaled oxidant gases (and particles) is the numerous antioxidant molecules normally present in lung lining fluid. Indeed, it is likely that inhaled O_3 and NO_2 react primarily with lining fluid components and may never directly reach the underlying cells,[43,44] achieving toxicity through intermediates formed when antioxidant defenses are overwhelmed. These defense molecules include glutathione

and ascorbate (both present at higher concentrations than in plasma), uric acid, and α-tocopherol. Iron-binding proteins present in lining fluid such as lactoferrin, transferrin, and ceruloplasmin inhibit metal-catalyzed oxidant Fenton reactions. Finally, enzymes with antioxidant activity are also detected in normal lining fluid (e.g., catalase, glutathione peroxidase, and extracellular superoxide dismutase [ec-SOD]).[45,46]

Despite, or perhaps because of, this large list of potential antioxidants, it has been difficult to gauge their relative functional importance. Nevertheless, some valuable data have emerged. Circumstantial evidence for the low-molecular antioxidants and mucin include the observation that cigarette smokers produce abnormally large amounts of mucus and have elevated amounts of uric acid, glutathione, and ascorbic acid in their bronchoalveolar fluids. This may, for example, contribute to their reported decreased sensitivity (assessed spirometrically) to O_3.[47] They are unable to withstand the continuous onslaught of oxidants in tobacco smoke, and excess oxidant damage contributes to the pathogenesis of smoking-associated lung disease.[48] Studies in animals also support a function for these molecules. When both ascorbate (by diet) and glutathione (pharmacologically) are depleted, guinea pigs show an increased sensitivity to the toxicity of inhaled fly ash particles.[49] Prior studies where only one antioxidant was depleted did not change responses to inhaled oxidants,[50] indicating the redundancy offered by having multiple antioxidants present.

For the enzymatic defenses, the best evidence is available for ec-SOD where both knockout and transgenic overexpressing mice have been studied. When exposed to hyperoxia, ec-SOD-null mice show reduced viability and an earlier onset of severe lung edema as compared to wild-type mice.[51] Conversely, overexpression of ec-SOD in the lungs of mice confers protection against hyperoxia-induced lung injury, further supporting a role for this antioxidant enzyme in protecting the lung against oxidative stress.[52] Studies of other enzymes using genetically altered mice have failed to provide a clear answer.[53] Determining the function, if any, of the low levels of catalase and glutathione peroxidase present in lining fluid is an especially thorny problem because of the redundancy already described, the recruitment of additional plasma antioxidants through increased vascular permeability following oxidant injury, and because these enzymes may actually function within lung cells (where they are found in greater abundance).

Finally, note that we have considered these antioxidants in the context of defense against inhaled environmental oxidants. Most of these exposures are the results of relatively recent industrialization. They were not present to exert selective pressure during evolutionary development of the mammalian respiratory tract. Instead, it is likely that these antioxidant defenses developed primarily to balance the oxidants used by host cells for killing infectious agents. The preparation of our lungs for modern environmental oxidant challenges may be considered a fortunate side effect of homeostatic mechanisms in innate immunity.

Surveillance by Cellular First Responders

Macrophages

Macrophages in the lungs include subsets in distinct anatomic compartments. Alveolar, interstitial, and airway macrophages have been characterized. The most numerous and best studied is the alveolar macrophage (AM). Normal adult lungs contain approximately 20×10^9 AMs,[54] of which bronchoscopic lavage routinely yields 10 to 20×10^6.[55] Although local proliferation may contribute some of the normal resident population, AMs are ultimately derived from bone marrow hematopoiesis. Increases in macrophage number in response to injury derive primarily from influx and differentiation of blood monocytes. Estimates for the life span of AMs in normal individuals range from one to several months.[56,57] The evidence for this includes the compelling observation that bone marrow transplant recipients of one gender eventually show repopulation of bronchoalveolar lavage (BAL) AMs by cells showing sex chromosomes of the other gender.[57]

The main function of the AM is phagocytosis and clearance of inhaled material, a task it does remarkably well—with some important exceptions. The classic work of Green and Kass[58] established that AMs ingest and kill inhaled bacteria in vivo. Subsequent in vitro studies have confirmed that AMs are avidly phagocytic and readily destroy ingested bacteria such as S. aureus, S. pneumoniae, and H. influenzae, as well as other pathogens and particles.[59] Depletion of AMs using clodronate-liposomes has allowed further experimental demonstration of the importance of AMs in early responses to bacteria, since marked decreases in bacterial clearance follow.[60,61] This same approach also reveals interesting complexities, including a beneficial effect of AM depletion in experimental tuberculosis (presumably by eliminating the intracytoplasmic "safe harbor" AMs provide)[62] and species differences, since diametrically opposite effects were seen in one study of AMs and clearance of Cryptococcus by rats and mice.[63] The former illustrates the principle that AMs can ingest, but fail to kill, certain microorganisms, such as Mycobacterium spp., Nocardia spp., and Legionella spp., which are then capable of replicating intracellularly. Ultimate eradication of these pathogens requires the development of cell-mediated immunity.

The process of phagocytosis has been analyzed in breathtaking detail, and key points are summarized in
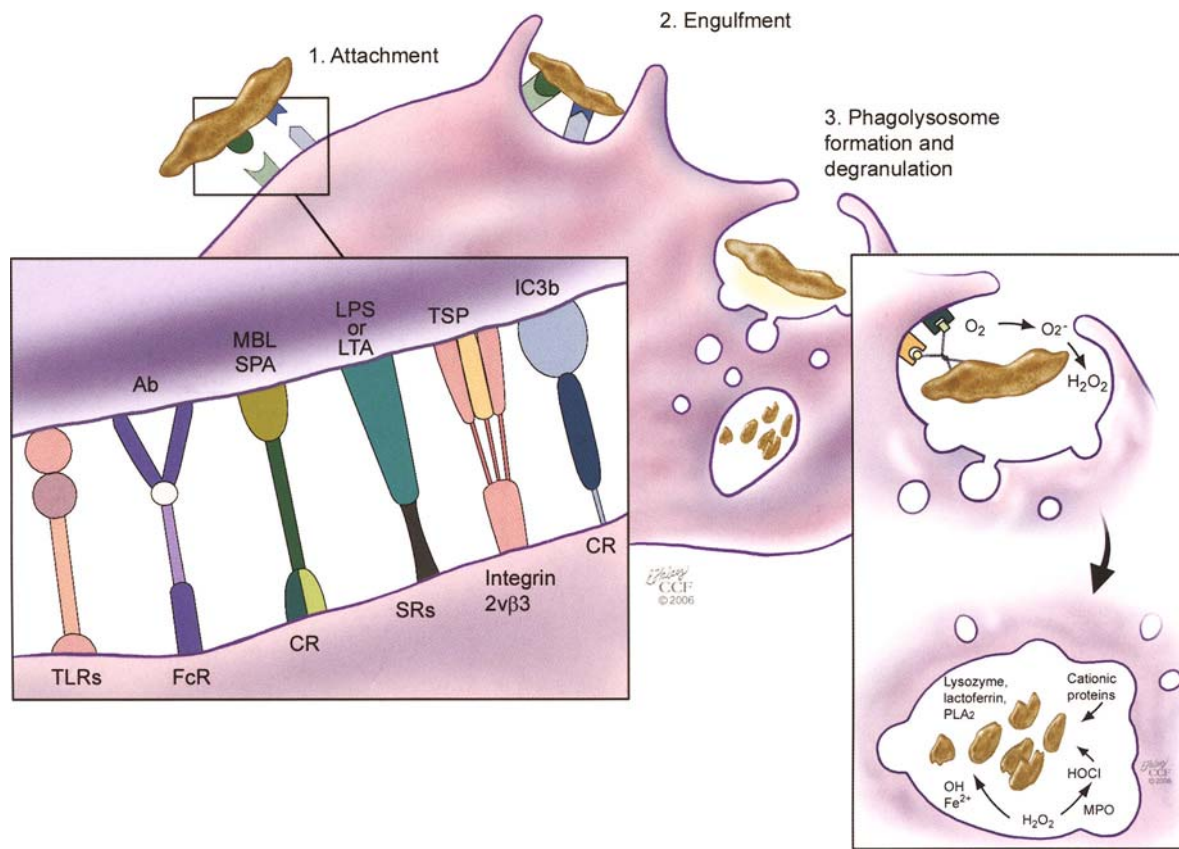
FIGURE 3.2. Phagocytosis. Innate defenses against inhaled pathogens begin with numerous pattern-recognition or opsonin receptors that mediate initial binding by macrophages and neutrophils, and which likely work together in a phagocytic synapse (see enlarged view of attachment step). Subsequent steps include internalization, killing, and degradation of pathogens by oxidants and other molecules. Ab, antibody; CR, complement receptor; FcR, immunoglobulin receptor; iC3b; inactivated complement 3b; LPS, lipopolysaccharide; LTA, lipotechoic acid; MBL, mannose binding lectin; PLA_2, phospholipase A_2; SPA, surfactant protein A; SRs, scavenger receptors; TLRs, toll-like receptors; TSP, thrombospondin.

Figure 3.2. A first and critical step is the recognition or binding of phagocytic targets. The AMs possess a broad array of membrane receptors that mediate binding of organisms and particles. Phagocytosis is initiated by these specific receptors that either recognize serum components (opsonins) or directly recognize molecular determinants on the target. These two mechanisms of particle recognition categorize the phagocytic process as either opsonin-dependent or opsonin-independent. The Fcg and CR3 are the best-characterized opsonin-dependent phagocytosis receptors. Fcg binds to IgG whereas CR3 binds the inactivated complement 3b (iC3b) fragment of complement. Recently other receptors such as collectin receptor, C1q, which binds the mannan binding protein (MBP), has been shown to mediate opsonin-dependent phagocytosis. IgG, iC3b, and MBP are opsonins that directly bind microorganisms and thus mark them for opsonin-dependent phagocytosis.

For innate, initial recognition of inhaled foreign material the AM also uses several pathogen-associated pattern receptors, which enable opsonin-independent phagocytosis.[64,65] The scavenger receptors constitute one important example. Scavenger receptors (SRs) represent a large family of cell surface glycoproteins that were identified during studies of the mechanisms for low-density lipoprotein (LDL) accumulation in atherosclerotic plaque macrophages. Subsequently, many other SRs on a variety of cells have been identified. The defining feature of the SRs is that they interact with a broad spectrum of ligands. Although the latter are mostly polyanionic molecules, there are a few exceptions to this rule, leading to the summation that they bind "most, but not all, polyanions" and to their designation as "molecular flypaper."[66] The SRs are divided into six subgroups, class A to F, based on their proposed tertiary structure. One example relevant to AM host defense function is the class A member MARCO, which can mediate AM uptake of unopsonized pneumococci and environmental particles in vitro and in vivo. The AMs express other SRs but their relative contributions to particle recognition and clearance remain

to be defined.[67] Other receptors that mediate opsonin-independent phagocytosis are found on AMs. These include a receptor for B-glucans on fungi, dectin-1,[68] the mannose receptor,[69] and others.[67] Finally, we must note lung macrophages (and other lung cells) express many of the Toll-like receptors now known to be critical in innate immune responses. The discovery and characterization of this large receptor family is a major recent accomplishment in immunology and has been extensively reviewed.[70,71] For our purposes, we note that capture of inhaled pathogens by pattern-recognition scavenger receptors facilitates activation of Toll-like receptors by pathogen-associated LPS or lipotechoic acid. The Toll-like receptors in turn trigger an elaborate intracellular signaling cascade that can lead to macrophage activation and release of proinflammatory mediators. The net effect is to increase the antimicrobial capacity of the macrophage and to recruit additional leukocytes if needed.

After recognition and activation of intracellular signaling, then what? Two tasks must be accomplished: internalization and killing. The cell biology of the internalization phase of phagocytosis has been dissected to a remarkable degree.[72,73] For killing of internalized bacteria and other pathogens, the AM has considerable, but not omnipotent, microbicidal machinery. It generates reactive oxygen species (using the respiratory burst) from reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that contribute to pathogen killing.[74] Other oxygen-independent bacterial killing mechanisms include proteases and phagolysosomal enzymes. In addition to reactive oxygen species, reactive nitrogen intermediates can contribute to pathogen killing.[75,76] Human AMs can be shown immunohistochemically to contain nitric oxide synthase (NOS) isoforms 2 and 3.[77,78] The high-output, inducible isoform NOS-2 appears to contribute to anti-mycobacterial defense,[79] but the role of reactive nitrogen intermediates in other antimicrobial responses of human macrophages remains controversial.[80,81]

After ingestion of particles, the AM functions ultimately to remove the offending material from the lung. This is achieved by movement of AMs to the mucociliary escalator and clearance to the oropharynx, or to a lesser degree, entry of macrophages into tissue compartments, lymphatics, and migration to thoracic lymph nodes.

The AM can also respond to pathogens by release of an extensive repertoire of inflammatory mediators that recruit additional help (i.e., the polymorphonuclear neutrophils, [PMNs]). These include lipid mediators (e.g., leukotriene B_4 [LTB_4]) and chemokines such as interleukin-8 (IL-8).[82,83] It is also worth considering that AMs also phagocytose many targets without generating oxidants or proinflammatory responses (e.g., in response to inert particles). This is a sensible adaptation to the need to ingest and clear frequently inhaled, nonpathogenic particles.

Epithelial Cells

Once considered merely passive bystanders, the epithelial cells of the airways and alveoli are now known to be very active participants in lung defenses against external challenges. The ciliated epithelial cells of the airways are an integral part of the mucociliary clearance system already discussed. Epithelial cells produce important components of the lining fluid in airway and alveolus, including mucus, surfactant proteins A and D, antimicrobial defensins, complement, and lysozyme. There is also evidence that they have some direct antibacterial function. For example, respiratory epithelial cells function in early clearance of *Pseudomonas aeruginosa* via interactions of cystic fibrosis transmembrane conductance regulator (CFTR) with the bacterium, which promotes epithelial internalization and clearance.[84]

Finally, the participation of epithelial cells in mediator networks that initiate and control lung inflammatory responses has been increasingly recognized. Airway epithelial cells secrete a large array of cytokines and other molecules (e.g., IL-1, -5, -6, and -8, and granulocyte-macrophage colony-stimulating factor [GM-CSF]).[27,85] By secreting these mediators, the airway epithelium is able to chemoattract and activate cells of the innate and adaptive immune system, which in turn immobilize and kill microorganisms. We must note, however, that the specific contribution of epithelial cells has not been fully tested since their selective ablation has not been feasible, in contrast to macrophages and PMNs, for example. Newer epithelial-specific gene targeting strategies may overcome this obstacle in future studies.[86–88]

Polymorphonuclear Neutrophils

After resident AMs, PMNs are the major second cellular defense against invading microorganisms in the lung. Under normal conditions, they are found primarily in the circulation, although a small number is present in lavage samples (<2%), possibly reflecting a low level of elicitation by ambient environmental exposures. Conveniently, at any given time, up to 40% of blood PMNs are marginated or in transit through the lung, facilitating recruitment when needed. The rapid and large movement of PMNs into the alveoli is achieved by the influence of several chemotactic factors released by AMs and other lung cells (e.g., IL-8, leukotrienes, complement fragments). These cause a rapid diapedesis, which is dominated by transit at the capillary level, in contrast to postvenular sites in other organs.

Polymorphonuclear neutrophils are also avidly phagocytic, especially for targets opsonized by serum antibodies or complement, which usually enter the alveolus along with PMNs after injury. The PMNs achieve killing of ingested microorganisms by generation of NADPH

oxidase-dependent reactive oxygen species (e.g., super-oxide and, after dismutation, hydrogen peroxide) and by phagolysosomal fusion. Reduced nicotinamide adenine dinucleotide phosphate oxidase is composed of a heterodimeric membrane-bound complex embedded in the walls of secondary granules (gp91phox and p22phox, forming cytochrome b558) and four cytosolic proteins (p47phox, p67phox, p40phox, and rac). During phagocytosis, the secondary granule fuses with the developing phagosome, depositing cytochrome b558 in the membrane. The cytosolic components associate with each other and then with the cytochrome to form the final NADPH oxidase. This complex transfers an electron from NADPH (thus oxidizing it) to molecular oxygen, producing superoxide. Superoxide dismutase converts the highly reactive superoxide into hydrogen peroxide. Fusion of azurophil granules with the phagolysosome permits entry of myeloperoxidase, which catalyzes hydrogen peroxide and chloride to form the oxidant HOCl. HOCl reacts with primary amines to generate highly toxic chloramines, which are powerful antimicrobial compounds active against most species of microorganisms. These metabolites of superoxide are potent microbicidal agents in vitro and are considered critical mediators of bacterial killing in vitro. An alternative hypothesis postulates that superoxide functions by creating electrochemical gradients that serve in turn to activate the primary granule proteins neutrophil elastase and cathepsin G inside the phagocytic vacuole.[89] Other microbicidal factors within the azurophil granules include defensins, bacterial permeability-increasing protein, lysozyme, and other proteases.

Clinical evidence for the importance of neutrophils in host defense of the lungs is abundant. Neutropenic patients often develop opportunistic infections by fungal or unusual pathogens in the lungs, underlining the important defense role of the PMN. Specific genetic defects are also illustrative. For example, defects in NADPH oxidase cause chronic granulomatous disease (CGD). It is noteworthy that only five microorganisms are responsible for the overwhelming majority of infections, including pneumonia, in CGD: *S. aureus, Burkholderia cepacia, Serratia marcescens, Nocardia* species, and *Aspergillus* species.[90] The selective profile of organisms that cause lung infections in neutropenic or CGD patients is telling. It reinforces the deduction that many other potential pathogens of low dose or low virulence are neutralized in these patients by other host defense mechanisms.

Mast Cells

Mast cells enjoy a distinct spatial distribution in the lung, being found preferentially in intraepithelial locations or around blood vessels and bronchioles. These are arguably ideal locations for surveillance of incoming challenges.[91]

Although early mast cell tumor necrosis factor (TNF) production is certainly their best-defined contribution during innate immune responses, the range of other functions that mast cells are capable of is impressive. First, we should recall their potential for degranulation, associated with the release of a plethora of preformed mediators, such as highly bioactive proteases, histamine, and proteoglycans. In addition, mast cells also produce a wide range of cytokines and chemokines, and release important lipid mediators, such as LTC_4 and LTB_4, in response to bacteria.[92] The profile of mast cell mediators produced is tightly regulated with respect to the type and amount, as well as temporal sequence. Although some pathogen products induce the release or generation of preformed mediators, lipid mediators and cytokines have a more selective effect.

Direct evidence of a role for mast cells in host defense against bacterial pathogens comes from studies of mast-cell–deficient mice. In both a model of cecal ligation and puncture and a model of *Klebsiella pneumoniae*–induced peritonitis, mice with normal numbers of mast cells survived bacterial challenge, whereas mast cell–deficient mice succumbed to infection. The ability to overcome infection was restored when mast cells were selectively reconstituted in the peritoneal cavity. The function of mast cells was closely linked to the ability to rapidly recruit neutrophils to the site of infection, supporting the view that mast cells serve to mobilize innate immune responses through early mediator production. The importance of TNF in this process was confirmed by studies in which mice were treated with anti-TNF antibodies. In some responses, the mast cell may be the major source of early TNF release, which is in keeping with observations of neutrophil recruitment after IgE-mediated mast cell activation.[93]

Natural Killer Cells

Natural killer (NK) cells are important in initial defenses against viral infection of the lungs. They arise from the same hematopoietic lineage as T cells, but differ in that they do not have to mature in the thymus and do not express rearranged antigen receptors. Instead, NK cells display families of cellular receptors that recognize virus-infected (and neoplastic) cells because of their altered expression of human leukocyte antigen (HLA) class I tissue antigens. The NK responses are mediated by inhibitory and activating receptors of two structural classes: killer Ig-like and lectin-like receptors.[94] When NK cell receptors fail to detect normal HLA class I, they initiate a program of activation that leads to lysis of the infected cell. The NK cells also release interferon-γ (IFN-γ), which in turn leads to recruitment of other immune cells. In experimental respiratory syncytial virus (RSV) infection, for example, there is a rapid antiviral NK cell IFN-γ

response that precedes and leads to recruitment of virus-specific, cytotoxic T lymphocytes. Local release of IL-12 and IL-15 by dendritic cells and macrophages contributes to stimulation of NK cells for rapid antiviral responses in the lung.[94,95]

Dendritic Cells

Dendritic cells (DCs), named for their characteristic long, branched processes, are specialized mononuclear phagocytes with important functions in antigen-presentation and initiation of adaptive immune responses. They are ubiquitous in the body, found in airways, alveolar parenchyma, and thoracic lymph nodes. Acting as sentinels in airways, they sample incoming pathogens and antigens through phagocytosis. When this is accompanied by a second, danger signal (e.g., pathogen patterns recognized by Toll-like receptors) they undergo a phenotypic and functional change from their basal immature state. This maturation promotes the processing of antigen and its presentation on the cell surface, and the migration of the dendritic cell to T-cell–rich areas of nearby lymph nodes. Here they can initiate or amplify adaptive immune responses by triggering proliferation and activation of antigen-specific T lymphocytes. This cursory summary does not do justice to the rich and detailed analysis of DCs and their role in this process, reviewed elsewhere for the interested reader.[96,97] For our purposes, we recognize this resident cell as an important transition between the innate response to inhaled antigenic material and the adaptive immune response that follows.

Adaptive Immunity

Having reviewed the initial elements of lung defense, we can now acknowledge the important role of adaptive immunity in dealing with pathogens that overcome the first, innate barriers. The adaptive immune response to pulmonary pathogens includes both humoral and cellular components. The benefit of humoral responses is well illustrated in the classic natural history of untreated pneumococcal pneumonia. Resolution of pneumonia begins with (and survival depends on) the appearance of IgG antipneumococcal antibodies produced by humoral immune response at about 1 to 2 weeks after the start of the infection.[98] Similarly, the natural history of viral lung infections illustrates the importance of cellular immune responses. Respiratory virus infections elicit CD4^{+}, CD8^{+}, and γδ T-cell responses, although the relative contributions of these subsets to viral clearance can be variable. From studies of influenza and parainfluenza virus infections in mice, we learn that CD8^{+} cytotoxic T cells play a key role in viral clearance. Neutralizing antibody is also generated late in the primary response, but does function

to clear primary infection unless the viral load is high. Typically, effector CD8^{+} T cells are first detectable in the lung on day 7 postinfection, and the number of CD8^{+} T cells peaks around day 10; optimal expansion of the CD8^{+} subset appears to depend on CD4^{+} T cells. The accumulation of CD8^{+} T cells in the lung results in clearance of the virus by day 10 postinfection and depends on either perforin or Fas mechanisms.[99]

It must be noted that cell-mediated immunity plays a role in recovery from influenza infection and may also prevent influenza-associated complications, but it does not seem to contribute significantly in preventing initial infection.[100] The humoral immune system produces antibodies against different influenza antigens, of which the hemagglutinin (HA)-specific antibody is the most important for neutralization of the virus and thus prevention of illness. The neuraminidase (NA)-specific antibodies are less effective in preventing infection, but they lessen the release of virus from infected cells.

Two observations provide compelling evidence of the importance of adaptive immunity to lung host defense. The first is that reinfection with the same organism is rare after recovery from a lung infection (or results in milder disease, e.g., RSV[101]). Indeed, this concept forms the basis for vaccination programs for influenza and infectious agents. The second is evidence from human primary and secondary immunodeficiency states, which reveal differing roles for the humoral and cellular branches of adaptive immunity.

Humoral immunodeficiencies, that is, those featuring defective antibody production, are relatively common, accounting for about 70% of all primary immunodeficiencies. Common effects include recurrent pneumonia, otitis media, sinusitis, and sepsis, caused by infections with pyogenic agents, such as *H. influenzae*, *S. pneumoniae*, and staphylococci. Hence, one generalization is that humoral (antibody) immunity is most important for successful defense against bacteria, especially encapsulated agents.[102] An important distinction is that most patients with defects involving predominantly humoral immunity generally recover from viral infections because of their normal cellular (T-cell) immune responses. Conversely, people with inadequate cellular immunity are highly susceptible to opportunistic infections with viruses, often presenting as progressive pneumonia caused by parainfluenza 3 virus, RSV, cytomegalovirus, varicella, or other opportunistic organisms, for example, *Pneumocystis jiroveci*. Since B-cell function is also T-cell dependent, T-cell immunodeficiencies are also accompanied by defects in antibody production. Such patients may also have infections with aggressive bacterial pathogens, similarly to those with primarily antibody deficiencies.

Secondary immunodeficiency states also illustrate the importance of adaptive immunity in the lungs. For example, infection is a common life-threatening complication faced

by immunosuppressed organ transplant recipients.[103] The respiratory tract is particularly vulnerable, representing a leading infectious site in lung, heart, and liver transplant recipients. As in primary T-cell deficiencies, these individuals experience suppression of lymphocyte-dependent immunity and increased incidence of viral and opportunistic pathogens. Similarly, the modern tragedy of AIDS, which primarily targets T-cell immunity, features enhanced susceptibility to opportunistic infection, in particular life-threatening infections of the lung.[104]

Finally, we can consider evidence from vaccination efforts, which also illustrate the power (and limitations) of adaptive immunity for lung defense. Vaccines are available to prevent two of the most common and most deadly causes of lower respiratory tract infections: influenza and pneumococcal disease. Influenza vaccines prevent influenza as well as several complications of influenza, via antibodies highly specific for the strain used for immunization (requiring new immunization programs for ever-changing influenza strains[105]). Pneumococcal polysaccharide vaccine prevents pneumococcal sepsis and systemic disease, but appears ineffective in reducing the incidence of local lung infection (pneumonia[41]), indicating that not all systemic antibody responses translate into a functional barrier against initiation of infection in the lungs.

Granulomatous Inflammation

Granulomatous inflammation occurs in response to certain infectious agents and persistent foreign material, and as part of disease of unknown etiology (e.g., sarcoidosis). It describes a distinct form of chronic inflammation, dominated by mononuclear phagocytes that take the form of macrophages, epithelioid cells, and multinucleated giant cells.[106] Typically these cells congregate and form well-demarcated focal lesions called granulomas, although a looser, more diffuse arrangement may be found. In addition, there is usually an admixture of other cells, especially lymphocytes, plasma cells, and fibroblasts. Understanding granuloma development and its role in lung defense is pertinent to a broad cross section of pulmonary pathology. Since it is most relevant to lung host defense, we will focus on granulomatous responses to mycobacterial infection. There are detailed reviews of the shared and unique features of pathogenesis of sarcoidosis, hypersensitivity pneumonitis, and other lung granulomatous disorders available for the interested reader.[107–109]

Fortunately for our review, the sequence of cellular events in granuloma formation is indeed best defined in responses to tuberculosis, the prototypical granulomatous disease (see Chapter 9). The process begins with activation of CD4$^+$ T cells by antigen-presenting cells that have ingested and processed antigenic mycobacterial components. This leads to proliferation and differentiation of naive CD4$^+$ T cells to T-helper-1 (Th1) cells, which release characteristic cytokines, especially IFN-γ. These mediators, in turn, activate macrophages for improved killing of intracellular bacteria and also account for the classic change of macrophage appearance to an enlarged cell with epithelioid morphology. The process also activates a number of cell surface molecules (e.g., macrophage fusion receptor, dendritic cell-specific transmembrane protein [DC-STAMP], CD47, CD44) thought to mediate cell-cell fusion and the formation of giant cells.[110–113]

The importance of this response in lung host defense is also best illustrated in tuberculosis, since it (usually) provides mycobacterial containment critical for successful protection against progressive infection.[114] Effective containment depends on various T-cell subpopulations, including CD4, CD8, γδ, and CD1 restricted T cells. Based on animal models, a hierarchy exists: CD4$^+$ T cells are most important, followed by CD8$^+$ T cells, with precise roles of γδ T cells and CD1 restricted T cells less well understood. There is also a temporal sequence to the involvement of different T-cell subtypes in the phases of granuloma formation.[115] It is clear, nevertheless, that CD4$^+$ T cells are the central organizers of the granuloma during every phase of granuloma formation. Mice genetically deficient in CD4$^+$ T cells form aberrant lesions that are unable to control bacterial numbers or prevent dissemination, establishing that CD4$^+$ T cells are important for initiation and construction of granulomas.[116] In humans, the requirement for CD4$^+$ T cells is demonstrated by the disseminated mycobacterial infections seen in patients during the late stages of AIDS when CD4$^+$ T-cell counts drop.[117]

It is worth emphasizing that while T cells are the major orchestrators of protection, the actual execution of antimicrobial action (i.e., killing of mycobacteria) is performed by the macrophages that ingested the mycobacteria in the first place. After cross-talk between T cells and macrophages via various cytokines, notably IFN-γ, TNF-α, and leukotriene A (LTA), macrophages are activated and have increased ability to kill the intracellular pathogens.[118,119] The importance of IFN-γ in human tuberculosis is clearly indicated by reports of severe and even fatal infections with mycobacteria in patients with defects in the IFN-γ receptor.[120] Specific changes in activated macrophages include increased phagocytic and microbicidal ability (through increased production of reactive oxygen species [ROS] and reactive nitrogen intermediates [RNI]) and increased release of cytokines, which promote fibroblast proliferation and collagen production. The established view has been that necrosis ensues upon vigorous activation of the macrophages by the adaptive immune response (T cells). This view is based in part on the first appearance of necrotic centers at 2 to 3 weeks after infection in a rabbit model, a time frame consistent with

generation of an adaptive response. However, this view has been challenged by findings in mycobacteria-infected guinea pigs where the development of the necrotic core was an early event and almost certainly preceded the emergence of the acquired immune response.[121]

Granuloma formation typically ends in fibrosis. Fibrosis serves to wall off the granuloma contents and limit spread of infection and organ damage. It was shown in early studies that healed primary lesions are usually sterile within 5 years.[122] Mediators implicated in the fibrosis process in granulomatous inflammation are numerous. Examples include TNF[123] and transforming growth factor-β (TGF-β),[124] among many others.[125] The Th2 cytokine IL-13 may also mediate fibrosis associated with granulomatous responses. Blockade of IL-13 in schistosome-infected mice reduces fibrosis without altering the Th2 cytokine profile.[126] It is worth noting that there is disagreement as to the relative primacy of macrophage-derived vs. T cell derived factors.[115,125]

Conclusion

The lung defenses we have surveyed provide a complex, multilayered response to the pathogens and other potentially injurious agents we breathe (Table 3.1). As the large size of this pathology book attests, this system is not perfect. Moreover, lung host defense must deal with the relentless evolutionary cleverness of microbes,[127,128] and common self-inflicted impairment due to alcohol and cigarette smoke.[129,130] Nevertheless, these defenses are remarkably successful, considering the frequency of contact with intruders that must be neutralized. We end by marveling at the details we know of this process, and with anticipation of future progress that will improve knowledge of lung pathology in general.

TABLE 3.1. Summary of lung host defenses

Anatomic
    Upper airway filtration
    Glottis
    Cough reflex
    Mucociliary clearance
Surveillance by soluble mediators
    Lysozyme
    Lactoferrin
    Antimicrobial peptides
      Defensins, cathelicidin
    Surfactant proteins
      SP-A, SP-D
    Immunoglobulins
      IgA, IgG
    Complement
    Antioxidants
      Uric acid, glutathione, α-tocopherol
      Extracellular superoxide dismutase
      Catalase, glutathione peroxidase
Surveillance by resident lung cells
    Macrophages
      Alveolar, airway, interstitial
    Epithelial cells
    Dendritic cells
    Mast cells
Recruited defenses
    Neutrophils
    Lymphocytes
    Monocytes
    Plasma mediators

References

1. McClellan R. Particle interactions in the respiratory tract. In: P Gehr, J Heyder, eds. Particle-lung interactions. New York: Marcel Dekker, 2000:3–66.
2. Bogaert D, De Groot R, Hermans PW. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004;4:144–154.
3. Gleeson K, Eggli DF, Maxwell, SL. Quantitative aspiration during sleep in normal subjects. Chest 1997;111:1266–1272.
4. Sessa R, Di PM, Schiavoni G, et al. Microbiological indoor air quality in healthy buildings. New Microbiol 2002;25:51–56.
5. Riley RL. Aerial dissemination of pulmonary tuberculosis. Am Rev Tuberc 1957;76:931–941.
6. Bell ML, Davis DL, Fletcher T. A retrospective assessment of mortality from the London smog episode of 1952: the role of influenza and pollution. Environ Health Perspect 2004;112:6–8.
7. Stone R. Air pollution. Counting the cost of London's killer smog. Science 2002;298:2106–2107.
8. Nel A. Atmosphere. Air pollution-related illness: effects of particles. Science 2005;308:804–806.
9. Donaldson K, Stone V, Seaton A, MacNee W. Ambient particle inhalation and the cardiovascular system: potential mechanisms. Environ Health Perspect 2001;109(suppl 4):523–527.
10. Bernstein JA, Alexis N, Barnes C, et al. Health effects of air pollution. J Allergy Clin Immunol 2004;114:1116–1123.
11. Tao F, Gonzalez-Flecha B, Kobzik L. Reactive oxygen species in pulmonary inflammation by ambient particulates. Free Radic Biol Med 2003;35:327–340.
12. Oberdorster G. Pulmonary effects of inhaled ultrafine particles. Int Arch Occup Environ Health 2001;74:1–8.
13. Dockery DW, Pope CA 3rd, Xu X, et al. An association between air pollution and mortality in six U.S. cities. N Engl J Med 1993;329:1753–1759.
14. Dockery DW, Pope CA 3rd. Acute respiratory effects of particulate air pollution. Annu Rev Public Health 1994;15:107–132.
15. U.S. Environmental Protection Agency. The ozone report: measuring progress through 2003. Research Triangle Park, NC: EPA, 2004.
16. Last JA, Sun WM, Witschi H. Ozone, NO, and NO$_2$: oxidant air pollutants and more. Environ Health Perspect 1994;102(suppl 10):179–184.

17. American Thoracic Society. Health effects of outdoor air pollution. Am J Respir Crit Care Med 1996;153:3–50.

18. Reynolds SM, Mackenzie AJ, Spina D, Page CP. The pharmacology of cough. Trends Pharmacol Sci 2004;25:569–576.

19. Marik PE, Kaplan D. Aspiration pneumonia and dysphagia in the elderly. Chest 2003;124:328–336.

20. Reynolds HY. Defense mechanisms against infections. Curr Opin Pulm Med 1999;5:136–142.

21. Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. J Clin Invest 2002;109:571–577.

22. Ganz T. Antimicrobial polypeptides. J Leukoc Biol 2004; 75:34–38.

23. Markart P, Korfhagen TR, Weaver TE, Akinbi HT. Mouse lysozyme M is important in pulmonary host defense against Klebsiella pneumoniae infection. Am J Respir Crit Care Med 2004;169:454–458.

24. Singh PK, Tack BF, McCray PB Jr, Welsh MJ. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. Am J Physiol Lung Cell Mol Physiol 2000;279:L799–805.

25. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol 2003;3:710–720.

26. Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol 2004;75:39–48.

27. Bals R, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. Eur Respir J 2004;23:327–333.

28. Moser C, Weiner DJ, Lysenko E, Bals R, Weiser JN, Wilson JM. Beta-Defensin 1 contributes to pulmonary innate immunity in mice. Infect Immun 2002;70:3068–3072.

29. Bals R, Weiner DJ, Meegalla RL, Wilson JM. Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. J Clin Invest 1999;103:1113–1117.

30. Hickling TP, Clark H, Malhotra R, Sim RB. Collectins and their role in lung immunity. J Leukoc Biol 2004;75: 27–33.

31. Wu H, Kuzmenko A, Wan S, et al. Surfactant proteins A and D inhibit the growth of Gram-negative bacteria by increasing membrane permeability. J Clin Invest 2003;111: 1589–1602.

32. LeVine AM, Kurak KE, Bruno MD, Stark JM, Whitsett JA, Korfhagen TR. Surfactant protein-A-deficient mice are susceptible to Pseudomonas aeruginosa infection. Am J Respir Cell Mol Biol 1998;19:700–708.

33. LeVine AM, Bruno MD, Huelsman KM, Ross GF, Whitsett JA, Korfhagen TR. Surfactant protein A-deficient mice are susceptible to group B streptococcal infection. J Immunol 1997;158:4336–4340.

34. LeVine AM, Hartshorn K, Elliott J, Whitsett J, Korfhagen T. Absence of SP-A modulates innate and adaptive defense responses to pulmonary influenza infection. Am J Physiol Lung Cell Mol Physiol 2002;282:L563–572.

35. LeVine AM, Whitsett JA, Hartshorn KL, Crouch EC, Korfhagen TR. Surfactant protein D enhances clearance of influenza A virus from the lung in vivo. J Immunol 2001; 167:5868–5873.

36. LeVine AM, Whitsett JA, Gwozdz JA, et al. Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung. J Immunol 2000;165:3934–3940.

37. Wright JR. Immunoregulatory functions of surfactant proteins. Nat Rev Immunol 2005;5:58–68.

38. Gross GN, Rehm SR, Pierce AK. The effect of complement depletion on lung clearance of bacteria. J Clin Invest 1978;62:373–378.

39. Hopken UE, Lu B, Gerard NP, Gerard C. The C5a chemoattractant receptor mediates mucosal defence to infection. Nature 1996;383:86–89.

40. Mueller-Ortiz SL, Drouin SM, Wetsel RA. The alternative activation pathway and complement component C3 are critical for a protective immune response against Pseudomonas aeruginosa in a murine model of pneumonia. Infect Immun 2004;72:2899–2906.

41. Jackson LA, Neuzil KM, Yu O, et al. Effectiveness of pneumococcal polysaccharide vaccine in older adults. N Engl J Med 2003;348:1747–1755.

42. Cunningham-Rundles C. Physiology of IgA and IgA deficiency. J Clin Immunol 2001;21:303–309.

43. Pryor WA, Squadrito GL, Friedman M. A new mechanism for the toxicity of ozone. Toxicol Lett 1995;82–83:287–293.

44. Postlethwait EM, Langford SD, Bidani A. Reactive absorption of nitrogen dioxide by pulmonary epithelial lining fluid. J Appl Physiol 1990;69:523–531.

45. Cross CE, van der Vliet A, O'Neill CA, Louie S, Halliwell B. Oxidants, antioxidants, and respiratory tract lining fluids. Environ Health Perspect 1994;102(suppl 10):185–191.

46. Comhair SA, Erzurum SC. Antioxidant responses to oxidant-mediated lung diseases. Am J Physiol Lung Cell Mol Physiol 2002;283:L246–255.

47. Frampton MW, Morrow PE, Torres A, Cox C, Voter KZ, Utell MJ. Ozone responsiveness in smokers and nonsmokers. Am J Respir Crit Care Med 1997;155:116–121.

48. MacNee W. Oxidants/antioxidants and COPD. Chest 2000; 117:303S–317S.

49. Norwood J Jr, Ledbetter AD, Doerfler DL, Hatch GE. Residual oil fly ash inhalation in guinea pigs: influence of absorbate and glutathione depletion. Toxicol Sci 2001;61: 144–153.

50. Kodavanti UP, Costa DL, Richards J, Crissman KM, Slade R, Hatch GE. Antioxidants in bronchoalveolar lavage fluid cells isolated from ozone—exposed normal and ascorbate-deficient guinea pigs. Exp Lung Res 1996;22:435–448.

51. Carlsson LM, Jonsson J, Edlund T, Marklund SL. Mice lacking extracellular superoxide dismutase are more sensitive to hyperoxia. Proc Natl Acad Sci USA 1995;92: 6264–6268.

52. Folz RJ, Abushamaa AM, Suliman HB. Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia. J Clin Invest 1999;103:1055–1066.

53. Ho YS. Transgenic and knockout models for studying the role of lung antioxidant enzymes in defense against hyperoxia. Am J Respir Crit Care Med 2002;166:S51–56.

54. Crapo JD, Young SL, Fram EK, Pinkerton KE, Barry BE, Crapo RO. Morphometric characteristics of cells in the alveolar region of mammalian lungs. Am Rev Respir Dis 1983;128:S42–46.

55. Reynolds HY. Immunologic system in the respiratory tract. Physiol Rev 1991;71:1117–1133.

56. van oud Alblas AB, van Furth R. Origin, kinetics, and characteristics of pulmonary macrophages in the normal steady state. J Exp Med 1979;149:1504–1518.

57. Thomas ED, Ramberg RE, Sale GE, Sparkes RS, Golde DW. Direct evidence for a bone marrow origin of the alveolar macrophage in man. Science 1976;192:1016–1018.

58. Green GM, Kass EH. The role of the alveolar macrophage in the clearance of bacteria from the lung. J Exp Med 1964; 119:167–176.

59. Jonsson S, Musher, DM, Chapman A, Goree A, Lawrence EC. Phagocytosis and killing of common bacterial pathogens of the lung by human alveolar macrophages. J Infect Dis 1985;152:4–13.

60. Dockrell DH, Marriott HM, Prince LR, et al. Alveolar macrophage apoptosis contributes to pneumococcal clearance in a resolving model of pulmonary infection. J Immunol 2003;171:5380–5388.

61. Broug-Holub E, Toews GB, van Iwaarden JF, et al. Alveolar macrophages are required for protective pulmonary defenses in murine Klebsiella pneumonia: elimination of alveolar macrophages increases neutrophil recruitment but decreases bacterial clearance and survival. Infect Immun 1997;65:1139–1146.

62. Leemans JC, Juffermans NP, Florquin S, et al. Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. J Immunol 2001;166:4604–4611.

63. Shao X, Mednick A, Alvarez M, van Rooijen N, Casadevall A, Goldman DL. An innate immune system cell is a major determinant of species-related susceptibility differences to fungal pneumonia. J Immunol 2005;175:3244–3251.

64. Janeway CA, Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002;20:197–216.

65. Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. Annu Rev Immunol 2005;23:901–944.

66. Krieger M, Acton S, Ashkenas J, Pearson A, Penman M, Resnick D. Molecular flypaper, host defense, and atherosclerosis. Structure, binding properties, and functions of macrophage scavenger receptors. J Biol Chem 1993;268:4569–4572.

67. Palecanda A, Kobzik L. Receptors for unopsonized particles: the role of alveolar macrophage scavenger receptors. Curr Mol Med 2001;1:589–595.

68. Herre J, Gordon S, Brown GD. Dectin-1 and its role in the recognition of beta-glucans by macrophages. Mol Immunol 2004;40:869–876.

69. Zhang J, Zhu J, Imrich A, Cushion M, Kinane TB, Koziel H. Pneumocystis activates human alveolar macrophage NF-kappaB signaling through mannose receptors. Infect Immun 2004;72:3147–3160.

70. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4:499–511.

71. Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol 2001;1:135–145.

72. Swanson JA, Hoppe AD. The coordination of signaling during Fc receptor-mediated phagocytosis. J Leukoc Biol 2004;76:1093–1103.

73. Stuart LM, Ezekowitz RA. Phagocytosis: elegant complexity. Immunity 2005;22:539–550.

74. Iles KE, Forman HJ. Macrophage signaling and respiratory burst. Immunol Res 2002;26:95–105.

75. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci USA 2000; 97:8841–8848.

76. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol 2004;2:820–832.

77. Kobzik L, Bredt DS, Lowenstein CJ, et al. Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localization. Am J Respir Cell Mol Biol 1993; 9:371–377.

78. van Straaten JF, Postma DS, Coers W, Noordhoek JA, Kauffman HF, Timens W. Macrophages in lung tissue from patients with pulmonary emphysema express both inducible and endothelial nitric oxide synthase. Mod Pathol 1998;11:648–655.

79. Nicholson S, Bonecini-Almeida M da G, Lapa e Silva JR, et al. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. J Exp Med 1996;183:2293–2302.

80. Weinberg JB. Nitric oxide production and nitric oxide synthase type 2 expression by human mononuclear phagocytes: a review. Mol Med 1998;4:557–591.

81. Fang FC, Vazquez-Torres A. Nitric oxide production by human macrophages: there's NO doubt about it. Am J Physiol Lung Cell Mol Physiol 2002;282:L941–943.

82. Peters-Golden M, Canetti C, Mancuso P, Coffey MJ. Leukotrienes: underappreciated mediators of innate immune responses. J Immunol 2005;174:589–594.

83. Zhang P, Summer WR, Bagby GJ, Nelson S. Innate immunity and pulmonary host defense. Immunol Rev 2000;173:39–51.

84. Pier GB. Role of the cystic fibrosis transmembrane conductance regulator in innate immunity to Pseudomonas aeruginosa infections. Proc Natl Acad Sci USA 2000;97:8822–8828.

85. Diamond G, Legarda D, Ryan LK. The innate immune response of the respiratory epithelium. Immunol Rev 2000;173:27–38.

86. Elias JA, Zheng T, Lee CG, et al. Transgenic modeling of interleukin-13 in the lung. Chest 2003;123:339S–345S.

87. van der Weyden L, Adams DJ, Bradley A. Tools for targeted manipulation of the mouse genome. Physiol Genomics 2002;11:133–164.

88. Sledz CA, Williams BR. RNA interference in biology and disease. Blood 2005;106:787–794.

89. Segal AW. How neutrophils kill microbes. Annu Rev Immunol 2005;23:197–223.

90. Rosenzweig SD, Holland SM. Phagocyte immunodeficiencies and their infections. J Allergy Clin Immunol 2004;113:620–626.

91. Abraham SN, Thankavel K, Malaviya R. Mast cells as modulators of host defense in the lung. Front Biosci 1997;2:d78–87.

92. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nat Immunol 2005;6:135–142.

93. Marshall JS, Jawdat DM. Mast cells in innate immunity. J Allergy Clin Immunol 2004;114:21–27.

94. French AR, Yokoyama WM. Natural killer cells and viral infections. Curr Opin Immunol 2003;15:45–51.

95. Yokoyama WM, Kim S, French AR. The dynamic life of natural killer cells. Annu Rev Immunol 2004;22:405–429.

96. Vermaelen K, Pauwels R. Pulmonary dendritic cells. Am J Respir Crit Care Med 2005;172:530–551.

97. Lambrecht BN, Hammad H. Taking our breath away: dendritic cells in the pathogenesis of asthma. Nat Rev Immunol 2003;3:994–1003.

98. Tuomanen EI, Austrian R, Masure HR. Pathogenesis of pneumococcal infection. N Engl J Med 1995;332:1280–1284.

99. Doherty PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. Effector CD4+ and CD8+ T-cell mechanisms in the control of respiratory virus infections. Immunol Rev 1997;159:105–117.

100. Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. Scand J Immunol 2004;59:1–15.

101. Openshaw PJ, Tregoning JS. Immune responses and disease enhancement during respiratory syncytial virus infection. Clin Microbiol Rev 2005;18:541–555.

102. Buckley RH. Pulmonary complications of primary immunodeficiencies. Paediatr Respir Rev 2004;5(suppl A): S225–233.

103. Kotloff RM, Ahya VN, Crawford SW. Pulmonary complications of solid organ and hematopoietic stem cell transplantation. Am J Respir Crit Care Med 2004;170: 22–48.

104. Boyton RJ. Infectious lung complications in patients with HIV/AIDS. Curr Opin Pulm Med 2005;11:203–207.

105. Whitney CG, Harper SA. Lower respiratory tract infections: prevention using vaccines. Infect Dis Clin North Am 2004;18:899–917.

106. Williams GT, Williams WJ. Granulomatous inflammation—a review. J Clin Pathol 1983;36:723–733.

107. Perez RL, Rivera-Marrero CA, Roman J. Pulmonary granulomatous inflammation: From sarcoidosis to tuberculosis. Semin Respir Infect 2003;18:23–32.

108. Mohr LC. Hypersensitivity pneumonitis. Curr Opin Pulm Med 2004;10:401–411.

109. Sawyer RT, Maier LA, Kittle LA, Newman LS. Chronic beryllium disease: a model interaction between innate and acquired immunity. Int Immunopharmacol 2002;2:249–261.

110. Vignery A. Macrophage fusion: the making of osteoclasts and giant cells. J Exp Med 2005;202:337–340.

111. Saginario C, Sterling H, Beckers C, et al. MFR, a putative receptor mediating the fusion of macrophages. Mol Cell Biol 1998;18:6213–6223.

112. Han X, Sterling H, Chen Y, et al. CD47, a ligand for the macrophage fusion receptor, participates in macrophage multinucleation. J Biol Chem 2000;275:37984–37992.

113. Yagi M, Miyamoto T, Sawatani Y, et al. DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. J Exp Med 2005;202:345–351.

114. Kaufmann SH. Protection against tuberculosis: cytokines, T cells, and macrophages. Ann Rheum Dis 2002;61(suppl 2):ii54–58.

115. Co DO, Hogan LH, Il-Kim S, Sandor M. T cell contributions to the different phases of granuloma formation. Immunol Lett 2004;92:135–142.

116. Mogues T, Goodrich ME, Ryan L, LaCourse R, North RJ. The relative importance of T cell subsets in immunity and immunopathology of airborne Mycobacterium tuberculosis infection in mice. J Exp Med 2001;193:271–280.

117. Barnes PF, Bloch AB, Davidson PT, Snider DE, Jr. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med 1991;324:1644–1650.

118. Ehrt S, Schnappinger D, Bekiranov S, et al. Reprogramming of the macrophage transcriptome in response to interferon-gamma and Mycobacterium tuberculosis: signaling roles of nitric oxide synthase-2 and phagocyte oxidase. J Exp Med 2001;194:1123–1140.

119. Nathan CF, Prendergast TJ, Wiebe ME, et al. Activation of human macrophages. Comparison of other cytokines with interferon-gamma. J Exp Med 1984;160:600–605.

120. Newport MJ, Huxley CM, Huston S, et al. A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection. N Engl J Med 1996;335:1941–1949.

121. Turner OC, Basaraba RJ, Orme IM. Immunopathogenesis of pulmonary granulomas in the guinea pig after infection with Mycobacterium tuberculosis. Infect Immun 2003;71: 864–871.

122. Opie E, Aronson J. Tubercle bacilli in latent tuberculosis lesions and in lung tissue without tuberculous lesions. Arch Pathol 1927;4:1–21.

123. Lukacs NW, Chensue SW, Strieter RM, Warmington K, Kunkel SL. Inflammatory granuloma formation is mediated by TNF-alpha-inducible intercellular adhesion molecule-1. J Immunol 1994;152:5883–5889.

124. Marshall BG, Wangoo A, Cook HT, Shaw RJ. Increased inflammatory cytokines and new collagen formation in cutaneous tuberculosis and sarcoidosis. Thorax 1996;51: 1253–1261.

125. Dheda K, Booth H, Huggett JF, Johnson MA, Zumla A, Rook GA. Lung remodeling in pulmonary tuberculosis. J Infect Dis 2005;192:1201–1209.

126. Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. J Immunol 2000;164:2585–2591.

127. Rhoades ER, Ullrich HJ. How to establish a lasting relationship with your host: lessons learned from Mycobacterium spp. Immunol Cell Biol 2000;78:301–310.

128. Flynn JL, Chan J. Immune evasion by Mycobacterium tuberculosis: living with the enemy. Curr Opin Immunol 2003;15:450–455.

129. Zhang P, Bagby GJ, Happel KI, Summer WR, Nelson S. Pulmonary host defenses and alcohol. Front Biosci 2002;7: d1314–1330.

130. Drannik AG, Pouladi MA, Robbins CS, Goncharova SI, Kianpour S, Stampfli MR. Impact of cigarette smoke on clearance and inflammation after Pseudomonas aeruginosa infection. Am J Respir Crit Care Med 2004;170: 1164–1171.