Murray & Nadel's Textbook of Respiratory Medicine

Murray & Nadel's Textbook of Respiratory Medicine

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We dedicate this textbook to Dr. Julius H. Comroe, Jr., who was our mentor during the formative years of our professional development. Dr. Comroe was one of the truly great academicians of his generation. He was an investigator of exceptional merit, an educator whose influence was worldwide, and a medical statesman of exemplary integrity and vision. In dedicating this book, we acknowledge especially Dr. Comroe's scholarly contributions and his commitment to the importance of basic science in the solution of clinical problems.

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Preface to the Sixth Edition

In this *Preface* to the *Sixth Edition of Murray & Nadel's Textbook of Respiratory Medicine*, the Editors are pleased to highlight the new features that enhance the readability and educational value of the book. Whereas advances in both the Fourth and Fifth Editions increasingly incorporated online resources, with the Sixth Edition, the textbook has become truly digital.

The Expert Consult eBook version of the Sixth Edition now provides easier navigation, more thorough and precise searching and retrieval capabilities, and extensive resource material. Via the eBook, readers will have access to nearly 200 videos and audio files and more than 600 new eFigures; there are also extensive cross references to other figures and videos throughout the book. Whereas Key Readings are listed for each chapter in both hardcover and electronic versions, the eBook contains the entire, extensive bibliography where the reader can access each reference by clicking the in-text citation, thereby opening the abstract and accessing direct links to PubMed. The eBook contains new and revised multiple choice questions from each chapter as rich sources of educational challenge and, using the eBook, readers will be able to take notes and highlight important content for later reference. Importantly, the Expert Consult eBook will feature updates, making it a living textbook.

New chapters have been created, former chapters divided, and still others consolidated; in all, the number of chapters has increased from 95 in the Fifth Edition to 106 in the Sixth, as a reflection of the growth in knowledge of scientific and clinical aspects of respiratory health and disease. For example, the chapters on asthma and COPD have both been split: each of these major pulmonary diseases now has one chapter encompassing its molecular phenotypes and pathogenesis and another chapter outlining diagnosis and management. In addition, a chapter on the genetics of asthma and COPD has been added. The section on sleep has been expanded from one to four chapters and sections on pleural disease and fungal disease have also been expanded. New chapters have been added on positron emission tomography, therapeutic bronchoscopy, interventional radiology, bronchiolitis, pulmonary hypertension due to lung disease, non-invasive ventilation, and extra-corporeal membrane oxygenation.

Two new positions have been created: an Editor-in-Chief, who has orchestrated this complex project, and an Editor of Thoracic Imaging, who has edited all clinical images and added hundreds more to the publication. Of the total of 227 authors, 44% are first-time authors to Murray & Nadel and more than 25% hold academic positions outside the United States.

As the partnership between the scientific and clinical applications of respiration has grown and evolved since 1988—when the *Textbook of Respiratory Medicine* was initially published—two guiding axioms have reinforced every edition: first, our staunch belief in the benefit of integrating basic science with the practice of respiratory medicine and, second, the value of having an extensive and inclusive bibliography of classic works and current relevant articles.

Technical advances in publishing have led to extraordinary improvements in how information is gathered, packaged, and displayed for optimum educational benefit. We want to congratulate our publisher, Elsevier, for ensuring that these opportunities were fully realized; moreover, we wish to compliment the entire, talented publishing staff that contributed to this Sixth Edition. Particular thanks go to Jennifer Shreiner, Senior Content Development Editor, who shepherded the project from beginning to end; to Helene Caprari, Content Strategist, for guiding the book through its various stages of production; and to Mary Pohlman, Senior Project Manager, for her proofing and copyediting prowess. Finally, we acclaim the superb work of all the authors and various contributors for bringing this textbook to life, in both hard copy and in its newest digital form.

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Preface to the First Edition

The rapid growth of knowledge of basic scientific principles and their application to respiratory medicine has resulted in a proliferation of monographs and texts dealing with selected aspects of pulmonary science and clinical medicine, but no single work has provided a comprehensive description of all that is currently known. The *Textbook of Respiratory Medicine* is an attempt to provide a well-balanced, authoritative, and fully documented book that integrates scientific principles with the practice of respiratory medicine. The text is sufficiently detailed and referenced to serve as the definitive source for interested students, house officers, and practitioners, both pulmonary specialists and generalists. It is written by leading experts, to guarantee that the material is authoritative and contemporary.

To deal with such an enormous amount of material, we have divided the book into three major sections. This organization should help guide interested readers from the intricacies of basic science to their application at the bedside. We begin in Part I with Scientific Principles of Respiratory Medicine. As implied, this is where the reader will find detailed information about the anatomy and development of the respiratory tract, respiratory physiology, pharmacology and pathology, and defense mechanisms and immunology. A strong foundation in these basic sciences will make possible a rational and scientific approach to the more specialized clinical material included in the subsequent sections. Part II, Manifestations and Diagnosis of Respiratory Disease, contains four chapters on the cardinal signs and symptoms of respiratory disorders and ten chapters on diagnostic evaluation, ranging from the history and physical examination to the newest and most sophisticated imaging, applied physiologic, and invasive techniques. Discrete clinical disorders are included in Part III, Clinical Respiratory Medicine. There are sections on Infectious Diseases, Obstructive Diseases, Neoplasms, Disorders of the Pulmonary Circulation, Infiltrative and Interstitial Diseases, Environmental and Occupational Disorders, Disorders of the Pleura, Disorders of the Mediastinum, Disorders in the Control of Breathing, Respiratory Manifestations of Extrapulmonary Disorders, and Respiratory Failure. All but one of the sections dealing with a generic clinical problem begin with a chapter entitled "General Principles and Diagnostic Approach." New challenges to adult respiratory medicine have sprung up, and these are reflected in chapters on subjects such as cystic fibrosis (previously a disease only of childhood!), environmental and occupational diseases, disorders of breathing, and respiratory problems associated with unusual atmospheres (high altitude, diving). The book ends with a novel and important section on Prevention and Control.

Putting together a *Textbook* of this scope and magnitude is no easy task and involves making certain decisions that all readers may not agree with. For example, while trying to keep the length of the book as manageable as possible, we decided to permit some overlap of content. Thus readers will find bronchodilators discussed in the chapter on airway pharmacology and again in the pertinent chapters on obstructive airway diseases. We have also welcomed differences of opinion among authors, provided the issues were clearly stated and the reasons for the author's position documented.

Our struggles were not as arduous as they might have been because we have had considerable help from many sources. First of all was the help from the 95 authors, who worked long and hard on their various contributions. The two editors worked in San Francisco, where they had the benefit of expert secretarial support from Ms. Dorothy Ladd and Mrs. Beth Cost. Special acknowledgment goes to Ms. Aja Lipavsky who, as editorial assistant, handled correspondence, proofing, permissions, and innumerable other details, and prepared the index. At W.B. Saunders in Philadelphia, the book was the brainchild of then-president John Hanley and was published with the guidance of J. Dereck Jeffers, William Lamsback, and the new president Lewis Reines. Production was supervised by Evelyn Weiman.

The long gestation of this book is over, parturition is near, and it will soon begin a life of its own. Like all expectant parents, we are concerned about how our offspring will make its way in the real world. We hope people will like it and find it useful.

John F. Murray, MD

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Videos

Murray & Nadel's Textbook of Respiratory Medicine

SECTION A

ANATOMY AND DEVELOPMENT OF THE RESPIRATORY TRACT

1

ANATOMY OF THE LUNGS

KURT H. ALBERTINE, PhD

INTRODUCTION

The lung has two essential, interdependent functions. One function is ventilation-perfusion matching to deliver oxygen to the body and to remove carbon dioxide that is produced by the body (Fig. 1-1). The second function is host defense against the onslaught of airborne pathogens, chemicals, and particulates. These essential functions are emphasized through the gross, subgross, histologic, and ultrastructural determinants of respiratory gas exchange in the normal human lung. Secondary functions of the lung also are important, such as surfactant synthesis, secretion, and recycling; mucociliary clearance; neuroendocrine signaling; and synthesis and secretion of a myriad of molecules by its epithelial and endothelial cells. The diversity of secondary functions emphasizes the importance of the lung in homeostasis. The chapter finishes with comparison of the lung of mice and humans, an important subject given the widespread use of murine models in lung research. Videos 1-1 to 1-5 provide views of lung movements related to changes in tidal volume, airway pressures, and respiratory rate.

GROSS AND SUBGROSS ORGANIZATION

The position of the lungs in the chest and in relationship to the heart is shown in Fig. 1-2. Figure 1-2A shows a midfrontal section through the thorax of a frozen human cadaver. Figure 1-2B shows a posterior-anterior chest radiograph of a normal human at *functional residual capacity* (FRC). The two illustrations represent the extremes of the approaches to lung anatomy. The cadaver lung (see Fig. 1-2A) shows the gross anatomic arrangements and relationships. The main distortion is that the lungs are at low volume. The vertical height of the lungs is only approximately 18 cm, which is well below that at FRC (see Fig. 1-2B). The diaphragm is quite elevated in Figure 1-2A, and is approximately 5 cm higher than its end-expiratory position in life. Another distortion is the abnormally wide pleural space; however, this fixation shrinkage artifact serves as a useful reminder that the lung is not normally attached to the chest wall. In life the separation between the parietal and visceral pleuras is only several micrometers.[1,2] The chest radiograph (see Fig. 1-2B) shows that the vertical height of the lung at FRC is approximately 24 cm, with the level of the bifurcation of the pulmonary artery approximately halfway up the lungs. The diaphragm is lower and flatter than in the cadaver.

In life the human lungs weigh 900 to 1000 g, of which nearly 40% to 50% is blood.[3,4] At end-expiration, the gas volume is approximately 2.5 L whereas, at maximal inspiration, it may be 6 L. Thus overall lung density varies from 0.30 g/mL at FRC to 0.14 g/mL at total lung capacity. But the density of the lung is not distributed uniformly, being approximately 1 g/mL near the hilum and 0.1 g/mL peripherally. If one likens each lung to a half cylinder, more than

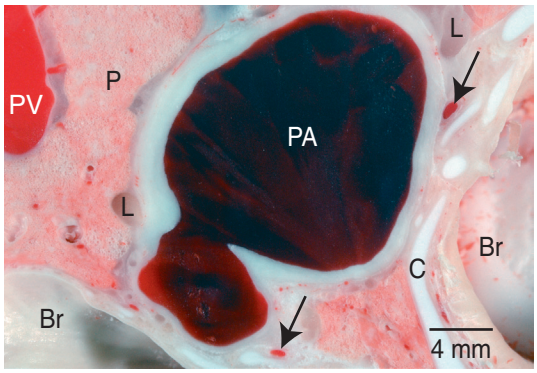

Figure 1-1 Frozen block of lung tissue. Air is brought into the lung via the bronchus (Br) outside of which is a plate of cartilage (C). Pulmonary arterial (PA) blood is dark purple because it is poorly oxygenated. Gas exchange across the lung's parenchyma (P) results in oxygenated pulmonary venous (PV) blood, which is crimson. Also present in the peribronchovascular connective tissue are bronchial arteries (*arrows*) and lymphatics (L). (Frozen sheep lung, unstained.)

50% of all the lung's alveoli are located in the outer 30% of the lung radius (hilum to chest wall). This is why the peripheral portion of the lung appears relatively empty in the chest radiograph (see Fig. 1-2). Variability in density also exists from top to bottom. In Figure 1-2 the blood vessels are more distended in the lower lung fields. The increasing distention of vessels from apex to base also illustrates the increase in vascular distending pressures at the rate of 1 cm H_2O/cm height down the lung.

The disposition of the various tissues that constitute the lung is summarized in Table 1-1. An amazing point is how little tissue is involved in the architecture of the alveolar walls.[5,6] But this is as it should be because the major physical problem of gas exchange is the slowness of oxygen diffusion through water.[7,8] Thus the alveolar walls must be extremely thin. In fact, the thickness of the red blood cell forms a substantial portion of the air-blood diffusion pathway. Advantage was taken of this fact to separate the carbon monoxide diffusing capacity measurement into two components: the capillary blood volume and the membrane diffusing capacity.[9] (For a discussion of diffusing capacity, see Chapters 4 and 25.)

The lung has two well-defined interstitial connective tissue compartments arranged in series, as described by Hayek[10] (Fig. 1-3). These are the parenchymal (alveolar wall) interstitium and the loose-binding (extra-alveolar) connective tissue (peribronchovascular sheaths, interlobular septa, and visceral pleura). The connective tissue fibrils (collagen, elastin, and reticulin) form a three-dimensional basket-like structure around the alveoli and airways (Fig. 1-4).[11] This basket-like arrangement allows the lung to expand in all directions without developing excessive tissue recoil. Because the connective tissue fibrils in the parenchymal interstitium are extensions of the coarser fibers in the loose-binding connective tissue, stresses imposed at the alveolar wall level during lung inflation are transmitted not only to adjacent alveoli, which abut each other, but also to surrounding alveolar ducts and bronchioles, and then to the loose-binding connective tissue supporting the whole lobule, and ultimately to the visceral pleural surface (see Fig. 1-3). These relations become more apparent in certain pathologic conditions. For example, in interstitial emphysema,[12] air enters the loose-binding connective tissue and dissects along the peribronchovascular sheaths to the hilum and along the lobular septa to the visceral pleura. Interstitial pulmonary edema liquid enters and moves along the same interstitial pathways (Fig. 1-5).[13]

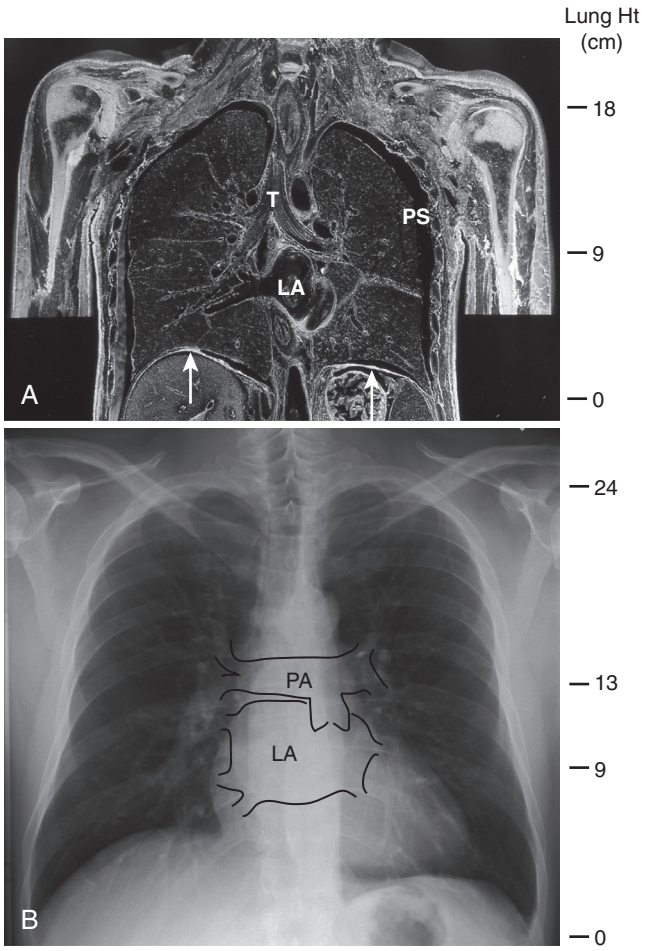

Figure 1-2 Comparison views of lung position in the chest and relationship to the heart. A, Midfrontal section through the thorax of a frozen cadaver of a 35-year-old human. The cadaver was prepared by routine embalming procedures, stored horizontally for 3 months in 30% alcohol, and frozen in the horizontal position for 1 week at –20° C. Frontal sections were cut with a band saw. Because the cadaver was preserved in the horizontal position, the weight of the abdominal organs compressed the contents of the thoracic cavity. The domes of the diaphragm (*arrows*) are elevated approximately 5 cm relative to their end-expiratory position in life. Pleural space (PS) width is artifactually enlarged; normally, in life, it is several micrometers in width. The trachea (T) is flanked on its left by the aortic arch and on its right by the azygos vein. The left pulmonary artery lies on the superior aspect of the left main-stem bronchus. Pulmonary veins from the right lung enter the left atrium (LA), which is located approximately 7 cm above the lung's base. These structures at the root of the lungs caused the esophagus to be cut twice as it follows a curved path behind them to reach the stomach. **B,** Chest radiograph of a normal human adult taken in the upright position at functional residual capacity. The lung height (cm) was measured from the costodiaphragmatic angle to the tubercle of the first rib. The main pulmonary artery (PA) and left atrium (LA) are outlined. The vascular structures, especially the pulmonary veins, are more easily seen near the bottom of the lung. This is partly because vascular distending pressures are greater near the bottom. The density of the lung is also graded, being higher at the bottom than the top and higher near the hilum than peripherally. (**A,** Reprinted with permission from Koritké JG, Sick H: *Atlas of sectional human anatomy*. Vol 1: Head, neck, thorax. Baltimore, 1988, Urban and Schwarzenberg, FT3a, p 83.)

Table 1-1 Components of Normal Human Lung

Component	Volume or Mass (mL)	Thickness (μm)	Reference No.
Gas	2400		8
Tissue	900		3, 4
Blood	400		4
Lung	500		8
Support structures	225		5
Alveolar walls	275		5, 6
Epithelium	60	0.18	5, 6
Endothelium	50	0.10	5, 6
Interstitium	110	0.22	5, 6
Alveolar macrophages	55		6

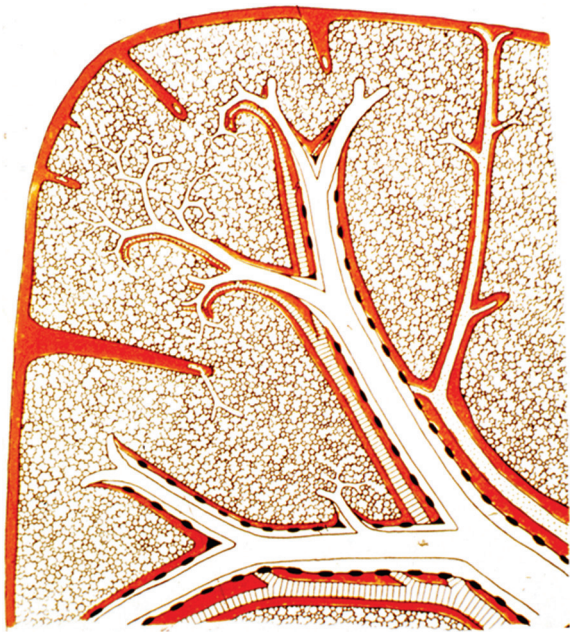

Figure 1-3 General plan depicting the interstitial connective tissue compartments of the lung. All of the support structures (airways, blood vessels, interlobular septa, visceral pleura) are subsumed under the loose-binding connective tissue. The alveolar walls' interstitium comprises the parenchymal interstitium. This organizational plan of the lung follows the general organization of all organs. (Reproduced with permission from Hayek H: *The human lung*, New York, Hafner, 1960, pp 298–314.)

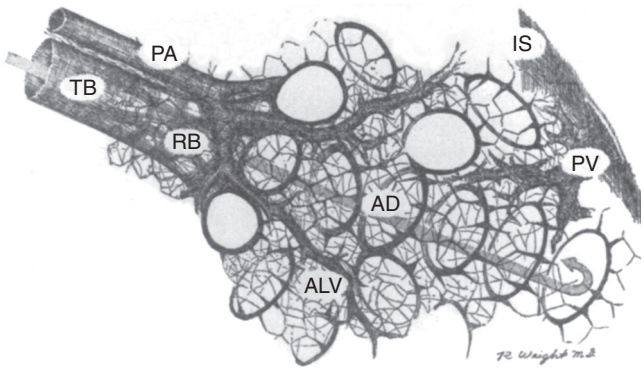

Figure 1-4 A drawing of the connective tissue support of the normal human adult lung lobule demonstrates the weave of fibers composing the "elastic continuum." AD, alveolar duct; ALV, alveolus; IS, interstitial space; PA, pulmonary artery; PV, pulmonary vein; RB, respiratory bronchiole; TB, terminal bronchiole. (Reprinted with permission from Wright RR: Elastic tissue of normal and emphysematous lungs. *Am J Pathol* 39:355–367, 1961.)

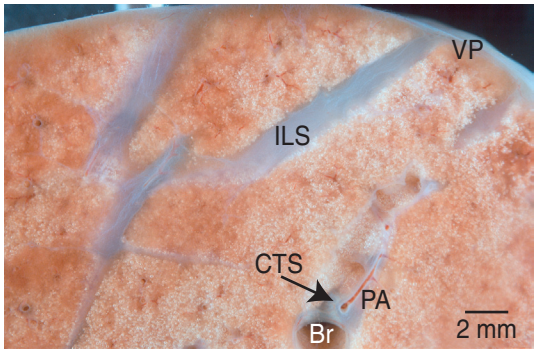

Figure 1-5 Interstitial pulmonary edema demonstrating the loose-binding (peribronchovascular) connective tissue spaces (CTS) that surround the bronchi (Br) and pulmonary arteries (PA). Interstitial edema also expanded the interlobular septa (ILS) that are contiguous with the connective tissue of the visceral pleura (VP). (Frozen sheep lung, unstained.)

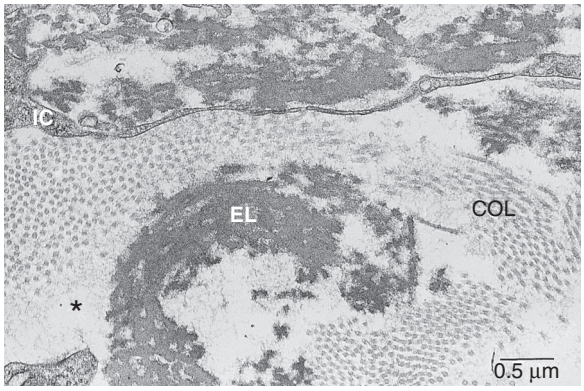

Figure 1-6 The interstitium. The connective tissue compartment of the lung contains interstitial cells (IC), fibrils of collagen (COL), and bundles of elastin (EL). The bulk of the interstitium, however, is occupied by matrix constituents (*) such as glycosaminoglycans. (Human lung surgical specimen, transmission electron microscopy.)

The bulk of the interstitium is occupied by a matrix of proteoglycans (Fig. 1-6).[14,15] Proteoglycans constitute a complex group of gigantic polysaccharide molecules (≈30 different core proteins, with great diversity of glycosaminoglycan side chains) whose entanglements impart a gel-like structure to the interstitium. That structural role, although essential, is not the sole role of these important molecules. A growing view is emerging of the lung's extracellular matrix components as regulators of lung physiology, helping in determining epithelial cell phenotype; binding of and subsequent signaling by cytokines, chemokines, and growth factors; and mediating cell proliferation, migration, differentiation, and apoptosis.[16-23] In disease states, degradation products of extracellular matrix components may activate the Toll-like receptor pathways (see later discussion); thus the degradation products may serve as endogenous sentinels of tissue damage and initiators of innate immune responses.[18,22-24] Within this gel-like interstitium reside several varieties of interstitial cells (contractile and noncontractile interstitial cells,[25,26] mast cells, plasma cells, and occasional leukocytes). The remainder of the interstitium is

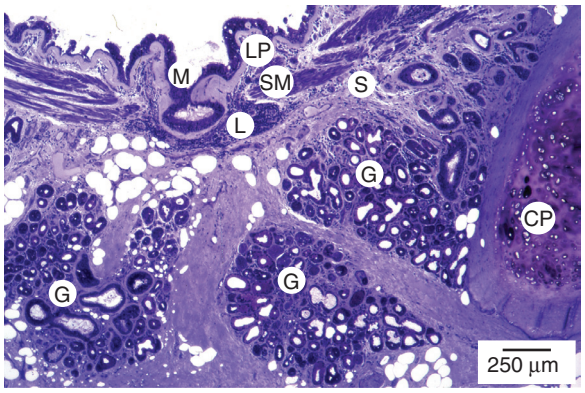

Figure 1-7 A bronchus. The bronchial wall is composed of mucosa (M), lamina propria (LP), smooth muscle (SM), and submucosa (S). Seromucous glands (G) are located between the spiral bands of smooth muscle and cartilaginous plates (CP). Diffuse lymphoid tissue (L) has infiltrated the lamina propria and submucosa. (Human lung surgical specimen, right middle lobar bronchus, 2-µm-thick glycol methacrylate section, light microscopy.)

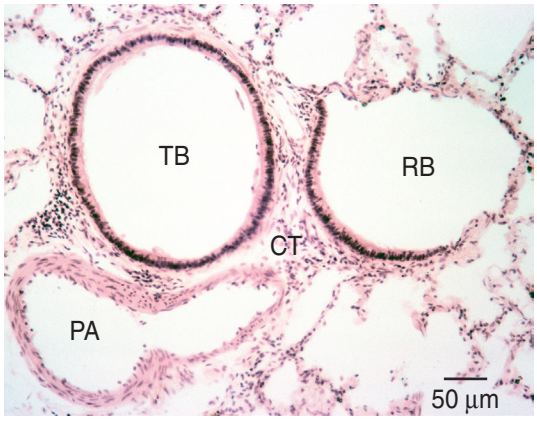

Figure 1-8 A terminal bronchiole and respiratory bronchiole. The wall of the terminal bronchiole (TB) is constructed of a single layer of ciliated cuboidal epithelium that rests over thin, discontinuous bands of smooth muscle and loose areolar connective tissue (CT). In contrast, the wall of the respiratory bronchiole (RB) is only partially lined by ciliated cuboidal epithelium (lower left side). The remainder of its wall is lined by squamous epithelium (upper right side). The connective tissue also surrounds the adjacent pulmonary arteriole (PA). (Human lung surgical specimen, 10-µm-thick paraffin section, light microscopy.)

composed of laminin, collagens, elastin and reticulin fibrils, fibronectin, and tenascin (see Fig. 1-6).

AIRWAYS

The airways, forming the connection between the outside world and the terminal respiratory units, are of central importance to our understanding of lung function in health and disease. Intrapulmonary airways are divided into three major groups: *bronchi* (Fig. 1-7), *bronchioles* (including the terminal bronchioles) (Fig. 1-8), and *respiratory bronchioles* (Fig. 1-9; see Fig. 1-8). By definition, bronchi have cartilage in their wall, whereas bronchioles do not. Respiratory bronchioles serve a dual function as airways and as part of the alveolar volume (gas exchange).

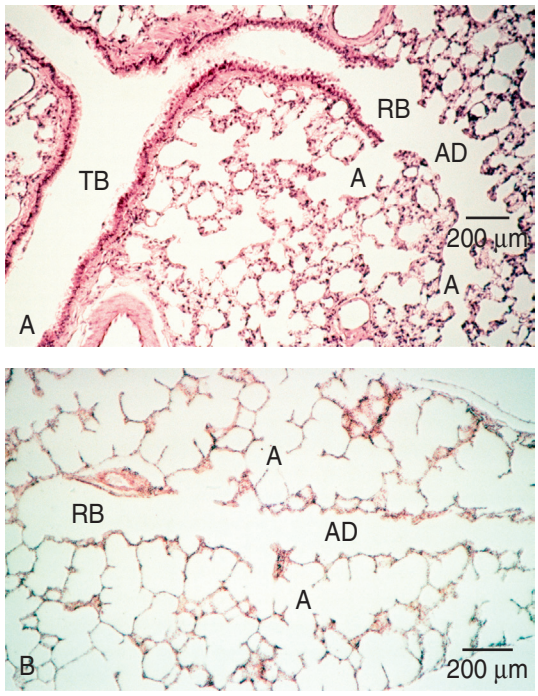

Figure 1-9 Longitudinal sections along bronchioles. A, Diameter remains relatively constant along the terminal bronchiole (TB), respiratory bronchiole (RB), and alveolar duct (AD). Alveoli (A) communicate with the gas-exchange ducts (RB and AD). **B,** This longitudinal section along a respiratory bronchiole (RB) and alveolar duct (AD) also shows that their diameter is relatively constant and that both gas-exchange ducts communicate with clusters of alveoli (A). (Human lung surgical specimens, 10-µm-thick paraffin section, light microscopy.)

The anatomic dead space, as measured by the single-breath nitrogen dilution technique, is approximately 30% of each tidal volume. Anatomically this dead space is accounted for principally by the volume of the extrapulmonary (upper) airway, including the nasopharynx and trachea, and the intrapulmonary bronchi.[27] The trachea and bronchi are cartilaginous, do not change shape significantly with ventilation, and do not participate in gas exchange. Bronchioles, approximately 1 mm in diameter or less, have no cartilage and are exceedingly numerous and short. They consist of approximately five branching generations and end at the terminal bronchioles. In contrast to the bronchi, the bronchioles are tightly embedded in the connective tissue framework of the lung and therefore enlarge passively as lung volume increases.[28] Histologically the bronchioles down to and including the terminal bronchioles ought to contribute approximately 25% to the anatomic dead space. In life, however, they contribute little because of gas-phase diffusion and mechanical mixing in the distal airways resulting from the cardiac impulse. By definition, the respiratory bronchioles and alveolar ducts participate in gas exchange and thus do not contribute to the anatomic dead space. The volume of the respiratory bronchiole-alveolar duct system is approximately one third of the total alveolar volume, and it is into this space that the fresh-air ventilation enters during inspiration.

Most airway resistance resides in the upper airway and bronchi. Normally the large airways maintain partial constriction. The minimal airway diameter in the human lung,

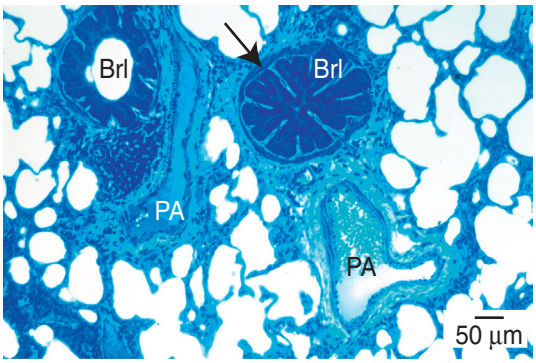

Figure 1-10 Cross sections of two bronchioles (Brl) that would contribute to increased airway resistance. On the left is a bronchiole that is partially narrowed, evident by the folded and thick epithelium. The bronchiole to the right is completely narrowed. Its lumen is obliterated by the infolded epithelium. This bronchiole's smooth muscle is thick (*arrow*), suggesting that the narrowing is related to constriction of the smooth muscle. Each bronchiole is flanked by a pulmonary arteriole (PA). (Sheep lung, 5-µm-thick paraffin section, light microscopy.)

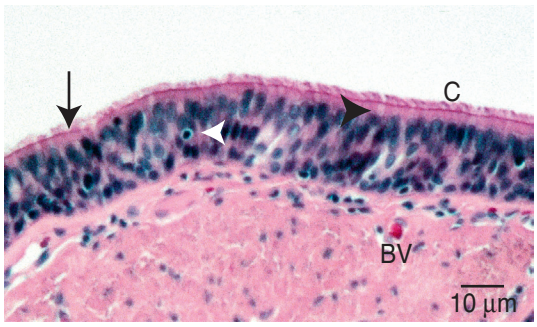

Figure 1-11 The bronchial mucosa consists of pseudostratified, columnar epithelium with cilia (C) and goblet cells (*black arrowhead*). The cilia, which form a thick carpet, move rhythmically and thereby propel liquid, mucus, cells, and debris centrally toward the pharynx. The dark band immediately beneath the cilia (*black arrow*) is produced by the basal bodies. By transmission electron microscopy, basal bodies are recognized as modified centrioles. A lymphocyte (*white arrowhead*) is intercalated among the epithelial cells. A bronchial blood vessel (BV) is located beneath the mucosal layer. (Human lung surgical specimen, 10-µm-thick paraffin section, light microscopy.)

approximately 0.5 mm, is reached at the level of the terminal bronchioles; succeeding generations of exchange ducts (respiratory bronchioles and alveolar ducts) are of constant diameter (see Fig. 1-9).[29,30] The functional significance of centralized resistance is that the terminal respiratory units (the physiologic alveoli) are regionally ventilated chiefly in proportion to their individual distensibilities (compliances) because most of their airway resistance is common. This is demonstrated normally by the finding that regional lung ventilation is dependent upon the initial volumes of the alveoli. Terminal respiratory units toward the top of the lung, which are more expanded at FRC, do not receive as great a share of the inspiratory volume as do the terminal respiratory units near the bottom of the lung.

The balance between anatomic dead space volume, for which the airway diameter ought to be as small as possible to maximize efficient alveolar ventilation (dead space–to–tidal volume ratio), and airflow resistance, for which the airway diameter ought to be as large as possible to minimize the work of breathing, requires a compromise. Normally, anatomic dead space is not maximal, nor is resistance minimal. In disease, by contrast, airways may narrow (Fig. 1-10), which increases resistance.

The cellular complexity of the airways is indicated by the nearly 50 distinct cell types found there, at least 12 of which are epithelial cells on the airway surface.[31] Nearly half of the epithelial cells in the normal human airway are ciliated at all airway generations (Fig. 1-11) down to bronchioles (Fig. 1-12).[32] Cilia move the superficial liquid lining layer (Fig. 1-13; see Fig. 1-11) continually toward the pharynx from deep within the lung. As the superficial lining liquid moves centripetally, the total perimeter of the airways decreases markedly.[5] If the lining liquid volume remained constant, the liquid layer ought to thicken but this does not happen, suggesting that much of the liquid is reabsorbed during its ascent along the airways.

The presence of apical junctional complexes between airway epithelial cells (see Fig. 1-13) has important functional implications for metabolically-regulated secretion into and absorption of electrolytes and water from the lining liquid. Apical junctional complexes consist of three

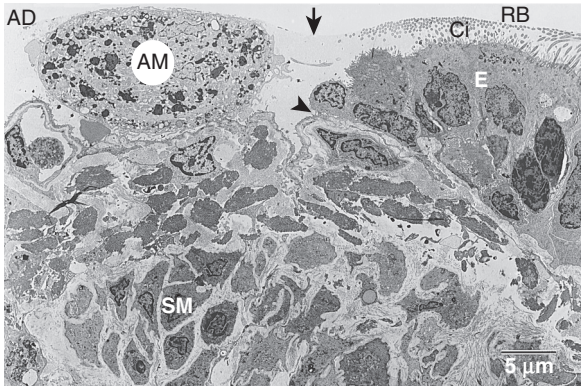

Figure 1-12 The respiratory bronchiole (RB)–alveolar duct (AD) junction is demarcated by an abrupt transition (*arrowhead*) from low cuboidal epithelial cells (E) with cilia to squamous epithelial cells. Submerged in the lining liquid (*arrow*) are an alveolar macrophage (AM) and cilia (Ci). Airway smooth muscle cells (SM) extend to this level of the airway tree. (Human lung surgical specimen, transmission electron microscopy).

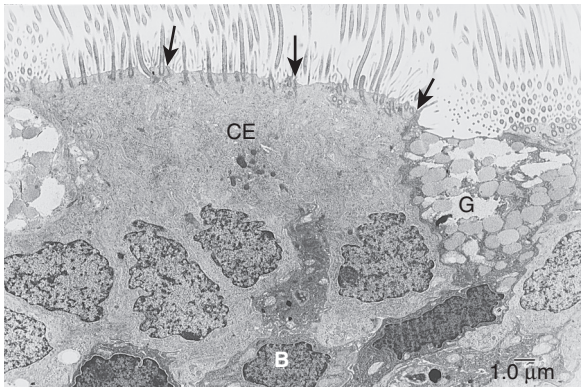

Figure 1-13 Cells constituting the bronchial epithelium are ciliated epithelial cells (CE), goblet cells (G), and basal cells (B). Goblet cells have abundant mucous granules in the cytoplasm, and their apical surface is devoid of cilia. Basal cells, as their name indicates, are located along the abluminal portion of the lining epithelium, adjacent to the basal lamina. The *arrows* at the apical surface of the airway cells indicate the location of junctional complexes between contiguous epithelial cells. (Human lung surgical specimen, transmission electron microscopy.)

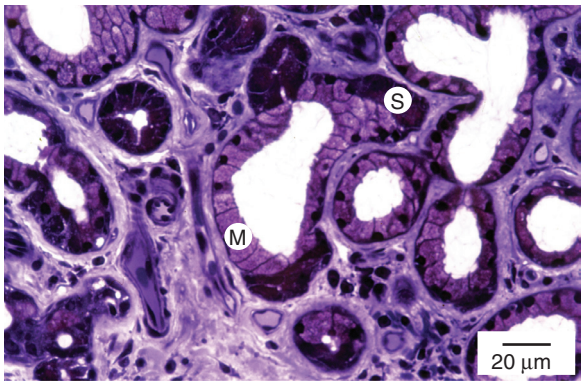

Figure 1-14 Submucosal glands shown at a higher magnification view than in Figure 1-7. These mixed, compound tubuloacinar glands contain mucus-secreting cells (M) and serous-secreting cells (S). The latter type form crescentic caps, or demilunes, over the ends of the acini. Mucus-secreting cells are the predominant glandular cell type.

elements: *zonula occludens* (tight junction), *zonula adherens*, and *macula adherens* (desmosome).[33] Tight junctions subserve two important functions: (1) restriction of passive diffusion by blocking the lateral intercellular space and (2) polarization of cellular functions (ion and water transport) between the apical and basolateral membranes.[34] Polarization of chloride and sodium transport allows the airway epithelium either to secrete or to absorb ions, with associated water movement.

Trapping of foreign material, such as particulates or bacteria, is accomplished by mucins. Mucins are complex glycoproteins that form gels, exemplified by MUC5A. MUC5A is present in the lung of humans.[35,36] Other mucins (e.g., MUC5B, MUC7)[37,38] become expressed by airway epithelial cells in diseases, such as cystic fibrosis. In that disease, MUC5B is produced by airway epithelial cells.[39] Normally MUC5B is produced by airway glandular cells,[37] but, in a variety of pulmonary diseases, its cell source is expanded.

Glands are limited to the submucosa of the bronchi. Airway glands secrete water, electrolytes, and mucins into the lumen (Fig. 1-14; see Fig. 1-7). Studies of the regulation of secretion in vivo and in vitro have shown that release can be modulated by neurotransmitters, including cholinergic, adrenergic, and peptidergic transmitters,[40,41] and by inflammatory mediators such as histamine,[42] platelet-activating factor,[43] and eicosanoids.[44] Goblet cells, which are mucin-secreting epithelial cells, also are present at most airway levels (see Fig. 1-13). Goblet cells decrease in number peripherally, normally disappearing at terminal bronchioles.[10,45] The absence of airway glands and goblet cells distal to ciliated epithelial cells makes sense because that arrangement should minimize the flow of mucus backward into alveolar ducts and alveoli.

Lymphocytes are frequently seen intercalated between airway epithelial cells (Fig. 1-15; see Fig. 1-11). These cytotoxic T lymphocytes undergo IgA class antibody responses.[46] T and B lymphocytes also accumulate in the lamina propria beneath the airway epithelium.[47]

Although most foreign material and immunologic stimuli are carried up the airways by mucociliary action, some are cleared by the lymphatics (discussed at the end of this chapter). In addition, lymphoid tissue is located in the lungs. Patches are distributed along the tracheobronchial

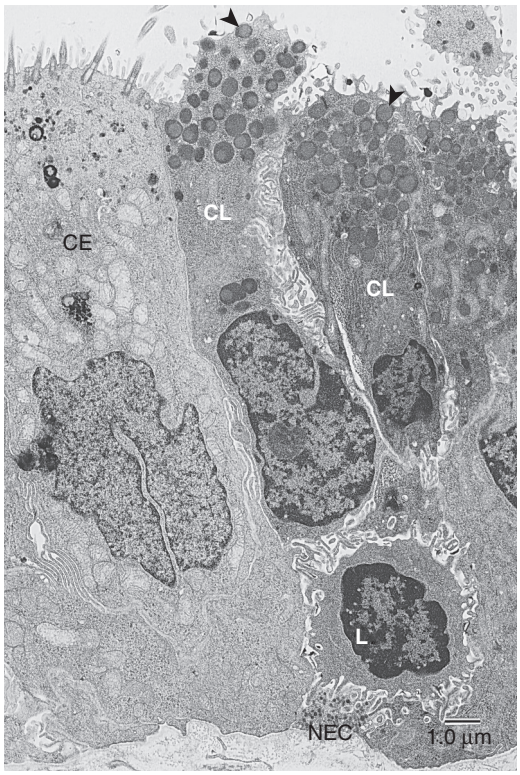

Figure 1-15 The terminal airway epithelium consists mainly of ciliated epithelium (CE) and nonciliated club cells (Clara) (CL). Club cells have the ultrastructural features of secretory cells; namely, they possess basally located rough endoplasmic reticulum, perinuclear Golgi apparatus, apically located smooth endoplasmic reticulum, and prominent membrane-bound granules (*arrowheads*). A lymphocyte (L) is intercalated among the epithelial cells. A small portion of a neuroendocrine cell (NEC) containing characteristic dense-cored vesicles is also visible at the base of the epithelial cells. (Human lung surgical specimen, transmission electron microscopy.)

tree (see Fig. 1-7) and, to a lesser extent, along the blood vessels.[48,49] These patches apparently develop in response to antigenic stimulation because they are not present at birth in humans or in germ-free animals.[48,49] Lymphocytes in these aggregates are principally B cells that express mainly IgA immunoglobulins.[47] The presence of lymphocytes along the airways provides a reminder that the respiratory system is constantly challenged by airborne immunologic stimuli. The tracheobronchial lymphoid tissue, including bronchus-associated lymphoid tissue, appears to provide an important locus for both antibody-mediated and cell-mediated immune responses. Another important locus of immune response is provided by the epithelial cells that line the airways and constitute the airway glands. Their importance stems from production of Toll-like receptors, whose role is identification of pathogen-associated molecular patterns.[50] Activation of Toll-like receptors leads to downstream signaling cascades that are involved in mucin production, leukocyte recruitment, antimicrobial peptide production, wound repair, and vascular formation.[51-55]

Some of the other cells associated with the airways are smooth muscle cells, mast cells, basal cells, and club cells (Clara). Smooth muscle cells form circular bands around the airway epithelium as far peripherally as the respiratory bronchioles (see Figs. 1-7 and 1-8). Smooth muscle tone is

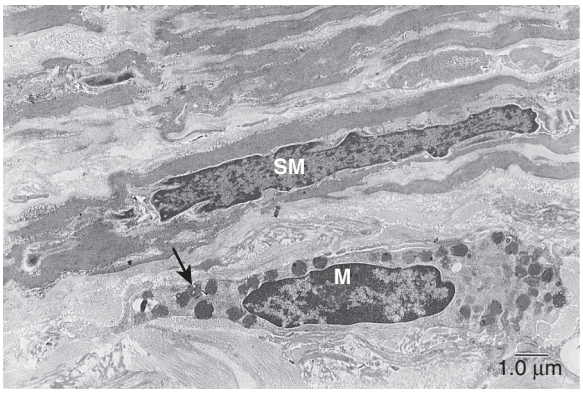

Figure 1-16 Mast cell (M) located adjacent to an airway. The mast cell flanks airway smooth muscle cells (SM). Granules in mast cells have heterogeneous morphologic characteristics, including whorled and scrolled contents (*arrow*). (Human lung surgical specimen, transmission electron microscopy.)

altered by the autonomic nervous system and by mediators released from mast cells, inflammatory cells, and neuroendocrine cells. During normal breathing, slight tonic contraction of small airway smooth muscle cells and reflex contraction of the larger airways stiffens them against external compression, as may result from forced expiration or coughing. The effector of these responses is the parasympathetic limb of the autonomic nervous system (vagus nerves). Therefore excessive vagal input causes severe contraction of airway smooth muscle and increases mucus secretion by submucosal glands, both of which limit airflow through conducting airways by decreasing airway lumen diameter and increasing airway resistance.

Mast cells in the human lung contain membrane-bound secretory granules that are characteristically filled by scrolled, crystalline, or particulate inclusions (Fig. 1-16). These granules contain a host of inflammatory mediators, including histamine, proteoglycans, lysosomal enzymes, and metabolites of arachidonic acid.[56] Not only can these mediators induce bronchoconstriction, they can also stimulate mucus production and induce mucosal edema by increasing permeability of bronchial vessels.

Basal cells are located along the basal lamina of airways (see Fig. 1-13). These small epithelial cells have been classically thought to be precursor cells for other airway epithelial cells, including ciliated cells.[31,57] However, more recent experiments suggest that columnar secretory cells or club cells may also differentiate into ciliated epithelial cells following tissue injury.[58,59]

Club cells (Clara), prominent in the terminal airways, are interspersed among the ciliated epithelial cells, are nonciliated, and have large apical granules (see Fig. 1-15).[60,61] Club cells have at least four functions in the lung. One function is serving as progenitor cells for themselves and for ciliated epithelial cells.[62,63] A second function is xenobiotic metabolism via the cytochrome P-450 monooxygenase system.[64-67] A third function is secretion: club cells are a source of surfactant proteins (SPs; SP-A, -B, and -D)[68-70] and also of lipids, proteins (club cell 10-kDa protein), glycoproteins, and modulators of inflammation (leukocyte protease inhibitor and trypsin-like protease).[71-73] A fourth function is liquid balance by influencing ion channels.[74,75]

BRONCHIAL CIRCULATION

The trachea (and esophagus), main-stem bronchi, and pulmonary vessels into the lung (see Fig. 1-1), as well as the visceral pleura in humans (see "The Pleural Space and Pleuras" toward the end of this chapter), are supplied by the bronchial (systemic) circulation.[45,76,77] Measurements of bronchial circulation, by microsphere studies in animals, indicate that flow is 0.5% to 1.5% of cardiac output and is predominantly to the large airways.[45,76,78-81] The bronchial arteries arborize into bronchial capillaries that form a network in the lamina propria, in the submucosa, and in the region external to the cartilage of bronchi, as well as in the lamina propria of neighboring pulmonary arteries.[82] Venous blood from the trachea and large airways enters bronchial venules, which converge to form bronchial veins that drain into the azygos or hemiazygos veins. Thus a substantial part of bronchial blood flow returns to the right side of the heart. Deeper in the lung, however, bronchial blood passes via short anastomotic vessels into the pulmonary venules, thus reaching the left side of the heart to contribute to the venous admixture.

The bronchial circulation has enormous growth potential, which is in contrast to the pulmonary circulation, which after childhood is unresponsive. In long-standing inflammatory and proliferative diseases, such as bronchiectasis or carcinoma, bronchial blood flow may be greatly increased.[76,83] Scar tissue and tumors larger than 1 mm in diameter receive their blood supply via the bronchial circulation.[84,85] The bronchial circulation is also the primary source of new vessels for repair of tissue after lung injury. As will be discussed near the end of this chapter, the bronchial circulation also supplies the visceral pleura of species that have thick visceral pleura, including humans.

PULMONARY CIRCULATION

In humans the pulmonary artery enters each lung at the hilum in a loose connective tissue sheath adjacent to the main bronchus (see Fig. 1-1). The pulmonary artery travels adjacent to and branches with each airway generation down to the level of the respiratory bronchiole (Fig. 1-17). The anatomic arrangements of the pulmonary arteries and the airways are a continual reminder of the relationship between perfusion and ventilation that determines the efficiency of normal lung function. Although the pulmonary veins also lie in loose connective tissue sheaths adjacent to the pulmonary artery and main-stem bronchus at the hilum, once inside the lung they follow Miller's dictum[45] that the veins will generally be found as far away from the arteries and airways as possible. Peripherally, in the respiratory tissue the pulmonary arteries branch out from the core of the terminal respiratory unit, whereas the veins occupy the surrounding connective tissue envelope (Fig. 1-18). Each small muscular pulmonary artery supplies a specific volume of respiratory tissue, whereas the veins drain portions of several such zones.

Considerable quantitative data about the pulmonary circulation are available for the human lung (Table 1-2).[86-88] Although most of the intrapulmonary blood volume is in

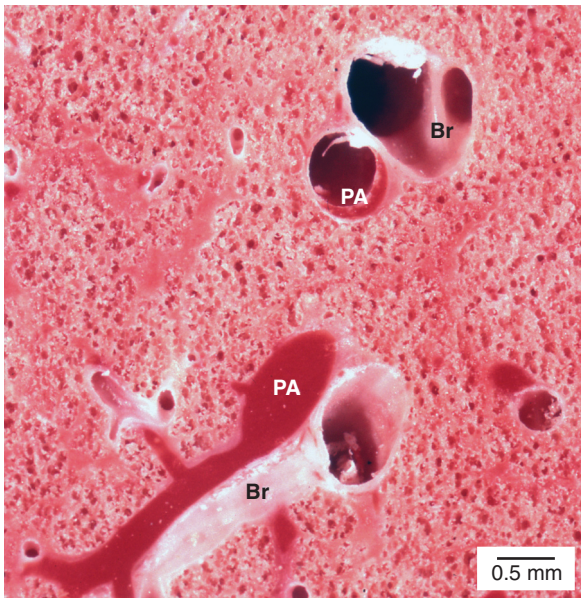

Figure 1-17 Divisions of the pulmonary artery (PA) travel beside the bronchi and bronchioles (Br) out to the respiratory bronchioles. Thus at all airway generations an intimate relationship exists with pulmonary arterial generations. Note that the loose-binding (peribronchovascular) connective tissue sheaths are not distended, compared to the interstitial edema cuffs in Figure 1-5. (Frozen normal sheep lung, unstained.)

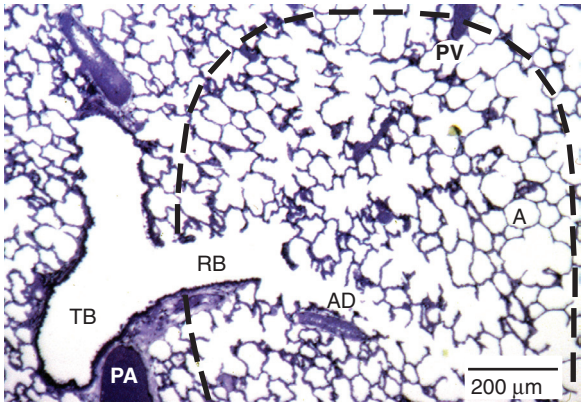

Figure 1-18 The terminal respiratory unit (the physiologist's alveolus) consists of the alveoli (A) and alveolar ducts (AD) arising from a respiratory bronchiole (RB). Each unit is roughly spherical, as suggested by the *dashed outline*. Pulmonary venules (PV) are peripherally located. PA, pulmonary artery; TB, terminal bronchiole. (Normal sheep lung, somewhat underinflated, 2-µm-thick glycol methacrylate section, light microscopy.)

Table 1-2 Quantitative Data on Intrapulmonary Blood Vessels in Humans

Vessel Class (with Diameter)	Volume (mL)	Surface Area (m^2)	Reference No.
Arteries (>500 µm)	68	0.4	86
Arterioles (13–500 µm)	18	1.0	86
Capillaries (10 µm)	60–200	50–70	87
Venules (13–500 µm)	13	1.2	88
Veins (>500 µm)	58	0.1	88

the larger vessels down to approximately 500 µm in diameter, nearly all of the surface area is in the smaller vessels. For example, the surface area of arterioles 13 to 500 µm in diameter exceeds that of the larger vessels by a factor of two, and the maximal capillary surface area is 20 times that of all other vessels.

Because the vertical height of the lung at FRC is 24 cm (see Fig. 1-2), the pressure within the pulmonary blood vessels varies by 24 cm H_2O over the full height of the lung. Thus, if pulmonary arterial pressure is taken as 20 cm H_2O (15 mm Hg, 1.9 kPA) at the level of the main pulmonary artery, which is halfway up the height of the lung, pressure in the pulmonary arteries near the top of the lung will be 12 cm H_2O, whereas pressure in pulmonary arteries near the bottom will be 36 cm H_2O. Pulmonary venous pressure, which is 8 cm H_2O at the level of the pulmonary artery in midchest (left atrial pressure), would be –4 cm H_2O near the top of the lung and +20 cm H_2O at the bottom. In the normal lung the blood volume is greater at the bottom because of increased luminal pressure, which expands those vessels and increases their volume. This effect of distention also decreases the contribution of the blood vessels at the bottom of the lung to total pulmonary vascular resistance.

From the time after birth through adulthood, the normal pulmonary circulation is a low-resistance circuit. The resistance is distributed somewhat differently, however, than in the systemic circulation, where the major drop in resistance is across the arterioles. Although the pressure drop along the pulmonary capillaries is only a few centimeters of water (similar to the pressure drop in systemic capillaries), the pulmonary arterial and venous resistances are low, so a relatively larger fraction of the total pulmonary vascular resistance (35% to 45%) resides in the alveolar capillaries at FRC.[89,90] (For further information about pulmonary circulation in health and disease see Chapters 6 and 58.)

Vasoactivity plays an important part in the local regulation of blood flow in relation to ventilation.[91,92] Because smooth muscle can be found in the pulmonary vessels on both the arterial and the venous side down to precapillary and postcapillary vessels,[93,94] any segment can contribute to active vasomotion.[95] In pathologic conditions, vascular smooth muscle may extend down to the capillary level.[96,97]

Theoretically, gas exchange may take place through the thin wall of almost any pulmonary vessel. At normal alveolar oxygen tensions, however, little oxygen and carbon dioxide is exchanged before the blood reaches the true capillaries.[98] In the pulmonary arterioles, because of their small volume (see Table 1-2), blood flow is rapid. As blood enters the vast alveolar wall capillary network, its velocity slows, averaging approximately 1000 µm/sec (or 1 mm/sec). Flow in the microcirculation is pulsatile because of the low arterial resistance.[99] Pulsations reach the microvascular bed from both the arterial and the venous sides. In fact, one sign of severe pulmonary hypertension is the disappearance of capillary pulsations.[100]

The capillary network is long and crosses several alveoli (Fig. 1-19) of the terminal respiratory unit before coalescing into venules. The vast extent of the capillary bed together with the length of the individual paths means a reasonable transit time for red blood cells, during which gas exchange can take place. The anatomic estimate of

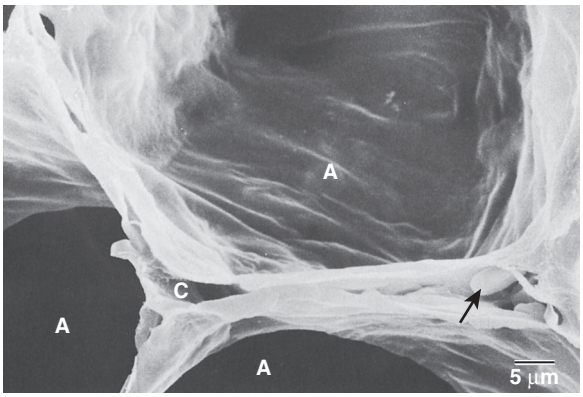

Figure 1-19 An alveolar capillary (C) is shared longitudinally along its path across three alveoli (A). The alveolar walls are flattened, and the wall junctions are sharply curved because the lung is fixed in zone 1 conditions. Some red blood cells remain in the capillary at an alveolar corner (*arrow*). (Perfusion-fixed normal rat lung, P_{AW} = 30 cm H_2O, P_{PA} = 25 cm H_2O, P_{LA} = 6 cm H_2O, scanning electron microscopy. P_{AW}, airway pressure; P_{LA}, left atrial pressure; P_{PA}, pulmonary artery pressure.)

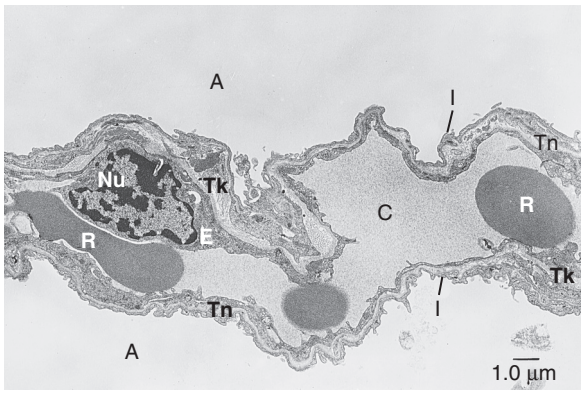

Figure 1-20 The thick (Tk) and thin (Tn) sides of an alveolar capillary (C) change as the capillary crosses between alveoli (A). The basal laminae of the capillary endothelium and alveolar epithelium fuse in the thin regions. The nucleus (Nu) of an endothelial cell (E) is visible above a red blood cell (R). I, alveolar type I cell. (Human lung surgical specimen, transmission electron microscopy).

approximately 0.5 to 1 second average transit time is essentially the same as that found using the carbon monoxide diffusing capacity method, in which one divides capillary blood volume by cardiac output to obtain mean capillary transit time.[101] In the normal lung sufficient time is available for equilibrium between the oxygen and carbon dioxide tensions in the alveoli and the erythrocytes in the pulmonary capillaries. Only under extreme stress (heavy exercise at low inspired oxygen tensions) or in severe restrictive lung disease would the red blood cells be predicted to pass through the microcirculation without enough time to reach diffusion equilibrium.[102]

Normally, capillary blood volume is equal to or greater than stroke volume. Under normal resting conditions, the volume of blood in the pulmonary capillaries is well below its maximal capacity, however. Recruitment can increase this volume by a factor of about three. Thus the normal capillary blood volume of 60 to 75 mL is one third of the capacity (200 mL) measured by quantitative histologic analysis.[5]

Anatomically, the pulmonary blood vessels can be divided into two groups in a manner similar to the connective tissue compartments: extra-alveolar and alveolar. *Extra-alveolar vessels* lie in the loose-binding connective tissue (peribronchovascular sheaths, interlobular septa). Extra-alveolar vessels extend into the terminal respiratory units. Arteries as small as 100 μm in diameter have loose connective tissue sheaths. This is in contrast to the bronchioles, which are tightly embedded in the lung framework from the bronchioles (1 mm in diameter) onward. *Alveolar vessels* lie within the alveolar walls and are embedded in the parenchymal connective tissue. They are subject to whatever forces operate at the alveolar level. They are referred to as alveolar vessels in the sense that the effective hydrostatic pressure external to them is alveolar pressure. Not all of the alveolar vessels are capillaries, however. Small arterioles and venules, which bulge into the air spaces, may be affected by changes in alveolar pressure. Likewise, not all of the capillary bed is alveolar under all conditions.[103] The corner capillaries in the alveolar wall junctions are protected from the full effects of alveolar pressure by the curvature and alveolar air-liquid surface tension.[104] This may account for the fact that, even under zone 1 conditions in which alveolar pressure exceeds both arterial and venous pressure, some blood continues to flow through the lung.[105] One has to go several centimeters up into zone 1 before blood flow stops completely. (For a discussion of distribution of pulmonary blood flow and lung zones, see Chapter 4.)

An important question is whether the normal human lung contains connections between the pulmonary arteries and veins that permit some portion of pulmonary blood flow to bypass the capillary network. Such vessels may develop congenitally or pathologically.[106] In the normal lung, however, functioning short circuits probably do not exist. (Pathologic arteriovenous communications are discussed in Chapter 61.)

Pulmonary capillaries are lined by continuous (nonfenestrated) endothelial cells (Fig. 1-20). These attenuated cells have an individual area of 1000 to 3000 μm^2 and an average volume of 600 μm^3.[107] These large, flat cells cover a total surface area of approximately 130 m^2.[107] Other structural features of pulmonary capillary endothelial cells are the large number of plasmalemmal vesicles and small number of organelles (see Fig. 1-20). Despite having relatively few organelles, pulmonary capillary endothelial cells do have organelles involved in protein synthesis, such as endoplasmic reticulum, ribosomes and Golgi apparatus, and endocytosis (caveolae, multivesicular bodies, and lysosomes).[108] The endocytic apparatus appears to participate in receptor-mediated uptake and transport (transcytosis) of albumin, low-density lipoproteins, and thyroxin.[109-113] Another route for passage of solutes and water is between adjacent endothelial cells (transcellular transport). However, that passage route is restricted by specialized junctional complexes called "tight junctions."[114,115]

In addition to its function in gas exchange, the pulmonary circulation is involved in a number of other functions important to homeostasis. The pulmonary vascular bed serves as a capacitance reservoir between the right and left sides of the heart. Consequently, the reservoir of blood in the pulmonary circulation is sufficient to buffer changes in right ventricular output for two to three heartbeats. The

pulmonary vascular bed also serves as a filter, trapping any embolic material from systemic vascular beds. For example, during intravascular coagulation or in processes involving platelet or neutrophil aggregation, the predominant site of sequestration is the lung. The main anatomic reason for this is that 75% of the total circulating blood volume is in the venous circuit, and the lung's microvascular bed is the first set of small vessels through which the blood flows. Moderate numbers of microemboli generally produce no detectable dysfunction because of the huge array of parallel pathways in the microcirculation. At most, microemboli temporarily block flow to a portion of or to an entire terminal respiratory unit. The fate of such emboli is not clear. Some are phagocytosed and removed into the lung tissue.[116] Some emboli can be degraded to a small size, pass through into the systemic circulation, and be removed by the reticuloendothelial system. One example of particulate matter that filters in the lung is the macroaggregated serum albumin used in lung-scanning procedures. (Further information about the pathophysiology of thromboembolic disorders is presented in Chapter 57.)

The endothelial cells of the pulmonary circulation are capable of a remarkable number of metabolic activities. This is not to say that endothelial cells in other organs do not have similar activities. But the central position of the lung, through which the entire cardiac output passes, places extra responsibility and extra importance upon its endothelial cells.[117-119] For example, angiotensin I, bradykinin, and prostaglandin E_1 are nearly completely inactivated during a single pass through the lungs. Pulmonary endothelial cells also express at least two subtypes of endothelin receptors (A and C).[120-122] Their expression coincides with rapid removal of endothelin, suggesting that the lung microcirculation participates in clearance of this potent vasoconstrictor peptide from the blood. Conversely, a potent vasodilator, nitric oxide, is generated locally in the lung, through expression of endothelial nitric oxide synthase.[123-129]

Endothelial cells may have a role in regulating vascular tone and reactivity. An indication of this regulatory role can be seen in the direct contacts between pulmonary endothelial cells in small arteries and veins and the surrounding smooth muscle cells. Such myoendothelial contacts have been described in the lungs of a number of small animals,[130-133] and we have seen them in the human lung (Fig. 1-21). Although their functional importance is unknown, they may have some bearing on endothelial-dependent vasoactivity.[134]

Regulation of vasoactivity by endothelial cells may be facilitated by site-specific phenotypes of endothelial cells (reviewed by Garlanda and Dejana[135] and Gebb and Stevens[136]). For example, endothelial nitric oxide protein is more evident in small pulmonary arterial vessels than in capillaries.[123-128] Presumably the more evident localization reflects the functional role of nitric oxide in regulating pulmonary artery smooth muscle tone. On the other hand, capillary endothelial cells appear to have more expression-activated message for leukocyte adhesion molecules than arterial endothelial cells.[135,136] Greater expression by capillary endothelial cells may contribute to sequestration of leukocytes in the capillary bed during acute inflammatory reactions. Another endothelial cell function that is site specific in the lung is Ca^{2+} transients that are induced by pressure elevations as small as 5 cm H_2O. The calcium transients seen in a subset of Ca^{2+} oscillating cells are referred to as "pacemakers" and are located in pulmonary venular capillaries.[137] The oscillations are propagated to adjacent endothelial cells. This endothelial response may be relevant in the pathogenesis of pressure-induced lung microvascular injury.

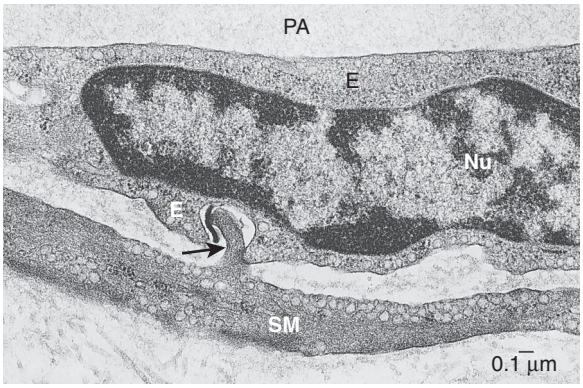

Figure 1-21 A myoendothelial cell contact (*arrow*) is made between a pulmonary arteriolar (PA) endothelial cell (E) and a subjacent vascular smooth muscle cell (SM). The distribution and functional significance of such contacts is unknown. One potential role may be to facilitate delivery of endothelium-derived relaxing factor to smooth muscle cells. Nu, nucleus of the endothelial cell. (Human lung surgical specimen, transmission electron microscopy).

TERMINAL RESPIRATORY UNITS

The "alveolus" of which the physician or pulmonary physiologist speaks is referred to as the "terminal respiratory unit" by the anatomist. The terminal respiratory unit consists of all the alveolar ducts, together with their accompanying alveoli, that stem from the most proximal (first) respiratory bronchiole (see Fig. 1-18). The terminal respiratory unit has both a structural and a functional existence and was first described by Hayek.[10] In the human lung this unit contains approximately 100 alveolar ducts and 2000 alveoli. At FRC the unit is approximately 5 mm in diameter, with a volume of 0.02 mL. In normal adult humans, there are approximately 150,000 such units in both lungs combined.[5] The acinus, an anatomic unit popular among pathologists, contains 10 to 12 terminal respiratory units.[138-140]

The functional definition of the terminal respiratory unit is that, because gas phase diffusion is so rapid, the partial pressures of oxygen and carbon dioxide are uniform throughout the unit.[141] Diffusion is the name for a thermodynamic process by which molecules express their kinetic energy. Net diffusion takes place when a concentration difference of a substance exists between two volumes. Thus oxygen in the alveolar duct gas will diffuse into the alveoli, because the incoming air has a higher oxygen concentration than the alveolar gas. Oxygen will also diffuse from the gas adjacent to the alveolar wall through the air-blood barrier into the red blood cells flowing in the capillaries (Fig. 1-22), where oxygen combines with hemoglobin. Carbon dioxide diffuses in the opposite direction. A key point about

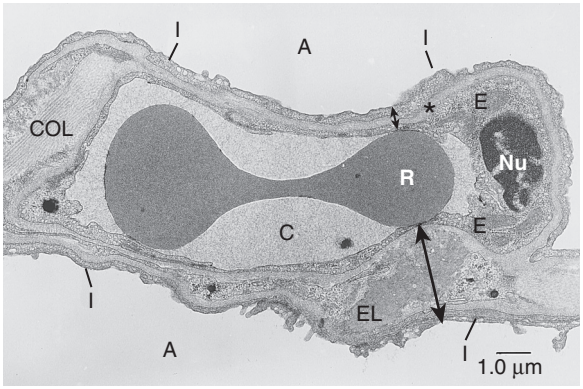

Figure 1-22 Cross section of an alveolar wall showing the path for oxygen and carbon dioxide diffusion. The thin side of the alveolar wall barrier (*short double-headed arrow*) consists of type I epithelium (I), interstitium (*) formed by the fused basal laminae of the epithelial and endothelial cells, capillary endothelium (E), plasma in the alveolar capillary (C), and finally the cytoplasm of the red blood cell (R). The thick side of the gas-exchange barrier (*long double-headed arrow*) has an accumulation of elastin (EL), collagen (COL), and matrix that jointly separate the alveolar epithelium from the alveolar capillary endothelium. As long as the red blood cells are flowing, oxygen and carbon dioxide probably diffuse across both sides of the air-blood barrier. A, alveolus; Nu, nucleus of the capillary endothelial cell. (Human lung surgical specimen, transmission electron microscopy.)

diffusion is that the process is much faster in the gas phase than in water. Thus the terminal respiratory unit size is defined in part by the fact that gas molecules can diffuse and equilibrate anywhere within the unit more rapidly than they can diffuse through the membrane into the blood. The main problem is that the solubility of oxygen in water is low relative to its concentration in gas. Water becomes a problem when edema liquid accumulates in alveoli and/or interstitium in the alveolar walls. Carbon dioxide is much more soluble in water (20 times the solubility of oxygen in water), and therefore carbon dioxide diffuses rapidly into the gas phase, even though the driving pressure for carbon dioxide diffusion is only one tenth that for oxygen entering the blood.

It is almost impossible to demonstrate that diffusion is limiting in the normal lung, except during heavy exercise while breathing gas containing very low oxygen concentrations.[102] Even then, diffusion limitation may not be as important as the reduced transit time of the red blood cells. However, apart from these observations during heavy exercise, most disorders of oxygenation are due to ventilation-perfusion inequalities.[142]

All portions of the terminal respiratory unit participate in volume changes with breathing.[143,144] Thus, if a unit were to increase its volume from FRC, the alveolar gas that had been in the alveolar duct system would enter the expanding alveoli, together with a small portion of the fresh air. Most of the fresh air would remain in the alveolar duct system. This does not lead to any significant gradient of alveolar oxygen and carbon dioxide partial pressures because diffusion in the gas phase is so rapid that equilibrium is established within a few milliseconds. But nondiffusible (suspended or particulate) matter would remain away from the alveolar walls and be expelled in the subsequent expiration.[145] This explains why it is difficult to deposit aerosols on the alveolar walls and why large inspired volumes and breath-holding are important for obtaining efficient alveolar deposition.

The anatomic alveolus is not spherical (Fig. 1-23; see Fig. 1-19). It is a complex geometric structure with flat walls and sharp curvature at the junctions between adjacent walls. The most stable configuration is for three alveolar walls to join together, as in foams.[5] The resting volume of an alveolus is reached at minimal volume, which is 10% to 14% of total lung capacity. When alveoli go below their resting volume, they must fold up because their walls have a finite mass. Most of the work required to inflate the normal lung is expended across the air-liquid interface to overcome surface tension; the importance of the air-liquid interface is demonstrated by the low pressure required to "inflate" a liquid-filled lung with more liquid.[146]

The phenomenon of terminal respiratory unit, or alveolar, stability is confused because not only is air-liquid interfacial tension involved, but each flat alveolar wall is part of two alveoli and both must participate in any change. Therefore atelectasis does not usually involve individual alveoli but rather relatively large units (Fig. 1-24).[147]

The alveolar walls are composed predominantly of pulmonary capillaries. In the congested alveolar wall, the blood volume may be more than 75% of the total wall volume. Alveoli near the top of the lung show less filling of the capillaries than those at the bottom.[148,149] This affects regional diffusing capacity, which is dependent on the volume of red cells in the capillaries (see also eFig. 25-10).

The transition from the cuboidal epithelium of the respiratory bronchiole to alveolar squamous epithelium is abrupt (see Fig. 1-12). Although Macklin[150] speculated that the permeability of the bronchiole-alveolar epithelial junctions may be special, no definitive difference has been demonstrated.[151] The controversy continues as to whether this region shows unique permeability features that might participate in clearance of particles or leakage of edema.[152-154]

The pleomorphic nature of the alveolar epithelium and the light and electron microscopic structure of its constituent cells have been described many times and will be only briefly summarized here. In normal mammals and other air-breathing species, including reptiles and amphibians, the alveolar epithelium is composed of cuboidal alveolar type II cells and flattened type I cells (Fig. 1-25). Alveolar type II cells outnumber type I cells (≈15% versus 8% to 10% of total peripheral lung cells, respectively), but type I cells account for approximately 90% to 95% of the alveolar surface area of the peripheral lung.[155] The two cell types have different functions and structure.

The alveolar type II cell is the major synthesizing and secreting factory of surfactant-associated proteins that affect adsorption of surfactant lipids to an air-liquid interface, surfactant recycling, and immunomodulatory functions. Alveolar type II cells also express receptors for several growth factors and secretagogues, enzymes, matrix proteins, and epithelial mucins.[156-161] The presence of various ion channels and transporters supports earlier evidence that alveolar type II cells are actively involved in liquid resorption and transepithelial water fluxes.[162] Alveolar type II cells are reported to express some species of aquaporin

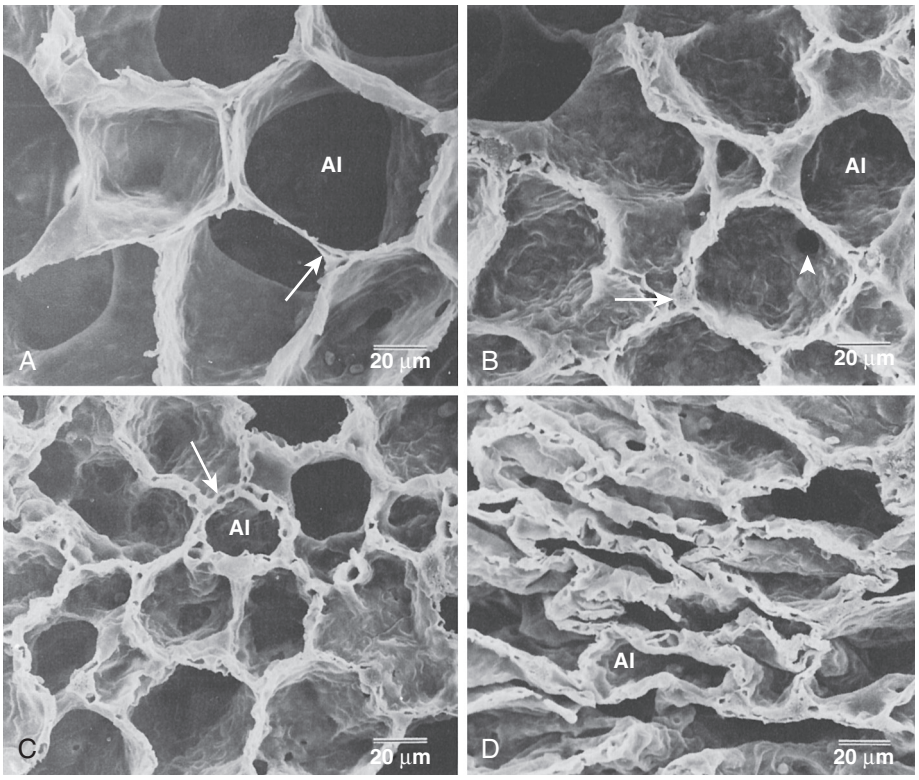

Figure 1-23 Alveolar shape changes at representative points along the air deflation pressure-volume curve of the lung. The four micrographs are at the same magnification. The air deflation pressures are as follows: **A,** 30 cm H_2O (total lung capacity; TLC); **B,** 8 cm H_2O (approximately 50% TLC); **C,** 4 cm H_2O (near functional residual capacity; FRC); and **D,** 0 cm H_2O (minimal volume). Vascular pressures are constant (P_{PA} = 25 cm H_2O and P_{LA} = 6 cm H_2O). Intrinsic alveolar shape (AI) is maintained from TLC to FRC (**A–C**). The alveolar walls are flat, and there is sharp curvature at the junctions between adjacent walls. Note the flat shape of the alveolar capillaries (*arrow*) at TLC (**A,** lung zone 1 conditions) compared to their round shape (*arrow*) at FRC (**C,** lung zone 3 conditions). The alveolar walls are folded, and alveolar shape is distorted at minimal lung volume (**D**). The *arrow* in **B** identifies an alveolar type II cell at an alveolar corner. The *arrowhead* in **B** identifies a pore of Kohn. P_{AW}, airway pressure; P_{LA}, left atrial pressure; P_{PA}, pulmonary artery pressure. (Perfusion-fixed normal rat lungs, scanning electron microscopy.)

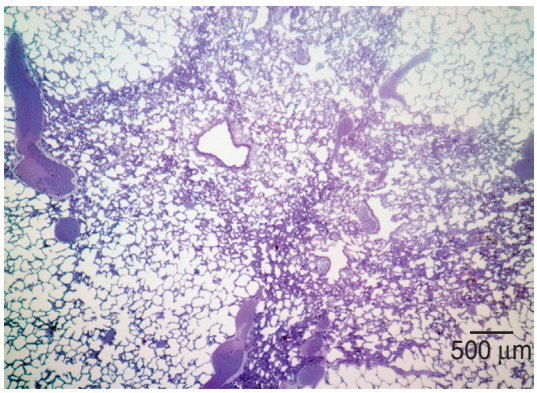

Figure 1-24 Histologic appearance of atelectasis. Atelectasis usually involves relatively large units of lung parenchyma, rather than individual alveoli. Alveolar walls in the atelectatic units are folded, distorting the shape of alveoli and capillaries, as shown in Fig. 1-23D. (Sheep lung injured by air emboli, 2-μm-thick glycol methacrylate section, light microscopy.)

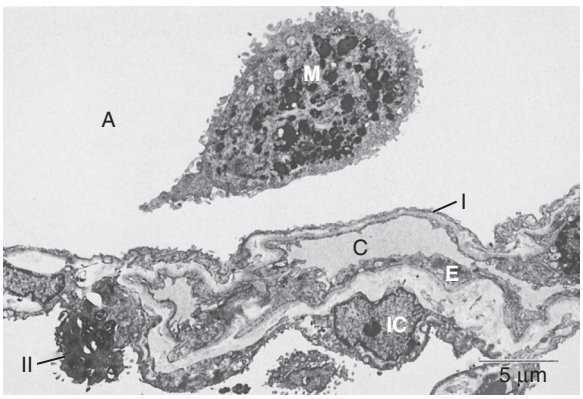

Figure 1-25 Cells of the terminal respiratory unit. An alveolar macrophage (M) is located in an alveolus (A). Alveolar macrophages are the air space scavengers that are cleared either up the mucociliary escalator or into the interstitium. These cells can be activated to express and secrete cytokines, which may interact with other cells. Cells of the alveolar wall are the lining alveolar type I and II cells (I and II, respectively) and the enclosed capillary (C), endothelial cells (E), and interstitial cells (IC). (Human lung surgical specimen, transmission electron microscopy.)

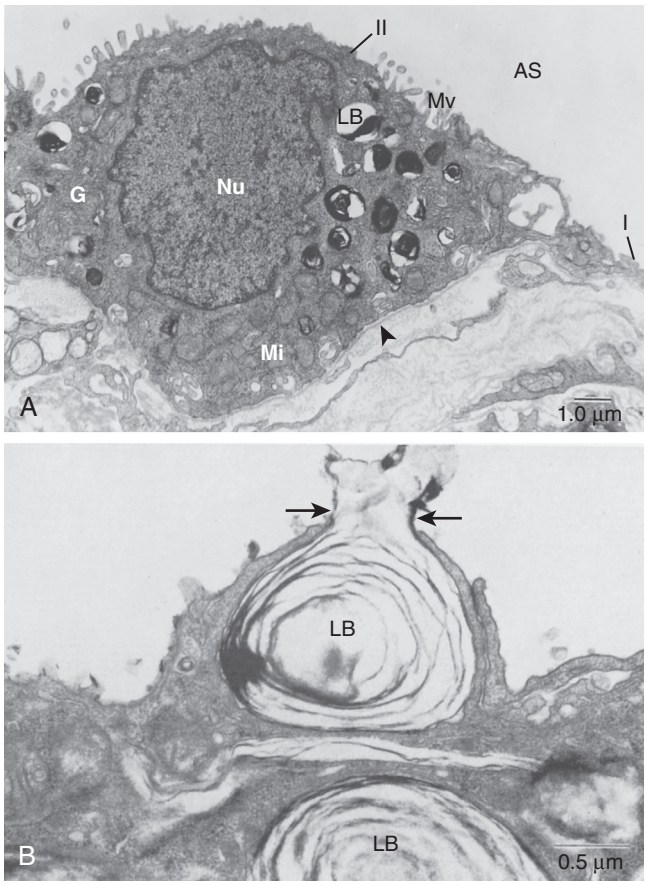

Figure 1-26 **A,** Alveolar type II (or granular) cells (II) are cuboidal epithelial cells that contain characteristic lamellar bodies (LB) in their cytoplasm and have stubby microvilli (Mv) that extend from the apical surface into the alveolar air space (AS). Other prominent cytoplasmic organelles in alveolar type II cells are mitochondria (Mi) and Golgi apparatus (G). Adjacent to the type II cell is a process of a type I cell (I). The abluminal surface of the epithelial cells rests on a continuous basal lamina (*arrowhead*). Nu, nucleus of an alveolar type II cell. **B,** The apical region of an alveolar type II cell has two lamellar bodies (LB), one of which has been fixed in the process of secretion by exocytosis (*arrows*). The lamellar osmiophilic bodies are believed to be the source of surface-active material (surfactant). Alveolar type II cells are usually located in the alveolar corners (see Fig. 1-23B). (Human lung surgical specimen, transmission electron microscopy.)

(AQP3, AQP1),[163,164] water channels that may facilitate transepithelial liquid fluxes.

The typical alveolar type II cell (e.g., human, rodent) is a small (300 μm^3), cuboidal cell with short stubby apical microvilli (Fig. 1-26). The distinguishing structural feature of an alveolar type II cell is its content of intracellular lamellar bodies, which are membrane-bound inclusions (diameter from <0.1 to 2.5 µm; mean, ≈1 µm) composed of stacked layers of cell membrane–like material (see Fig.1-26). These bodies contain pulmonary surfactant and are composed of phospholipid species similar to those of lavaged surfactant.[165] Lamellar bodies also contain various proteins, including SP-A, SP-B, and SP-C but probably not SP-D, typical lysosomal enzymes, an H^+ transporter, a unique α-glucosidase, and other molecules.[166-168] Alveolar type II cells also internalize and recycle surfactant lipids and proteins, but the cellular pathways are not well characterized in terms of participating organelles, signaling mechanisms, and general molecular regulation. Multivesicular bodies, organelles generally involved in endocytosis, are unusually abundant in alveolar type II cells and also express the ABC-type transporter membrane protein.[169]

Alveolar type I cells have extensive, attenuated cytoplasmic processes that form a large, thin surface area for gas exchange (see Fig. 1-25). The enormous surface area of these cells presents a logistical problem for transport of new proteins and other substances within the cell over long distances and most likely contributes to the vulnerability of the type I cell to injury. Under normal conditions, alveolar type I cells attach via tight junctions to neighboring alveolar type II cells to form a relatively impermeable seal between alveolar air and alveolar wall interstitial spaces. Although the cells express connexin proteins used to form gap junctions,[170] such junctions have not been consistently observed by electron microscopy. Lectin-binding and histochemical studies show that the chemical nature of the alveolar type I cell apical membrane differs markedly from that of type II cells, and this concept is confirmed by the identification of novel type I cell proteins. The alveolar type I cell protein aquaporin-5 is of particular interest because this water channel has the highest water permeability known, at least in vitro.[171] Type I cells also express epithelial Na^+ channels and membrane Na^+,K^+-ATPase.[172,173] These observations collectively imply that type I cells may play a role in pulmonary water flux, although this is not yet proven.[174,175]

Alveolar type I cells contain many small, non–clathrin-coated vesicles, or caveolae, that are open either to the alveolar lumen or interstitium or are detached from the surface as free vesicles in the cytoplasm.[176] Immunohistochemistry shows that the vesicles contain caveolin-1 protein.[177] Likewise, biochemical analyses[178] show high concentrations of caveolin protein and messenger RNA in lung where it is expressed mainly by type I and vascular endothelial cells. Caveolin-1 is a scaffolding protein that organizes specialized membrane phospholipids and proteins into vesicles. Caveolin-1 can bind free cholesterol and modulate the efflux of cholesterol from the cell when intracellular concentrations rise,[179] and, in other cell systems, its expression is tightly linked to the availability of free cholesterol. Caveolae appear to sequester various proteins into the vesicles; such proteins include growth factor receptors, signaling molecules such as G proteins, Ca^{2+} receptors and pumps, and, in endothelial cells, endothelial nitric oxide synthase. The general effect of sequestration of receptors and signaling molecules into caveolae is to maintain them in a functionally quiescent state.

Trapping and clearance of particulate matter impinging on the alveolar surfaces is vital and takes place in the alveolar surface liquid. Within this liquid are suspended alveolar macrophages (see Fig. 1-12). The cytoplasm of alveolar macrophages contains numerous storage granules that are blackened by ingested particulate matter that reach the alveoli (see Fig. 1-25). Alveolar macrophages actively express and secrete cytokines, such as tumor necrosis factor-α and transforming growth factor-α, that are important for innate immunity. Some of these alveolar macrophages penetrate into the lung interstitium and can be seen as deposits of black pigment within interstitial foci. The majority of alveolar macrophages that reach the terminal airways via the slow, upward flow of alveolar lining liquid

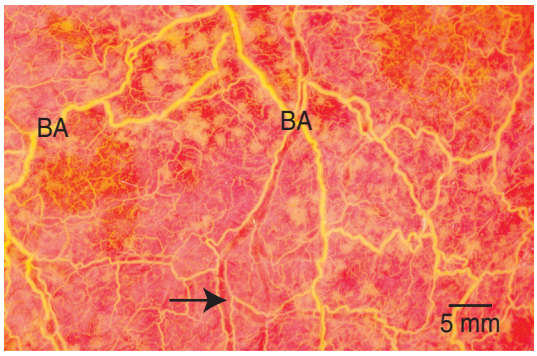

Figure 1-27 Surface view of the visceral pleura. Yellow latex polymer (Microfil) was perfused through the bronchial artery trunk to fill the bronchial arteries (BA) that supply the visceral pleura. Bronchial arterioles flank lymphatics (*arrow*) that constitute the superficial lymphatic plexus of the lung. (Sheep whole lung, macroscopic view).

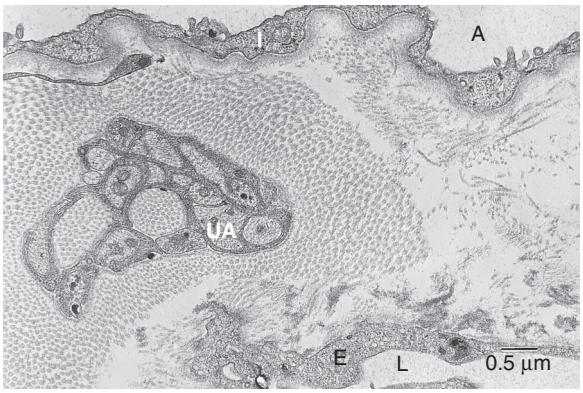

Figure 1-28 Unmyelinated axons (UA) known as C fibers are shown situated in the interstitium of a respiratory bronchiole, between an alveolar type I cell (I) lining an alveolus (A) and an initial lymphatic (L). Although the presence of small clear vesicles is suggestive of cholinergic (autonomic) axons, unequivocal identification as either motor or sensory fibers is not possible in random thin sections. E, lymphatic endothelial cell. (Human lung surgical specimen, transmission electron microscopy.)

are expelled with the surface film as it is pulled up onto the mucociliary escalator.[180-182]

LYMPHATICS

Another route for clearance of particulate matter and liquid from the lung is the pulmonary lymphatic system. Lymphatics of the lung are subdivided into two principal groups based on their location: a deep plexus and a superficial plexus.[10,45,183,184] Both plexuses are made up of initial and collecting lymphatics, with communications between the two.[10,45,153,183] The *deep plexus* is situated in the peribronchovascular connective tissue sheaths of the lung (see Fig. 1-1).[10,45,153,183] Lymphatics in the deep plexus are distributed around the airways, extending peripherally to the respiratory bronchioles and next to branches of the pulmonary arteries and veins.[10,45,153,183] The *superficial plexus* is located in the connective tissue of the visceral pleura (Fig. 1-27). This plexus is prominent in the lung of species with thick visceral pleura, including humans (see "The Pleural Space and Pleuras").[10,45,183] Lymphatics are not found in the alveolar walls.

Lymph is propelled centripetally toward the lung's hilum or pulmonary ligament to reach regional lymph nodes. In the human, pulmonary lymph flows to extrapulmonary lymph nodes located around the primary bronchi and trachea.[10,45,183]

INNERVATION

Innervation of the human lung consists of sensory (afferent) and motor (efferent) pathways.[183,185-187] The sensory pathways originate in relation to the airway epithelium, submucosa, interalveolar septa, and smooth muscle. Mapping the complete distribution of the mucosal sensory nerve endings has been hampered by the lack of dependable morphologic methods that identify intraepithelial sensory axons. Ultrastructural techniques have shown that axons, when found, resemble known sensory endings in other organs (<1 µm in diameter, electron lucent, and containing microtubules and smooth endoplasmic reticulum).[188] Fibers of this pathway include myelinated, slowly adapting stretch receptors (Hering-Breuer reflex) and irritant receptors, but most are unmyelinated, slow-conducting C fibers located in the terminal respiratory units, either along the bronchioles or within the alveolar walls (Fig. 1-28). There has been speculation about the function of C fibers since Paintal first suggested that they played a role in sensing parenchymal connective tissue distortion, as during pulmonary vascular congestion and interstitial edema.[189-192] The speculation has been neither proven nor disproven.

Chemosensory cells are also present in the upper and lower airways.[193,194] This sensory role is subserved in the human lung by ciliated airway epithelium of the upper and lower airways, which have functional components for bitter taste receptors.[195] In addition, solitary epithelial brush cells with a chemosensory function are present in upper and lower airways.[196] The afferent fibers travel in the vagus nerves and terminate in the vagal nuclei in the medulla oblongata.[197]

Submucosal sensory nerve endings, in contrast, are more reliably identifiable because the axon can be stained with methylene blue or silver nitrate. Furthermore, studies of axonal transport indicate that the peripheral processes of sensory ganglia project to the submucosa.[198] Ultrastructural observations of these fibers reveal axonal terminals containing numerous membranous inclusions and mitochondria, which are characteristic of mechanoreceptors.

The motor pathways reach the lung through the sympathetic and parasympathetic nervous systems. Preganglionic contributions to the sympathetic nerves arise from the upper four or five thoracic paravertebral ganglia, whereas the preganglionic parasympathetic nerves originate in the brain stem motor nuclei associated with the vagus nerves. Postganglionic sympathetic nerve fibers terminate near an airway, innervating vascular smooth muscle cells and submucosal glands. Postganglionic parasympathetic fibers extend from ganglia mainly located external to the smooth muscle and cartilage. Some submucosal ganglia exist, but they are generally smaller and have fewer neurons.

Mucosal motor nerve endings also exist.[199] Characteristic ultrastructural features are axonal profiles containing many small, agranular vesicles and few mitochondria.

Unfortunately, the source and function of these axons is unknown. A goblet cell secretomotor role is doubtful because goblet cells in isolated epithelial strips do not secrete glycoproteins when bathed in drugs that mimic neurotransmitters.[200] Alternatively, a role may be the release of mucus by direct response to mechanical and chemical signals. Another effector role of nerves in the lung is epithelial ion transport, a process that is stimulated by catecholamines,[201] acetylcholine,[202] and neuropeptides.[203] This role is further supported by the presence of α-adrenergic, β-adrenergic, and muscarinic receptors throughout the airway epithelium.[204]

Submucosal tracheal gland efferent nerve endings consist of cholinergic, adrenergic, and peptidergic axonal profiles.[205,206] Discrimination among these axonal types is partially aided by their ultrastructural appearance: cholinergic axons have small, agranular vesicles; adrenergic axons have small, dense-cored vesicles; peptidergic axons have many large, dense-cored vesicles. One must realize, however, that these descriptive definitions are not absolutely reliable.

The lung also contains a component of the diffuse neuroendocrine system called the amine uptake and decarboxylation system.[207,208] Despite the growing recognition that a diffuse neuroendocrine system is located in the lung, we do not understand its normal functional role, although one can postulate that these cells release hormones that affect smooth muscle.[209,210] This system is composed of single neuroendocrine cells and clusters of such cells, known as neuroepithelial bodies, distributed along the airway epithelium to the region of alveolar ducts.[211-214] The neuroepithelial bodies are preferentially located at airway bifurcations. Pulmonary neuroendocrine cells are ultrastructurally characterized by dense-cored vesicles in their cytoplasm (Fig. 1-29). The dense-cored vesicles are considered to be the storage sites of amine hormones (serotonin, dopamine, norepinephrine) and peptide hormones (bombesin, calcitonin, leu-enkephalin).[215] Neurons are also associated with the airway epithelial and neuroendocrine cells; they appear to be the storage sites for vasoactive intestinal peptide[215,216] and substance P.[217,218]

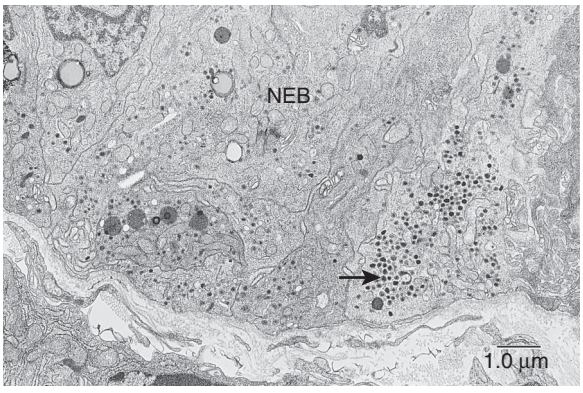

Figure 1-29 Neuroepithelial body (NEB) located in a peripheral airway. Neuroepithelial bodies contain aggregates of neuroendocrine cells. A characteristic ultrastructural feature of neuroendocrine cells is the presence of small (0.1 to 0.3 µm in diameter) dense-cored vesicles in their cytoplasm (*arrow*). Each dense-cored vesicle is bounded by a unit membrane. (Human lung surgical specimen, transmission electron microscopy.)

THE PLEURAL SPACE AND PLEURAS

As stated at the outset of this chapter, the primary function of the lung is ventilation-perfusion matching, ensuring efficient gas exchange between alveolar air and alveolar capillary blood. This vital function is met, in part, by extensive and rapid movement of the lung within the pleural space and its pleural liquid.[219,220] Online supplemental digital videos linked to this chapter provide a glimpse of the view that surgeons have during dissection through the intercostal muscles: the lungs glide along the deep surface of the translucent endothoracic fascia and parietal pleura (see Videos 1-1 to 1-5). The pleural space also serves as an outlet into which pulmonary edema liquid can escape.[221,222] The pleural liquid also serves to couple the lung to the chest wall.[223] What are the anatomic features of the pleural space and pleuras that contribute to these functions?

An important anatomic fact is that the pleural space is a real space (Fig. 1-30); it is not a potential space.[2,223] The pleural space surrounds the lung, except at its hilum, where the parietal pleura and visceral pleura are contiguous.[10,116] Separations are present between the parietal and visceral pleuras along the interlobar fissures and costodiaphragmatic recesses. The normal volume of pleural liquid is 0.1 to 0.2 mL/kg body weight in most mammals.[223,224] This specific volume is distributed across a pleural surface area of approximately 1000 cm^2 per lung and pleural space width of 10 to 20 µm (see Fig. 1-30).[2,223] Normally there is little or no contact across the pleural space because the microvilli that extend from the parietal and visceral mesothelial cells are only 3 to 5 µm long.[1,2,225,226]

Visceral pleural anatomy is characterized by a single layer of mesothelial cells that have microvilli extending from their surface into the pleural space.[225] However, the thickness of the visceral pleura is not uniform across species (Fig. 1-31). Visceral pleural anatomy is characterized as

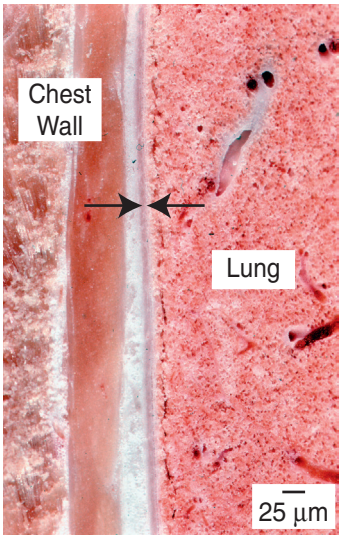

Figure 1-30 The pleural space is a real space. The dark band delimited by the opposed *arrows* is the pleural space, which is located between the chest wall and lung. (Frozen sheep chest wall and lung, unstained.)

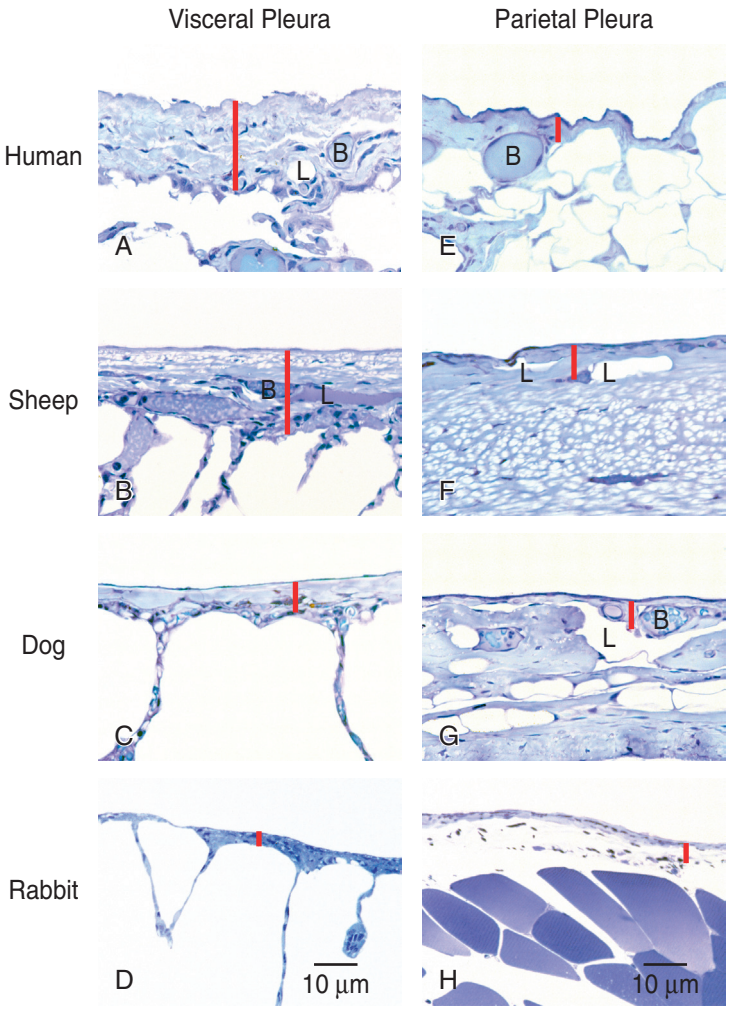

Figure 1-31 Comparative histologic features of the visceral and parietal pleuras among humans, sheep, dogs, and rabbits. The eight panels are shown at the same magnifications. **A–D,** Visceral pleura. **E–H,** Parietal pleura. The most obvious feature of the visceral pleura is its greater thickness (*longer red vertical bars*) in humans and sheep compared to the thinner visceral pleura of dogs and rabbits (*shorter red vertical bars*). The parietal pleura is thinner and consistently so among all the same species. Both the visceral and parietal pleuras are lined by a single layer of mesothelial cells that have microvilli extending from their surface into the pleural space. Subjacent to the mesothelial cell lining layer is loose areolar connective tissue. Among species with "thick" visceral pleura, the loose areolar connective tissue is traversed by bronchial microvessels (B), lymphatics (L), and nerves. By comparison, among species with "thin" visceral pleura, the loose areolar connective tissue is devoid of microvessels, other than the subjacent pulmonary microvessels at the perimeter of the most superficial alveoli. Lymphatics and nerves are infrequent. In the parietal pleura's loose areolar connective tissue are systemic blood microvessels (B), lymphatics (L), and nerves. This histologic organization is consistent among species. (Human, sheep, dog, and rabbit lung, 2-µm-thick glycol methacrylate sections, light microscopy.)

"thick" or "thin."[227] Species with a thick visceral pleura (range is 25 to 100 µm) are humans, sheep, cows, pigs, and horses.[225] Species with a thin visceral pleura (range 5 to 20 µm) are dogs, rabbits, rats, and mice.[228] The variability in thickness is related to the connective tissue layer beneath the visceral pleural mesothelial cells. The other anatomic difference among species with thick or thin visceral pleura is their arterial blood supply. Species with a thick visceral pleura has an arterial blood supply from the systemic circulation, via bronchial arteries (see Fig. 1-27).[45,183,225,227] By comparison, species with a thin visceral pleura has an arterial blood supply from the pulmonary circulation. The reason for this striking difference in visceral pleural anatomy among mammals is not known.

Parietal pleural anatomy is also characterized by a single lining layer of mesothelial cells with microvilli extending from their surface.[226] The thin subjacent loose areolar connective tissue layer contains systemic blood vessels, lymphatics, and nerves. Unlike the situation with the visceral pleura, this thin histologic organization of the parietal pleura is consistent among species, including humans (see Fig. 1-31).[10,116,226,229,230]

The unique anatomic features of the parietal pleura are the lymphatic stomata.[226,230-233] They are openings (≈1 to 3 µm in diameter) between parietal mesothelial cells (Fig. 1-32). Tracer studies revealed that India ink and chicken red blood cells (which are nucleated and therefore easily identifiable) are cleared almost exclusively from the pleural space by the stomata, which are located over the intercostal spaces in the distal half of the thorax, and along the sternum and pericardium of experimental animals that have been studied.[226,229] The openings are continuous with

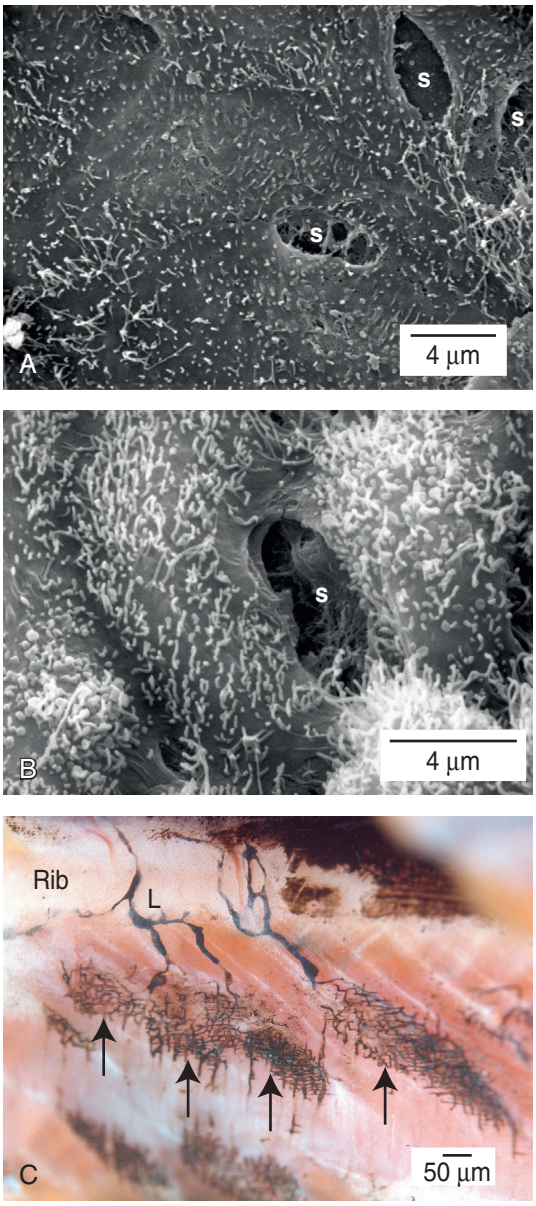

Figure 1-32 Surface view of lymphatic stomata, initial lymphatics, and collecting lymphatics of the parietal pleura. A and **B** are scanning electron micrographs that show the unique structure of lymphatic stomata (S). Stomata are apertures between the pleural space and the initial lymphatics in the parietal pleura. Three stomata are visible in a low-magnification field of view in **A**. Stomata are located over intercostal muscles. **B** shows a different stoma at a higher magnification. Microvilli are not present at the aperture of stomata, which are lined by mesothelial cells. **C** shows a portion of the parietal pleura where colloidal carbon is seen in four beds of initial lymphatics (*arrows*) that are located over an intercostal space. Colloidal carbon is also in collecting lymphatics (L) that cross a rib, where the collecting lymphatics drain into lymphatic vessels that accompany the intercostal vessels. (Rabbit, macroscopic view of the parietal pleura after colloidal carbon was placed in the pleural space in situ.)

the lumen of lymphatic capillaries. Physiologic studies showed that protein and particulate matter in the pleural space are cleared almost exclusively by the parietal pleural system of stomata and lymphatics.[231,234] The lymphatics convey the pleural liquid to regional lymph nodes along the sternum and vertebral column; from there, lymph is carried to the thoracic duct and right lymphatic duct. In this regard, normal pleural liquid is cleared by mechanisms that are consistent with normal interstitial liquid turnover in tissues throughout the body.

COMPARISON OF THE LUNG OF MICE AND HUMANS

A point made in the previous section is that species variations are significant in visceral pleural structure and blood supply. This point raises a question of what other species variations are found in the lung. For the purpose of this chapter, comparison is made between mouse and human, owing to the fantastic discoveries about genetic and molecular regulation of lung biology by making mouse constructs to identify normal lung structure and function, as well as to study the impact of disease on lung structure and function. Key structural features of mouse and human pulmonary morphology are summarized in Table 1-3.[235-237] This table reveals that many anatomic and developmental differences are present that may be helpful to keep in mind.

In the mouse lung, in contrast to the human, the walls of intrapulmonary conducting airways do not have cartilage, which may affect the distribution of airway resistance compared to the human lung (Fig. 1-33). In addition, in the mouse lung, respiratory bronchioles are essentially absent, whereas the human lung has approximately 150,000 respiratory bronchioles (see Fig. 1-33). Thus the mouse lung has fewer airway generations and a significantly smaller total surface area for gas exchange than the human lung. Another potential impact of the fewer airway generations, as well as narrower conducting airways, is that the deposition of inhaled particulates may have a different distribution in the lungs of mice compared to those of humans. Also, because the mouse lung has fewer airway generations, the parenchyma makes up a larger proportion of total lung volume in mice (≈18%) compared to humans (≈12%).

Another notable species difference is the distribution of various cell types. In the upper airway of the human lung, the principal secretory cells are goblet cells (see Fig. 1-13), whereas in the upper airway of the mouse lung, the principal secretory epithelial cells are the club cells (Clara). Club cells in the human lung are found in the terminal airways (see Fig. 1-15). In addition, in the upper airway of the human lung, additional secretory cells are mucous and serous epithelial cells in submucosal glands (see Figs. 1-14 and 1-33), which are not found in the upper airway of the mouse lung (see Fig. 1-33). Thus different cell types contribute to airway secretions in the two species.

Lastly, the lung's developmental stage at full term is different between mice and humans. In mice, lung development at full term is at the saccular stage. In humans, lung development at full term is at the beginning of the alveolar stage. This timing difference is helpful to keep in mind when developmental comparisons are made (see also Chapter 2).

In general, those who use animal models should recognize that, even in the normal setting, important differences in the structure, cellular composition, and development may affect the applicability of findings to the human lung.

Table 1-3 Comparative Anatomy of Mouse and Human Lungs

Anatomic Feature of the Lung	Mouse	Human
Visceral pleura thickness	5-20 μm	25-100 μm
Visceral pleura arterial supply	Pulmonary	Systemic (bronchial)
Lobes	4 right; 1 left	3 right; 2 left
Airway generations	13-17	17-21
Airway branching pattern	Single	Dichotomous
Main bronchus diameter	≈1 mm	≈10-15 mm
Intrapulmonary airway cartilage	No	Yes
Tracheal epithelium thickness	11-14 μm	50-100 μm
Tracheal club cells (Clara)	≈50%	None
Tracheal goblet cells	Absent	Present
Tracheal submucosal glands	Absent	Present
Proximal intrapulmonary airway thickness	8-17 μm	40-50 μm
Proximal intrapulmonary airway club cells	≈60%	None
Proximal intrapulmonary airway goblet cells	Absent	Present
Proximal intrapulmonary airway submucosal glands	Absent	Present
Terminal bronchiole diameter	≈10 μm	≈600 μm
Terminal bronchiole thickness	≈8 μm	Not determined
Terminal bronchiole club cells	≈70%	None
Respiratory bronchioles	Absent (or one)	Present (≈150,000)
Lung parenchyma–total lung volume ratio	≈18%	≈12%
Alveolar diameter	30-80 μm	100-200 μm
Air-blood barrier thickness	≈0.32 μm	≈0.68 μm
Pulmonary venule location	Next to bronchioles	Along interlobular septa
Developmental stage at full term	Saccular	Alveolar

Adapted from references 235–237.

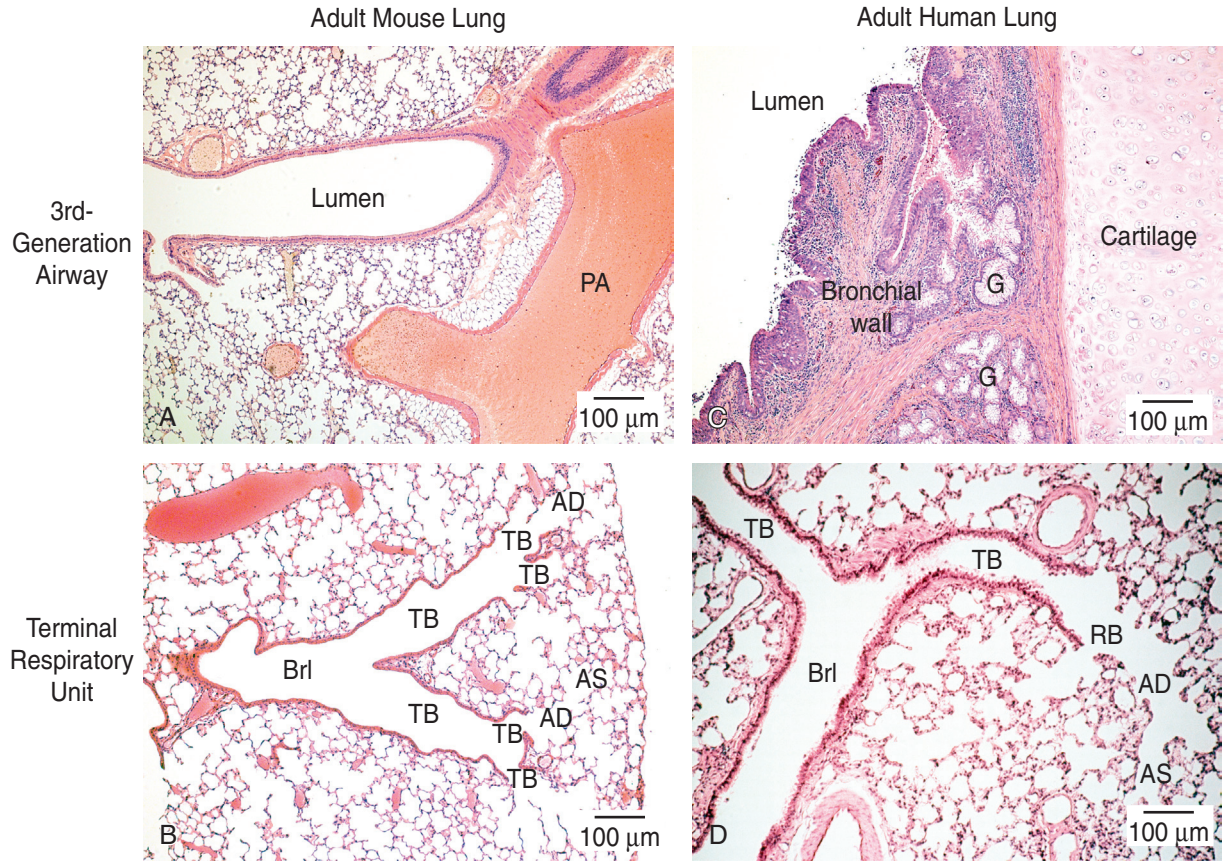

Figure 1-33 Comparison of lung morphologic features between adult mice (left column) and humans (right column). The four panels are the same magnification, as shown by the scale bar in each panel. The upper row compares 3rd-generation, intrapulmonary airways between mouse (**A**) and human (**C**). The mouse's airway lumen is narrower than the same-generation airway (bronchus) in the human. Absent from the wall of the mouse's airway wall are cartilage and submucosal glands (G), both of which are obvious in the wall of the bronchus of the human airway. The lower row compares terminal respiratory units between mouse (**B**) and human (**D**). The mouse's terminal respiratory units do not have respiratory bronchioles; therefore terminal bronchioles (TB) open directly into alveolar ducts (AD). By comparison, the human's terminal respiratory units have respiratory bronchioles (RB), which open into alveolar ducts (AD) and air space (AS). Brl, bronchiole; PA, pulmonary artery. (Mouse and human lung tissue, 5-μm-thick paraffin-embedded sections, light microscopy.)

Key Points

- The primary function of the lung is ventilation-perfusion matching for efficient gas exchange between alveolar air and alveolar capillary blood.
- The anatomic arrangements of the pulmonary arteries beside the airways are a reminder of the relationship between perfusion and ventilation that determines the efficiency of normal lung function.
- The major physical problem of gas exchange is the slowness of oxygen diffusion through water. Thus the alveolar walls must be extremely thin. Because of that thinness, the thickness of the red blood cell forms a substantial portion of the air-blood diffusion pathway.
- The airways form the connection between the outside world and the terminal respiratory units; therefore the airways are of central importance to our understanding of lung function in health and disease.
- The terminal respiratory unit consists of all the alveolar ducts, together with their accompanying alveoli, that stem from the most proximal (first) respiratory bronchiole, and contains approximately 100 alveolar ducts and 2000 alveoli. The functional definition of the terminal respiratory unit is that, because gas-phase diffusion is so rapid, the partial pressures of oxygen and carbon dioxide are uniform throughout the unit.
- Smooth muscle cells form circular bands around the airway epithelium as far peripherally as the respiratory bronchioles. Tone in the smooth muscle is altered by the autonomic nervous system and by mediators released from mast cells, inflammatory cells, and neuroendocrine cells.
- Because smooth muscle is in the pulmonary vessels on both the arterial and the venous side down to precapillary and postcapillary vessels, any segment can contribute to active vasomotion and therefore pulmonary vascular resistance.
- Normally, capillary blood volume is equal to or greater than stroke volume. Thus, under normal resting conditions, the volume of blood in the pulmonary capillaries is well below its maximal capacity. Recruitment can increase capillary blood volume threefold.
- The endothelial cells of the pulmonary circulation manifest a remarkable number of metabolic activities.
- The type II cell is the major synthesizing and secreting factory of the alveolar epithelium and implements epithelial repair via its ability to proliferate.
- The clearance of particulate matter impinging on the alveolar surfaces is dependent on the slow turnover and movement of the alveolar surface liquid, as well as on the phagocytic function of the macrophages and the clearance function of the pulmonary lymphatics.

ACKNOWLEDGMENTS

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Complete reference list available at *ExpertConsult*.

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2 LUNG GROWTH AND DEVELOPMENT

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INTRODUCTION

Multicellular life requires the use of oxygen for the generation of high-energy compounds (e.g., adenosine triphosphate) to sustain the metabolic activities of complex organisms. Because multicellular organisms depend on oxygen, they have evolved systems for its efficient acquisition and distribution. A benchmark in the adaptation of vertebrates living on land was the development of a gas exchange system that provided a sufficient amount of oxygen to meet the metabolic requirements of cellular respiration. As organisms increased in size, the surface area required for adequate gas exchange became significantly larger; for example, the surface area of the adult human lung epithelium has been estimated to be 70 m^2.[1] The problem of generating such a large surface area in a confined space has been solved in the basic structure of the lung, where branched epithelial tubules conduct air to millions of alveoli that lie closely apposed to the lung microvasculature. The epithelium lining the surface of the lung is continuously exposed to biologic and chemical hazards from the environment, which has also necessitated the development of an innate defense system in the lung. Early embryologic experiments established that lung morphogenesis is critically dependent on reciprocal interactions between the lung endoderm and its surrounding splanchnic mesoderm, which supplies progenitors of endothelial cells, smooth muscle cells, mesothelial cells, and fibroblasts. As we will discuss, these interactions are complex and highly regulated in time and space. Disruptions in the lung developmental program, be it for genetic or epigenetic reasons, can lead to compromised structure and function. A better understanding of the molecular mechanisms controlling lung development will optimize therapeutic strategies to treat the diseased or malformed lung. Several additional recent reviews are also available.[2-6]

STAGES OF LUNG DEVELOPMENT

Lung development has traditionally been divided into five stages that are primarily based on histologic appearance (Fig. 2-1). After lung bud formation the basic branching pattern of the pulmonary tree and an associated vascular plexus is established during the **embryonic** and **pseudoglandular** stages. The epithelial branching program, which is under genetic control, is stereotyped and uses three geometrically distinct local modes of branching that proceed in three different sequences.[7] Human lung development begins with the emergence of the laryngotracheal groove from the floor of the foregut endoderm during the fourth week of gestation. A few days later the caudal end of the primordium enlarges and bifurcates, giving rise to the left and right bronchial buds (see Fig. 2-1A). These buds elongate caudally during the fifth week of gestation, when a second round of branching takes place, resulting in three secondary buds in the right lung and two in the left. These buds will become the primary lobes of the left and right lung. A third round of branching gives rise to bronchial tubules that will become the bronchopulmonary segments in the mature lung. Concurrent with these events in the distal region, the cranial portion of the primordium gives rise to the trachea and larynx, which separate from the esophagus by the end of this stage. The lung epithelium at this stage is tall columnar and shows no morphologic evidence of differentiation. At the molecular level, however, some aspects of epithelial differentiation have already begun; for example, the most distal epithelial cells express messenger RNA for the lung-specific marker *surfactant protein* (SP) C.[8] The lung mesenchyme, which is derived from splanchnic mesoderm, is loosely organized at the beginning of this stage and appears to lack vascular structures. In situ hybridization studies probing for the *vascular endothelial growth factor* (VEGF) receptor FLK1 have demonstrated, however, that vascular precursors are closely apposed to the distal epithelium at the time of bud induction.[9] These cells form a vascular plexus (see Fig. 2-1B) by a process termed "vasculogenesis," wherein vessels are formed de novo by the organization of vascular precursors. By the end of the embryonic stage, pulmonary arteries and veins connect this plexus to the atria; the pulmonary arteries and veins grow into the lung by angiogenesis, with new branches arising from preexisting vessels.

Dichotomous and lateral branching of the lung epithelium continues during the **pseudoglandular** stage, which lasts from week 5 to week 17 of gestation. This results in the final pattern of the pulmonary tree, which comprises 22 to 23 generations of bronchial tubules. Terminal bronchioles branch distally to give rise to the acinar tubules and buds that will eventually form pulmonary acini in the adult

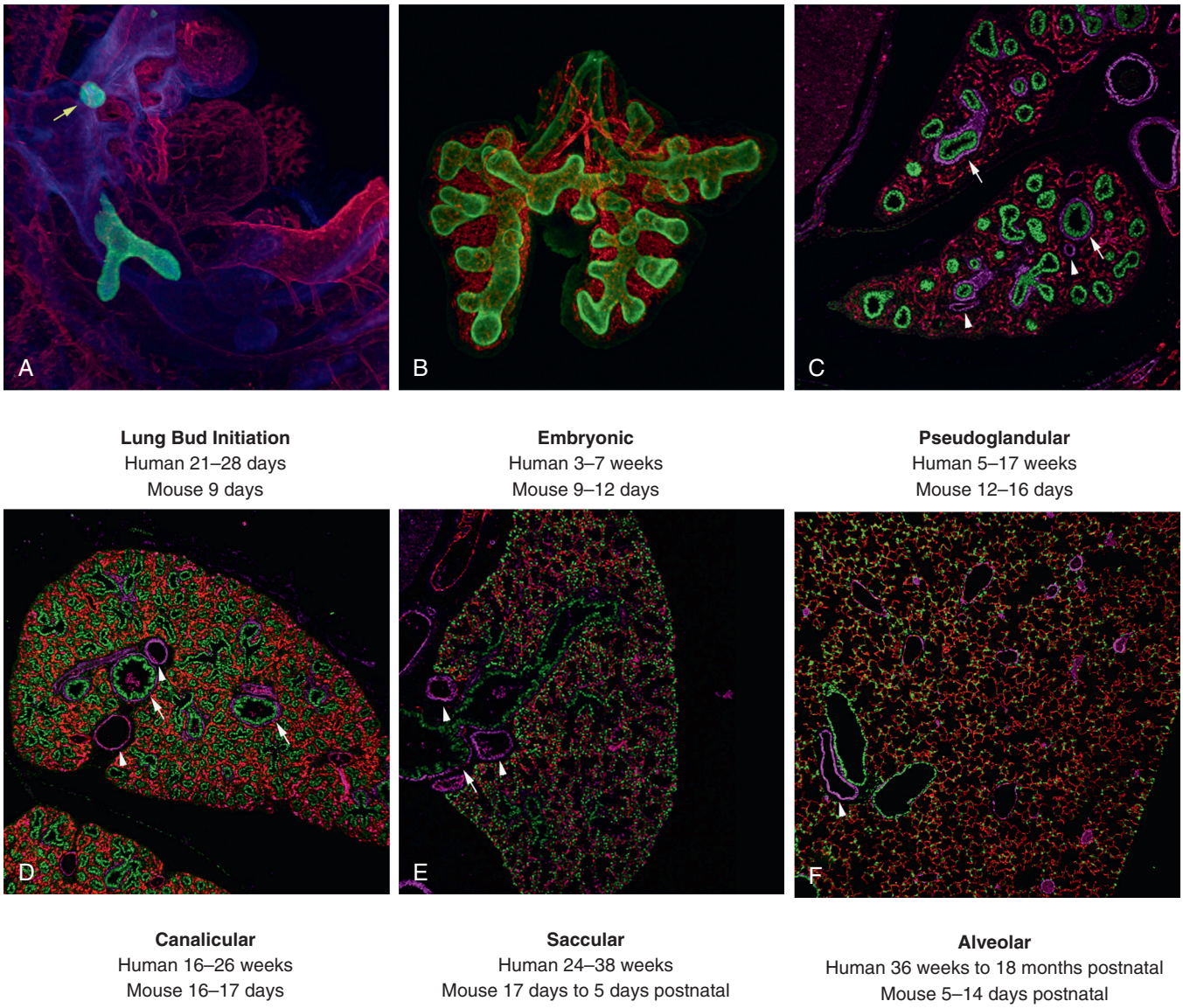

Figure 2-1 Morphology of the stages of lung development. Mouse lungs from the indicated developmental stages were stained with an antibody against Nkx2-1 to identify epithelial cells in the respiratory lineage (*green*), an antibody against endomucin to identify vascular cells (*red*), and an antibody against α-smooth muscle actin to identify smooth muscle cells (*magenta*). An antibody against E-cadherin identifies the foregut endodermal epithelium (*blue*) (**A**). All images of sectioned lungs (**C-F**) are at the same magnification. **A,** Lung buds originate as a pair of outpocketings from the ventral foregut endoderm on day E9.5 in the mouse; the lung endoderm stains positive for Nkx2-1, as does the primitive thyroid rudiment (*yellow arrow*). **B,** During the embryonic stage, dichotomous and lateral branching of the lung epithelium continues. Vascular precursors are already present and form a plexus surrounding the epithelium. **C,** During the pseudoglandular stage, the lung primarily consists of epithelial tubules surrounded by a relatively thick mesenchyme. Proximal epithelial cells show tall columnar morphologic characteristics, whereas more distal epithelial cells are cuboidal. The vasculature branches in parallel with the epithelium, and smooth muscle cells surrounding airways (*white arrows in all panels*) and vessels (*white arrowheads in all panels*) are evident. **D,** During the canalicular stage, epithelial acini appear, and the vasculature becomes more abundant and closely apposed to the epithelium. **E,** During the saccular stage, type I cell differentiation increases air space size. The vasculature has continued to expand, fully investing the lung parenchyma. Fusion of the epithelial and endothelial basal laminae brings capillaries and type I epithelial cells into close association. **F,** During the alveolar stage, the formation, lengthening, and thinning of secondary septa markedly increase the epithelial surface area. The capillaries, which until now have existed as a double septal network, have fused into one. (Confocal images generated by Jamie Havrilak, Graduate Program in Molecular and Developmental Biology, Cincinnati Children's Hospital Medical Center.)

(see Fig. 2-1C). Morphologic differences in the epithelium are apparent. The proximal epithelium is initially populated by relatively undifferentiated columnar, glycogen-rich cells, but ciliated, nonciliated, goblet, mucous, basal, and neuroendocrine cells are identifiable by the end of this stage. The distal epithelium is populated by distal epithelial cells, the precursors of alveolar type II cells, which are cuboidal columnar and contain copious amounts of glycogen. Smooth muscle cells differentiate in the mesenchyme and surround the epithelium perpendicular to the long axis of the tubules; this proceeds in a proximal-to-distal manner. The pulmonary vasculature branches in parallel with the

airway epithelium (see Fig. 2-1C), and pulmonary lymphatics initiate as buds from the veins.[10]

Patterning of the pulmonary tree is completed at the beginning of the **canalicular** stage (16 weeks to 26 weeks; see Fig. 2-1D), and the cells constituting the proximal epithelium continue to differentiate as ciliated, nonciliated, and secretory cells. Among the latter are club cells (Clara), identifiable by the presence of the cell-specific *club cell secretory protein* (*Clara*) (CCSP). Acinar tubules and buds, which are lined by cuboidal epithelial cells, expand and differentiate to form pulmonary acini consisting of respiratory bronchioles, alveolar ducts, and alveoli. Nascent type II cells containing increasing amounts of surfactant-associated proteins and phospholipids become prominent in the distal epithelium. Differentiation of squamous type I cells from type II cells begins. A dramatic expansion of the pulmonary capillary bed (vascular canals) in the lung parenchyma gives this stage its name (see Fig. 2-1D). These vessels surround the developing acini and come in direct contact with the epithelium, giving rise to the primordial air-blood barrier.

During the **saccular** stage, which persists from week 24 until term, the terminal acinar tubules in the lung periphery continue to branch and air space size increases. Alveolar type II cells undergo significant maturation, as evidenced by increased synthesis of SP-A, SP-B, SP-C,[11] and SP-D[12] and of surfactant phospholipids.[13] Glycogen stores, which serve as a substrate for phospholipid synthesis,[14] decrease, while the number of lamellar bodies increases. Squamous type I cells continue to differentiate and constitute an increased proportion of the distal lung surface, thereby increasing the effective area for gas exchange (see Fig. 2-1E). Septal walls consist of a central connective tissue core with a capillary network on each side. Subsequent fusion of the basal laminae of the distal epithelium and endothelium brings capillaries into close association with type I cells, which decreases the diffusion distance between air spaces and capillaries to allow more efficient gas exchange (see Fig. 2-1E).

The transition from the canalicular to saccular stage of lung development marks the threshold of viability for preterm infants who have access to neonatal intensive care support.[15,16] Before 22 weeks' gestation there is insufficient surface area in the distal pulmonary tree to support safe, reliable oxygenation and ventilation, even when surfactant replacement therapy and sophisticated mechanical ventilation techniques are available. Survival at 23 weeks' gestation ranges from 15% to 30%. Mortality decreases with each additional week of gestation; by 25 weeks, survival exceeds 60%, although significant morbidity in the form of *bronchopulmonary dysplasia* (BPD) and neurodevelopmental compromise persists.[17]

As the threshold of viability is crossed, *respiratory distress syndrome* (RDS) becomes the primary source of morbidity and mortality for the preterm infant. RDS is a consequence of deficient surfactant production, leading to terminal airway atelectasis and epithelial injury. The subsequent capillary leak produces the hyaline membranes that are classically associated with this disease. Surfactant replacement therapy has dramatically improved RDS survival rates and reduced morbidity. Term infants affected by RDS often have comorbid conditions such as maternal diabetes, which delay maturation of the surfactant production system.

Genetic mutations leading to SP-B deficiency result in a clinical presentation indistinguishable from the early stages of RDS. Affected infants, however, are typically full term and have only a transient response to surfactant replacement therapy, which leads to early neonatal death or the development of severe neonatal chronic lung disease. The only definitive treatment is lung transplantation. Lethal respiratory failure in a mouse model of SP-B deficiency can be reversed with targeted expression of an *SPB* transgene, demonstrating the potential for gene therapy.[18] Mutations of *SPC* produce a spectrum of pulmonary disorders during infancy, including interstitial pulmonary fibrosis.[19] As with SP-B deficiency, surfactant replacement therapy has little or no benefit, with lung transplantation as the only documented potential cure. Other genetic defects of the surfactant production system also lead to fatal surfactant deficiency in the neonate. Infants deficient in ABCA3, which transports surfactant phospholipids to lamellar bodies, have normal SP-B expression but develop unexplained lethal respiratory failure and death within 1 month of birth.[20]

The final stage of lung development is the **alveolar** stage, which lasts from week 36 of gestation through the first 18 months of postnatal life. As the name implies, true alveoli are generated from terminal saccules during this stage. Interstitial tissue in primary septa is reduced, while secondary septa markedly lengthen and thin (see Fig. 2-1F). Concomitant with these changes is the fusion of the double septal capillary network into one (see Fig. 2-1F). This remodeling requires an initial burst of interstitial fibroblast proliferation, which subsequently slows down, and the cells synthesize increased amounts of collagen and elastin. Septation results in a marked increase in the number of alveoli from approximately 30 million at term to 300 million in the adult. Increased numbers of type II and type I cells accompany alveolar expansion, with type I cells now covering 95% of the alveolar surface area.[21]

BPD, which is typically restricted to infants born before 32 weeks' gestation, represents a particularly challenging complication.[21a] The condition is only associated with preterm birth and is defined by a characteristic appearance on chest radiograph and persistent requirement for supplemental oxygen beyond 36 weeks after conception.[22] In the era of surfactant replacement therapy, BPD is distinguished by alveolar simplification due to the apparent arrest of the alveolarization during the third trimester.[23] Compromised oxygenation and ventilation may worsen as infant somatic growth progresses and attendant metabolic demands outstrip pulmonary function. Respiratory morbidity is not restricted to infants born before 32 weeks' gestation. Late-preterm infants born between 32 and 37 weeks' gestation are more likely than term infants to require respiratory support, including positive-pressure ventilation, after birth.[24] Given the dramatic increase in alveolar number during the late third trimester, it follows that late-preterm infants may have a smaller margin of safety when making the transition to extrauterine life.

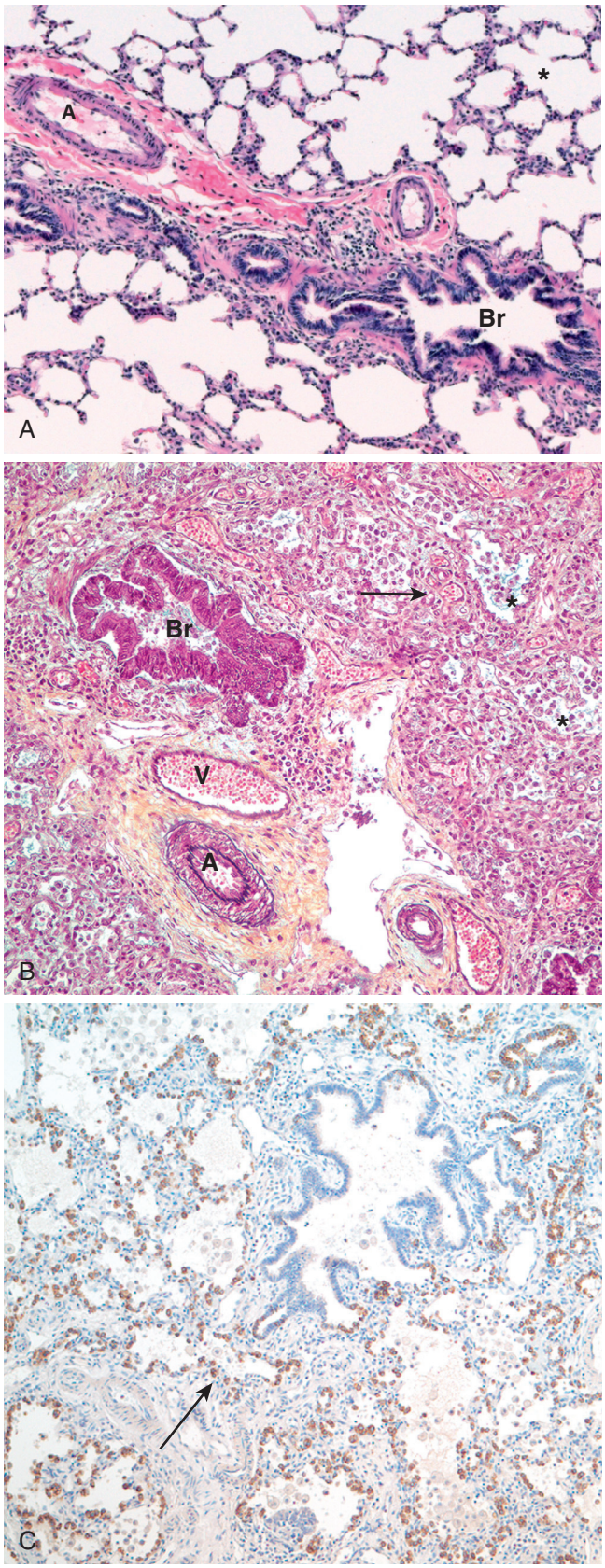

Figure 2-2 Histopathologic features of human alveolar capillary dysplasia and extralobar pulmonary sequestration. A, Section of normal infant lung stained with hematoxylin and eosin demonstrates a typical bronchovascular bundle incorporating a small bronchus (Br) and artery (A), without an accompanying vein. A typical alveolar network with abundant air spaces (*asterisk*) is present. **B,** Pentachrome-stained section from a full-term newborn with alveolar capillary dysplasia. There is abundant mesenchyme separating rudimentary, dysplastic terminal air spaces (*asterisk*). Alveolar capillaries (*arrow*) are sparse in number and distended. There is misalignment of the pulmonary vasculature. A pulmonary artery (A) and prominent muscularis and bronchus (Br) are accompanied by an anomalous pulmonary vein (V). The paucity of alveolar capillary structures accounts for the profound pulmonary hypertension in these patients. (×10 original magnification.) **C,** Immunohistochemistry of extralobar pulmonary sequestration from a full-term neonate demonstrates expression of prosurfactant protein C in the epithelium (*brown-staining cells*). The sequestration has the histologic appearance of primitive lung at the canalicular stage of development with an abundance of mesenchyme separating nascent air space structures (*arrow*). (Micrographs courtesy of Dr. Susan Wert, Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, and Dr. Gail Deutsch, Division of Pathology, Seattle Children's Hospital.)

TISSUE INTERACTIONS AND LUNG DEVELOPMENT

A basic tenet of lung development is that it requires inductive interactions between the endodermal epithelium and the mesodermal mesenchyme. Reciprocal inductive interactions involve one cell type signaling to another cell type and then responding to signals sent back; both cell types are thus signaling and responding to each other. This is particularly evident in the embryonic and pseudoglandular stages, where it has been shown conclusively that lung epithelium must be associated with lung mesenchyme in order to survive[25] and branch.[26] The factors that drive branching morphogenesis are diffusible, because embryonic lung epithelium branches when separated from lung mesenchyme by a filter that prevents direct cell-cell contact but allows diffusion of soluble factors.[27] Importantly, these experiments also showed that survival of lung mesenchyme is dependent on the presence of lung epithelium, underscoring that induction is reciprocal. The fate of the entire respiratory endoderm, from the trachea to the bud tips, however, is not fully committed during the embryonic stage. Reciprocal recombination experiments have shown that distal lung mesoderm can reprogram tracheal endoderm to branch and differentiate like lung,[28,29] and that tracheal mesoderm can reprogram lung endoderm to differentiate like trachea.[30,31]

Bronchopulmonary sequestration may represent an intriguing manifestation of aberrant pulmonary endoderm-mesoderm interaction. These masses of abnormal lung tissue, which may be contained within the lung or in an extrapulmonary location within the abdomen, may be in direct communication with gastrointestinal tract structures, suggesting ectopic induction of embryonic foregut. The histopathologic appearance of these lesions includes typical cellular components of pulmonary parenchyma along with inflammatory and fibrotic components (Fig. 2-2C).[32]

Normal lung function requires the precise alignment of the distal epithelium and the vasculature to meet the

respiratory requirements of the developing organism. Certain lethal congenital malformations of the lung, such as *alveolar capillary dysplasia with misalignment of pulmonary veins* (ACD/MPV), are due to a perturbation in the relationship between vascular and airway development. ACD/MPV is characterized by a paucity of alveolar capillaries, thickened pulmonary mesenchyme, and misalignment of the pulmonary veins, which reflect the reciprocal relationship required for airway and vascular development (see Fig. 2-2B). ACD/MPV is associated with mutations in the transcription factor FOXF1, which is expressed in the lung mesenchyme.[33] The alveolar simplification of BPD is also accompanied by a relative paucity of alveolar capillaries that is reminiscent of ACD/MPV. Evidence that *vascular endothelial growth factor A* (VEGF-A), which is produced by lung epithelial cells, can reverse the alveolar defect further reinforces the concept of interdependent development of vascular and airway structures.[34]

MOLECULAR REGULATION OF LUNG DEVELOPMENT

Elucidation of the factors that regulate lung growth and development has been the focus of an intense research effort.[34a] This stems not only from a desire to understand the basis of pulmonary pathologic conditions present at birth, but also from the possibility that understanding how the lung develops will provide insight into how the lung repairs itself following injury or disease. Given the morphogenetic precision required to generate a lung that can function effectively in gas exchange, coupled with the fact that the lung contains over 40 differentiated cell types,[1] it is not surprising that the molecular regulation of lung development is proving to be very complex. Identifying the factors involved provides only part of the story. When, where, how much of, and for how long these factors are expressed must also be considered. The fact that there is crosstalk between some of the identified pathways significantly increases the level of complexity.

DIFFUSIBLE MEDIATORS OF LUNG DEVELOPMENT

Fibroblast Growth Factors and Fibroblast Growth Factor Receptors

In both humans and mice, the *fibroblast growth factor* (FGF) family comprises 22 structurally related molecules[35]; among these, FGF1, 2, 7, 9, 10, and 18 have been localized to the developing lung. FGFs bind and signal through high-affinity, ligand-dependent transmembrane receptors (*fibroblast growth factor receptors* [FGFRs]) that contain an intracellular tyrosine kinase domain. There are four FGFRs, all of which are expressed in the lung. Alternative messenger RNA splicing results in two isoforms each for FGFR1, FGFR2, and FGFR3 that have distinct ligand specificities.[36] FGFR activation is modulated by heparin or heparan sulfate.[37]

FGF1 and *FGF2* are not critical for lung development, because the single deletion of either gene or the double ablation of both has no effect on lung development. FGF10 is an ideal candidate for mediating tissue interactions in the lung, because it is expressed in the mesenchyme, whereas its primary receptor, FGFR2b, is expressed by epithelial cells. Ablation of either *FGF10*[38] or *FGFR2b*[39] results in complete pulmonary agenesis caudal to the trachea. The basis for this phenotype comes from the ability of FGF10 to induce lung epithelial budding by chemoattraction[40,41]; in the absence of FGF10, primary buds cannot form. FGF10 affects the expression of many target genes in early lung epithelium,[42] including other important signaling molecules such as *bone morphogenetic protein 4* (BMP4)[40] and Notch family members.[43]

Like FGF10, FGF9 acts as a mediator of reciprocal tissue interactions, because it is expressed in the epithelium and mesothelium, whereas its receptor (FGFR2c) is found in the mesenchyme. FGF9 controls lung mesenchyme size by regulating cell proliferation.[44] The observation that the amount of available mesenchyme appears to control lung branching[25] is consistent with the finding that the lungs of *Fgf9*-null mice are severely hypoplastic, with decreased amounts of mesenchyme and reduced Fgf10 expression.[45] The observation that *Fgf18*-null mice exhibit reduced alveolar size resulting from reduced cell proliferation during the saccular stage[46] suggests a role for FGF18 in late lung development. FGF7 has been shown to stimulate lung epithelial cell proliferation,[47] as well as surfactant protein gene expression and surfactant phospholipid synthesis in type II cells.[48,49] Transgenic overexpression of Fgf7 in the developing mouse lung epithelium results in lesions resembling congenital cystic adenomatoid malformations[50]; examination of human congenital cystic adenomatoid malformations, however, shows that FGF7 expression is actually decreased and FGF10 expression is unchanged.[51] Although intraperitoneal injection of neutralizing antibodies against Fgf7 inhibits postnatal lung growth and alveolus formation,[52] mice with a targeted deletion of *Fgf7* have no apparent lung phenotype.[53] Other FGFs are also required during alveologenesis, because mice with deletions of both *Fgfr3* and *Fgfr4* fail to form normal alveoli.[54]

The Sprouty (SPRY) proteins, which antagonize FGFR signaling, modulate the effects of FGFs in the developing lung. Although single deletion of either *Spry2* or *Spry4* has no effect on lung development, mice null for both genes have defects in multiple organs, including the lung.[55]

Retinoic Acid

Retinoic acid (RA), the active derivative of vitamin A, is essential for the normal development of many tissues, including the lung. Maternal vitamin A deficiency results in severe respiratory phenotypes in offspring, including tracheoesophageal fistula, lung hypoplasia, and lung agenesis.[56] RA signals through RAR and RXR nuclear receptors, both of which have α, β, and γ isoforms, and these are expressed in the lung from the outset of development. Mice with double deletions of *Rara*/*Rarb* or *Rara*/*Rxrb* show the same lung abnormalities as those seen in vitamin A–deficient embryos.[57] The mechanism by which RA controls lung morphogenesis is not fully resolved. Data from cultured early embryonic foreguts suggest that RA allows activation of *Wingless* (Wnt) signaling by inhibiting *Dickkopf1* (DKK1); this affects FGF10 expression in the mesoderm, as

well as maintenance of lung progenitor cell fate.[58] RA further affects lung bud induction by inhibiting *transforming growth factor-β* (TGF-β) activity in the prospective lung field, which in turn allows expression of FGF10.[59]

RA also enhances perinatal alveolus formation in rodents,[60] which has led to its clinical use for the prevention of BPD.[61] The effect is modest but significant; about 15 infants must be treated to prevent one case of BPD.[62] The mechanism is not understood in detail but is likely related to maintenance of alveolarization after preterm delivery, reducing the potential for alveolar simplification.

Sonic Hedgehog

The hedgehog signaling pathway plays an important role in the development of multiple organs.[63] *Sonic hedgehog* (SHH) is highly expressed in the developing lung epithelium,[64] and its primary receptor, *patched 1* (PTCH1), is found in mesenchymal cells,[65] suggesting that SHH is part of an epithelial-mesenchymal inductive loop. Shh is initially expressed throughout the epithelium but becomes restricted to subsets of cells from day E16.5 onward.[66] *Shh*-null mice form lungs, indicating that Shh is not required for lung specification and bud induction; however, these lungs are severely hypoplastic,[67] suggesting that Shh is involved in regulating branching morphogenesis. *Shh* deletion profoundly affects lung growth and patterning, but the specification of epithelial cell types appears to be unaffected.[68] Because Shh serves as a survival factor for lung mesenchymal cells,[69] the lung hypoplasia seen in *Shh*-null embryos may be due to a decrease in mesenchymal mass. Shh is also a negative regulator of Fgf10, and *Shh*-null embryos exhibit expanded Fgf10 expression.[68] A clinical syndrome with a respiratory phenotype that is consistent with disruption of SHH signaling is Smith-Lemli-Opitz.[70] Smith-Lemli-Opitz syndrome is phenocopied by mutations in Δ-7-dehydrocholesterol reductase (*DHCR7*), which is involved in cholesterol synthesis[71]; cholesterol modification of SHH is required for effective signaling.[72,73]

SHH levels are modulated by its binding to PTCH1.[73] In the absence of ligand, PTCH1 represses *Smoothened* (SMO) and prevents activation of the hedgehog signaling pathway. SHH also up-regulates PTCH1 expression, and any PTCH1 in excess of that involved in controlling signaling binds SHH and sequesters it, creating a negative feedback loop that restricts its spread. Another molecule regulating SHH levels is *hedgehog interacting protein* (HHIP), a membrane-bound protein that binds all mammalian hedgehog proteins and, like PTCH1, is up-regulated in response to SHH.[74] Targeted deletion of *HHIP* results in lung hypoplasia[75] that may be due to a loss of FGF10 expression at the prospective sites of bud formation as a result of increased SHH signaling.

Transforming Growth Factor-β Superfamily

The TGF-β superfamily comprises activins, inhibins, the BMPs, müllerian inhibiting substance, and TGF-β1, 2, and 3. TGF-β1 treatment of cultured embryonic lung explants[76] or misexpression of TGF-β1 targeted to the lung in vivo[77] severely inhibits branching morphogenesis. This is likely due to the ability of TGF-β1 to inhibit FGF10 expression.[59] TGF-β1 signals through a heteromeric complex of type I (TGF-βrI) and type II (TGF-βrII) receptors and exerts its effects on downstream target genes via the Smad family of proteins.[78] Inhibition of TGF-βrII in cultured embryonic lungs increases lung branching,[79] as does attenuation of SMAD2/3,[80] underscoring the inhibitory nature of TGF-β1 on lung morphogenesis. *Tgfb1*-null mice show no apparent lung phenotype, although it should be noted that 50% of these mice die on E10.5, just after the onset of lung development.[81] Most *Tgfb2*-null mice die shortly before or during birth with a wide range of developmental defects. The lungs of neonates have dilated conducting airways and collapsed terminal and respiratory bronchioles.[82] Deletion of *Tgfb3* results in retarded development and differentiation of the lung epithelium, mesenchyme, and vasculature.[83] The fact that TGF-β3 appears to promote morphogenesis contrasts with the inhibitory function of TGF-β1, suggesting that these ligands affect distinct aspects of lung development.

Of the four BMPs expressed in the developing lung (BMP3, 4, 5, and 7), BMP4 has been the focus of the most studies. Bmp4 is expressed in the ventral foregut mesenchyme before lung bud induction and then is expressed in the distal epithelium and proximal mesenchyme after the lung has formed. In the mouse, epithelial expression declines in the distal epithelium before birth but begins in the capillary endothelium. Bmp4 expression is up-regulated by Fgfs in the epithelium and by Shh in the mesenchyme. Specific deletion of *Bmp4* or *BMP receptor 1a* (*Bmpr1a*) from the distal lung epithelium results in reduced proliferation, increased apoptosis, and cystic morphogenesis.[84] Early endodermal deletion of both *Bmpr1a* and *Bmpr1b* results in reduced ventral Nkx2.1 expression, which is replaced by expanded expression of dorsal Sox2.[85] These data support a model in which BMP4 promotes the proliferation and survival of undifferentiated lung progenitor cells.

Wnts and β-Catenin

Members of the Wnt family of secreted glycoproteins are critically involved in cell fate determination, proliferation, survival, and motility in organogenesis.[86] Wnt ligands bind their receptors to activate a pathway that ultimately stabilizes β-catenin, which then interacts with nuclear *T-cell factor/lymphoid enhancer factor* (TCF-LEF) transcription factors to modulate transcription of downstream target genes.[87] Wnts1, 2, 2b, 5a, 7b, and 11 are expressed in the lung. Their secretion is mediated by the transmembrane protein *Wntless* (WLS); deletion of *WLS* from the lung endoderm disrupts branching morphogenesis and pulmonary endothelial differentiation.[88] Canonical Wnt signaling plays a critical role in lung development, because endodermal deletion of β-catenin abrogates specification of lung progenitors and leads to complete lung agenesis.[89] The ligands responsible for lung progenitor specification are likely Wnt2/2b, because their dual deletion phenocopies exactly the endodermal loss of β-catenin.[90] Proximal-distal airway patterning and epithelial cell differentiation are disrupted when Wnt signaling is inhibited after specification of lung progenitors, either by targeted epithelial deletion of β-catenin[91] or by misexpression of the Wnt antagonist Dkk1.[92] In addition to its role in specifying lung endoderm, Wnt2 also activates a signaling network necessary for smooth muscle differentiation.[93] Inactivation of Wnt5a results in a foreshortened trachea, distended distal airways, and retarded lung maturation.[94] Mice null for *Wnt7b* die at birth from respiratory failure. Early proliferation is reduced

in both epithelial and mesenchymal tissue compartments, leading to lung hypoplasia, although cell fate specification and overall tissue architecture are unchanged.[95] Constitutive activation of Wnt signaling in the developing lung epithelium with hyperactive β-catenin results in lungs that lack fully differentiated cell types and instead contain multiple intestinal and nonlung secretory cell types.[96] Taken together, these observations indicate that the temporospatial regulation of Wnt signaling must be tightly regulated to ensure normal lung morphogenesis and differentiation.

Platelet-Derived Growth Factor

The *platelet-derived growth factor* (PDGF) family consists of five different disulphide-linked dimers built up of four different polypeptide chains encoded by four different genes. PDGF-A, which homodimerizes with itself or heterodimerizes with PDGF-B, plays an important role in lung development. PDGF-A is expressed in distal lung epithelium, whereas its receptor, PDGFRA, is expressed in nearby mesenchymal cells, indicative of a paracrine signaling loop between the epithelium and mesenchyme. Deletion of *PDGF-A* results in arrested alveolus formation and postnatal death.[97] The lungs lack the differentiated alveolar myofibroblasts that produce elastin, which is critical for alveolus formation.

Vascular Endothelial Growth Factor

VEGF-A, C, and D are all found in the lung. The temporal and spatial expression of VEGF-A during lung development implies a central role in the maturation and organization of the pulmonary vascular network. VEGF-A is expressed in epithelial and mesenchymal compartments during the embryonic and pseudoglandular stages, becoming more restricted to the epithelium as development progresses into the canalicular stage.[9,98] VEGF-A exists as three isoforms (120, 164, and 188) that have distinct functions in vascular development.[99] Genetic studies in mice demonstrate the importance of local tissue concentrations of Vegf-a to effect appropriate vascular development and distal airway structures. Increased expression of Vegf164 in distal epithelium disrupts assembly of the vascular plexus and arrests airway branching without affecting endothelial cell proliferation or survival, indicating that crosstalk between the developing epithelium and vasculature is required for normal morphogenesis.[100] Vascular ablation in the early lung causes significant alterations in stereotypic branching of the epithelium.[101] VEGF-A expression is controlled by multiple mediators, such as FGFs and SHH.[102]

Glucocorticoids

Glucocorticoids exert potent effects on a variety of different tissues, with a common theme that they induce the precocious appearance of normal developmental events. The effects of glucocorticoids on lung function have been a topic of intense interest since the observation that dexamethasone accelerates lung maturation in premature lambs.[103] Glucocorticoid receptors are present on the developing pulmonary epithelium as airway branching progresses during the pseudoglandular stage of lung development. Epithelial expression persists through the saccular and alveolar stages, accompanied by the onset of expression within the mesenchymal compartment.[104] Exogenous glucocorticoids stimulate morphologic maturation and many aspects of surfactant phospholipid biosynthesis.[105] Targeted disruption of the glucocorticoid receptor in mice leads to respiratory distress and early neonatal death[106]; the lungs of these animals are atelectatic with blunted alveolarization. Although the number of type II cells is increased by 30%, the relative expression of Sp-a and Sp-c is decreased by 50%. The number of type I cells is decreased by 50%, as are the type I cell markers T1α and aquaporin-5, suggesting that glucocorticoids facilitate the differentiation of type II cells into type I cells.[107] Somewhat paradoxically, however, mice null for corticotropin-releasing hormone show deficits in septal thinning, air space formation, and content of Sp-a and Sp-b but have no deficit in surfactant phospholipid biosynthesis.[108]

Given the broad distribution of pulmonary glucocorticoid receptors in the developing lung, it is not surprising that the therapeutic effects of glucocorticoid treatment are complex. Clinical experience suggests that glucocorticoids have contrasting biologic effects depending upon whether treatment is directed toward the fetal lung or the preterm neonatal lung. Women in preterm labor are routinely treated with glucocorticoids to reduce the incidence and severity of neonatal RDS.[109] Antenatal steroid treatment accelerates fetal lung maturation by inducing mesenchymal thinning and enhancing pulmonary function, presumably through stimulation of surfactant production. Morphometric studies in sheep suggest that antenatal steroid treatment may also induce some blunting of alveolarization.[110] Glucocorticoid treatment has also been employed to treat preterm infants experiencing severe BPD. Although early studies and anecdotal reports suggested that steroid treatment could reverse the fibrosis and scarring associated with BPD and significantly improve pulmonary mechanics,[111] subsequent studies demonstrated no clear improvement in long-term pulmonary outcome and increased risk for neurodevelopmental impairment.[112]

TRANSCRIPTIONAL REGULATION OF LUNG DEVELOPMENT

The diffusible molecules mediating tissue interactions in the developing lung initiate signaling cascades that lead to changes in gene expression. The diversity of cell types found in the lung, which all differentiate under tight spatial and temporal control, makes regulation of gene expression by transcription factors in the developing lung highly complex. Although no lung-specific transcription factors have yet been found, research over the last decade has identified several transcription factors in addition to those described earlier that are crucial to normal lung development.

NKX2-1

NKX2-1 (also known as *"thyroid transcription factor 1"* [TTF1]) is found in the presumptive respiratory region of the foregut endodermal epithelium before lung bud induction. NKX2-1 is expressed in the forebrain, thyroid, and lung, where it interacts with multiple partners to influence several key aspects of development.[113] Mice null for *Nkx2-1* develop tracheoesophageal fistulas, with main-stem bronchi connecting to hypoplastic, cystic lungs.[114] Whereas differentiation of the most proximal epithelium is somewhat preserved in *Nkx2-1*–null lungs, markers of distal epithelial

differentiation, including the surfactant proteins, are completely lacking. Haploinsufficiency for the *NKX2-1* gene in humans leads to brain-lung-thyroid syndrome, which is characterized by benign hereditary chorea, respiratory disease, and congenital hypothyroidism.[115-118] The respiratory phenotypes include RDS at birth, as well as recurrent pulmonary infections and interstitial lung disease later in childhood. The control of NKX2-1 expression in lung development is not fully understood.

GLI Genes

Three *GLI* genes (1, 2, and 3) code for zinc finger transcription factors that are the principal effectors of hedgehog signaling. All three *Gli* genes are expressed in distinct but overlapping domains in lung mesenchyme, with expression being highest in the distal tips.[119,120] The analysis of compound mutant mice has demonstrated the complexity of how *Gli* genes affect lung development. Embryos expressing different combinations of *Gli* genes show a range of lung defects, the most striking of which is the absence of lungs, trachea, and esophagus in $Gli2^{-/-}$, $Gli3^{-/-}$ compound mutants.[121] The presence of a single *Gli3* allele ($Gli2^{-/-}$, $Gli3^{+/-}$) is sufficient to allow formation of hypoplastic lungs in which the left and right lungs do not separate, and the embryos have tracheoesophageal fistulas. The phenotype seen in *Gli2*/*Gli3* double-null embryos is more severe than that seen in *Shh*-null animals; this suggests that the *GLI* genes may lie downstream in signaling pathways other than SHH, or that the other hedgehog proteins (Indian and desert) may be active in the lung. Mutations in the human *GLI3* gene cause Pallister-Hall and Greig syndromes, which affect development of several organ systems, including the lung.[122]

FOX Family

The FOX family of transcription factors contains more than 50 members, all of which share a winged-helix DNA binding domain. FOXA1 and FOXA2 are closely related proteins found in the foregut endoderm and its derivatives. Their spatial and temporal expression patterns are similar in the lung. Mice lacking *Foxa2* do not form endoderm and hence cannot form lungs[123]; targeted deletion of *Foxa2* in lung epithelial cells, however, demonstrates that it is required for alveolarization and epithelial cell differentiation.[124] Deletion of *Foxa1* in mice delays some aspects of sacculation and alveolarization prenatally and perinatally, but these differences normalize by 2 weeks of age,[125] suggesting compensation by *Foxa2*. Deletion of both genes inhibits cell proliferation, branching morphogenesis, and epithelial cell differentiation,[126] indicating that *FOXA1/2* play a central role in lung development.

Foxa1 is expressed in lung mesenchyme and controls genes involved in epithelial-mesenchymal interactions, because a haploinsufficiency results in defective branching, lobation, and epithelial differentiation in the mouse lung.[127] In humans, *FOXF1* mutations are associated with ACD/MPV.[33] Foxj1 controls expression of left-right dynein, which is required for correct anchoring of basal bodies; deletion of *Foxj1* causes situs inversus, the loss of motile cilia in airway epithelial cells, sinusitis, and bronchiectasis.[128] Although these features are associated with Kartagener syndrome in humans, no mutations in the *FOXJ1* gene have been directly linked to this disorder. FOXP1 and FOXP2, which are known transcriptional repressors, are expressed in the lung epithelium; both genes are expressed distally, but only FOXP1 is expressed proximally. $Foxp2^{-/-}$ mice show impaired alveolarization, an effect exacerbated in compound mutant $Foxp2^{-/-}$, $Foxp1^{+/-}$ mice, which have hypoplastic lungs and die at birth. Foxp1 acts cooperatively with Foxp4 to restrict goblet cell specification, thereby regulating the balance of cell types in the airway epithelium.[129]

GATA6

GATA6, a zinc finger transcription factor that is required for visceral endoderm differentiation,[130] is the only GATA family member expressed in the distal epithelium of the developing lung. Mice bearing a dominant-negative Gata6/engrailed fusion protein under control of the Sftpc promoter show reduced numbers of proximal airway tubules. Lung epithelial cell differentiation is also affected, with these mice completely lacking detectable alveolar type I cells.[131] Loss of Gata6 in the lung epithelium causes a loss of differentiation and the precocious appearance of bronchioalveolar stem cells that is the result of increased Wnt signaling.[132] GATA6 regulates expression of WNT7B and also interacts with NKX2-1 to control expression of SP-A, B, and C.[113]

SOX Family

Members of the SOX family of transcription factors function as key regulators of cell fate and differentiation. Of the 20 known SOX proteins, SOX2, 4, 9, 11, and 17 are found in the developing lung. Sox2 is highly expressed in nonbranching epithelium but repressed by Fgf10 in epithelial cells that are actively invading the surrounding mesenchyme,[133] suggesting that silencing of Sox2 is required for the epithelium to branch. The repression of Sox2 by Fgf10 may be mediated by BMP signaling.[85] Overexpression of Sox2 in lung epithelial cells inhibits lung branching by forcing the cells to commit prematurely to a differentiation program, thereby rendering the cells incompetent to respond to branching signals.[134] Sox11 is also expressed throughout the developing lung epithelium, and mice null for *Sox11* have significant lung hypoplasia.[135] Sox17 expression in the lung is dynamic, being first detected in the mesenchyme during the embryonic stage, then in the conducting airway epithelium during the canalicular stage. Because its misexpression in the distal epithelium disrupts branching and causes the ectopic expression of proximal airway markers, Sox17 is thought to play a key role in specifying differentiation of airway epithelial cells.[136]

POSTTRANSCRIPTIONAL GENE REGULATION IN LUNG DEVELOPMENT

Micro-RNAs (miRNAs) are small noncoding RNA molecules that modulate physiologic and pathologic processes by inhibiting gene expression through RNA translation repression or messenger RNA degradation. Functionally mature miRNAs are generated by a series of ribonuclease III cleavage steps. Key enzymes in miRNA biogenesis include DROSHA, which cleaves primary miRNAs into precursor miRNAs in the nucleus, and DICER1, which cleaves precur-

sor miRNAs to the mature form in the cytoplasm. Mature miRNAs are incorporated into the large multiprotein RNA-induced silencing complex, which represses RNA translation or induces messenger RNA degradation. miRNAs regulate key biologic processes important in lung development, including cellular proliferation, apoptosis, and differentiation. miRNA profiling reveals that the lung has a specific miRNA expression profile that is conserved across species (including mouse and human) and regulated specific to developmental stage, sex, and cell type.[137-140] miRNAs have a critical role in controlling organogenesis. Loss- and gain-of-function studies, as well as differing expression profiles between patients or animal models with lung disease and normal controls, implicate miRNAs in the pathogenesis of many lung diseases, including chronic obstructive pulmonary disease, lung cancer, pulmonary inflammatory disease, idiopathic pulmonary fibrosis, asthma, and cystic fibrosis (for reviews see references 141 to 144). Additionally, studies in model systems have identified a critical role for miRNA-mediated regulation in lung development. Inactivation of Dicer1, a key enzyme in miRNA biogenesis, targeted to the developing lung epithelium results in neonatal death because of arrested airway-branching morphogenesis, increased cell death, and altered expression of critical epithelial-mesenchymal signaling molecules.[145] Specific miRNAs that have been identified to influence lung development include the miR302/367 cluster that directs lung endoderm development by coordinating proliferation, differentiation, and apical-basal polarity of lung progenitor cells,[146] the miR17-92 cluster that is required for lung growth as well as for promoting proliferation and inhibiting differentiation of lung epithelial progenitor cells,[147,148] miR127, which regulates terminal bud size and number,[149] and miR221 and miR130a, which have opposing effects on airway and vascular morphogenesis.[137] The recent discovery of heterozygous germline loss-of-function *DICER1* mutations in familial *pleuropulmonary blastoma* (PPB), a rare pediatric lung tumor that often arises during lung development, provides evidence that the *DICER1*/miRNA pathway controls human lung development and suppresses tumorigenesis.[150] The majority of carriers with *DICER1* mutations are phenotypically normal, suggesting that loss of one *DICER1* allele is compatible with normal development and insufficient for tumor formation. PPB is composed of both epithelial and mesenchymal cells.[151,152] In a subset of patients, overgrowth of the mesenchymal cells results in a sarcoma that is associated with a poorer prognosis. Interestingly, the protein DICER1was found to be lost in the epithelial tumor component but retained in the mesenchymal cells by immunohistochemistry, suggesting that loss of DICER1 specifically in the lung epithelium promotes PPB formation.[150] Consistent with this notion, *Dicer1* gene ablation targeted to the developing murine pulmonary epithelium results in a PPB-like phenotype (Fig. 2-3). Because PPB often arises in the setting of an inherited tumor predisposition syndrome characterized by increased incidence of other neoplasms, including cystic nephroma, ovarian sex cord–stromal tumor, embryonal rhabdomyosarcoma, and multinodular goiter, the DICER1/miRNA pathway functions that control lung development are probably also operative in other organs.[153-155]

Key Points

- The lung epithelium begins as two buds from foregut endoderm. Subsequent branching morphogenesis and alveolarization leads to a mature organ containing over 300 million alveoli.
- The pulmonary vasculature develops in parallel with the branching epithelium.
- Lung development requires reciprocal interactions between the epithelium and mesenchyme derived from splanchnic mesoderm.
- Tissue interactions are mediated by an array of diffusible signaling molecules. Variations in the temporal and spatial expression of these mediators add complexity to these interactions.
- Diverse classes of transcription factors that lie downstream of diffusible mediators further regulate morphogenesis and effect the differentiation of individual cell types.

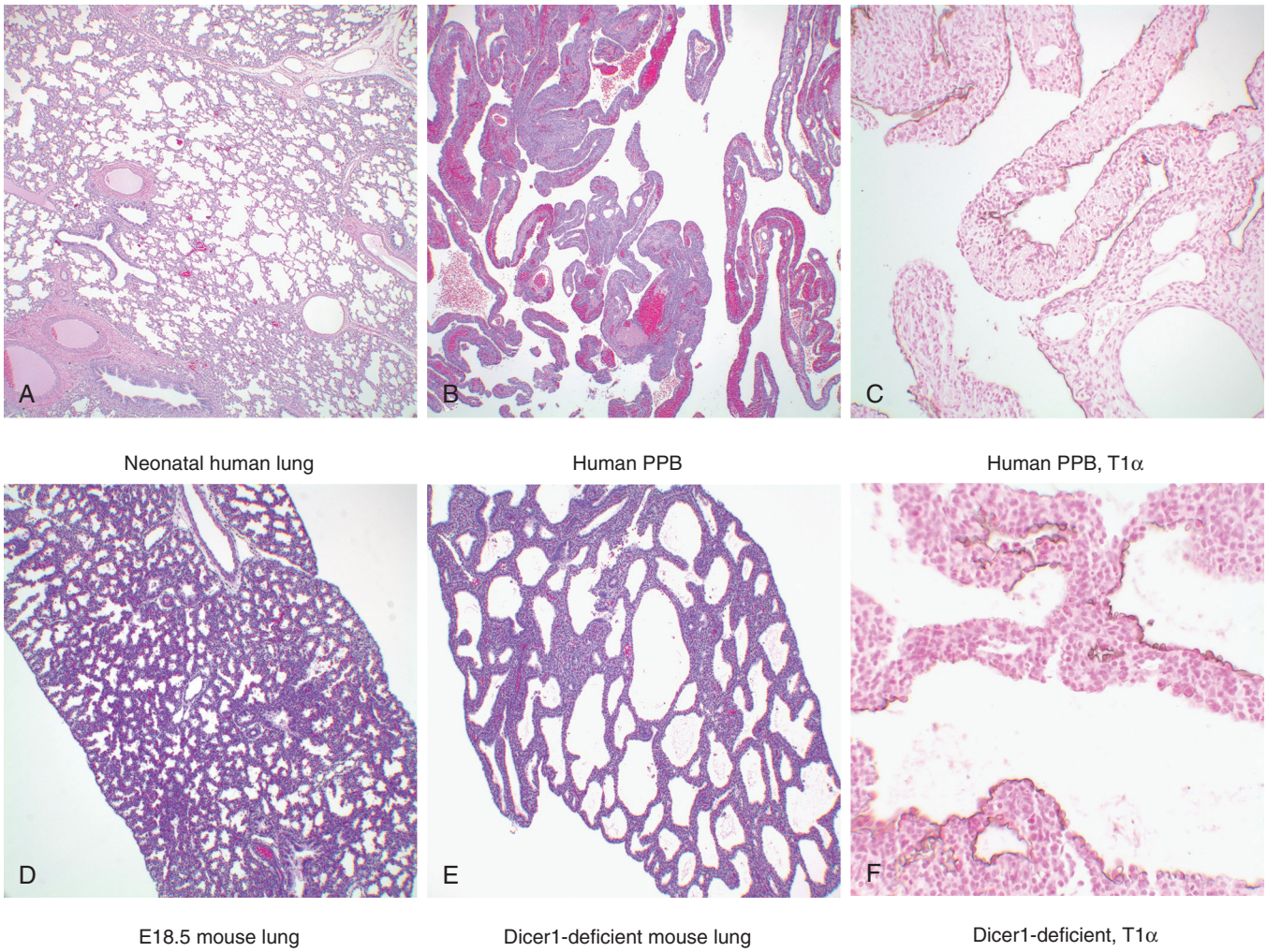

Figure 2-3 Mouse lungs with Dicer1 loss mimic pleuropulmonary blastoma (PPB) in neonates. Human PPB (**B** and **C**), adjacent normal neonatal lung (**A**), Dicer1-deficient mouse embryonic day 18.5 lungs (**E** and **F**), and normal lungs from Dicer1-proficient littermates (**D**) were compared by hematoxylin and eosin staining. PPB and Dicer1-deficient murine lung sections were also immunostained for the type I cell marker, T1α (**C** and **F**), to determine the phenotype of the epithelial cells lining the cysts. **A** and **D**, The neonatal human lung adjacent to the tumor shows normal morphologic characteristics for the alveolar stage of lung development, and the mouse lung shows morphologic characteristics typical of the saccular stage of development. **B**, Early-stage type I PPB is characterized by epithelium-lined cysts with intervening septa containing mesenchymal cells. **E**, The Dicer1-deficient mouse lungs have morphologic characteristics similar to those of human PPB, including epithelium-lined cysts separated by septa containing mesenchymal cells. Many of the epithelial cells lining the PPB cysts have a type I cell phenotype as determined by expression of T1α (**C**). Similarly, the Dicer1-deficient epithelial cells lining the cysts in the murine model also express T1α (**F**). (**A** and **B,** ×4 original magnification; **C,** ×20 original magnification; **D** and **E,** ×10 original magnification; **F,** ×40 original magnification.)

Complete reference list available at *ExpertConsult*.

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3 GENETICS OF LUNG DISEASE

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INTRODUCTION

The human genome comprises approximately 3.2 billion base pairs. With the exception of identical twins, each human being has a unique DNA sequence. There are at least 10 million locations in the genome where DNA sequence varies between individuals. These locations are referred to as "polymorphic" when at least two variants (also known as "alleles") are present at a frequency greater than 1%. Most human diseases are the result of the interplay between these genetic polymorphisms and environmental exposures. The first step in any investigation of the genetic causes of a disease or phenotype is to determine the relative importance of these two causes of the disorder among the population of interest. To begin this process, one must determine the heritability of the disease of interest. Heritability is defined as the percentage of phenotypic variation that is due to variation in genetic factors. Often the first step is to determine if the trait, disease, or phenotype aggregates in families, but this will not prove that the trait of interest is genetic because traits can aggregate in families for purely environmental reasons, such as cigarette smoking, or because the prevalence of the trait is high, such as obesity. The most direct way to estimate the contribution of genetic variation to a disease is to measure heritability. Heritability can be estimated using families. For example, in twin studies a greater concordance of the phenotype between identical (monozygotic) twins than fraternal (dizygotic) twins can provide evidence of heritability of that phenotype. For lung disorders, heritabilities range from 20% to 90% depending on the type of lung disease, the mode of inheritance, and the degree of environmental influence.

There are two primary types of genetic disorders, monogenic (due to variation in a single gene) or complex (due to variation in multiple genes). Monogenic disorders demonstrate high heritability, segregate in families in a predictable way, and are caused by variation in a single major gene with less obvious environmental influence. The single gene usually has specific variation in the coding region of the gene that leads to an abnormal protein that causes an obvious clinical phenotype. Often the phenotype has multiple components, suggesting multiple effects of the gene variant(s). This is called "pleiotropy," in which one variant has many effects. There are currently over 10,000 monogenic disorders that have been identified and are characterized in the Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim). Positional cloning (linkage mapping followed by association mapping; described later) has been the primary means of the identification of these genetic variants until recently. With the completion of the Human Genome Project and the rapid advancement of genotyping technologies, attention has turned to identification of genetic variation associated with complex genetic disorders. Those efforts initially used positional cloning but now primarily rely on genetic association studies.

In contrast to monogenic disorders, complex genetic disorders are caused by variation in multiple genes and multiple environmental exposures, with each genetic variant having a much smaller effect than those seen in monogenic disorders. Because of the multiple gene-gene and gene-environment interactions, there is no obvious mendelian mode of inheritance in families for complex traits. One of the most prominent hypotheses for the genetic basis of common disease is the common disease/common variant hypothesis. This hypothesis suggests that key genetic determinants of common diseases have a relatively high allele frequency (i.e., 5% to 40%) and modest effect sizes. Given the modest effect sizes (odds ratios on the order of 1.1 to 1.4), large sample sizes are necessary to identify the genetic variants associated with complex traits despite the high allele frequency expected in these disorders. It is likely that there is a range of allele frequencies that predispose to complex diseases, with a corresponding range of effect sizes, but large-scale studies to evaluate the evidence for rare variation as a contributor to complex disease are only beginning to emerge.

The dichotomy described earlier between monogenic and complex disease is somewhat artificial because the clinical phenotype of many monogenic disorders varies as a result of the specific mutation present, other modifier genes, and environmental exposures. As genes for complex traits begin to be identified, their role in monogenic disorders is also being elucidated.

SCOPE OF THE PROBLEM

Some complex genetic disorders, such as age-related macular degeneration, are oligogenic, in which a small number of genes, three to five, explain the bulk of the clinical phenotype. However, for most complex traits, literally hundreds of genes with small effects are likely involved in disease causation. Thus a series of challenges have faced complex trait geneticists in the genome era of medicine.

The field of human genetics has continued to expand as the type of genomic variation that can be measured expands. Parallel advances in data analysis strategies are necessary to ensure efficient and valid inferences based on the ever-increasing volume of data that can be collected on large numbers of individuals. This cyclical pattern of advancement has been typical of the last several decades and is likely to be typical going forward. For example, initial problems relating to genotyping reliability and completeness for common variants (those with frequency > 5% in a given population) that were present at the time of release of the initial sequence of the genome have been largely resolved, as have the methods necessary to detect and control for population stratification (confounding by allele frequency differences in cases and controls, discussed later) and to account appropriately for the hundreds of thousands or millions of tests conducted in a genome-wide association study. Since 2010, large efforts have been focused on resequencing technologies, which sequence the same site in multiple individuals to capture sequence variation and thus capture uncommon and rare variation (frequency ≤ 5%). As we are able to measure a larger variety of genomic data (e.g., transcriptomic and epigenetic data) on ever-increasing numbers of individuals, the major analytic challenges will be in developing methods for integration of the different types of data. For all types of genetic variation, the ability to determine if genetic effects are real or not requires replication of results in independent populations, a process that can be difficult with the presence of phenotypic heterogeneity across populations. In particular, varying genetic backgrounds and environmental influences can result in variability in the effects of genetic variants across populations. Finally, the ultimate challenge of finding and verifying the functional variants in putative disease genes is still a laborious process without a clear-cut methodology for success.

POTENTIAL IMPACT OF HUMAN GENETICS

Genetics has the potential, because of its hypothesis-free nature, to identify novel mechanisms of disease pathobiology and hence to identify novel targets for a therapeutic intervention or disease prevention. In addition, genetics has the potential to predict specific subgroups of patients with a different clinical course or response of their disease, or differences in treatment. Finally, genetics has the potential to allow for early detection of susceptible individuals at risk for a specific disease phenotype or to allow avoidance of environmental factors that are known to cause the disease or to institute preventive therapy before disease develops. These genetic insights are still just beginning to be applied, and it will take time for genetics to become routinely used at the bedside.

MOLECULAR CHARACTERIZATION OF GENETIC VARIATION

Molecular genetics is elegant in its simplicity. Just four base pairs (two purines [adenine and guanine] bind to two pyrimidines [thymine and cytosine]) code for 20 amino acids that form the molecular building blocks of complex proteins. However, the assemblage of inherited genes (genotypes), control mechanisms, resultant proteins, and post-translational modifications have the capacity to create a complex panoply of unique biologic, physiologic, or visible traits of an organism (phenotypes). The relationship between these rather simple molecular characteristics and the vast array of complex phenotypes is, in part, explained by a number of seminal discoveries that were made more than 50 years ago.

Gregor Mendel[1] was the first to demonstrate that discrete traits could be inherited as separable factors (genes) in a mathematically predictable manner. Mendel's laws describe the relationship between genotype and phenotype and established the concept that each gene has alternative forms (alleles). Charles Darwin[2] made the observation that evolution represents a series of environmentally responsive "genomic" upgrades. Thomas Morgan[3] established the concept of linkage by using *Drosophila* to discover that genes were organized (and inherited) on individual chromosomes, and that genetic material was recombined or exchanged between maternal and paternal chromosomes during meiosis and that the frequency of recombination could be used to establish the relative genomic distance between genes. However, it was not until 1944 that Avery, MacLeod, and McCarty while working with *Pneumococcus* discovered that DNA was identified as the essential molecule that transmitted the genetic code.[4] The double-helix structure of DNA was discovered by Watson, Crick, Chargaff, Franklin, and Wilkins in 1953,[5] and over the next 50 years genetics assumed a central role in understanding the biologic and physiologic differences between and among species and between states of health and states of disease. In aggregate, these seminal discoveries led to a number of fundamental principles in molecular genetics that provide the basic mechanisms that link the four base pairs (adenine [A] binding to thymine [T] and guanine [G] binding to cytosine [C]) to health and disease.

GENOMIC MAPS

Over the past several decades genomic maps have evolved from karyotypes (microscopic visualization of chromosomes during metaphase) to restriction enzyme sites to genetic maps to maps with specific base pair sequence. In fact, to date there are hundreds of vertebrate, invertebrate, protozoan, plant, fungal, bacterial, and viral genomes that have been sequenced and are available on the National Center for Biotechnology Information (NCBI) Web site (www.ncbi.nlm.nih.gov). These genomic maps have not only been essential for identifying which genes and sequence changes cause disease or enhance the risk for adverse outcomes, these species-specific maps have also led to a very clear understanding of molecular evolution and have provided essential tools for understanding aspects of molecular