Chapter 2
Morphological and Morphometric Properties of the Blood-Gas Barrier: Comparative Perspectives

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Abbreviations

BGB	Blood-gas barrier
CL	Capillary loading
DLO_2	Total morphometric diffusing capacity of the lung
DtO_2	Morphometric diffusing capacity of the tissue barrier (BGB) of the lung
FM	Surface (free) macrophage
OLB	Osmiophilic lamellated body
PCBV	Pulmonary capillary blood volume
RSA	Respiratory surface area
St	Surface area of the blood-gas barrier
PCBV	Pulmonary capillary blood volume
τht	Harmonic mean thickness of the blood-gas barrier
τht_{min}	Minimum harmonic mean thickness of the blood-gas barrier
τt	Arithmetic mean thickness of the blood-gas barrier

2.1 Introduction

> Efficient gas exchange in the lung depends, as we have said repeatedly, on designing a very thin barrier of very large surface area between air and blood. (Weibel 1984)

Energy is pivotal to maintaining the intricate cellular processes such as ion pumping and enzymatic activities which drive life. Oxidative phosphorylation involves breaking down of organic molecules such as sugars in the presence of oxygen (O_2),

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generating adenosine triphosphate (ATP), the molecular factor which cells utilize to operate physiological processess. The means of acquiring O_2 by simple physical diffusion began early in the evolution of aerobic life and was forthwith conserved. In some cases, occasioning catastrophic demises, shifts in the levels of O_2 in the biosphere, for example, as occurred in the Paleozoic's Permian–Triassic eras (Graham et al. 1995), have been important drivers in the progress and the diversification of life. Change in structural complexity, that is, from simple (unicellular) to complex (multicellular) forms, transition from aquatic life to terrestrial one and with it switch from water to air breathing, and realization of highly energetically costly lifestyles such as flight are some of the most decisive events which were directly impelled by the presence and concentrations of O_2 in the environment. Paradoxically, while O_2 is vital to life, it is also an environmental toxin and a mutagen (Auten and Davis 2009; Cabelli 2010). Brand (2007) stated that "if oxygen were invented today, it would be banned by the FDA (Federal Drug Agency)," and, respectively, Canfield (2014), Lane (2002), and Kleiber (1961) termed O_2 "the signature feature of Earth," "the molecule that made the world," and "the fire of life."

Possibly only exceeded by the gastrointestinal system, among the various organs of the body, the lung presents the largest surface area to the external environment which continuously and intimately interacts with air. For example, over a 24-h period, the human lung is ventilated ~25,000 times with ~20,000 L of air (Burri 1985a; Brain 1996). By weight, daily, more than 20 kg of air enters and leaves the human body, a load that considerably exceeds that of food and water consumed over the same period (Brain 1996). Gas exchange occurs by passive diffusion across a very thin tissue partition, the blood-gas barrier (BGB). Although inherently passive, the lung is a mechanically dynamic organ. The mammalian lung is, for example, ventilated with air from contractions of the diaphragm and the intercostal muscles, which attach to the ribs and perfused with blood by the contractions of the heart muscle. Regarding its construction, the BGB presents structural attributes that are at variance with each other. While a critical mass of cellular and connective tissue components is necessary to confer strength, the parts must be arranged to form the thinnest possible partitions which produce greatest conductance of O_2. This is brought about by a proximal–distal progressive reduction of the bulk of structural components which separate the airway and vascular systems. The epithelium lining the airways changes from a complex pseudostratified ciliated one with mucus cells in the upper airways (trachea and bronchi) to columnar and cuboidal ones in the lower parts of the airway system (the terminal bronchi and the bronchioles) to a simple squamous one on the respiratory surface (Fig. 2.1). Corresponding structural changes occur on the vascular side of the gas exchanger, where the thickness of the walls of the blood vessels become smaller, terminating in blood capillaries which are lined by endothelial cells only.

The structural properties of the BGB (thin and vast) present great challenges for efficient ventilation and perfusion of the lung (Weibel 2013). In the human lung, the thickness of the BGB (harmonic mean thickness, τht) is 0.62 μm, which is a fraction of the thickness of a foolscap paper, and the surface area is 140 m^2, which is approximately that of the surface of a tennis court (Gehr et al. 1978).

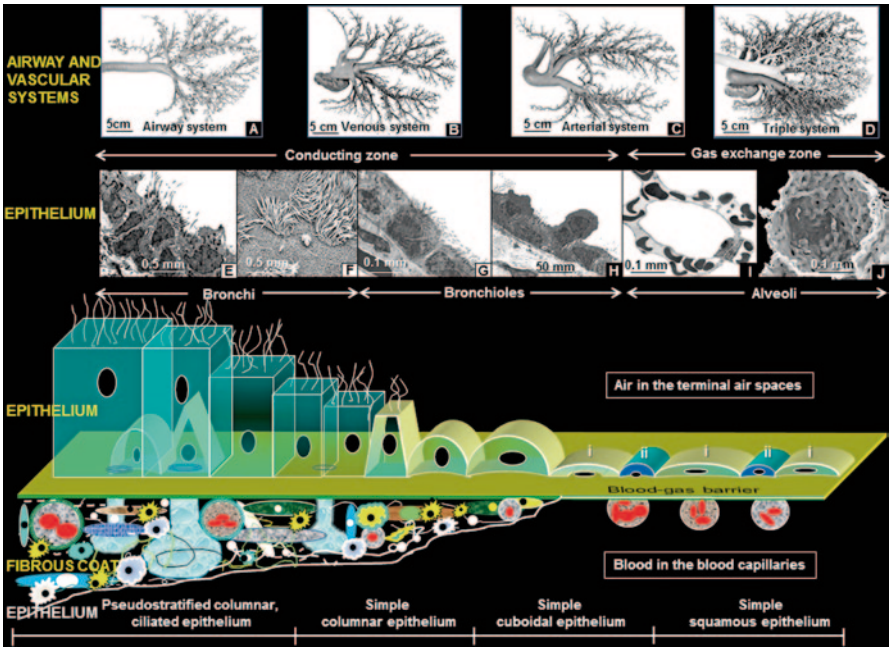


Fig. 2.1 Proximal–distal diminution of the quantity of the tissue components separating the pulmonary airway and vascular systems. At the gas exchange level, the partition (the blood-gas barrier) is very thin. *Inserts:* **a**–**d** Casts of the airway (**a**) and vascular systems (**b**, **c**) of the lung of the pig, *Sus scrofa,* showing how the systems bifurcate and terminate in very small respiratory units and how closely the systems mirror (relate to) each other (**d**). **e**–**j** Transmission and scanning electron micrographs showing changes in the epithelial complexities in a mammalian lung at various parts of the airway system. **e** The complex pseudostratified ciliated epithelium with mucus cells (upper airways—trachea and bronchi). **f** ciliated and non-ciliated cells of the epithelium of the upper airways. **g**, **h** Columnar and cuboidal epithelial cells of the lower parts (terminal bronchi and bronchioles) of the airway system. **i**, **j** Epithelial cells lining the gas exchange (alveolar) surface. *i* type-1 cell, *ii* type-2 cell. Concept from Weibel (1984)

2.1.1 Cellular Organisation of the Lung Parenchyma

At the gas exchange level, the lung parenchyma comprises motley of cellular and connective tissue components. The number, size, and location of these constituents are important for optimal functions of the organ which are gas exchange and metabolic activity. More than 40 different kinds of cells have been reported in the lung, with ~20 of them found on the respiratory surface (Breeze and Wheeldon 1977; Andrews 1981; Burri 1985b; Weibel 1985). The gas exchange surface is formed mainly by the type-1 and type-2 cells (pneumocytes; Fig. 2.2a) while endothelial cells delimit the blood capillaries (Fig. 2.2b). The type-2 cells are a highly specialized group of cells (Fig. 2.3a–e). By the process of exocytosis, they secrete surfactant (Pattle 1976; Daniels et al. 1994; Bastacky et al. 1995), a complex surface-active phospholipid substance which stabilizes the alveoli by lowering surface

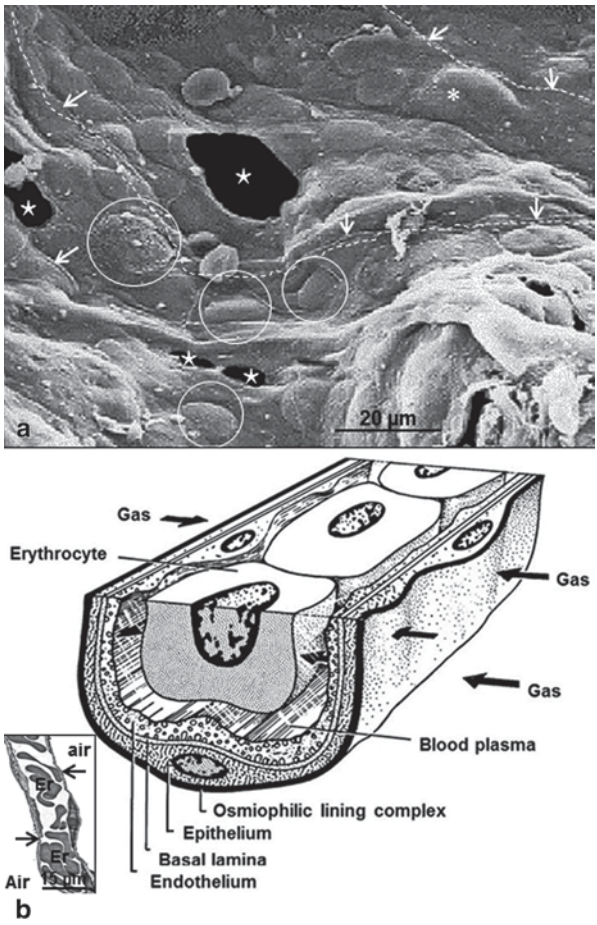


Fig. 2.2 **a** Alveolar surface showing boundaries of a squamous type-I cell *(dashed line)* with the intercellular junctions marked with arrows. The type-II cells are *encircled*. *Stars:* interalveolar pores (formerly the Pores of Kohn), *asterisk:* likely cell body (perikaryon) of the type-I cell. **b** A schematic diagram of a blood capillary showing the various structural components. *Arrows:* gas (oxygen) diffusing across the blood-gas barrier and the plasma layer. *Insert:* Longitudinal section of a blood capillary showing erythrocytes *(Er)* which are separated from air by a blood-gas barrier *(arrows)*

tension on the respiratory surface. The surfactant is removed from the respiratory surface mainly by the type-II cells themselves and recycled and by the surface macrophages, which break it down (Rooney et al. 1994; Clements 1997; Veldhuizen et al. 1998; Veldhuizen and Haagsman 2000; Fehrenbach 2001): The macrophages account for ~10–20 % of the removal of the surfactant.

Although the surfactant occurs in different forms and quantities on the respiratory surface of the vertebrate lungs (Daniels et al. 1994; Bernhard et al. 2001a, b; Maina 1998, 2011), differences in the degrees of differentiation and location of the pneumocytes occur in different taxa. For example, in the lungs of the lungfishes, Dipnoi (Fig. 2.3a, b), amphibians (Fig. 2.3c), and the less-derived reptilian species, the cells are not morphologically different. They combine features of the fully differentiated type-1 and type-2 cells in being cuboidal in shape, having microvilli on their free surface, and containing various organelles of which the characteristic ones are the intracellular inclusions called osmiophilic lamellated bodies (OLBs; Fig. 2.3a–e; Rooney et al. 1994; Vanhecke et al. 2010). The precursors of the

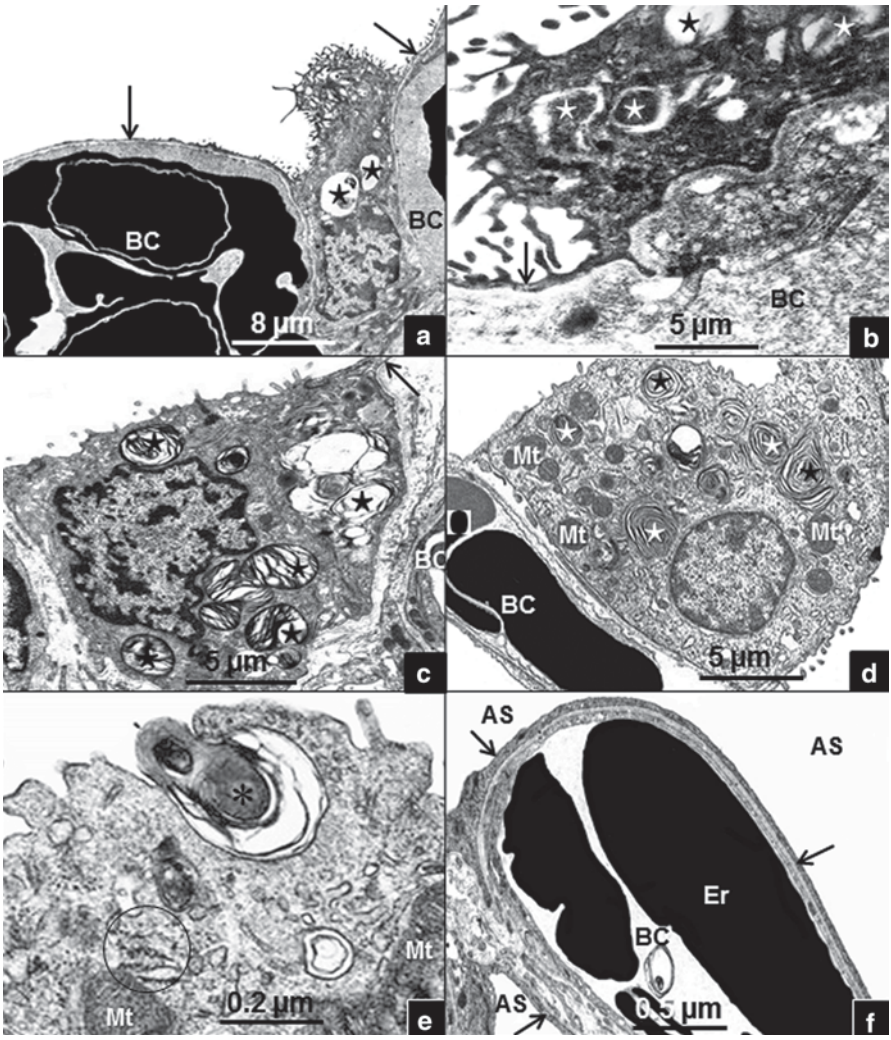


Fig. 2.3 a, b Type-II cells of the lung of the lungfish, *Protopterus aethiopicus,* containing osmiophilic lamellated bodies *(stars)*. Cuboidal/columnar in shape, the cells also have thin cytoplasmic extensions *(arrows)*. **c** Type-II cell of the reptilian lung showing osmiophilic lamellated bodies *(stars)*. *Arrow:* cytoplasmic extension. **d** A type-II cell of a mammalian lung showing osmiophilic lamellated bodies *(stars)* and mitochondria *(Mt)*. **e** Close-up of a type-II cell of a mammalian lung shown secreting surfactant by exocytosis *(asterisk)*. *Circled:* rough endoplasmic reticulum. **f** A pulmonary blood capillary showing the extremely thin type-I cells *(arrows)* fronting an air space *(AS)*. *BC* blood capillary, *Er* erythrocytes

surfactant (the OLBs) consist of large tightly packed concentric layers. In the lungs of the more-derived reptilian species (e.g., crocodiles), birds, and mammals, the pneumocytes are morphologically and ultrastructurally different: The type-1 cells have very thin and long cytoplasmic extensions which lack organelles (Fig. 2.3f),

and the type-2 ones are cuboidal in shape and, in addition to having OLBs, contain intracytoplasmic organelles such as mitochondria, rough endoplasmic reticulum, and Golgi complexes (Fig. 2.3d, e). Indicating its importance in lung function, the surfactant has been conserved for ~300 million years (Orgeig and Daniels 1995; Sullivan et al. 1998; Power et al. 1999).

In the human lung, the type-1 cells have an average volume of ~1,500 μm^3, cover a mean surface area of ~5,000 μm^2 (Crapo et al. 1982, 1983), and the cytoplasmic extensions extend to a distance of ~50 μm from the cell body (perikaryon). The type-2 cells (Fig. 2.2a) are small (~550 μm^3 in volume), form ~12% of the total cell population, and cover only ~4% of the alveolar surface (Haies et al. 1981; Crapo et al. 1982). Morphological specializations and strategic location of the pneumocytes and the connective tissue and other cellular components contribute to formation of thin BGB. The more metabolically active cuboidal and better-organelles-endowed cells and hence thicker type-2 cells cover much less of the respiratory surface while the type-1 cells which are metabolically inert and extremely thin (Figs. 2.2a, 2.3f) line most of it. While comprising ~10% of the total number of cells in the mammalian lung, the type-1 cells cover as much as 96% of the alveolar surface (Haies et al. 1981; Crapo et al. 1982, 1983). The location of the epithelial cell bodies (perikarya) away from the respiratory surface (Fig. 2.4a) and placing of connective tissue elements such as collagen (Fig. 2.4b, c), elastic tissue, and even nerve axons (Fig. 2.4d) in non-gas exchanging areas of the parenchyma contribute to thinning of the BGB. In the avian lung, the type-2 cells do not exist on the air capillaries (the respiratory units of the lung): Practically all the perikarya of the type-1 ones are confined to the atria and the infundibulae, non-respiratory parts of the parabronchial mantle (Fig. 2.5a, b). The location of the perikarya of the cells, normally the thickest part of the cell, away from the air capillaries contributes to thinner BGB than would otherwise be the case. In the mammalian lung, the interalveolar septum

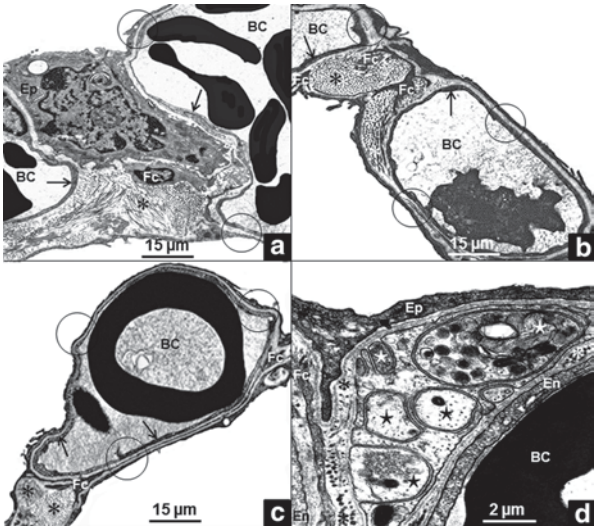


Fig. 2.4 a–c Placement of a type-II epithelial cell (*Ep,* **a**), collagen fibers (*asterisks,* **a**–**c**), and fibrocytes (*Fc,* **a**–**c**) away from the thinnest parts of the blood-gas barrier *(circles)*. *Arrows:* endothelial cells. **d** Lung of the black mamba, *Dendroaspis polylepis,* showing axonal profiles *(stars)* offset from a blood capillary *(BC)*. *Asterisk:* collagen fibres, *En* endothelial cell

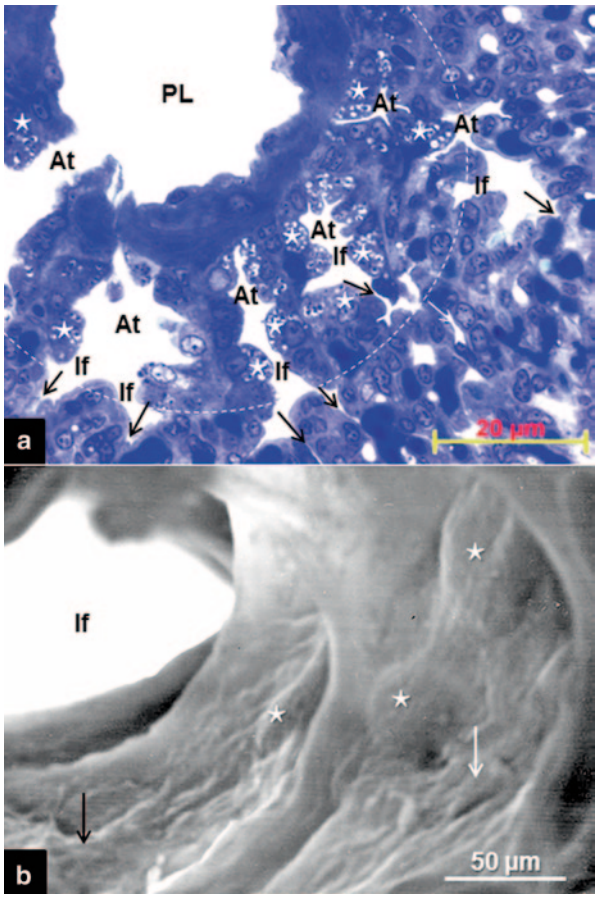


Fig. 2.5 **a** Location of type-II cells *(stars)* in the atria *(At)* and infundibulae *(If)*. The air capillaries, the respiratory units, are shown by *arrows*. *PL* parabronchial lumen. *Dashed line:* approximate limit of the distribution of the type-II cells in the exchange tissue. **b** Close-up of an infundibulum *(If)* showing type-II cells *(stars)* and macrophages *(arrows)* on the surface

displays distinctively thick (supporting) and thin (respiratory) sites (Fig. 2.6a): Connective tissue elements such as collagen, elastic tissue, and cells such as fibrocytes and interstitial macrophages are found on the supporting side while the respiratory area comprises an epithelial cell, an interstitium, and an endothelial cell (Weibel and Bachofen 1997; Weibel 1984). Such functionally distinct sites are lacking in the avian lung (Fig. 2.6b).

The endothelial cells which line the pulmonary blood capillaries connect through intercellular junctions (Fig. 2.7a, b). Their thin cytoplasmic extensions contain numerous micropinocytotic vesicles. Compared to the epithelial cells, the endothelial cells are less permeable to solutes and are more selective to some ions (Effros et al. 1986). The endothelial cells constitute ~41 % of the entire population of lung cells (Crapo et al. 1983) and are considerably involved in the metabolic functions of the lung (Bakhle 1975; Bakhle and Vane 1977; Becker 1984). The type-3 cells, the so-called brush cells, have only rarely been described in the lung (Dormans 1985; Goniakowska-Witalinska and Leuweryns 1991; Meyrick and Reid 1968; Gomi 1982).

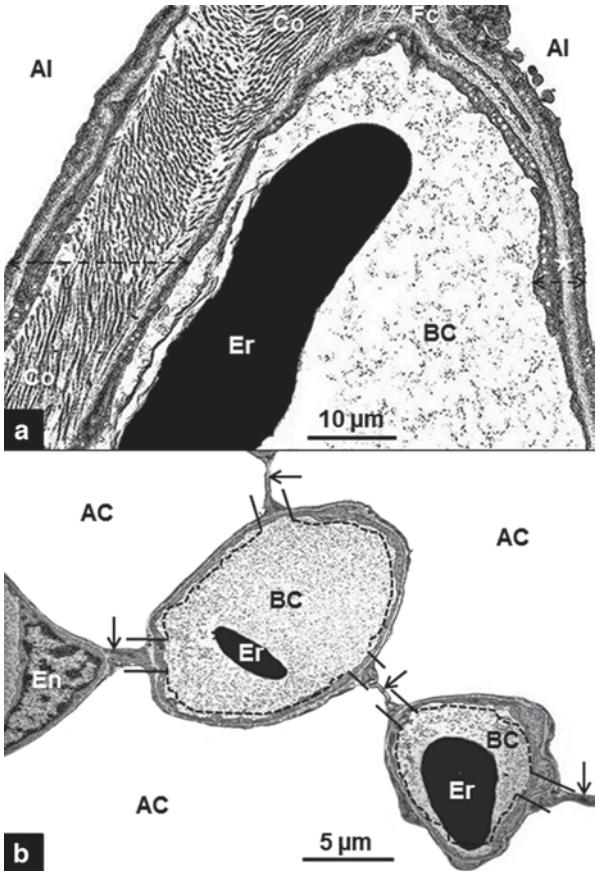


Fig. 2.6 **a** Interalveolar septum in a mammalian lung showing a thick (supporting) side *(asterisk and dashed arrow)* and a thin (gas exchange) side *(star* and *dashed arrow)*. The thick side contains a large amount of collagen *(Co), alveolus (Al)*. **b** Exchange tissue of the avian lung showing air capillaries *(AC)* and blood capillaries *(BC):* thick and thin sides of the blood-gas barrier do not exist. Practically all the blood-gas barrier *(dashed line)* is involved in gas exchange while only a small part, the areas where blood capillaries interconnect *(areas between the lines),* is not involved in gas exchange. *Arrows:* epithelial–epithelial cell connections, *En* endothelial cell, *Er* erythrocyte, *Fc* fibrocyte

They are characteristically pyramidal in shape; their free surface has large blunted microvilli, numerous intracytoplasmic glycogen granules, and plentiful microfilaments which constitute a cytoskeletal system. The type-3 cells have been associated with chemoreceptive, absorptive, and secretory roles (Dormans 1985). A rare "mitochondria-rich cell" was identified in the lung of the turtle *Pseudemys scripta* by Bartels and Welsch (1984), and neuroepithelial cells have been described in lungs of several amphibian species (Rogers and Cutz 1978; Goniakowska-Witalinska et al. 1990; Cutz and Jackson 1999).

The motile surface (free) macrophages (FMs) protect the respiratory surface by ingesting and destroying or sequestering pathogenic microorganisms and injurious particulates (Fig. 2.8a–f; Nguyen et al. 1982; Brain and Hyg 1988; Nganpiep and Maina 2002). By keeping the respiratory surface "clean," the FMs ensure that the surface of the BGB remains uncluttered and therefore thin. The cells are rare in healthy amphibian and reptilian lungs, abundant in the mammalian lungs, and scarce in those of birds (Nganpiep and Maina 2002; Kiama et al. 2008; Maina 2005). The efficiency of the FMs and other phagocytic cells and immune-competent cells in

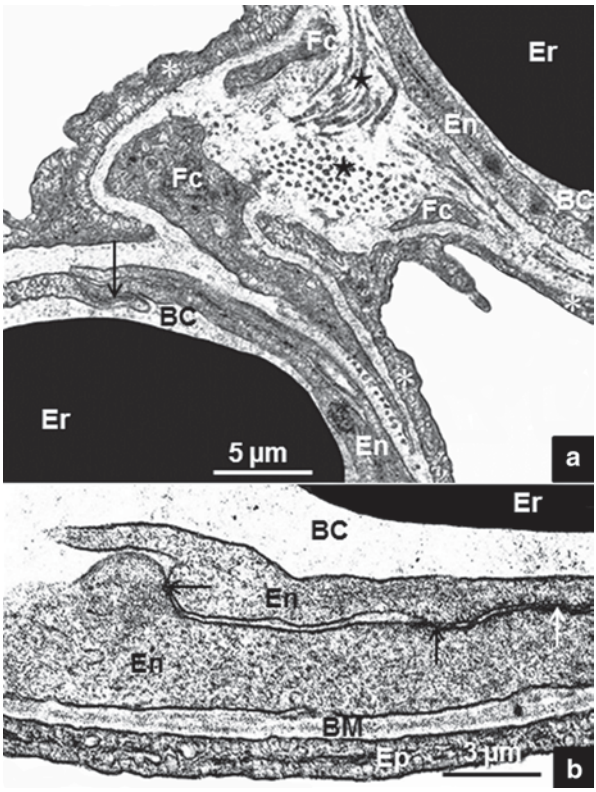


Fig. 2.7 **a** Interconnection of endothelial *(En)* cells of the pulmonary blood capillaries *(arrow)* and location of connective tissue elements between adjacent blood capillaries *(BC)*. *Stars:* collagen, *asterisks:* epithelial cells. **b** Close-up of a blood-gas barrier *(BC)* showing an endothelial cell junction *(arrows)*. *Ep* epithelial cell, *BM* basement membrane, *Er* erythrocyte, *Fc* fibrocyte

the lung is so high that in, for example, healthy human beings, below the larynx, the respiratory surface is reportedly sterile (Skerret 1994). The surface macrophages of the lung derive from the bone marrow. They circulate in blood as monocytes before ultimately settling on the surface of the lung after passing through the blood capillary endothelium by the process of diapedesis.

2.1.2 Structure of the Blood-Gas Barrier

Basically, the BGB comprises an epithelial cell which fronts air, an interstitial space which contains various cellular and connective tissue elements, including extracellular matrix, and an endothelial cell which lines the blood capillaries (Fig. 2.9a, b; Weibel 1973; Meban 1980; Maina and King 1982; Maina and West 2005). In the lungs of the air-breathing vertebrates, the "tripartite, laminated" structural design of the BGB has been greatly conserved (Maina and West 2005). Among other roles which include regulation of movement of molecular factors, maintenance of normal cellular cytoarchitecture, prevention of passage of noninflammatory cells, and control of morphogenesis, proliferation, and differentiation of cells, the basement

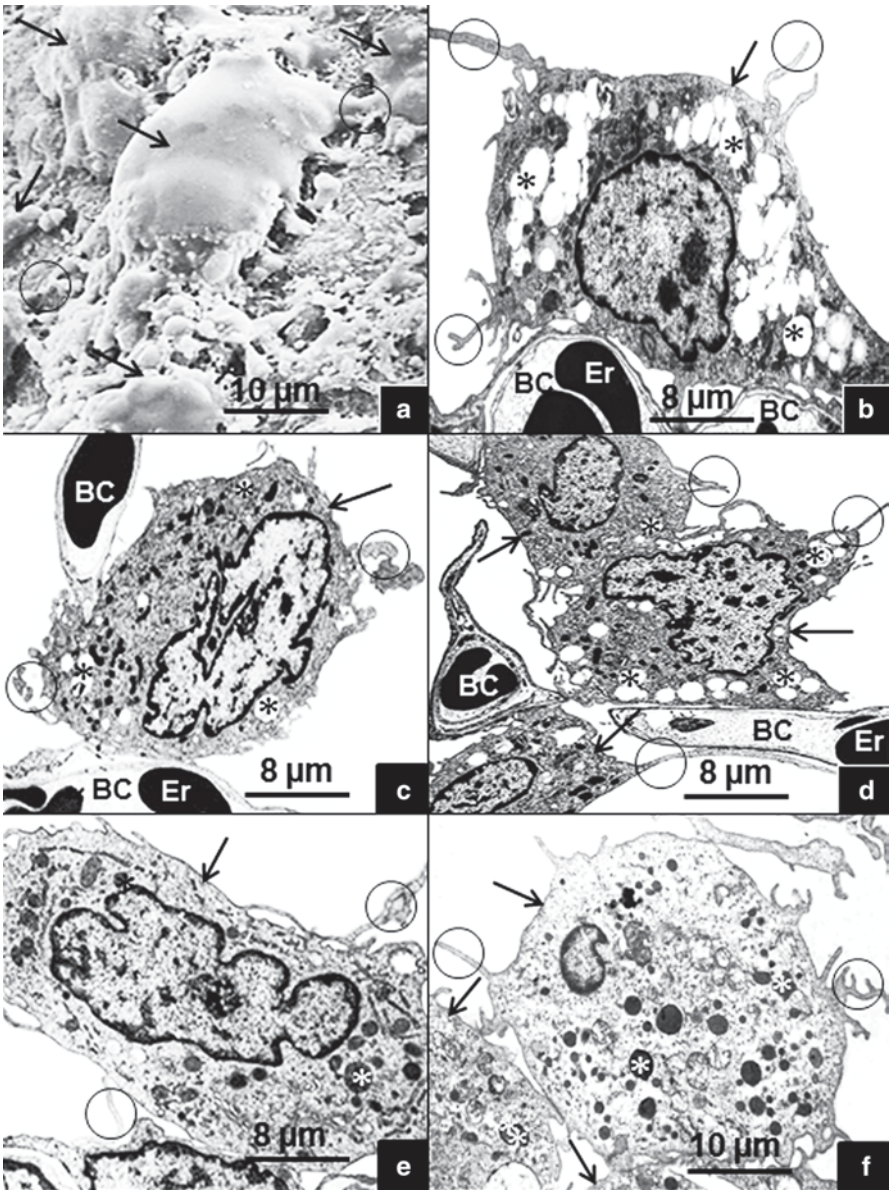


Fig. 2.8 Free (surface) macrophages *(arrows)* of lungs of various air-breathing vertebrates. **a** Amphibian lung—tree frog, *Chiromantis petersi*. **b**–**e** Mammalian lungs. **f** Avian lung, domestic fowl, *Gallus gallus* variant *domesticus*. The cells are large and move using cytoplasmic extensions, filopodia *(circles)*. *Asterisks:* vesicular bodies, *BC* blood capillary, *Er* erythrocytes

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