

Surfactant and the Glycocalyx

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Gas exchange in the lung takes place via the air-blood barrier in the septal walls of alveoli. The tissue elements that oxygen molecules have to cross are the alveolar epithelium, the interstitium and the capillary endothelium. The epithelium that lines the alveolar surface is covered by a thin and continuous liquid lining layer. Pulmonary surfactant acts at this air-liquid interface. By virtue of its biophysical and immunomodulatory functions, surfactant keeps alveoli open, dry and clean. What needs to be added to this picture is the glycocalyx of the alveolar epithelium. Here, we briefly review what is known about this glycocalyx and how it can be visualized using electron microscopy. The application of colloidal thorium dioxide as a staining agent reveals differences in the staining pattern between type I and type II alveolar epithelial cells and shows close associations of the glycocalyx with intraalveolar surfactant subtypes such as tubular myelin. These morphological findings indicate that specific spatial interactions between components of the surfactant system and those of the alveolar epithelial glycocalyx exist which may contribute to the maintenance of alveolar homeostasis, in particular to alveolar micromechanics, to the functional integrity of the air-blood barrier, to the regulation of the thickness and viscosity of the alveolar lining layer, and to the defence against inhaled pathogens. Exploring the alveolar epithelial glycocalyx in conjunction with the surfactant system opens novel physiological perspectives of potential clinical relevance for future research.

Keywords: lung ; alveoli ; air-blood barrier ; air-liquid interface ; alveolar lining layer ; surfactant ; glycocalyx

1. Introduction

The architecture of the lung is optimized to serve its main function, gas exchange. A large surface area for air and blood (about 120 m²) with minimal distance (about 2 µm) is distributed over hundreds of millions of alveoli. The wall separating neighbouring alveoli contains three tissue compartments that constitute the air-blood barrier: alveolar epithelium, capillary endothelium, and the interstitium in-between. The basic knowledge about the structure and function of the alveolar epithelium seems to be well established. It is a continuous layer constituted by a mosaic of two cell types with specific differentiation. While alveolar epithelial type I (AEI) cells are specialised lining cells, alveolar epithelial type II (AEII) cells are specialized secretory and progenitor cells. AEI cells, which are less frequent than AEII cells, cover the vast majority of the alveolar surface with their branched thin squamous cell extensions ^[1]. Interspersed are single cuboidal AEII cells, which are easily recognized by their characteristic secretory organelles, the surfactant-storing lamellar bodies. Epithelial renewal and repair of both cell types is provided by AEII cells (for review, see ^{[2][3]}).

Soon after the first demonstration of a continuous alveolar epithelium in the mammalian lung by electron microscopy (EM) ^{[4][5]}, it became obvious that this epithelium is not naked. It is covered by a fluid alveolar lining layer consisting of two phases: a surface film and an aqueous hypophase ^{[6][7]}. Later cryo-EM studies have confirmed the existence of a thin and continuous alveolar lining layer ^[8]. Surfactant, the secretory product of AEII cells, is a central component of this layer, i.e., it exerts its functions at the air-liquid interface of lung alveoli (for review, see ^{[2][9][10][11][12][13]}).

2. Surfactant

Surfactant is complex, both biochemically and ultrastructurally. It consists of about 90% lipids (mainly saturated phospholipids) and about 10% proteins (including the surfactant proteins SP-A, SP-B, SP-C and SP-D). All surfactant components are synthesized, stored, secreted and to a large extent recycled by AEII cells. Intracellular surfactant (at least lipids and the hydrophobic SP-B and SP-C) is assembled in lamellar bodies prior to secretion. Intraalveolar surfactant includes the surface film and morphologically distinct subtypes in the hypophase. These subtypes largely correspond to different stages in surfactant metabolism and activity. According to current models, freshly secreted surface-active lamellar body material transforms into tubular myelin, which is a potential precursor of the surface film. Additional multilayered surface-associated surfactant reservoirs have also been suggested. Inactive surfactant is usually present as small unilamellar vesicles. These can either be taken up by AEII cells for recycling or degradation or taken up by alveolar

macrophages for degradation. Thus, the hypophase of the alveolar lining layer provides both a substrate on which surfactant acts as well as a reservoir in which intermediates of surfactant metabolism (precursors and remnants) are located.

The functions of surfactant are biophysical as well as immunomodulatory, with its individual components contributing to these functions in different and specific ways. The hydrophobic SP-B and SP-C are assigned mainly biophysical relevance whereas the hydrophilic SP-A and SP-D, which belong to the protein family of collectins, are considered to serve mainly immunomodulatory functions. Regarding its biophysical functions, surfactant stabilizes alveolar dimensions and thus prevents alveolar collapse by a surface-area dependent reduction of alveolar surface tension. Moreover, the low surface tension at the alveolar air-liquid interface secured by surfactant also prevents intraalveolar edema formation. Surfactant is therefore essential for normal alveolar micromechanics and lung function by keeping alveoli homogeneously open, dry and clean (reviewed in [2][11][13]).

It is remarkable that early studies on AEL cells, the surfactant system and the alveolar lining layer (even before these now common names were coined) already suggested the presence of carbohydrates in this context. In 1954, Charles Clifford Macklin not only predicted the presence of an “alveolar mucoid film”, but also estimated (“arbitrarily”) its thickness to be 200 nm (confirmed more than 40 years later [8]) and suggested its components to be “acid mucopolysaccharides and myelinogens”, i.e., glycosaminoglycans and phospholipids [14]. After the actual discovery of surfactant by John Clements [15], Bolande and Klaus, based on light microscopic findings, suggested that, besides phospholipids, “a mucopolysaccharide fraction may also be present” in the alveolar lining layer [16]. Similarly, Groniowski and Biczyskova studied the alveolar lining layer by EM and found evidence indicating “the existence of another component of the acidic mucopolysaccharide nature” [17]. In that sense, we have to reintroduce the glycocalyx into our concepts of the alveolar lining layer and the surfactant system.

The spatial interaction between surfactant and the glycocalyx at the alveolar epithelial surface may generate a “win-win” situation, where surfactant benefits from glycocalyx components such as hyaluronan, warranting its biophysical function. Conversely, surfactant may contribute to the integrity and function of the alveolar epithelial glycocalyx. It is unknown how the content of the alveolar hypophase is sensed, e.g., with respect to its volume, viscosity and surfactant pool size. This remains to be investigated both under normal and challenge conditions such as acute lung injury and pulmonary infection. Integrating the glycocalyx into our picture of the lung alveolus also offers attractive therapeutic perspectives, e.g., by adding glycocalyx components to exogenous surfactant preparations. Moreover, engineering of synthetic glycocalyx [18] provides a very promising approach for the preservation and/or reconstitution of the unique micro-environment on top of the alveolar surface. New developments in EM should be included in such studies, e.g., volume correlative light and electron microscopy techniques [19][20]. It seems timely to explore the structure and function of the alveolar epithelial glycocalyx, in particular its relations to the surfactant system. Given its potential importance, it should no longer remain an enigma.

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