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Doing Lung Stereology. Where is the Point?

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Quantitative microscopy is essential in experimental morphology and pathology, including the phenotyping of genetically altered organisms. The gold standard for obtaining quantitative structural data at all microscopic levels is provided by state-of-the-art stereology. Stereology offers a toolkit of unbiased sampling rules and estimation tools based on geometric probes (points, lines, planes, disectors) which allows for quantitative characterization of 3D objects on the basis of measurements made on 2D sections. Stereological methods are accurate, efficient, simple, and transparent.

Here, an update on stereology of the lung, with special emphasis on the surfactant system, is given [1-6]. Stereological data on lung structure are indispensable to make statistically valid comparisons, e.g. between wildtype and gene-manipulated mouse models of lung disease.

The pulmonary surfactant system has biophysical and immunomodulatory functions that are important for normal lung function. Surfactant consists of about 90% lipids and about 10% proteins, including the surfactant-associated proteins SP-A, -B, -C, and -D. Only at the EM level does surfactant show its morphological complexity. Surfactant is synthesized, stored, secreted, and to a large extent recycled by type II alveolar epithelial cells. The vast majority of intracellular surfactant material (all lipids and the hydrophobic SP-B and -C) is assembled in specific storage organelles, lamellar bodies, prior to secretion.

The hydrophilic surfactant proteins SP-A and SP-D are members of the collectin subfamily of C-type lectins. Collectins, pattern-recognition proteins with extended collagen-like domains linked to C-type lectin domains, participate in the innate immune response by both binding a wide variety of glycoconjugates and modulating immune cell responses. Lungs from collectin gene-manipulated mice can be analyzed by stereology in order to detect emphysema-like pathology and alterations in the surfactant system. The Euler number of the network of alveolar openings, estimated using physical disectors at the light microscopic level, is an unbiased and direct estimate of alveolar number. Surfactant-producing alveolar type II cells can be counted and sampled for local size estimation with physical disectors at a high magnification light microscopic level. The number of their surfactant storing lamellar bodies can be estimated using physical disectors at the EM level. By immunoelectron microscopy, surfactant protein distribution can be analyzed with the relative labeling index.

These design-based stereological methods allow for a complete quantitative lung phenotype analysis of gene-manipulated mouse models at the light and electron microscopic level.

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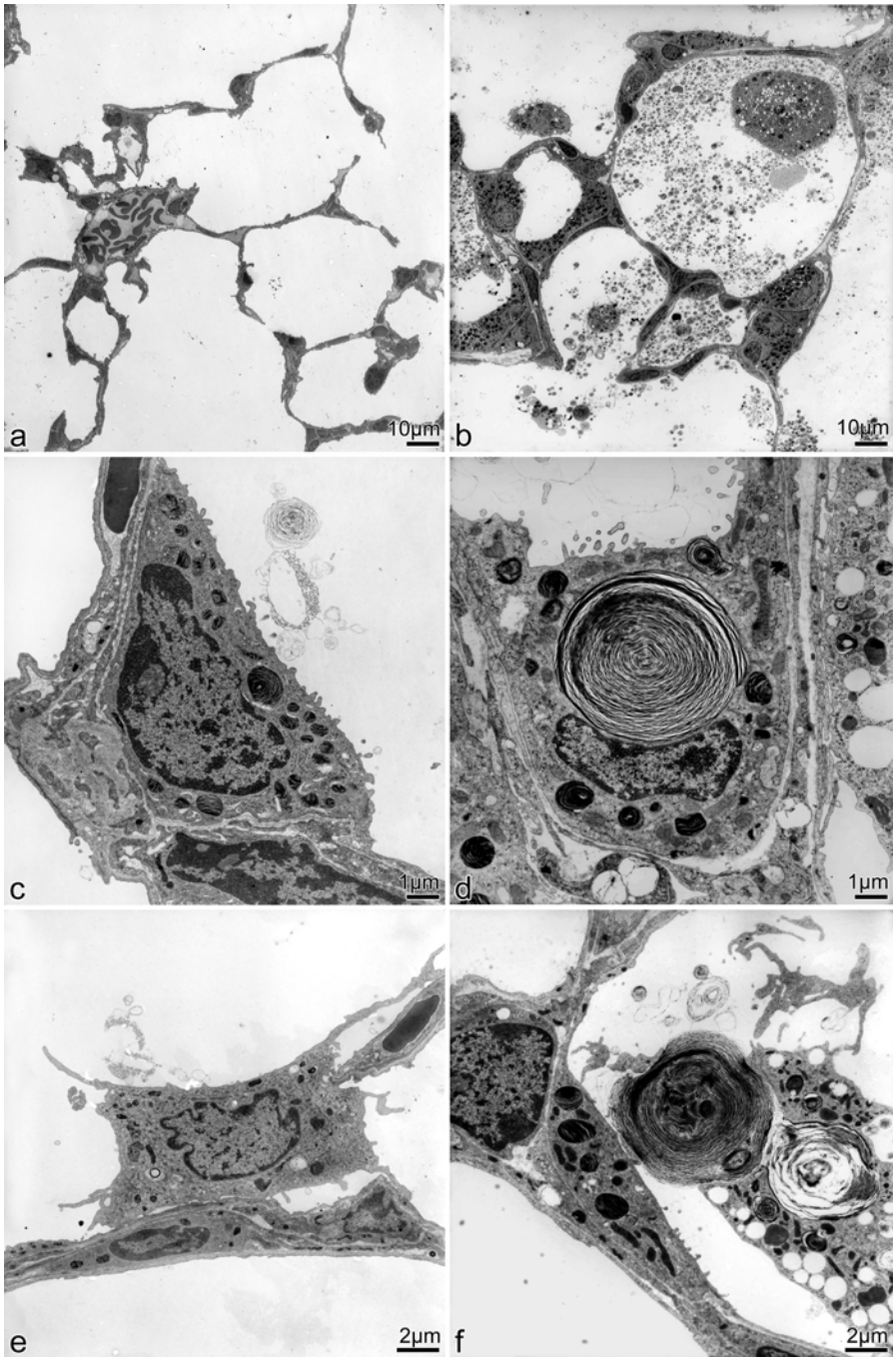

Fig. 1. Electron micrographs from lungs of wildtype (a, c, e) and lung collectin (SP-A/SP-D) double deficient mice (b, d, f) demonstrating qualitative findings for parenchymal architecture (a, b), type II cells (c, d), and alveolar macrophages (e, f). Parenchymal architecture shows enlarged airspaces, thickened alveolar septae, intraalveolar surfactant accumulation, occurence of foamy alveolar macrophages, and type II cell hyperplasia in SP-A/SP-D deficient mice (b). Type II cells of SP-A/SP-D deficient mice occasionally show giant lamellar bodies (d). Alveolar macrophages of SP-A/SP-D deficient mice are filled with surfactant material taken up from the alveolar space (f).

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