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Abstract

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Estimation of absolute number of alveolar epithelial type 2 cells in mouse lungs: a comparison between stereology and flow cytometry

Accurate estimation of the absolute number of a particular cell-type in whole organs is increasingly important in studies on organogenesis, and the remodelling and repair of diseased tissues. The unbiased estimation of the absolute number of cells in an organ is complicated, and design-based stereology remains the method of choice. This has led investigators to explore alternative approaches – such as flow cytometry – as a faster and less labour-intensive replacement for stereology. To address whether flow cytometry might substitute stereology, design-based stereology was compared with microfluorosphere-controlled flow cytometry, for estimation of the absolute number of alveolar epithelial type 2 cells (AEC2) in the lungs of two mouse strains: wild-type C57BL/6J mice and *Sftpc*-YFP mice. Using design-based stereology, ≈ 10.7 million and ≈ 9.0 million AEC2 were estimated in the lungs of wild-type C57BL/6J mice and *Sftpc*-YFP mice, respectively. Substantially fewer AEC2 were estimated using flow cytometry. In wild-type C57/BL6J mouse lungs, 59% of the AEC2 estimated by design-based stereology were estimated by flow cytometry (≈ 6.3 million), using intracellular staining for pro-surfactant protein C. Similarly, in *Sftpc*-YFP mouse lungs, 23% of the AEC2 estimated by design-based stereology were estimated by flow cytometry (≈ 2.1 million), using yellow fluorescent protein fluorescence. Our data suggest that flow cytometry underestimates AEC2 number, possibly due to impaired recoverability of AEC2 from dissociated lung tissue. These data suggest design-based stereology as the method of choice for the unbiased estimation of the absolute number of cells in an organ.