Characterization of a Marker of Differentiation for Tracheal Ciliated Cells Independent of Ciliation

Moira L. Aitken, Manuel Villalon, Maricela Pier, Pedro Verdugo, and Mark Nameroff

Abstract

Although morphologic features have been used to follow cell lineage and differentiation, an objective assessment of differentiation can be best established by characterizing the expression of specific proteins that form the phenotypic profile of differentiated cells. Thus, specific markers or probes are required to unequivocally identify the various types of cells resulting from differentiation in a cell lineage. We report characterization of an IgM monoclonal antibody (5B4/H3), which recognized a surface antigen of approximately 130 kD unique to ciliated cells. The antibody reacted with the lumenal surface of the ciliated cells in transmission electron micrographs, in immunohistochemical staining of trachealsections, and in cultured monolayers of tracheal epithelial cells. Flow cytometry, performed on enzymatically dispersed tracheal epithelial cells tagged with 5B4/H3 and fluorescent-labeled goat anti-mouse IgA/IgG/IgM, produced a population of fluorescent ciliated cells and a mixed nonfluorescent, nonciliated cell population. Ciliated cells were followed *in vitro* by time-lapse video microscopy for 48 to 72 h. Some of the ciliated cells lost their cilia under these culture conditions, but these cells were still found to react with the 5B4/H3 antibody. The antigen detected by this antibody remained on the surface of the cells after they lost their cilia. These results indicate that 5B4/H3 recognized a cell surface antigen that is specific to the ciliated cells and is independent of cell morphology. This marker will be useful in tissue culture studies of airway epithelial lineage, or differentiation, in which cell morphology is variable and cannot be used as a reliable marker of differentiation.