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Commentary

A Useful *In Vitro* Model for Transport Studies of Alveolar Epithelial Barrier

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Simplified *in vitro* models have been extremely useful for mechanistic characterization of transport functions of the respiratory epithelial tract lining the distal lung (i.e., the alveolar epithelium). For example, *in vitro* models have permitted more detailed characterization of important biological features identified in the lung *in vivo:* presence of functional tight junctions, pathways responsible for active ion transport in alveolar epithelial cells, and expression of cell-specific phenotypic markers. Ideally, a good *in vitro* model should accurately correlate with observations of transport/biological phenomena *in situ*/*in vivo.* For example, some *in vitro* models of the intestinal epithelial barrier (including Caco-2 and T84 cell lines) generally reflect the *in vivo* transport properties of intestinal absorption and/or secretion of ions and solutes (1–3). However, alveolar epithelial barrier studies using cell lines versus primary cultured pneumocyte monolayers suggest that the latter may be preferred for *in vitro* study of transepithelial transport functions.

Monolayers of rat pneumocytes in primary culture exhibit phenotypic (4) and morphologic (5) characteristics of Type I cells *in vivo,* making this model particularly relevant for *in vitro* studies of transport phenomena occurring in the respiratory epithelium lining the distal airspaces of the lung, where greater than 95% of the surface area is lined by Type I cells. Of importance with regard to the primary cultured monolayer model is the consistent presence of well-formed, functional tight junctions that limit the passive permeation of hydrophilic solutes in a size-dependent manner (6–8). These monolayers have been reported to severely restrict the translocation via paracellular routes of hydrophilic solutes whose molecular weight is greater than 40 kDa (6,7). Thus, macromolecules greater than 40 kDa are expected to traverse the barrier strictly via transcellular pathways, including nonspecific fluid phase endocytosis, adsorptive endocytosis, and/or receptor-mediated endocytosis (6–8). Similar transport characteristics have also been found to be present in primary cultured human alveolar epithelial cell monolayers (9).

The transepithelial electrical resistance of rat alveolar epithelial cell monolayers is greater than 1000 ohm-cm^2 under serum-free culture conditions and greater than 2000 ohm-cm^2 when serum is present in the culture medium. Factors reported to influence the "tightness" of rat alveolar epithelial cell monolayers in primary culture include: epidermal growth factor and hypoxia (5% oxygen exposure for 24–48 h) (10), which increase resistance, and hyperoxia (95% oxygen exposure for 24–48 h) and beta-agonists (e.g., terbutaline), which decrease resistance (11–14). Removal of calcium from bathing fluids rapidly decreases monolayer resistance to zero (Kim, unpublished data), as has been observed in other epithelial barriers (15).

Primary cultured alveolar epithelial cell monolayers express numerous transport-related proteins, including apical amiloride-sensitive sodium channels (13,14,16), basolateral sodium pumps (12–14,17–20), and several basolateral sodium cotransport and exchange mechanisms (21–23). In addition to polarized expression of sodium transporters, there is evidence for the presence of organic cation transporters (24), proton-dependent peptide transporters (25), and others (13). These alveolar epithelial ion transporters are thought to play important roles in alveolar fluid homeostasis contributing to efficient gas exchange in the distal region of the lung. Many of these transport activities have been verified in whole lung studies (26–32). Recent reports utilizing primary cultured rat pneumocyte monolayers relevant to the study of drug transport include: passive permeation of lipophilic solutes (33) and dextrans (6), osmotic water flow (34), and active and passive permeation of amino acids (35), peptides (35,37), and proteins (7,8,38). In addition, other cell biological characteristics (e.g., expression of caveolae/caveolins (39)) have been investigated using primary cultured pneumocyte monolayers. These reports collectively suggest that the primary cultured pneumocyte monolayer may be of great use for mechanistic studies of biochemical and cell biological functions of alveolar epithelium, as well as for investigation of transport functions

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of the air-blood barrier of the distal lung (including drug targeting/delivery).

A number of immortal cell lines (e.g., L-2, A549, H441, and MLE-15) have been utilized for study of physiology and cell biology of alveolar epithelium over the years by many laboratories. Of particular importance for transport studies is the fact that these respiratory epithelial cell lines in general do not exhibit significant transepithelial electrical resistance when cultivated to confluence on permeable substrata (40–43). Lack of functional tight junctional complexes is most likely the cause for the absence of significant electrical resistance of monolayers of immortal cell lines. One recent report (44) suggested that A549 cell monolayers grown on collagen-coated permeable filters could develop transmonolayer resistance of ~600 ohm-cm^2, although very high diffusion rates of a series of hydrophilic solutes simultaneously measured are inconsistent with these measurements. Moreover, using the same culture approach, it was more recently reported (43) that A549 transmonolayer resistance was close to zero even after six days of culture to confluence. Overall, low values for transepithelial resistance make it very difficult to interpret other transepithelial transport data (including drug trafficking) obtained from utilization of immortal cell lines.

To illustrate the problem further, passive solute transport data for flow across A549 cell monolayers (44) lead to the calculation of an equivalent pore radius of ~17 nm. This is about three times greater than that estimated for rat alveolar epithelial cell monolayers (6) and for intact alveolar epithelium in isolated perfused rat lungs (31). The number of equivalent pores per unit area estimated for A549 cell monolayers comes out to be 1.7×10^8 pores/cm^2, which is about a thousand-fold greater than that estimated for rat alveolar epithelial cell monolayers. These data reflect the very rapid transport rates, approaching free diffusion (even for immunoglobulins that have solute radius of ~16 nm), reported for A549 cell monolayers, likely reflecting the absence of restricted diffusion at paracellular tight junctions.

The lack of functional tight junctions in these immortal cell line monolayers renders interpretation of data pertaining to epithelial cell transport and/or polarity difficult. For example, a report contending that transferrin is preferentially taken up from the basolateral fluid of A549 cells grown on permeable supports appeared recently (43), but the interpretation is confounded by the fact that labeled transferrin added to basolateral fluid may have crossed the barrier relatively quickly via leaky paracellular pathways. Similarly, a recent report (45) dealing with polarized secretion of bioactive substances (e.g., fibrinogens) into basolateral fluid of A549 cell monolayers grown on permeable substrata is equally problematic in that secreted substances may have diffused across the monolayers through leaky junctions.

In summary, primary cultured pneumocyte monolayers appear to be a reliable model for study of alveolar epithelial transport processes and cell biology, since many of the transport processes and other characteristics present in the primary culture model are likely representative of those in the respiratory epithelium lining the distal region of intact lung. Moreover, primary cultured pneumocytes exhibit phenotypic transition into Type I-like cells, making this *in vitro* model even more attractive for identification and characterization of alveolar epithelial transport processes. By contrast, although some phenotypic features of alveolar epithelial cells may be found in immortal cell lines, these cells generally do not appear capable of replicating the transepithelial transport properties of intact alveolar epithelium sufficiently to allow their utilization *in vitro* for the study of pulmonary drug delivery/design/targeting.

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