Monolayers of Human Alveolar Epithelial Cells in Primary Culture for Pulmonary Absorption and Transport Studies

Katharina J. Elbert,[1,5] Ulrich F. Schäfer,[1] Hans-Joachim Schäfers,[2] Kwang-Jin Kim,[3] Vincent H. L. Lee,[4] and Claus-Michael Lehr[1,6]

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Purpose. To develop a cell culture model of human alveolar epithelial cells in primary culture for the *in vitro* study of pulmonary absorption and transport.

Methods. Type II pneumocytes isolated from normal human distal lung tissue by enzyme treatment and subsequent purification were plated on fibronectin/collagen coated polyester filter inserts, and cultured using a low-serum growth medium. Characterization of the cell culture was achieved by bioelectric measurements, cell-specific lectin binding, immunohistochemical detection of cell junctions, and by assessment of transepithelial transport of dextrans of varying molecular weights.

Results. In culture, the isolated cells spread into confluent monolayers, exhibiting peak transepithelial resistance of 2,180 ± 62 $\Omega \times cm^2$ and potential difference of 13.5 ± 1.0 mV (n = 30–48), and developing tight junctions as well as desmosomes. As assessed by lectin-binding, the cell monolayers consisted of mainly type I cells with some interspersed type II cells, thus well mimicking the situation *in vivo*. The permeability of hydrophilic macromolecular FITC-dextrans across the cell monolayer was found to be inversely related to their molecular size, with P_{app} values ranging from 1.7 to 0.2 × 10^{-8} cm/sec.

Conclusions. A primary cell culture model of human alveolar epithelial cells has been established, which appears to be a valuable *in vitro* model for pulmonary drug delivery and transport studies.

KEY WORDS: human alveolar epithelium; primary cell culture; lectin binding; histochemical characterization; drug transport.

INTRODUCTION

The absorption of drugs administered via the pulmonary route can be studied in different models: *in vivo* model, isolated perfused lung model, and *in vitro* cell culture model. Intact lung models most closely resemble the *in vivo* situation, but due to their complexity they do not allow to distinguish between permeation barriers presented by the alveolar epithelium and other tissues of the lung. Furthermore, the characterization of alveolar epithelial transport mechanisms is rendered difficult by the inaccessibility of the distal airways for precise dosing and sampling, along with the unknown experimental surface area.

Cell culture systems of isolated alveolar epithelial cells on the other hand provide a means to study the mechanisms of binding and transport processes at the level of the alveolar epithelium. The lack of systemic blood flow and respiratory motility in the cell culture system might influence the correlation between drug transport in culture to that *in vivo* to a yet undetermined extent. Nevertheless, the alveolar epithelium constitutes the major barrier to macromolecular drug absorption into pulmonary circulation (1,2). Tight monolayers of alveolar epithelial cells are considered to provide a useful model for transport studies of drugs applied via pulmonary routes to be absorbed into the systemic compartment.

In vivo, the alveolar epithelium consists of two distinct epithelial cell types: the cuboidal type II cells produce the lung surfactant and serve as progenitor cells for the second cell type, the type I cells, in case of lung injury (3,4). Type I cells cover 93% of the surface of the alveolar spaces, and appear as very thin cells with protruding nuclei, thus providing a short diffusion path for gas exchange. Alveolar macrophages as part of the immune system reside on the surface of the alveolar epithelium (5).

Although several lung epithelial cell lines obtained from bronchio-alveolar carcinoma or immortalized by transfection have been described, their characteristics share only limited similarity to the distal lung epithelium *in vivo*. Existing lung epithelial cell lines show mainly alveolar type II cell properties, and fail to develop substantial transepithelial electrical resistance, indicating the lack of tight junction-mediated epithelial barrier formation (6–8). Therefore, lung cell lines available to date do not appear to be suitable models for transport studies, while they may be useful tools to investigate type II pneumocyte biology.

Alveolar epithelial cells in primary culture on the other hand provide a tight epithelial absorption barrier resembling the pulmonary barrier *in vivo*, since in culture they differentiate into cell monolayers of high transepithelial resistance, and display morphological and functional properties similar to those of the *in vivo* situation (9).

Primary cultures of alveolar epithelial cells for use in transport studies have so far been isolated from rat and recently also from rabbit (10–12). However, due to species differences, transport characteristics observed in a human primary cell culture model are expected to more closely represent the situation in human than culture models derived from rodents. Alveolar epithelial cells from human lungs have previously been isolated and cultured by several researchers, in order to study their phospholipid secretion (13–15), xenobiotic metabolism (16), interaction with extracellular matrix components (17), morphology in mixed culture (18), release of arachidonic acid metabolites (19), polyamine uptake characteristics (20), and expression of MHC molecules (21). In none of these studies, however, the formation of a tight epithelial barrier in culture has been investigated. Therefore, our aim was to develop an alveolar epithelial cell culture system of human origin suitable for drug transport studies. Here we present evidence that human alveolar

[1] Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, 66123 Saarbrücken, Germany.
[2] Department of Thoracic and Cardiovascular Surgery, Saarland University, 66424 Homburg, Germany.
[3] Will Rogers Institute, Pulmonary Research Center, Department of Medicine, University of Southern California, Los Angeles, California 90033, USA.
[4] Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, California 90033, USA.
[5] Present address: Advanced Inhalation Research Inc., Massachusetts 02139, USA.
[6] To whom correspondence should be addressed. (e-mail: lehr@rz.uni-sb.de)

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epithelial cells grown in primary culture are capable of forming a tight epithelial barrier morphologically similar to the epithelium *in vivo*, which is an essential prerequisite for the utilization in *in vitro* drug transport studies.

MATERIALS AND METHODS

Materials

Specimen of distal portions of normal lung tissue were obtained from patients undergoing lung resection for bronchiogenic carcinoma (all males, average age was 66 years). Informed written consent was obtained from every patient prior to the operation. Trypsin (type I), DNase I, Percoll, DME/F12 powdered medium, nitroblue tetrazolium, 5-bromo-4-chloro-3-indolylphosphate disodium salt (BCIP), hematoxylin solution Gill No. 3, α-naphthyl acetate esterase kit, mouse monoclonal anti-human desmosomal protein antibody, FITC-labeled anti-rabbit Ig-antibody, FITC labeled anti-mouse Ig-antibody, propidiumiodide, and FITC-dextrans were obtained from Sigma (Deisenhofen, Germany). A549 cells were obtained from BioWhittaker (Walkerville, MD, USA) via Boehringer Ingelheim Bioproducts (Ingelheim, Germany). Aqueous mounting medium was from Polysciences (Eppelheim, Germany), and rabbit monoclonal anti-human ZO-1 antibody was obtained from Zymed (South San Francisco, CA, USA). Fetal bovine serum (FBS), amphotericin B, and penicillin-streptomycin stock solution were from Life Technologies (Eggenstein, Germany). Magnetic beads were obtained from Miltenyi Biotec (CD-14 microbeads; Bergisch Gladbach, Germany) and Dynal (M-450 CD-14 Dynabeads; Hamburg, Germany). Small airway growth medium (SAGM) was obtained from BioWhittaker (Walkersville, MD, USA) via CellSystems (Remagen, Germany). Human fibronectin and rat tail type I collagen were obtained from Collaborative Biomedical Products (Heidelberg, Germany). Polyester filter inserts (Transwell Clear, 0.45 μm pores, 6.5 mm diameter) were obtained from Corning-Costar (Bodenheim, Germany). Lectins labeled with different fluorescent markers were obtained from Vector Laboratories (Burlingame, CA, USA).

Isolation of Type II Pneumocytes

Type II alveolar epithelial cells were isolated using a modification of the methods described by Bingle *et al.* (18), Hoet *et al.* (20), and Cunningham *et al.* (21). Briefly, after removing visible bronchi, the lung tissue was chopped into pieces of 0.6 mm thickness using a McIlwain tissue chopper, washed with BSS (balanced salt solution; 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 10 mM HEPES, 5.5 mM glucose, pH 7.4) three times to partially remove macrophages and blood cells. The tissue was incubated in 0.5% trypsin twice for 15 minutes at 37°C in a shaking waterbath. The partially digested tissue was minced in the presence of 40% FBS in DME/F12 medium and DNase I (350 units/ml), and triturated for 5 minutes by repeatedly pipetting the cell suspension slowly up and down. After filtration through gauze and a 40 μm cell strainer, the cells were incubated with a 1:1 mixture of DME/F12 medium and SAGM, containing 5% FBS and 350 units/ml DNase I, on tissue-culture treated plastic petri dishes in a humidified incubator (5% CO_2, 37°C) for 90 minutes in order to let macrophage attach on the plastic surface. The non-adherent cells were layered on a discontinous Percoll density gradient (densities 1.089 and 1.040 g/ml) and centrifuged at 25 × g for 20 minutes. The cell layer at the interface of the two gradients was collected, and washed 4 × with BSS to remove the Percoll. To remove remaining alveolar macrophages, the cell suspension was then incubated with magnetic beads coated with anti-CD-14 antibodies at room temperature for 20 minutes under constant mixing. After the removal of the beads using a magnet and assessment of cell viability by trypan-blue exclusion, the purified type II pneumocyte suspension was suspended in SAGM supplemented with 1% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin, and plated on polyester filter inserts coated with fibronectin-collagen according to the method of Lechner *et al.* (22), at a cell density of 400,000 cells/cm^2. The cells were maintained in a humidified atmosphere (5% CO_2, 37°C) under liquid-covered conditions, and growth medium (0.2 ml on the apical and 0.8 ml on the basolateral side) was changed daily starting from 60 hours after plating the cells. The transepithelial electrical resistance (TEER) and the potential difference (PD) were measured every day starting from day 2 in culture using an EVOM voltohmmeter (WPI, Berlin, Germany) with the temperature of the culture plate maintained at 37°C. TEER and PD were corrected for the respective background values of contributed by filter and medium.

Evaluation of Alveolar Type II Epithelial Cell Purity

Type II pneumocytes were identified by staining for alkaline phosphatase (23). Freshly isolated cells were spun down on glass slides, air-dried, and stained for 20 minutes at room temperature. Enzyme substrate solution was prepared by adding 7 mg of nitroblue tetrazolium (dissolved in 400 μl DMF) to 10 ml of 0.2 M Tris-HCl buffer (pH 9.0), containing 3 mg of BCIP. Following incubation with the enzyme substrate solution, the specimen was washed, counterstained with hematoxylin solution for 2 minutes and mounted in aqueous mounting medium. Air-dried cytospin preparations of freshly isolated cell suspension were also stained for non-specific esterase activity to determine the number of macrophages, using naphthol AS-D chloroacetate as substrate (24).

Lectin and Immunohistological Staining

Freshly isolated cells as well as cells after 8 days in culture were stained with lectins specific for alveolar epithelial type II cells (Maclura pomifera agglutinin, MPA) and type I cells (Ricinus communis agglutinin, RCA; and tomato lectin, TL) (25–27). Origin, carbohydrate specificities, and the blocking sugars of those lectins are listed in Table 1. Unfixed cells grown on filter support were allowed to equilibrate in bicarbonated Krebs-Ringer-buffer (KRB; 114 mM NaCl, 5 mM KCl, 1.65 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, 10 mM HEPES, 20 mM $NaHCO_3$, 10 mM glucose, 1.1 mM $MgCl_2$, and 1.25 mM $CaCl_2$, pH 7.4) for 30 minutes at 37°C prior to incubation with FITC-MPA and rhodamine-RCA, or FITC-TL (all 50 μg/ml in KRB) for 1 h in a humidified atmosphere (5% CO_2, 37°C). The cells were then rinsed three times with KRB, methanol fixed, incubated with propidiumiodide for nuclear counterstain (where appropriate), and examined using a laser scanning confocal imaging system (MRC-1024, Bio-Rad, München, Germany) connected to an argon ion laser (American Laser Corp., Salt

Table I. Origin, Carbohydrate Specificities, and Blocking Sugars of the Lectins Used

Lectin	Origin	Carbohydrate specificity	Inhibitor
MPA	*Maclura pomifera*	GalNAc, Gal-GalNAc, α-Gal	GalNAc
RCA-I	*Ricinus communis*	GalNAc, Gal-GlcNAc, β-Gal	GalNAc
TL	*Lycopersicum esculentum*	GlcNAc	$(GlcNAc)_{2-3}$

Note: The abbreviations used: GalNAc, N-acetylgalactosamine; Gal, galactose; GlcNAc, N-acetylglucosamine.

Lake City, UT, USA) and an Axiovert 100 microscope (Carl Zeiss, Oberkochen, Germany). Binding specificity of each lectin was checked by coincubation with the appropriate sugar. Freshly isolated cells were spun down on glass slides, methanol fixed, and then lectin-stained as described above. To verify the cell specificity of the lectins used for cell type characterization, pieces of normal human lung tissue were fixed with Bouin's solution and embedded in paraffin. Sections of 4μm thickness were dewaxed, rehydrated and then lectin-stained as described above.

For detection of intercellular junctions, alveolar epithelial cells cultured on filter inserts were fixed with methanol, and incubated with rabbit anti-human ZO-1 (zonula occludens protein I) antibody at a dilution of 1:10 at 4°C overnight, followed by incubation with FITC-labeled anti-rabbit Ig-antibody (dilution 1:25) for 1 h at room temperature. Alternatively, mouse anti-human desmosome antibody (dilution 1:100), followed by FITC-labeled anti-mouse IgG-antibody (dilution 1:100) was used. Nuclei were counterstained using propidiumiodide, and the specimen were observed by confocal laser scanning microscopy.

FITC-dextrans Transport Studies

Confluent human alveolar epithelial cell monolayers on day 8 or 9 in culture exhibiting TEER above 1,400Ω × cm^2 (47 filters in total from 6 different preparations) and A549 cells grown to confluent monolayers (passage numbers 88–95) were used in the transport studies. Fluxes of FITC-labeled dextrans of molecular weights of 4, 10, 20, 40, and 70 kD at 37°C were measured across the monolayers in apical-to-basolateral and in basolateral-to-apical directions. Following 3 h equilibration with KRB containing 0.2% bovine serum albumin, transport experiments were started by spiking either apical (0.2 ml) or basolateral fluid (0.8 ml) with a stock solution of the respective FITC-dextran, yielding a final dosing concentration of 1 mg/ml.

Samples from the respective receiver fluid were taken at 0.5, 1, 2, 3, 4, and 5 hours, replaced with an equal volume of fresh KRB and assayed in a fluorescence plate reader (Cytofluor II, PerSeptive Biosystems, Wiesbaden, Germany) at excitation and emission wavelengths of 485 and 530 nm, respectively. Donor samples were also taken and analyzed similarily. At the end of the 5 hour transport experiment, TEER and PD were measured to monitor cell monolayer integrity.

Cumulative appearance of drug in the receiver compartment was plotted as a function of time. Apparent permeability coefficients P_{app} were calculated using the equation:

$$P_{app} = (1/(AC_0))(dQ/dt),$$

where A, C_0 and Q are the diffusion area, the initial donor concentration, and the amount of drug permeated across the cell layer, respectively.

RESULTS

Cell Isolation, Morphology, and Bioelectric Properties

Cell yields were 2.4 ± 0.9 × 10^6 cells/g lung tissue at 81 ± 1% alveolar type II epithelial cell purity, as assessed by staining for alkaline phosphatase (Fig. 1A), and a viability of 91 ± 4% (n = 7). Macrophages were estimated to constitute 10.0 ± 3.7% (n = 3) of the total cell population upon seeding.

In culture, the isolated cells spread to form confluent monolayers, exhibiting protruding nuclei surrounded by thin cytoplasmic extensions, similar to type I cells *in vivo*, by day 6–8 in culture (Fig. 1B). The alkaline phosphatase activity appeared to be decreased in cells cultured for 8 days, compared to that in freshly isolated cells.

Cross sections of human alveolar epithelial cells grown on a Transwell filter membrane for 7 days (Fig. 1C, glutaraldehyde/osmium tetroxide fixed and embedded in resin, semithin sections stained with methylene blue and viewed under a light microscope) verified that indeed monolayers of very thin cells had formed. Multilayered cells were not found at earlier or later time points of the growth period also (not shown).

From day 3 in culture onwards the cell layers began to exhibit measurable TEER and PD, showing peak values of 2180 ± 62 Ω × cm^2 and 13.5 ± 1.0 mV (apical side being negative), respectively, on day 8 (n = 30–48, from six different preparations (Fig. 2).

Characterization by Lectin Binding and Immunohistology

Freshly isolated type II pneumocytes, double-stained with FITC-MPA and rhodamine-RCA, showed preferential MPA binding, while RCA-stained cell remnants could also be seen, sometimes attached to MPA-positive cells (Fig. 3A). after 8 days in culture, most of the seeded type II cells acquired a flattened morphology, exhibiting prominent binding of alveolar type I cell specific RCA (Fig. 3B). MPA-binding type II cells, albeit few in numbers, are also present in the monolayer, interspersed with the type I-like cells. In a few cases round cells sitting on the epithelial monolayer, without contact to the filter membrane, could be seen, most likely representing alveolar macrophages. Tomato lectin (TL) as another type I cell-specific lectin was found to bind to the vast majority of cells on day 8 in culture. Coincubation with the respective inhibitory sugar blocked lectin-binding nearly completely (data not shown). The lectin binding observed in sections of human lung tissue confirmed the type I pneumocyte specificity of RCA and TL, and the type II pneumocyte specificity of MPA (data not shown).

The presence of tight junctions was demonstrated by visualization of ZO-1 protein. The cultured alveolar epithelial cell monolayer showed intensive staining of tight junctional belts

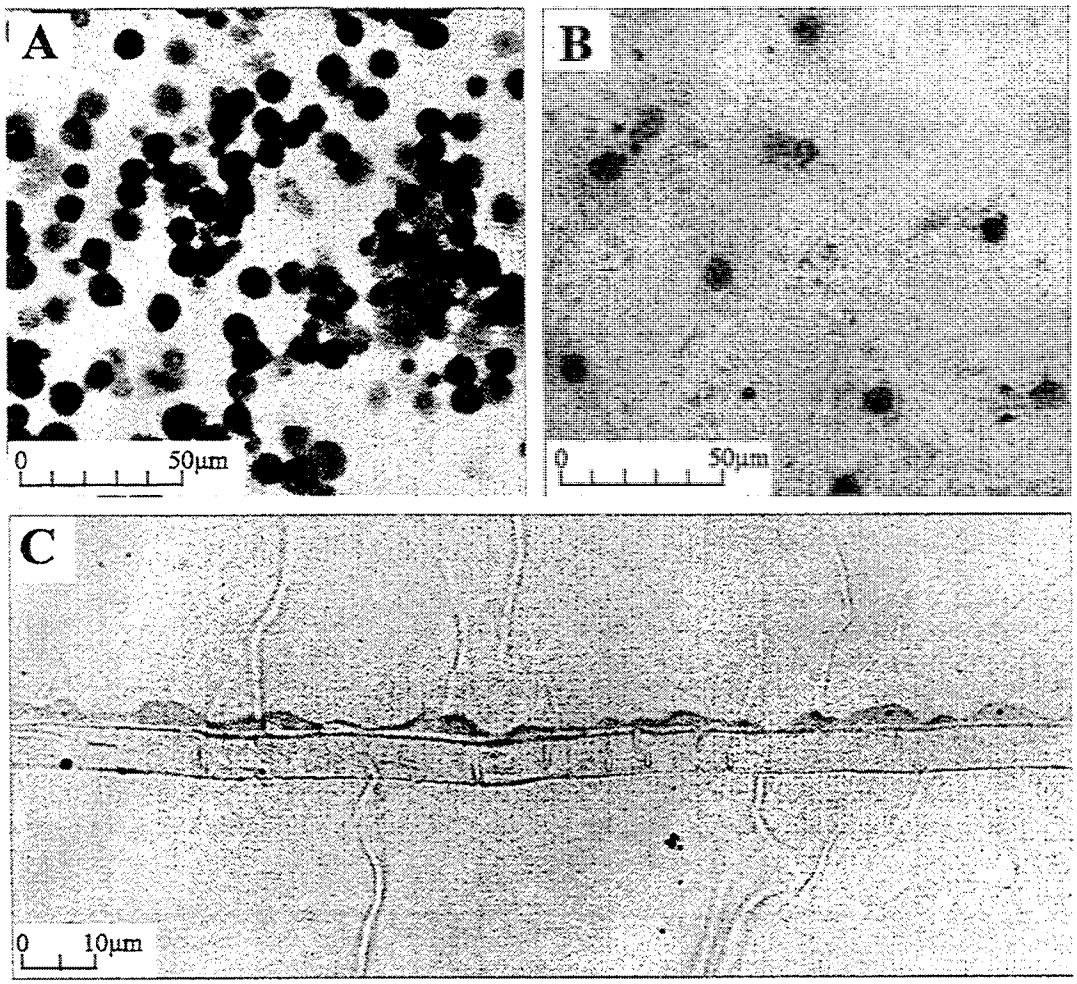

Fig. 1. Freshly isolated human alveolar epithelial cells (panel A) and human alveolar epithelial cells on day 8 in culture (panel B), stained for alkaline phosphatase. Cell nuclei are counterstained with hematoxylin. Panel C shows a cross section of a human alveolar epithelial cell monolayer grown on a Transwell filter membrane for 7 days (glutaraldehyde/osmium tetroxide fixed and embedded in resin, semithin sections stained with methylene blue and viewed under a light microscope).

surrounding flattened (type I-like) as well as smaller cuboidal (type II) cells (Fig. 4A). Desmosomes, appearing as chain of pearl-like dots between neighboring cells, could be detected as well (Fig. 4B).

Transport Studies

The flux of the hydrophilic macromolecular model drugs FITC-dextrans across human alveolar epithelial cell monolayers was found to be linear when plotted against time and appears to be inversely related to their molecular size (Fig. 5). The transport of the FITC-dextrans in the apical-to-basolateral direction was not significantly different from that in the basolateral-to-apical direction (Table 2, $p > 0.2$). TEER of the cell monolayers increased by 32%, while PD declined by 31% during the 5 hours of experiment.

DISCUSSION

Culture Conditions

Our study demonstrates that it is possible to obtain tight and differentiated alveolar epithelial cell monolayers of human origin by culturing alveolar type II pneumocytes isolated from resected lung tissue. Isolation and purification of alveolar epithelial type II cells, and culture in low-serum growth medium (containing 1% FBS) supported the development of confluent monolayers consisting of flattened, type I-like cells, exhibiting bioelectric parameters similar to those reported for rat alveolar epithelial cell monolayers (TEER of >2,000 $\Omega \times cm^2$, and PD > 10 mV (10). When DME/F12 1:1 mixture containing 10% FBS, a growth medium often employed in airway cell cultures, was used, transepithelial resistance did not rise above 180 $\Omega \times cm^2$, and proliferation of fibroblasts was observed in the cultures (data not shown). In contrary to rat alveolar epithelial cells, which are known to be able to attach and spread on uncoated tissue culture-treated filter membranes (10), we observed that fibronectin-collagen-coated filter surfaces favored the attachment of human alveolar epithelial cells and differentiation in comparison to uncoated filters. On uncoated tissue-cultured treated filters surfaces, the human cells attached to a lesser extent, and failed to develop high TEER and PD values in culture in most cases. These observations are in

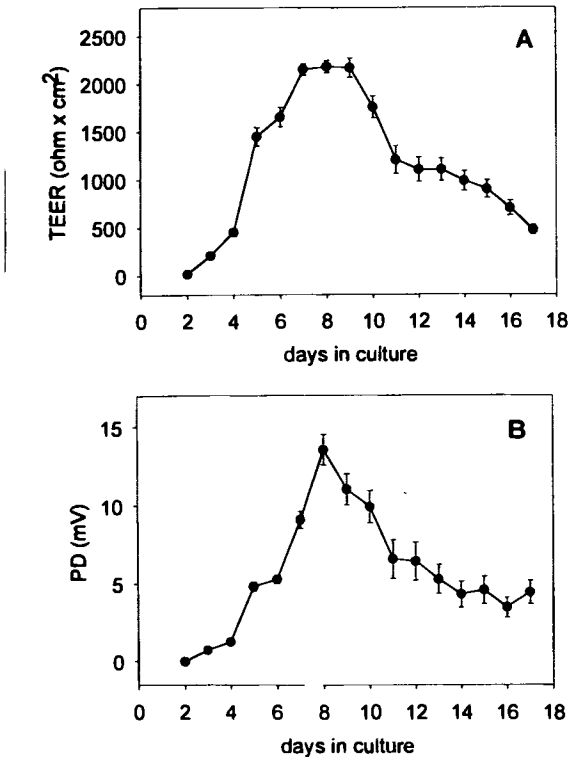

Fig. 2. Time courses of transepithelial resistance (TEER, plot A) and potential difference (PD, plot B) in human alveolar epithelial cells in culture. Each point represents the mean ± S.E. value of 30–48 determinations from six different cultures.

agreement with data obtained by other groups, reporting species differences for the substratum adherence of human and rat alveolar epithelial cells in culture. Robinson *et al.* found that human pneumocytes adhere poorly to tissue culture-treated plastic, but well to extracellular matrix (13). Similarly, Papadopoulos *et al.* observed an approximately three times higher adherence of human alveolar type II cells to collagen and fibronectin substrata when compared to uncoated tissue culture plastic. Furthermore, collagenous substrata were found to promote cell spreading in culture (17). Nevertheless, some cultures we prepared developed into monolayers of differentiated, flattened cells, exhibiting high bioelectric values, even when seeded on uncoated tissue culture-treated filter membranes. This irregularity might be attributed to the variability in healthiness of the resected lung tissue, as the degree of inflammatory changes due to smoking and to obstruction by tumor, and consequently the individual level of cell activation may be different from patient to patient. Our data suggest that partially compromised epithelial cells may benefit from a preexisting layer consisting of the extracellular matrix components collagen and fibronectin, while less compromised cells may be able to depend solely on their endogenous synthesis and release of matrix proteins for attachment and spreading.

Similarly, we found that the variability in tissue healthiness, which varied from donor to donor, may have an influence on the optimal seeding density. In preliminary experiments we tested seeding densities of 2×10^5, 4×10^5, and 6×10^5 cells/cm^2, and found that in some cases seeding even at the lowest density (2×10^5 cells/cm^2) still resulted in tight cell monolayers. In most preparations a seeding density of at least 4×10^5 cell/cm^2 was required for the establishment of monolayers tight enough for transport studies. In all cases, however, seeding at high densities (at or above 6×10^5 cells/cm^2) did not further enhance bioelectric properties, but on the contrary resulted in lower and/or less persistent TEER and PD values. Thus, the seeding density of 4×10^5 cell/cm^2 was regarded as optimal and used for generating all the data presented here.

Morphological Characteristics and Lectin Binding of Cultured Cell Monolayers

Morphological observations and lectin histochemical characterization employing known type I and type II cell-specific lectin markers on human alveolar epithelial cells grown for 8 days in culture suggest the differentiation of seeded type II cells to type I like cells in culture. The flattening and spreading of cytoplasm of the seeded pneumocytes in culture (Fig. 1), the reactivity of cells in culture with cell-specific lectin markers (Figs. 3A and B), and the re-establishment of an intact epithelial barrier in culture provide evidence for the differentiation of type II to type I-like cells. Immunohistochemical evidence for the expression of tight junctional protein ZO-1 as well as desmosomes (Figs. 4A and B) further corroborate the development of a tight epithelial monolayer suitable for transport studies.

Permeability of Cell Monolayers to Model Drugs

To assess the permeability of this human alveolar epithelial barrier in culture to macromolecular compounds, we studied the transport of the hydrophilic, paracellularly transported model drugs (FITC-dextrans with molecular weights ranging from 4 to 70 kD) across the cultured cell monolayer. The transport of FITC-dextrans is dependent on molecular size (Fig. 5) and shows no directionality, with apparent permeability coefficients P_{app} ranging from 0.2 to 1.7×10^{-8} cm/sec (Table 2). These P_{app} values are strikingly similar to those reported for dextrans translocated across rat alveolar epithelial cell monolayers, which exhibit similar TEER values of >2,000 $\Omega \times$ cm^2 in culture (11) (Table 3). The lack of directionality in dextran fluxes as well as the observed semi-linear relationship between diffusion coefficients and dextran P_{app} values are suggestive of diffusion through aqueous pores. Since the P_{app} values for dextrans found in rat and human alveolar epithelial cells as well as the size of the cells (height and number of cells per cm^2) are similar, it is likely that dextrans in human alveolar epithelial monolayers probably permeate via the same equivalent pore system with ~6 nm radius. Studies with a wider range of hydrophilic molecules will be required to determine an accurate pore size and number, in order to compare the pore characteristics of our cell culture model to equivalent pore models reported for lung epithelial barriers by others (29,30). Further studies investigating other mechanisms of transport, such as carrier mediated or endocytotic processes, need to be carried out in for further characterization of our cell culture model. Comparison of permeability data observed in *in vitro* systems with data obtained from lung *in vivo* experiments is difficult because of the complex anatomical structures in the intact mammalian lung, and by differences in experimental setup (e.g., method of dose application, and effective surface

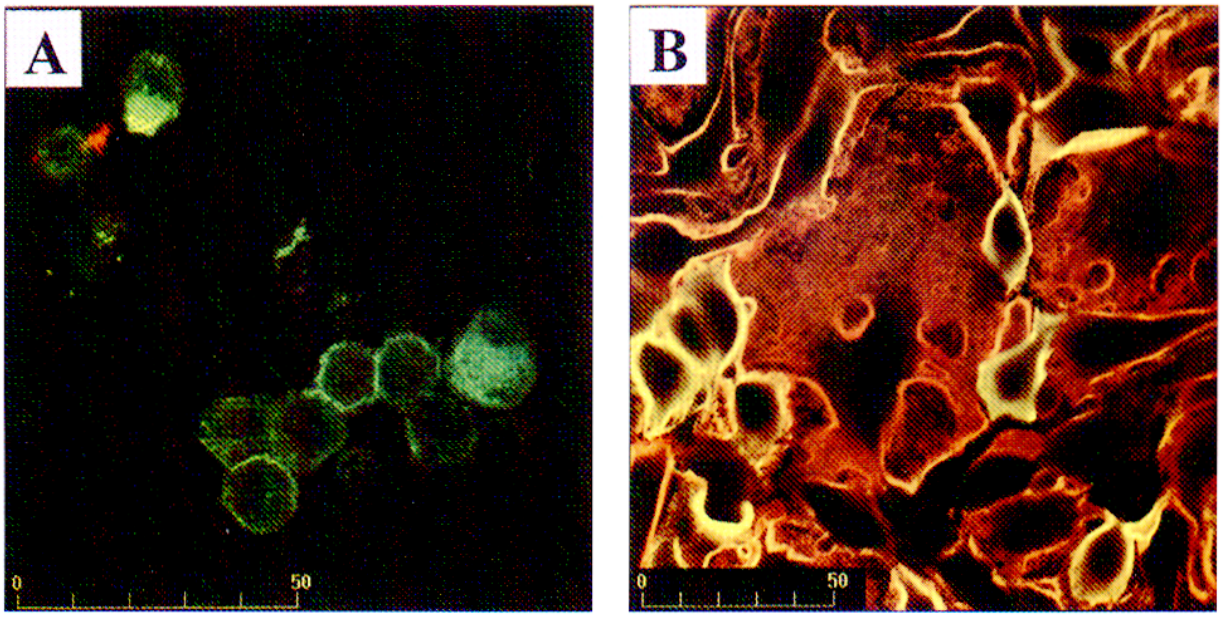

Fig. 3. Freshly isolated human alveolar epithelial cells (panel A) and human alveolar epithelial cells on day 8 in culture (panel B), double-stained with FITC-MPA (green fluorescence) and rhodamine-RCA (red fluorescence). (Bar = 50 μm).

area). The P_{app} value for FD-70 reported in our study is about an order of magnitude lower than that of $\sim 8 \times 10^{-8}$ cm/sec found by Theodore *et al.* for 60–90 kD dextrans in the distal respiratory tract of dogs (28). In contrast, Morita *et al.* observed clearance rates of 7.2, 2.0, and 0.4% for FITC-dextrans of 4, 10, and 70 kD, respectively, in rat lungs *in vivo* within two hours (31). Considering the surface area of rat lung to be $\sim 4 \times 10^3$ cm^2, these values would translate into P_{app} values in the order of 10^{-9} cm/sec, which is about an order of magnitude lower than those found for human and rat (11) alveolar epithelial cell monolayers. The P_{app} values reported for FITC-dextrans across the human lung cancer cell line A549 (32) are similar to those in A549 cells obtained in the present study. It is interesting to note that these values are 150–300 fold higher than the corresponding values across the human and rat primary culture models. It is likely that the higher P_{app} across A549 cells is due to the much lower transepithelial resistance of A549 cells. This emphasizes the better utility of our human primary cell

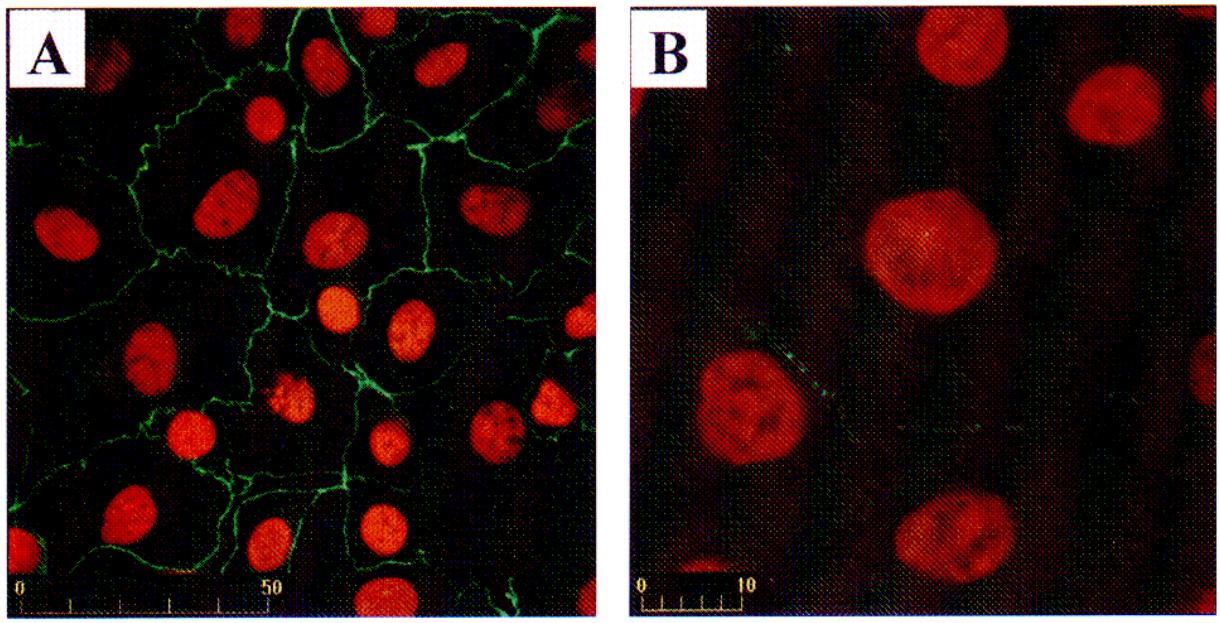

Fig. 4. Human alveolar epithelial cells on day 9 in culture, immunostained for ZO-1 protein (panel A, green fluorescence) and desmosomal protein (panel B, green fluorescence). Cell nuclei are counterstained with propidiumiodide (red fluorescence). (Bars = 50 μm and 10 μm, respectively).

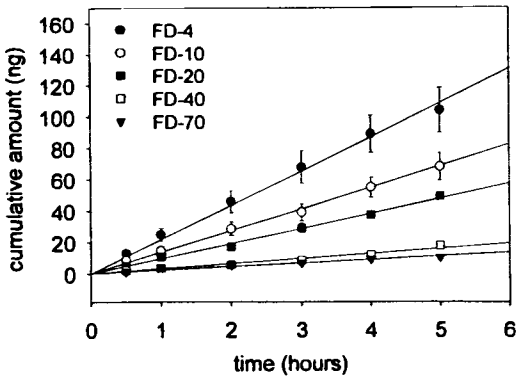

Fig. 5. Time courses of FITC-dextran transport in apical-to-basolateral direction across human alveolar epithelial cells on day 8–9 in culture. Each point represents the mean ±S.E. value of 6–9 determinations from six different cultures.

Table III. Comparison of FITC-Dextran Permeability Characteristics Among Different Alveolar Epithelial Cell Culture Models

Drug	P_{app} (cm/sec) × 10^{-8} [a]			
	Human alveolar epithelial cell monolayers	Rat alveolar epithelial cell monolayers[b]	A549 cells[c]	A549 cells[d]
FD-4	1.71 ± 0.25	1.29 ± 0.06	340 ± 30	254 ± 1
FD-10	1.05 ± 0.11	1.16 ± 0.09	220 ± 2	149 ± 11
FD-20	0.80 ± 0.04	1.13 ± 0.17	140 ± 6	113 ± 12
FD-40	0.29 ± 0.03	0.35 ± 0.06	71 ± 3	40 ± 6
FD-70	0.19 ± 0.07	0.15 ± 0.01	60 ± 5	21 ± 4

[a] Mean ± S.E.
[b] From Matsukawa *et al.* (11).
[c] From Kobayashi *et al.* (32).
[d] Present study.

culture model for *in vitro* drug transport studies compared to the A549 cell culture model. Since FITC-dextran fluxes across the human alveolar epithelial cell monolayer in primary culture were of similar magnitude as the dextran fluxes across the rat alveolar epithelial cell monolayers, our data suggest that at least paracellular transport across the alveolar epithelium does not show a species difference between human and rat. Further work will help to clarify whether a species difference exists on the other hand for transcellular simple diffusion, carrier-mediated transport, and endocytotic processes.

In summary, human alveolar epithelial type II cells isolated from normal human lung tissue were shown to undergo morphological and histochemical changes, differentiating from type II to type I-like cells. The cells spread and developed into confluent tight monolayers, which react with type I cell-specific lectin markers, and exhibit tight junctions as well as desmosomes. This primary cell culture system appears to provide an *in vitro* model useful for drug absorption and transport studies of the human alveolar epithelial barrier, enabling the investigation of epithelial transport systems present in the human distal lung.

ACKNOWLEDGMENTS

This paper is dedicated to Professor B.C. Lippold, Düsseldorf, on occasion of his 60th birthday.

Table II. Permeability Characteristics of FITC-Dextrans (FD) of Varying Molecular Weights Across Primary Cultured Human Alveolar Epithelial Cell Monolayers

Drug	MW (daltons)	P_{app} (cm/sec) × 10^{-8} in human alveolar epithelial cell monolayers[a]	
		AB	BA
FD-4	4,400	1.71 ± 0.25	1.12 ± 0.25
FD-10	12,000	1.05 ± 0.11	N.D.
FD-20	19,500	0.80 ± 0.04	N.D.
FD-40	42,000	0.29 ± 0.03	N.D.
FD-70	71,200	0.19 ± 0.07	0.10 ± 0.02

Note: The abbreviations used: AB, apical-to-basolateral; BA, basolateral-to-apical; N.D., not determined.
[a] Mean ± S.E. (n = 6–9).

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