

Respiratory virus infection of differentiated primary bronchial/tracheal and small airway epithelial cells

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Summary

Background: The continuing emergence of viruses causing significant respiratory illness in humans highlights the need for *in vitro* models of the human airway epithelium to better understand virus biology and pathogenesis. In this study, we investigate the ability of ATCC® Primary Cell Solutions™ Primary Human Bronchial/Tracheal Epithelial Cells (ATCC® PCS-300-010) and Primary Human Small Airway Epithelial Cells (ATCC® PCS-301-010) to differentiate into airway epithelium using an air-liquid interface culture system, as well as their susceptibility to a range of respiratory viruses, including several isolates of the 2009 H1N1 influenza pandemic. Results were compared using varying media conditions and between two different human cell donors. Differentiation into the appropriate cell types that populate the airway epithelium was determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Methods: Cells were differentiated at an air-liquid interface for 28 days and cell differentiation was determined by qRT-PCR performed on RNA extracted at 7-day intervals. After 28 days of differentiation, the cells were infected with respiratory viruses, including 5 isolates of the 2009 H1N1 influenza A virus, influenza B virus, respiratory syncytial virus, parainfluenzavirus 3, rhinovirus 16, adenovirus type 5, and coronavirus. Infection was monitored for 4 days, and resultant viral yields were determined by plaque assay for influenza viruses and 50% tissue culture infective dose (TCID₅₀) for all other viruses.

Results: Up-regulation of genes involved in cilia formation, secretory protein production, and cell-cell adhesion was observed after differentiation in both the primary human bronchial/tracheal and small airway epithelial cells. Viral susceptibility after differentiation was demonstrated for both types of primary cells, although differences in viral growth kinetics between the cell types was observed. Media conditions were shown to have a pronounced impact on gene expression and viral infectivity. Finally, differences were also observed between primary cells isolated from different human donors.

Conclusions: This work demonstrates the ability of ATCC® Primary Cell Solutions™ Primary Human Bronchial/Tracheal Epithelial Cells and Primary Human Small Airway Epithelial Cells to be used as *in vitro* models that more closely represent the human airway epithelium than standard cell culture. These model systems may be useful tools for the investigation of viral infections of the respiratory epithelium. Optimization of media conditions is critical for proper differentiation of these cells at an air-liquid interface. Differences in viral infectivity were observed between primary bronchial/tracheal cells isolated from different human donors.

Introduction

The airway epithelium is a continuous pseudostratified layer of cells that line the respiratory tract. It is composed of 3 major cell types: basal, ciliated, and secretory. A critical function of the airway epithelium is to act as a protective barrier from infection by microbial agents and other inhaled particles. Proper differentiation into the cell types populating the airway epithelium is critical for maintaining major defense functions such as mucociliary clearance and secretion of factors mediating immunity (1, 3). Viral infections of the respiratory tract occur when a virus is able to bypass these protective barriers and gain entry into airway epithelial cells by attachment to receptors on the cell surface (3). Special culture systems have been developed whereby primary human airway epithelial cells can be stimulated to differentiate into pseudostratified epithelium with mucin secretion and functional cilia. The formation of an epithelium from undifferentiated primary airway epithelial cells is dependent on creation of an air-liquid interface using a collagen-coated semi-permeable membrane which allows the basal surface of the cells exposure to differentiation media while the apical surface remains exposed to air (2). In this study, we investigate the ability of ATCC® Primary Cell Solutions™ Primary Human Bronchial/Tracheal Epithelial Cells (ATCC® PCS-300-010) and Primary Human Small Airway Epithelial Cells (ATCC® PCS-301-010) to differentiate into airway epithelium using the air-liquid interface culture system, as well as their susceptibility to infection by a range of respiratory viruses including several isolates from the 2009 H1N1 influenza pandemic.

Materials and Methods

Cell differentiation Primary Human Bronchial/Tracheal Epithelial Cells (ATCC® PCS-300-010) and Primary Human Small Airway Epithelial Cells (ATCC® PCS-301-010) were maintained in Airway Epithelial Cell Basal Medium (ATCC® PCS-300-030) supplemented with either Bronchial Epithelial Cell Growth Kit (ATCC® PCS-300-040) or Small Airway Epithelial Cell Growth Kit (ATCC® PCS-301-040) according to product recommendations. To compare cells from a different human donor, Human Bronchial/Tracheal Epithelial Cells (FC-0035) and Human Small Airway Epithelial Cells (FC-0016) were obtained from Lifeline Cell Technologies® (Frederick, MD) and grown under the same conditions as the ATCC® cells. Cells were differentiated by seeding 50,000 cells onto a collagen-coated semi-permeable membrane supported in a 24-well plate (Corning® HTS Transwell®-24 Well Plate 3378, 0.4mm pore size). Three days after seeding, growth media was removed and 0.5ml of cell type-specific differentiation medium was applied to the basal chamber of each well, allowing for the formation of an air-liquid interface (ALI) to promote cell differentiation. Differentiation media was changed every 2-3 days for 28 days before infection.

Viruses Influenza viruses were obtained from either the ATCC® or BEI Resources collection (Manassas, VA). Influenza viruses were produced either in cell culture (ATCC® CCL-34™ MDCK cells) or embryonated chicken eggs. Other respiratory viruses were obtained exclusively from the ATCC® collection. Virus details are listed below.

ATCC/BEI #	Virus	Strain	Source
VR-1535	Influenza B	B/Lee/40	Cell culture
VR-1737	Influenza A (H1N1)	A/Virginia/ATCC2/2009	Cell culture
NR-13658	Influenza A (H1N1)	A/California/04/2009	Cell culture
NR-13659	Influenza A (H1N1)	A/California/04/2009	Egg
NR-13663	Influenza A (H1N1)	A/California/07/2009	Egg
NR-14694	Influenza A (H1N1)	A/New York/18/2009	Egg
VR-5	Human adenovirus 5	Adenoid 75	Cell culture
VR-93	Human parainfluenza virus 3	C 243	Cell culture
VR-283	Human rhinovirus 16	11757	Cell culture
VR-740	Human coronavirus	229E	Cell culture
VR-26	Human respiratory syncytial virus A	Long	Cell culture

Infection and titration Differentiated cells were infected with virus at day 29 post-ALI. All influenza viruses were inoculated at 5,000 PFU/well while the other respiratory viruses were inoculated at 50,000 IU/well except coronavirus, which was inoculated at 5,000 IU/well. To infect, the apical surface the cells were washed once with 100µl PBS before applying 100µl of virus dilution and incubating for 1 hour at 37°C, 5% CO₂. Virus was removed and the apical surface washed once with 100µl PBS before incubating the plates at 37°C, 5% CO₂ for 4 days. Virus was harvested every 24 hours post-infection by applying 200µl of differentiation medium to the apical surface of the cells, incubating at 37°C, 5% CO₂ for 20 minutes before harvesting. Four wells were inoculated per virus so that each well was harvested only once. Viruses were titered by plaque assay on MDCK (ATCC® CCL-34™) cells for influenza viruses and by 50% tissue culture infective dose (TCID₅₀) for all other respiratory viruses. Limit of detection is 10^{1.45} TCID₅₀ or PFU/ml.

Quantitative RT-PCR (qRT-PCR) RNA was extracted from cells at day 0, 7, 14, 21, and 28 post-ALI using TRIzol® Reagent (Invitrogen™ 15596-018) according to manufacturer's instructions. Quantitative RT-PCR was performed using High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems™ 4374966) to create cDNA, followed by TaqMan® Gene Expression Master Mix (Applied Biosystems™ 4369016) and pre-made primers from Applied Biosystems™ for qRT-PCR. The pre-made primers target *MUC5B* (Hs00861588_m1), *TUBA1A* (Hs00362387_m1), *PCDH1* (Hs00170174_m1), *SCGB1A1* (Hs00171092_m1), and *SFTPB* (Hs01090667_m1). Beta-actin was used as a control to normalize gene expression levels (Human ACTB Endogenous Control, Applied Biosystems™ 4333762F). Fluorogenic signal was detected using the CFX96™ Real-Time PCR Detection System (Bio-Rad).

Results

Gene expression: Analysis of the expression of genes involved in cilia formation, secretory protein production, and cell adhesion in differentiating primary bronchial/tracheal epithelial cells

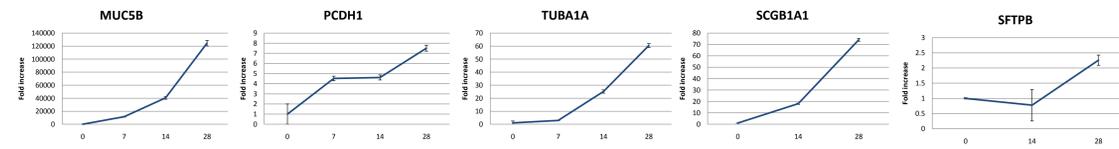


FIG 1. RNA was extracted at 7-day intervals from differentiating primary bronchial/tracheal cells and qRT-PCR was performed to determine relative gene expression levels. Up-regulation of genes involved in cilia formation, secretory protein production, and cell-cell adhesion were seen in both cell types. Gene expression was normalized to β-actin.

Gene	Product	Function
MUC5B	mucin-5B	Component in the mucus matrix of the airway
TUBA1A	tubulin alpha-1A	Structural protein in the axonemal microtubules of cilia
PCDH1	protocadherin-1	Cell-cell adhesion (likely involved in cell-cell adhesion of ciliated columnar cells)
SCGB1A1	Clara cell secretory protein	Protective protein secreted by Clara cells of the bronchiolar epithelium
SFTPB	surfactant protein B	Protective protein secreted by airway secretory cells

Viral infection: Differentiated primary bronchial/tracheal and small airway epithelial cells show varying susceptibility to respiratory virus infection

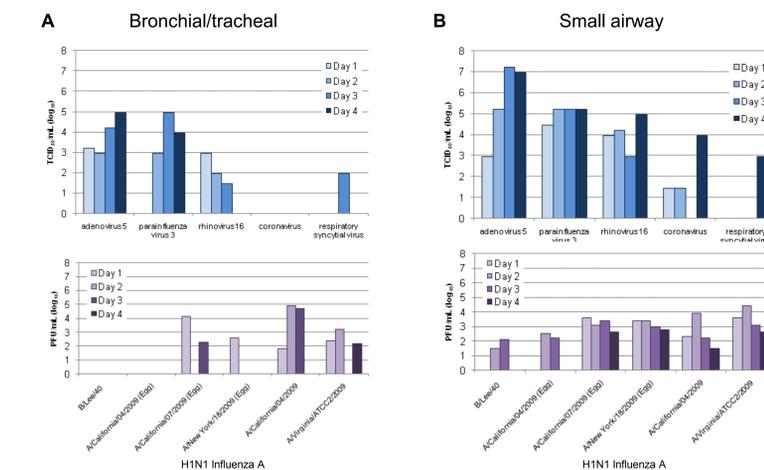


FIG 2. Cells were differentiated in an air-liquid interface culture system for 28 days before infection. Virus was harvested from the apical surface every 24 hours for 4 days and titered by plaque assay for influenza viruses or TCID₅₀ for all other respiratory viruses. Titration data is shown for viral infection of differentiated primary bronchial/tracheal epithelial cells (A) and differentiated primary small airway epithelial cells (B). In general, the primary small airway epithelial cells produced higher titer virus and the infections persisted for a longer period of time.

Media conditions: Media used during differentiation significantly affects gene expression levels and susceptibility to infection in primary bronchial/tracheal epithelial cells

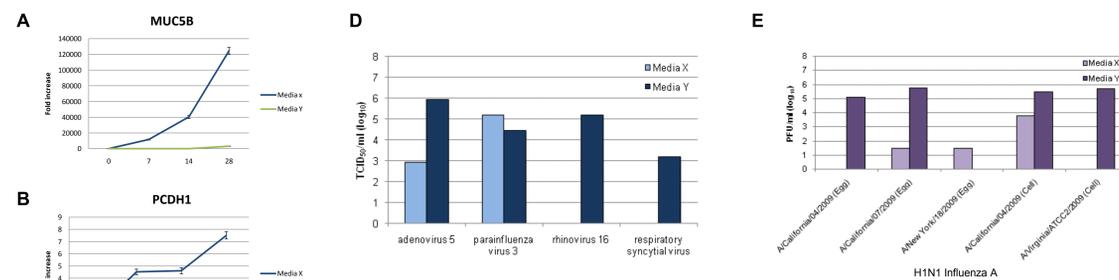


FIG 3. In all graphs, Media X represents differentiation media where optimal differentiation of primary bronchial/tracheal cells was seen. Media Y represents differentiation media where the cells did not properly differentiate into mucociliary cell types. Quantitative RT-PCR data is shown (A, B and C) comparing gene expression levels in primary bronchial/tracheal cells differentiated in the two different media formulations. Cells were differentiated for 28 days in either media condition before infection with respiratory viruses. Virus was harvested two days post-infection and titered using plaque assay or TCID₅₀ (D and E). Overall, cells grown in sub-optimal media are generally more susceptible to infection and produce more infectious virus by day 2 post-infection. Exceptions are parainfluenzavirus 3 (D) and one isolate of H1N1 influenza A (E).

Results

Cell donors: Differentiated bronchial/tracheal epithelial cells from two different human donors show varying viral infectivity

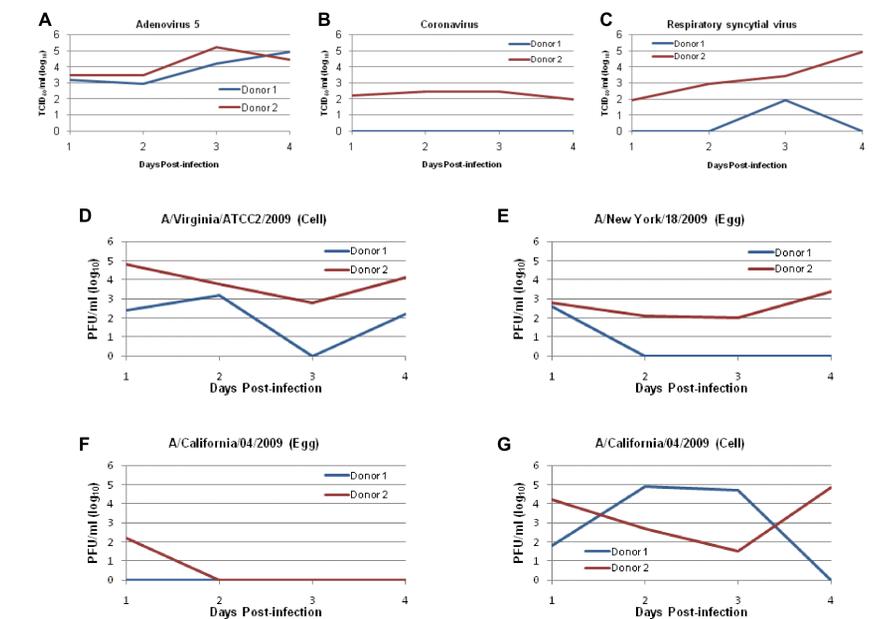


FIG 4. Primary bronchial/tracheal cells isolated from two different human donors were differentiated for 28 days before infection with respiratory viruses. Adenovirus 5 is shown as an example of similar infectivity between the two donors (A), while coronavirus (B) and respiratory syncytial virus (C) show greater ability to infect differentiated cells from Donor 2 than Donor 1. Variability between donors was also observed after infection with the H1N1 influenza A isolates (D-G). In one case, a significant difference in susceptibility was seen between the same isolate (A/California/04/2009) propagated in either embryonated chicken eggs (F) or cell culture (G) prior to use in this study.

Conclusion

This work demonstrates the potential for ATCC® Primary Cell Solutions™ Primary Human Bronchial/Tracheal Epithelial Cells and Primary Human Small Airway Epithelial Cells to be used as *in vitro* models to better understand respiratory virus biology. We found that primary cell differentiation was marked by up-regulation of genes involved in the formation of cilia, secretory proteins, and cell-cell adhesion. Overall, differentiated primary bronchial/tracheal and small airway epithelial cells were susceptible to respiratory viral infections, though differences were observed between each virus. We found that cell differentiation and viral infectivity was greatly effected by media conditions. In general, properly differentiated cells are less susceptible to viral infection. This indicates that the differentiated primary cells are able to act as a barrier to infection similar to their function *in vivo*. Finally, we show evidence that infectivity can vary greatly between the same cells isolated from two different human donors. This provides a caution for researchers wishing to reach general conclusions from results obtained from a single donor. Our work demonstrates the potential of using primary cell models of the airway epithelium to study viral infections of the respiratory tract. This work is particularly relevant now that primary cells are easy to obtain and can significantly enhance research performed using cell culture.

References

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