Comparison of respiratory virus infection between human nasal epithelial cell monolayer and air-liquid interface 3D culture

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Introduction

Viral infection has been implicated in exacerbations of COPD and Asthma[1)], and Human Rhinovirus (HRV) is often detected in patients during exacerbations[2)3)]. Therefore, appropriate *in vitro* model is required to evaluate infectious mechanism and the efficacies of new therapy.
In this study, we compared the replication of virus, such as human rhinovirus (HRV16), respiratory syncytial virus (RSV A2) and influenza virus (WSN33, H1N1) in 3D culture air-liquid interface human nasal epithelial cells (NEC-3D) and NEC monolayer culture (NEC-M).

Methods

Virus
HRV16 and RSV-A2 were obtained from ATCC and WSN33, H1N1 was obtained from HPA, UK.

Cells
Nasal epithelial cells (NEC) were purchased from Epithelix Sárl and cultured as monolayer. Air-liquid interface cultured nasal epithelial cells were also obtained from Epithelix Sárl (MucilAir™)

Infection
Before infection, cells were washed with media to remove mucus. HRV16 (approx. 1MOI, 5.9 x 10^5 $TCID_{50}$), RSVA2 (approx 0.1 MOI, 5.7 x 10^5 $TCID_{50}$) and WSN33 (approx. 1 MOI, 5.7 x 10^5 $TCID_{50}$) were infected to ALI cultured insert. HRV16 (approx. 1MOI, 2.8 x 10^4 $TCID_{50}$), RSVA2 (approx. 0.1 MOI, 2.4 x 10^4 $TCID_{50}$) and WSN33 (approx. 1 MOI, 2.8 x 10^4 $TCID_{50}$) were infected to nasal epithelial monolayer culture (96 well plate).
The cells were washed out with PBS after 1hr virus absorption. The cells were incubated further up to 10 days. Supernatants were collected everyday and kept at -80ºC. For RSV, the supernatant was mixed with sucrose solution (25%) before frozen.

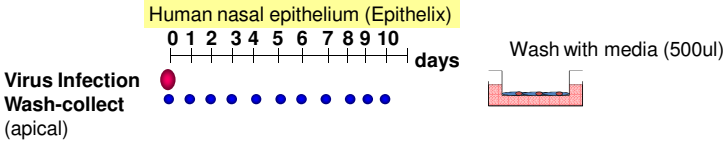
Virus titration assay

Extracellular HRV and RSV load has been determined by CPE assay in Hela cells and Hep2G cells, respectively. Twenty µl of supernatant was collected and 10-fold serial dilutions of the supernatant were prepared with DMEM containing 1% FCS. All titrations were performed by infecting confluent Hela/Hep2G cell monolayers in 96 well plate with serially diluted supernatant (1/10 – 1/100000) and then assessing each dilution's cytopathic effects at 3 days after infection by visual inspection. The cells were then stained with Crystal Violet for confirmation. The amount of virus required to infect 50% of Hela/Hep2G cells was calculated in each treatment and shown as log,$TCID_{50}$ (50% tissue culture infection dose)U/20µl.

Extracellular H1N1 was also determined by CPE assay in MDCK cells. Twenty µl of supernatant was collected and 10-fold serial dilutions of the supernatant were prepared with DMEM in the FCS-free DMEM containing 1.5 µg/ml L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) treated trypsin.

IL-8 assay

The levels of IL-8 were determined by ELISA (R&D systems).



Results

HRV16 replicated in NEC monolayer and the $TCID_{50}$ was 3 at 5days post-infection. In contrast HRV16 replicated better in 3D-NEC and $TCID_{50}$ reached to 4.5 at 4 days post-infection, and peaked at 6 days (6.3 $TCID_{50}$) (Figure 1). RSV A2 replicated well in both cells with peak $TCID_{50}$ of 4 in monolayer and 5.3 in 3D-NEC (Figure 2). Influenza WSN33 only weakly replicated in NEC monolayer with peak $TCID_{50}$ of 2, but it replicated well in 3D-NEC and the peak $TCID_{50}$ was 6.8 at 5 days post-infection (Figure 3). All virus induced IL-8 production and the induction level was less than 10 fold compared with basal IL-8 level in NEC monolayer, but it was induced by more than 400 fold in 3D-NEC (Figure 4).

Figure 1

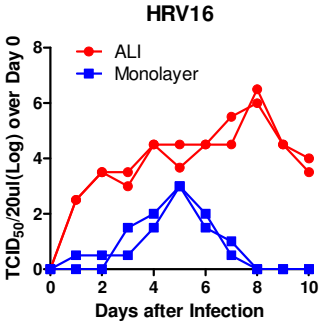


Figure 2

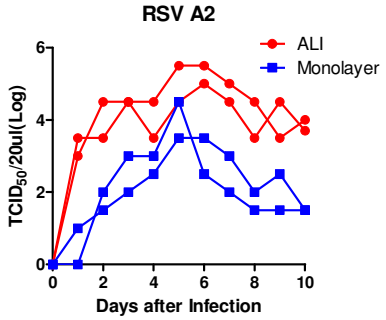


Figure 3

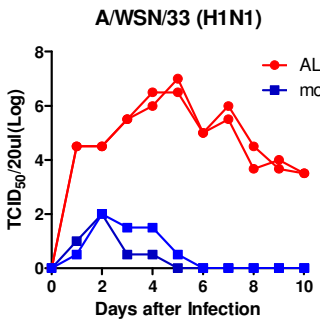
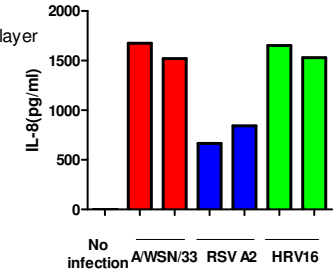


Figure 4



Conclusions

All respiratory virus used in this study, particularity HRV16 and H1N1, replicated more in NEC-ALI 3D than NEC-Monolayer. IL-8 production was also higher in NEC-ALI 3D than NEC-Monolayer.
This unique and robust 3D culture cell system (MucilAir™) will be a better *in vitro* model for evaluation of respiratory virus infection.

Bibliography

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