Evaluation of in vitro 3D human nasal epithelium (MucilAir™) to study the mechanism of action of influenza A and B viruses

Marion Janona1, Samuel Constant2, Julien Burlaud-Gaillard3, Isabelle Legastelois1, Sophie Buffin1

1Research Department, Sanofi Pasteur, Marcy L’Étoile, France; 2Epithelix Sàrl, Geneva, Switzerland; 3INSERM U966 & Plateforme IBiSA de Microscopie Electronique, Université François Rabelais et CHRU de Tours, Paris, France

INTRODUCTION

1. Influenza virus causes disease by infecting nasopharynx mucosa. Therefore, appropriate in vitro models are useful to evaluate the mechanism of viral infection and the efficacy of new therapies or vaccines.
2. In this study, we used commercially available, in vitro reconstituted 3D human upper airway epithelia (MucilAir™, Epithelix), fully differentiated and containing goblet, basal and ciliated cells showing cilia beating. This system is constituted of primary human epithelia cells freshly isolated from nasal biopsies seeded onto a semi-porous membrane.
3. This human standardized nasal epithelium displays specific defense mechanisms comparable to the in vivo situation, such as mucus production, mucociliary clearance, and secretion of defensive molecules.

METHODS

Viral inoculation from the apical side with a MOI of 0.1 under a volume of 100µL.
Incubation of the viral inoculum for 3 hours then removal of the inoculum.
Incubation at 34°C in a 5% CO2 incubator.

RESULTS

COMPARISON OF THE KINETICS OF FOUR STRAINS PRESENT IN THE 2016-2017 INFLUENZA VACCINE

(A) TEER measurements
- During infection with the A/H3N2 strain, the TEER decreased while it remained the same for the A/H1N1 strain.
- For both B strains, a huge decrease of the TEER was measured until 72h p.i.

(B) CCID50 infectious titers from the apical wash
- For the A strains, infectious titers were negative 24h post-infection but became positive at 48h and increased until 72h to reach 10⁶ to 10⁷ CCID50 mL⁻¹.
- For the B strains, positive titers were obtained as soon as 24h p.i. and plateaued between 48h and 72h reaching 10⁶-10⁷ CCID50 mL⁻¹.

CONCLUSIONS

- In this study we showed that the 3D human upper airway epithelia (MucilAir™) from Epithelix can support a productive A and B infection with the A/H3N2 strain-infection starting one day after the B strain-infection. Of note, infectious titers were also higher for the B strains compared to the A strains.
- B/Phuket infection caused a severe damage of the epithelium, as observed by histology. Epithelial damage was also evidenced by the dramatic decrease in TEER after infection with this strain. Electron microscopy confirmed the presence of virus budding from the ciliated cells, with viral particles in a single file all along the cilia.
- This 3D model, mimicking the in vivo upper airway epithelium infected by influenza A and B viruses, will be used to study and compare the mechanism of action of different strains of influenza viruses.

Cell Culture Infectious Dose 50 % (CCID50) Hemagglutination Unit (HAU) Multiplicity Of Infection (MOI) Post-Infection (p.i.) Scanning Electron Microscopy (SEM) Transmission Electron Microscopy (TEM) Trans-Epithelial Electrical Resistance (TEER)

Study funded by Sanofi Pasteur