EPITHELIX
In vitro respiratory solutions

Novel fully primary human airway epithelium-alveolar macrophages in vitro co-cultures models to study host pathogen interactions

Bernadett Boda, Carole Bertinetti, Ophélie Verbeke, Gowsinth Gunasingam, Song Huang and Samuel Constant

Epithelix Sàrl, 18 chemin des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland

Introduction

Being the body's first line of defense against airborne pathogens like bacteria and viruses, the respiratory epithelium acts as a physical barrier as well as an effective mucociliary escalator. Moreover, the airway epithelium is also a powerful immunoregulator that orchestrates innate and adaptive immune responses during bacterial or viral infections. Here we established a new co-culture model using human pulmonary macrophages (MΦ) and well characterized, standardized human airway epithelium in air-liquid interface culture, such as MucilAir™, SmallAir™.

1. Methods

1. 1 Isolation of pulmonary macrophages

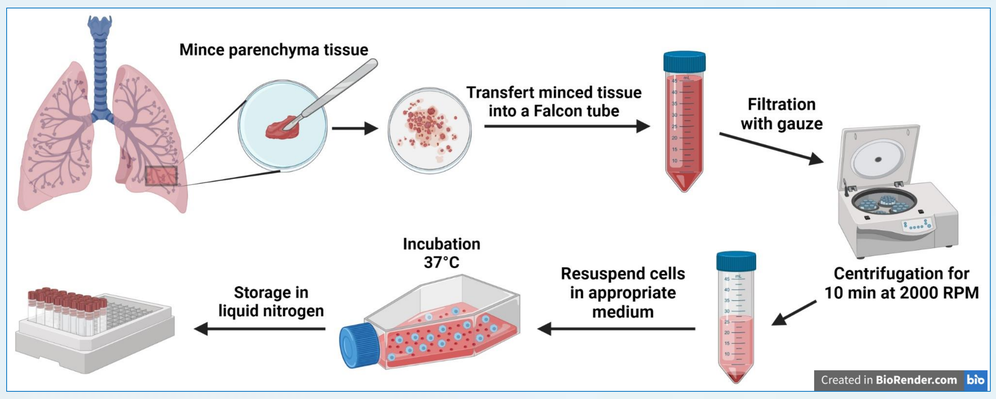


Figure 1. Human pulmonary macrophages were isolated from lung provided by biobanks with informed consent and with approval from local ethics commissions.

1. 2 Characterization of pulmonary macrophages

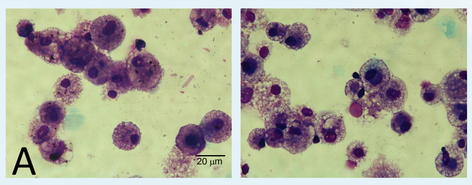
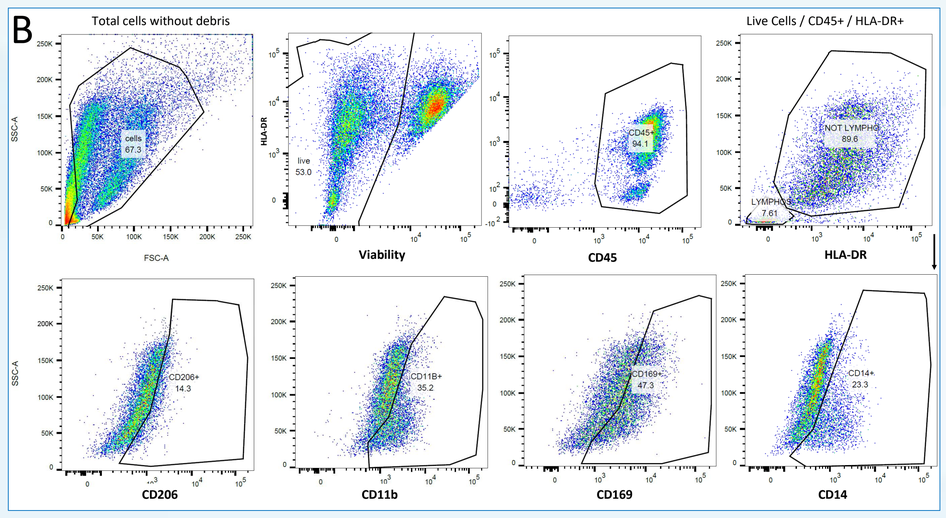


Figure 2. Characterization of pulmonary macrophages. A. Cellular morphology of macrophages from two different donors (donor 1 and 2) using cytospin and Giemsa staining. B. Flow cytometry of pulmonary macrophages. The panel of macrophage markers was designed according to Bharat *et al*., 2016. Example from a healthy donor. (donor 2)



2. Results

2.1 Co-culture of airway epithelium and pulmonary macrophages

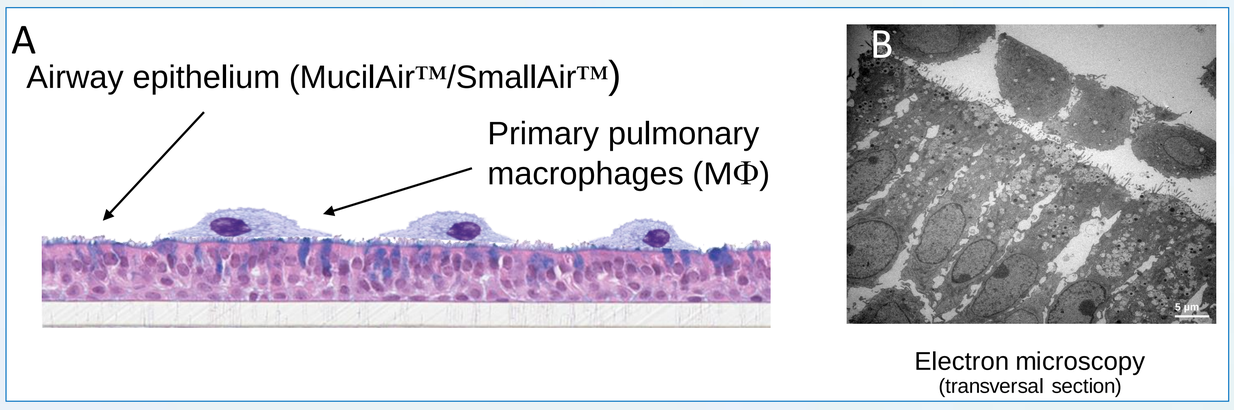


Figure 3. Co-culture of human airway epithelium and pulmonary macrophages. A. Schema of the apical addition of macrophages on a MucilAir™ transversal section. B. SmallAir™ co-cultured on the apical side with 50 000 macrophages (donor 2). Co-culture was fixed at 24 hours for electron microscopy.

2.2. Phagocytic activity of the macrophages in co-culture

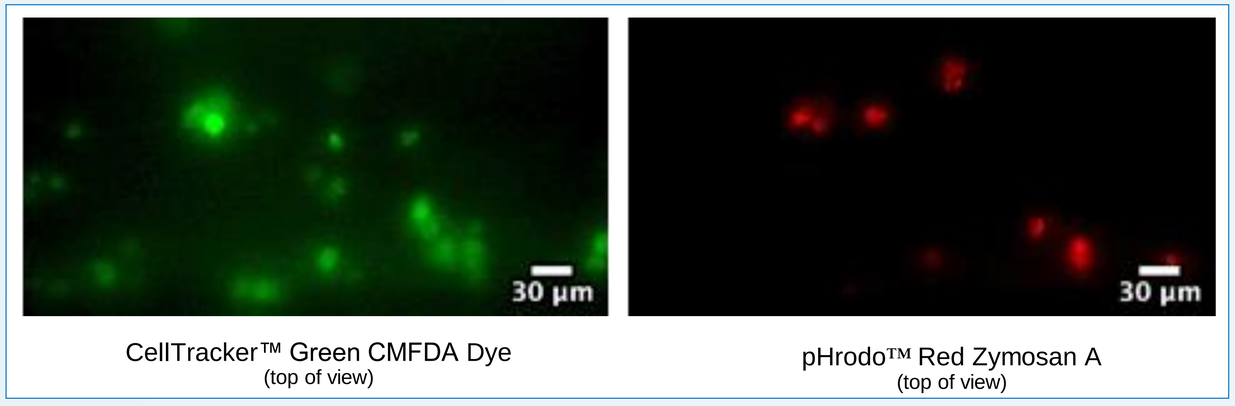


Figure 4. SmallAir™ co-cultured with macrophages marked by CellTracker™ Green dye and challenged with pHrodo™ Zymosan A to test phagocytic activity at 24 hours of co-culture. Ingested bioparticles are shown in red.

2.3 MucilAir™-MΦ infected with Methicillin Sensitive *Staphylococcus aureus* (MSSA) on the apical side

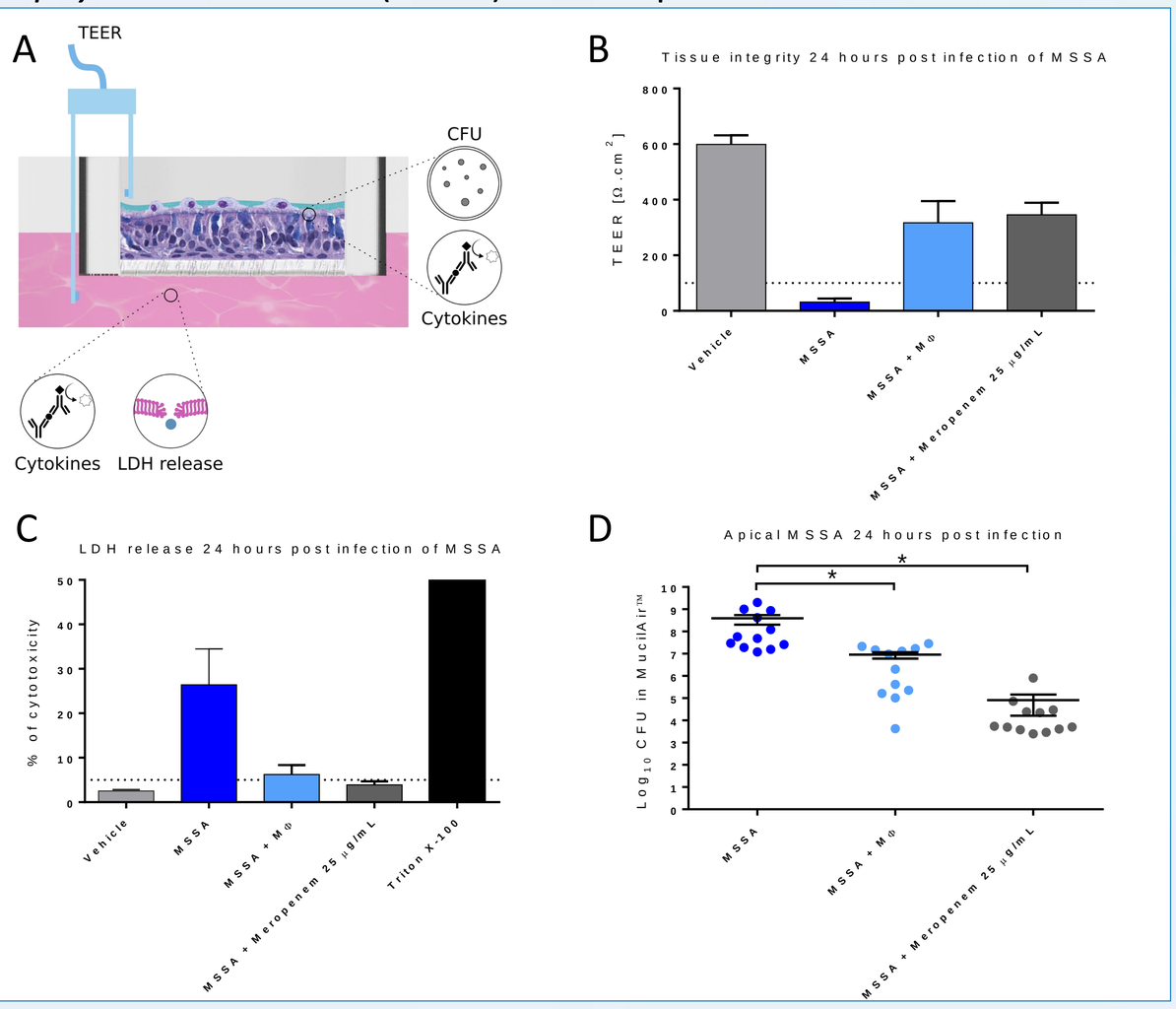


Figure 5. Nasal MucilAir™-Pool-MΦ (donor 2) was infected with 10^6 CFU of MSSA (ATCC 29213) in 10 µL. A. General schema for endpoint measurements. Co-culture was compared to infected MucilAir™-Pool and infected MucilAir™-Pool treated with vehicle or appropriate antibiotic. Endpoints were assessed at 24 hours post infection (4 independent experiments, n=12, mean±SEM). B. Transepithelial electrical resistance (TEER). C. Lactate dehydrogenase (LDH) assay (Triton X-100: 100 % toxicity. D. Enumeration of bacteria at the apical side. One-way ANOVA with Dunnett's posttest, $p<0.05$ (GraphPad, Prism).

2.4 MucilAir™-MΦ infected with *Streptococcus pneumoniae* 19F (clinical strain, Sp19F) on the apical side

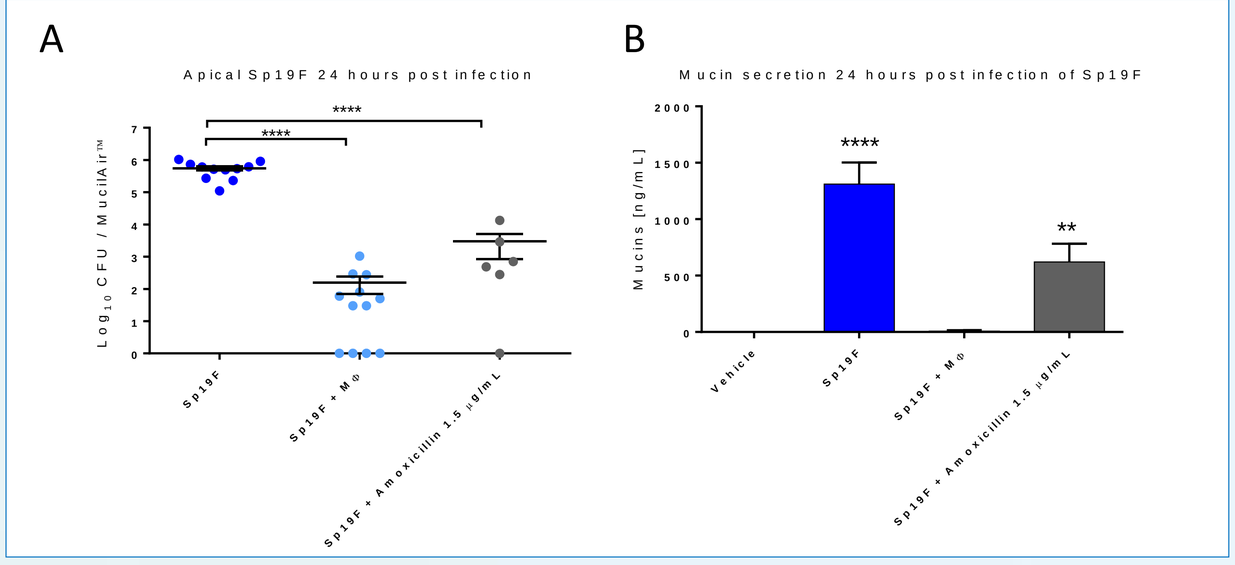


Figure 6. Nasal MucilAir™-Pool-MΦ (donor 2) was infected with $3x10^4$ CFU of Sp19F in 10 µL. The number of bacteria (A) and the secretion of mucins (B) were measured at 24 hours post infection on the apical side (4-2 independent experiments, n=12-6, mean±SEM). One-way ANOVA with Dunnett's posttest, $p<0.05$ (GraphPad Prism).

Summary

- Pulmonary macrophages are CD45+/HLADR+ and show donor specific expression of CD206, CD169, CD11b, CD68 and CD14 markers
- Macrophages co-cultured with MucilAir™/SmallAir™ are viable and have phagocytic activity in the co-culture
- Macrophages in co-culture decrease the growth of *Staphylococcus aureus* (MSSA) by more than 1 log and prevent bacterium-induced toxicity
- Macrophages in co-culture decrease the growth of *Streptococcus pneumoniae* (Sp19F) by more than 3 log and prevent bacterium-induced increase of mucin secretion

Conclusion

Novel fully primary human airway epithelium-alveolar macrophages *in vitro* co-cultures models are promising research tools to study infectious diseases.