In Vitro ALI Co-Culture Model Using Primary Human Airway Epithelium & Non-Autologous Neutrophils to Study Host-Pathogen and Immunoglobulin Interactions

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Introduction

Polymorphonuclear neutrophils (PMN) play an important role in infectious and inflammatory diseases. They serve as first line of defense against pathogens such as viruses and bacteria. The project's aim was firstly to develop an *in vitro* primary cell-based co-culture model using human airway epithelium (MucilAir™) and heterologous PMNs. Secondly, to investigate the potential of immunoglobulin purified from human plasma to reduce the level and consequences of pathogen infection in the conducting airways of the respiratory tract.

1. Methods

1. 1 Isolation of Human Neutrophils

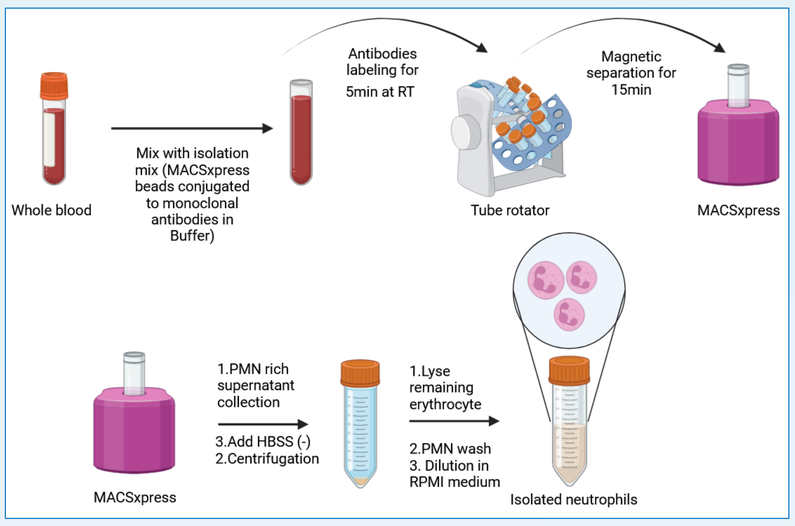
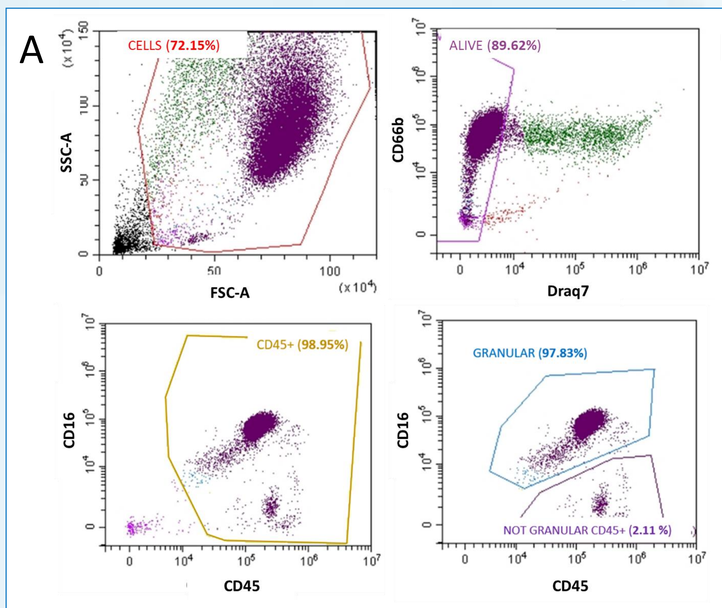


Figure 1. Human neutrophils isolated from fresh blood with informed consent and with approval from local ethics commissions, using negative selection with magnetic beads MACSxpress®.

1. 2 Characterization of Neutrophils



Good purity (97%) and viability (89%) of neutrophils were obtained after MACSxpress® isolation and lysis.

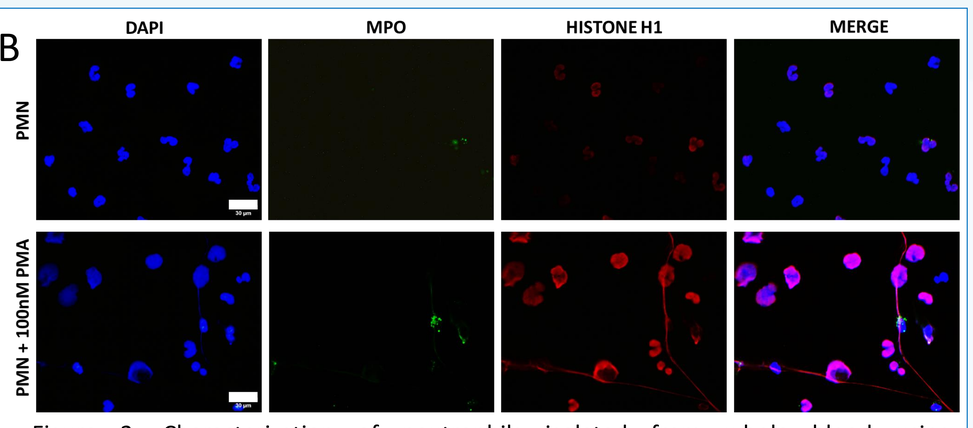
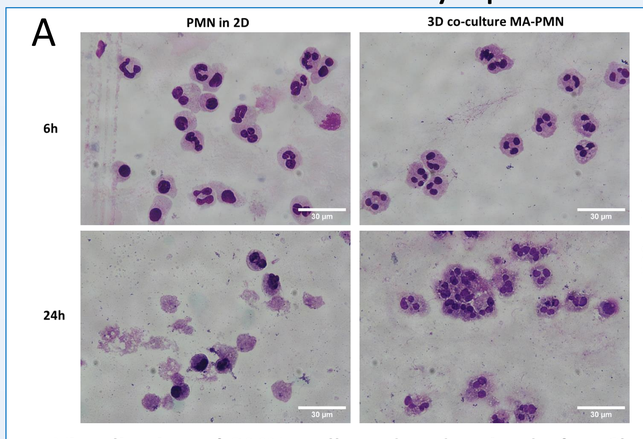


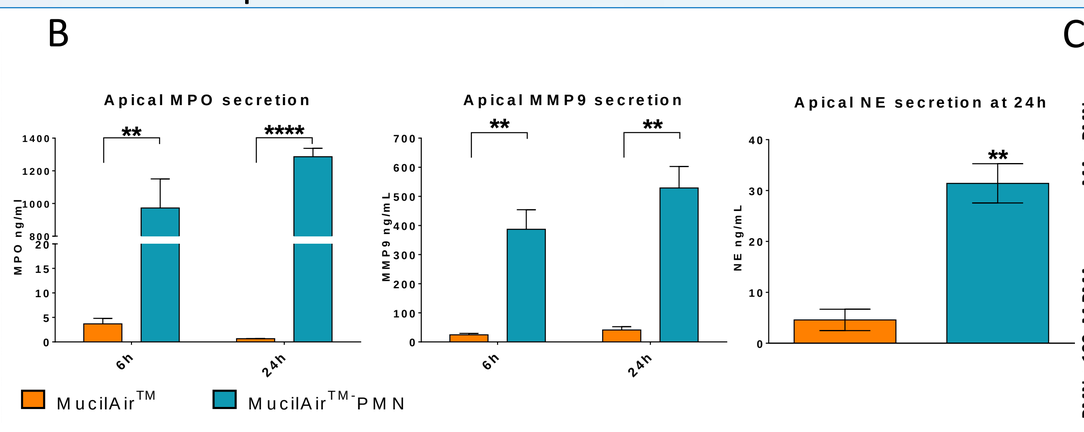
Figure 2. Characterization of neutrophils isolated from whole blood using MACSxpress®. A. Flow cytometry data on neutrophils (donor #1). Display of all events measured by SSC-A (granularity) and FCS-A (relative size). Dead cells are excluded using Draq 7. Display of CD45+ (leucocyte marker) and CD16+ (neutrophil markers). B. Neutrophil extracellular Traps (NET) Formation in 2D at 2h. PMN (donor #1) were incubated for 2h with Phorbol myristate acetate (PMA) at 100 nM. MPO (Myeloperoxidase) is stained in green, Histone H1 in red and nucleus is stained with DAPI. Scale bar = 30 µM. Magnification x40.

2. Results

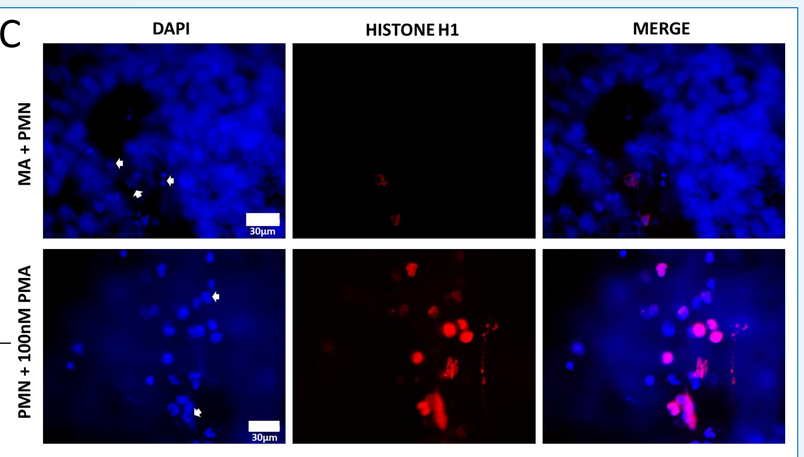
2.1 Co-culture of airway epithelium and Neutrophils



- **Visualization of PMNs collected and stained after 6h of co-culture confirmed the presence of intact cells with segmented nuclei.**



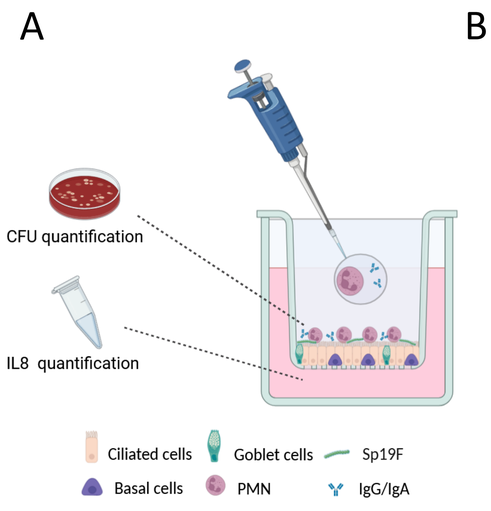
- **PMN enzymes (MMP9, MPO, NE) were released on the apical sides of the co-culture**

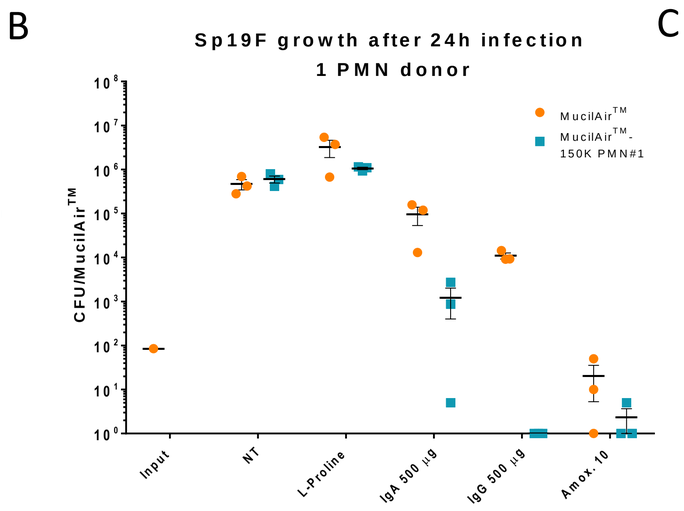


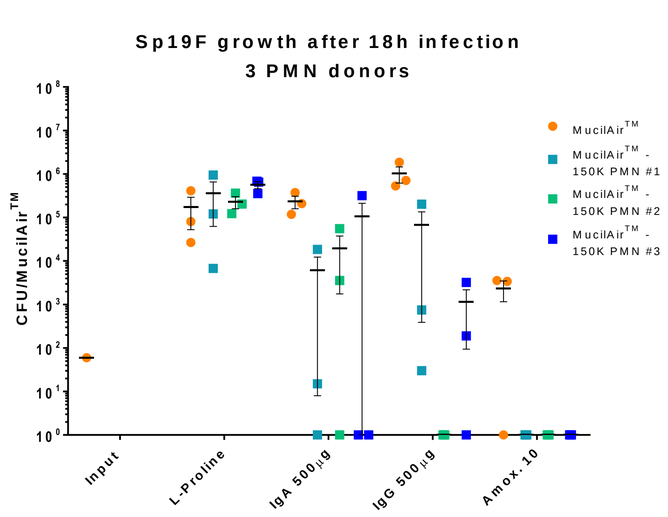
- **PMNs are able to undergo NET formation when stimulated in co-culture.**

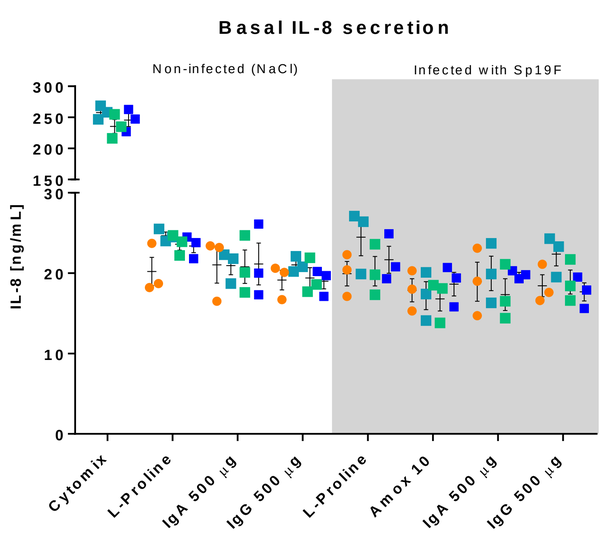
Figure 3. Characterization of the co-culture bronchial MucilAir™ – PMN. A. Morphology of PMN in 2D culture or in 3D co-culture with MucilAir™. PMNs (donor #1) were cultured alone in RPMI medium (PMN in 2D) or seeded on the apical side of MucilAir™ (co-culture MA-PMN in 3D). Cells were collected after 6 h or 24h and sprayed on a slide by cytospinning and stained by May Grunwald Giemsa staining. B. Effect of PMN (donor #1) on cytokines secretion in 3 donors of Bronchial MucilAir™. The bar graphs show the mean value of triplicate data originates from 3 MA donors in each group (n=3 donors, mean ± SEM). Apical washes were performed and MMP9 (Matrix metalloproteinase-9, 6h and 24h), MPO (6h and 24h) and NE (Neutrophil elastase, 24h) levels were quantified (n=3). Statistical analyses were performed using student unpaired t-test (GraphPad Prism software) (**p < 0.01,***p < 0.001, ****p < 0.0001). C. NET Formation in 3D. 150 000 PMNs (donor #1) were mixed with PMA 100 nM and deposited on apical surface of MucilAir™. Histone H1 is stained in red, and nucleus stained with DAPI. Fluorescent microscopy images of human primary PMNs are shown, white arrow = example of neutrophil nucleus, scale bar = 30 µM, magnification x40.

2.2 Effect of IgG on MucilAir™- Neutrophils infected with *Streptococcus pneumoniae* 19F (clinical strain, Sp19F) on the apical side









- **In presence of plasma-derived IgG or IgA (500 µg), and PMN a trend of Sp19F growth inhibition was noted. The activity of Ig varies with the PMN donor.**
- **The presence of Sp19F does not trigger IL-8 secretion, with or without neutrophils and plasma-derived IgG or IgA (500 µg).**

Figure 4. Bronchial MucilAir™- PMN was infected with 10^2 CFU of *Streptococcus Pneumoniae* 19F (Sp19F) in 10 µL. A. General schema for endpoint measurements. Experiments were done with MucilAir™ using 1 PMN donor #1 at 24h (B) or 3 PMN donors #1, #2, #3 at 18h (C) (n=3, mean ± SEM). B. Enumeration of bacteria at the apical side after 24h. C. Enumeration of bacteria at the apical side after 18h and effect of Sp19F infection on basal IL-8 secretion on Bronchial MucilAir™- PMN co-culture. L-Proline solution: vehicle for Igs, Amox: Amoxicillin 10 µg/mL in basal.

Summary

- Neutrophil morphology, viability and purity were analyzed using CD16 and Giemsa staining, and function was tested by stimulating NETosis.
- Neutrophil enzymes (MMP9, MPO, NE) were released and NETosis can be induced in the co-culture.
- The simultaneous presence of plasma-derived IgG or IgA (500 µg) and PMN tends to reduce the growth of Sp19F.

We have developed the basis for a novel complex epithelial co-culture model involving immune components. This model is a promising tool to study respiratory infections and related treatments.