Development of primary human airway models with dendritic cells cultured at air-liquid interface

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Introduction

As the first line of defence against airborne pathogens, pollutants, irritants or sensitizers the airway epithelium acts as key barrier through mucociliary clearance and innate immune defence mechanisms. After initial epithelial response, dendritic cells initiate adaptive immune response by presenting antigens to effector cells. Here, we report the development of a human 3D co-culture model including upper airway epithelia (MucilAir^{TM}) and heterologous monocyte derived-dendritic cells (moDCs).

Methods

1) Monocytes derived dendritic cells (moDCs) preparation

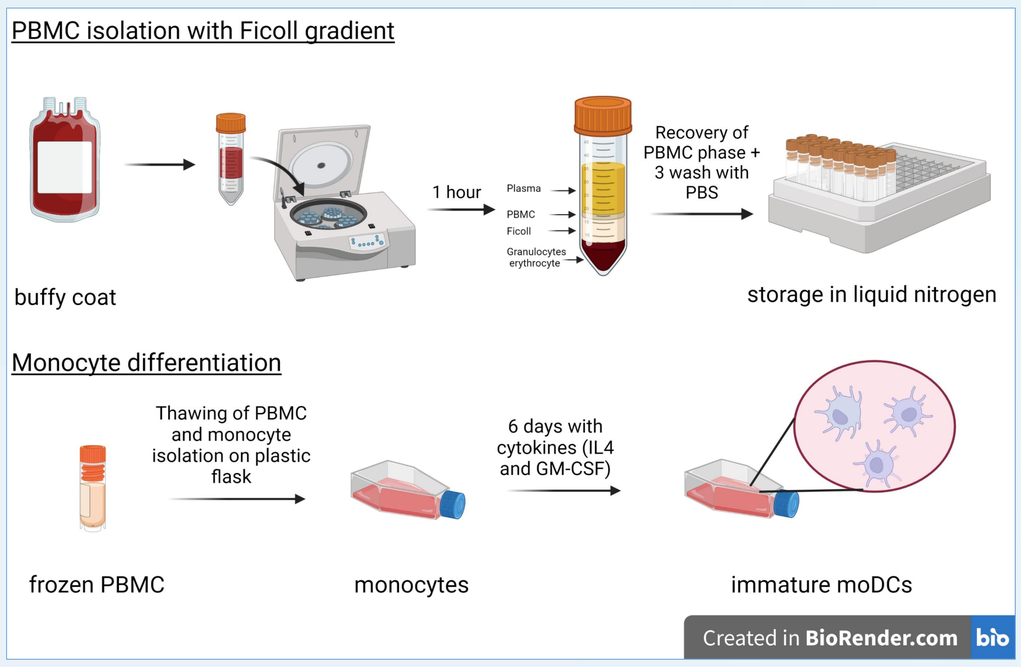

Figure 1. Buffy coat were obtain from Transfusion Center (Hôpitaux Universitaires Genève) with informed consent and with approval from local ethics commissions. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque™.

2) Characterization of immature moDCs

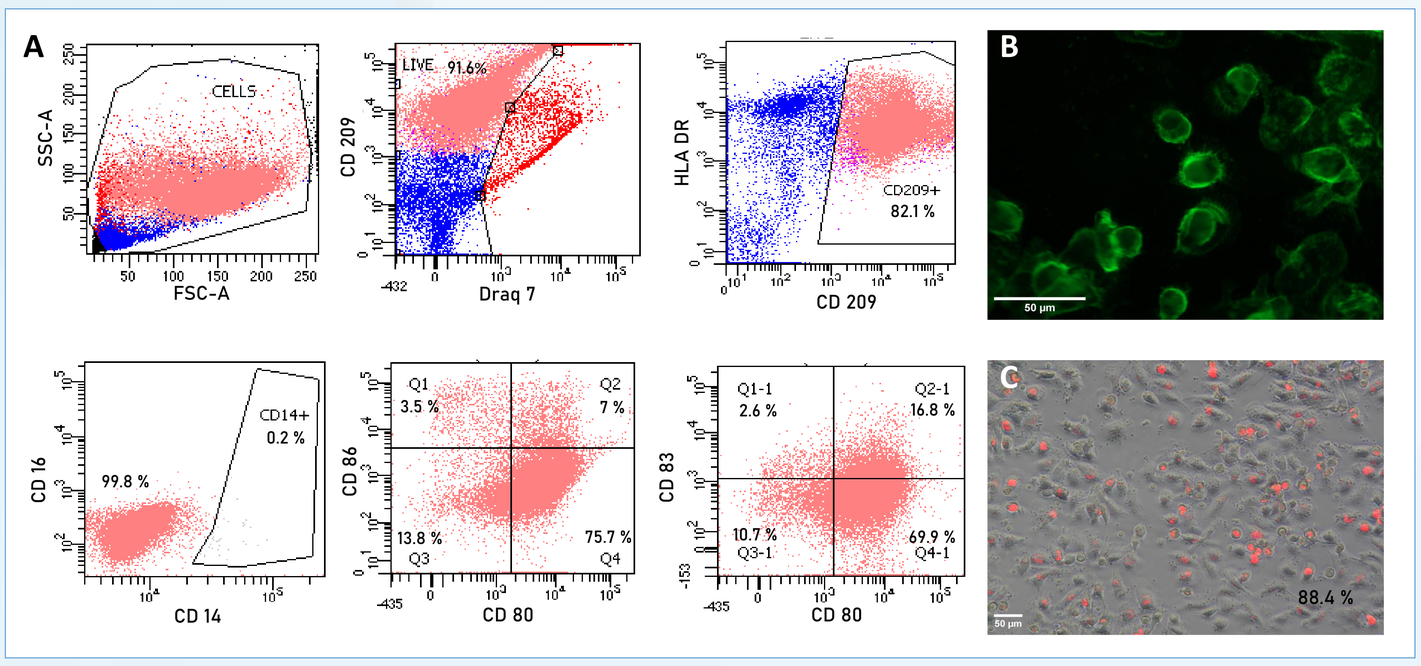

Figure 2. Dendritic cell markers and phagocytic assay in 2D. Flow cytometry of donor 013 **(A)** and CD86 immunostaining of donor 007 **(B)** were performed after 6 days differentiation from monocytes. **(C)** pHrodo™ Red Zymosan Bioparticles™ was added after differentiation of donor 015 and imaged at 24 hours in a culture plate. Ingested particle are shown in red.

Results

1) Establishment of co-culture model at air-liquid interface

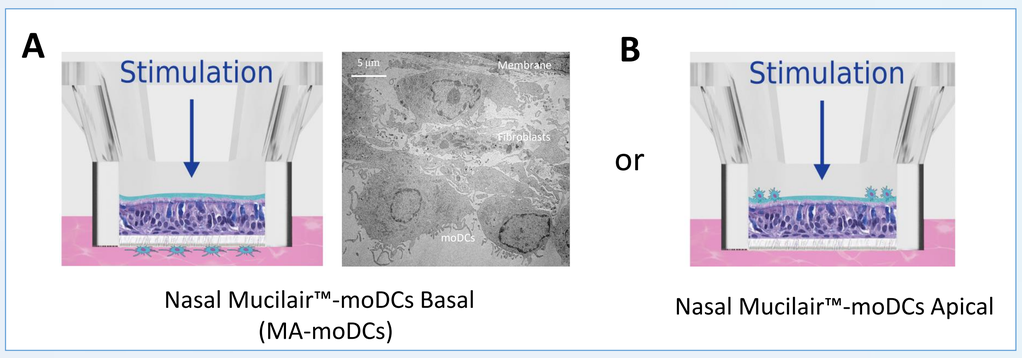

Figure 3. Co-culture of human airway epithelium and 50’000 or 100’000 moDCs. **(A)** Mucilair™ with moDCs on basal side below the transwell membrane (left), electron microscopy image from triple co-culture, containing human lung fibroblasts on basal side (right). **(B)** Mucilair™ with moDCs on the apical side, on the epithelium.

2) Stability of co-culture model

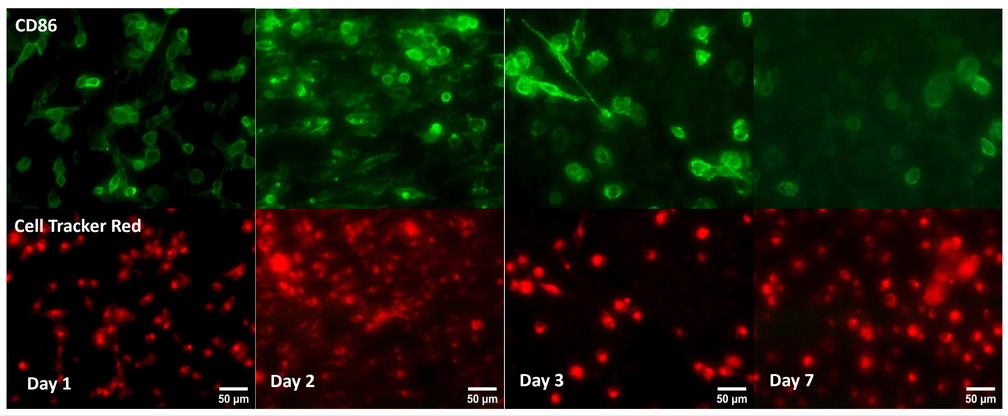

Figure 4. Mucilair^{TM} co-cultured with moDCs at basal side marked by a live cell dye, CellTracker™ Red CMTPX. Integrity and functionality of epithelial was fully preserved for 7 days. Co-cultures were fixed and immunostained for CD86.

3) Phagocytic assay on co-culture model

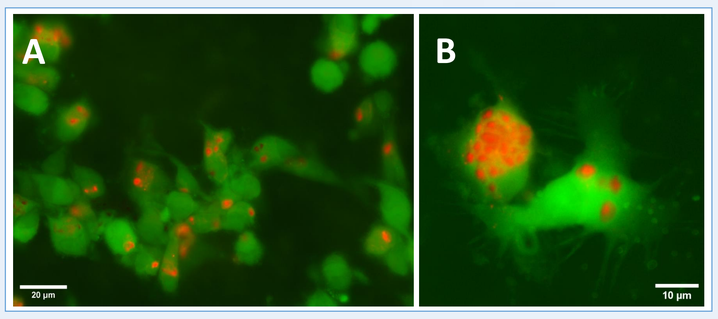

Figure 5. pHrodo™ Red Zymosan Bioparticles™ assay. **(A)** Mucilar^{TM} co-cultured with moDCs (donor 010) at basal side marked by a live cell dye (green). Zymosan was added on basal side 2 hours after model assembly and imaged at 24 hours (red). **(B)** Higher magnification.

4) Cytokine secretion 24 hours after apical simulation of MA and MA-moDCs (50’000 cells)

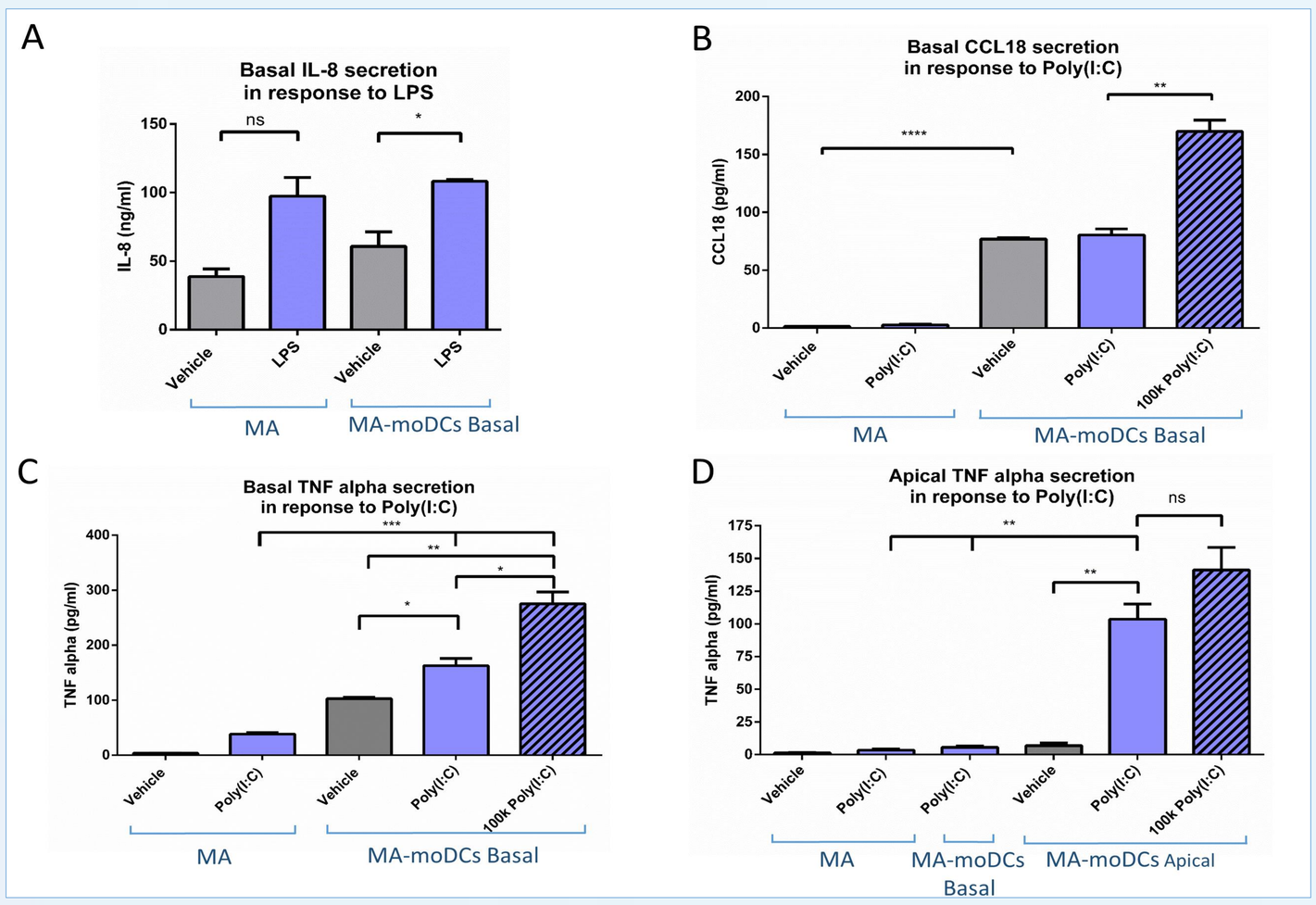

Figure 6. Quantification of cytokines by ELISA methods. **(A)** Basal IL-8 secretion after LPS stimulation (0.2 mg/ml, 30 µl). (n=2, donor 007, mean±SEM) **(B)(C)(D)** Basal CCL18, basal TNF alpha and apical TNF alpha secretion after Poly(I:C) (1.5 mg/ml, 20 µl) exposure (n=3, donor 015, mean±SEM). Saline solution (0.9 % NaCl) was used as Vehicle.
Unpaired Student t test,*p<0.05,**p<0.005,***p<0.0005,****p<0.0001 (GraphPad Prism).

Summary

- Immature monocyte derived dendritic cells are CD209+/HLA-DR+/CD80+ and CD14-
- Co-culture Mucilair™-moDCs is viable up to one week
- Co-culture responds to different stimuli and shows a significantly higher TNF alpha secretion in response to Poly(I:C) apical challenge
- The MucilAir™- moDCs co-culture is a new promising immunocompetent model that needs further investigations to determine application fields