**Abstract**

The airway epithelium contributes to innate immune responses and can also influence adaptive immune responses by instructing dendritic cells (DCs). Under steady state conditions, DCs are instructed by epithelia to adopt a tolerogenic phenotype. However, dysregulation of cross-talk between airway epithelial cells and DCs is known to contribute to inflammatory respiratory conditions, e.g. asthma [1]. The contribution of airway epithelial cell crosstalk with DCs to Chronic Obstructive Pulmonary Disease (COPD) pathophysiology is not well understood. We hypothesized that dysregulated airway epithelial cell interaction of DCs contributes to an aberrant immune response to respiratory infections contributing to COPD exacerbations.

We developed an in vitro co-culture system to study the effect of human rhinovirus (HRV) 16-infected COPD airway epithelium on human monocyte-derived dendritic cells (moDCs) from healthy donors. In contrast to moDCs cultured in vitro with airway epithelial cells induced significant changes in moDC inflammatory gene expression and increased their ability to induce Th2 differentiation, which was further enhanced by HRV16 infection. Therefore, our co-culture system recapitulates key features of known COPD relevant biology. In addition, the gene expression changes induced by COPD epithelial airway overlapped significantly with those induced by mature moDCs activated with LPS and IFNγ, but also affected novel biomolecular pathways specific to co-culture with COPD epithelium. These findings suggest that COPD airway epithelial cell instruction of DCs, as compared to healthy airway epithelia, results in aberrant inflammatory responses that could contribute to viral exacerbations, and furthermore highlight DCs as potential targets in the design of novel therapies for COPD.

**Introduction**

COPD Stable State

**Viral Bacterial Infection**

COPD Progression

**Increased information and mass 
Increased infections and response and airway remodeling**

COPD Exacerbation

**Increased information and lung damage 
New pathogens Chronic inflammation**

**Methods**

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

- Primary COPD and healthy human bronchial epithelial cells (HBE) were expanded in flasks and seeded in 96 transwell filter plates with 0.4μm pore size in an air liquid interface (ALI) culture system. After the cells formed a confluent epithelial layer, apical media was removed and the cells were basolaterally cultured with differentiation media for 18 days to fully form airway epithelium in cell culture.

- Monocytes were harvested from healthy blood donors with a CD14+ isolation kit (Miltenyi) and differentiated into DCs using a standard 0.4μM/505 CFU method. Successful differentiation was confirmed by detecting DC surface markers [e.g. CD209/DC-505G] via flow cytometry after 6 days in culture. These healthy moDCs were seeded basolaterally into the airway culture to create a co-culture system enabling cross-talk via soluble factors. Immediately prior to co-culture, the airway was treated with PBS or infected with human rhinovirus type 16 (HRV16) to understand how virus affects cell response. As a control the moDCs were also cultured alone in 3 separate conditions: i) untreated, ii) infected with HRV16, and iii) LPS/IFNγ matured. The same media, plate, and seeding conditions were used for both mono- and co-cultured moDCs. To assess how healthy or COPD airway can affect the ability of moDCs to instruct T cells, the moDCs were recovered from both mono- and co-culture and function was gauged by an agglutination macrophage migration inhibitory factor (MIF) assay. Naive CD4+ T cells were isolated from healthy blood donors with a naive CD4+ isolation kit (Miltenyi), pulsed with CFSE, and combined in a 10:1 ratio with moDCs.

**Results**

Figure 2: COPD airway epithelia infected with HRV conditions healthy moDC to drive increased T cell proliferation and elevated pro-inflammatory cytokine responses. Co-cultures and controls were set up as described in the methods for 4 healthy moDC donors (indicated by color) with 3 COPD or 3 healthy airway donors. The moDC were recovered and cultured in an allogenic MUR with naive CD4+ T cells, then after 5 days T cell proliferation (A) and cytokine production (B) were assessed (one way ANOVA with Tukey’s multiple comparison: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

A. moDC induction of T cell proliferation was enhanced by co-culture with COPD airway moDC co-cultured with both mock and HRV-infected COPD airway significantly increased T cell proliferation compared to healthy airway co-culture and monocyte moDC.

B. moDC induction of T cell cytokines was enhanced by healthy airway co-culture, with HRV-infected COPD airway showing the strongest effect. moDC co-cultured with HRV-infected COPD airway significantly increased IL-1β, IL-6, IL-2, and TNF secretion relative to mono-culture. Disease effects were observed with HRV-infected COPD airway co-culture significantly increasing IL-6 compared to HRV-infected healthy airway. HRV-infected COPD airway co-culture also showed a strong trend towards increased IFNγ and IL-2 compared to HRV-infected healthy airway. TNF was significantly increased by co-cultured moDC relative to mono-culture, with a greater effect of COPD airway (1.84-2 fold increase induced by COPD co-culture vs 1.47-1.66 fold increase induced by healthy).

**Conclusions and Future Directions**

- We have established a co-culture model, which independent of disease, demonstrates that airway epithelia exerts an immunomodulatory effect on healthy moDC function.

- moDC conditioning with COPD airway epithelia, as compared to healthy airway epithelia, led to 1) enhanced naive T cell proliferation and 2) elevated pro-inflammatory T cell cytokine production, thus recapitulating key features of known COPD relevant biology.

- In addition, moDC conditioned with COPD airway, as compared to healthy airway and monoco-cultured moDC, exhibited an altered transcription profile.

- Next steps: Future studies will focus on understanding the transcriptional profiles seen in moDC conditioned with COPD airway, and how these relate to the functional differences observed in our primary cell-based assays.

**References**

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