Severe Asthma-on-Chip: A Novel In Vitro Platform to Model Viral-Induced Exacerbations in Asthma

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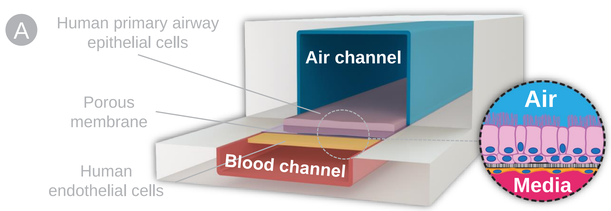
Abstract

New therapies for severe asthma, particularly treatments which can reduce exacerbations remain a great unmet medical need. Advanced pre-clinical models are needed to further elucidate complex mechanisms that underlie asthma exacerbation for the development of novel therapeutics. Recently, we have developed a 3D microphysiological **human Airway Chip** containing a **fully differentiated mucociliary bronchiolar airway epithelium** underlined by a microvascular endothelium which experiences fluid flow[1]. When infected with human Rhinovirus (HRV), a leading cause of asthma exacerbation in children and adults, the Airway Chip demonstrated induction of a pro-inflammatory response characterized by ciliated cells death, goblet cells hyperplasia and release of cytokines including IFN-α2, IFN-λ1, CXCL10 and CXCL11, as well as recruitment and extravasation across the endothelium of circulating human neutrophils. To recapitulate viral-induced asthma exacerbation and model molecular responses observed in severe asthma, we then infected IL-13-treated Airway Chip with HRV. HRV challenge of IL-13-treated cultures resulted in altered interferon response and increase of neutrophil recruitment when compared with IL-13 or HRV stimulation alone. Neutrophil recruitment could be pharmacologically inhibited by MK-7123, a CXCR2 antagonist (10 µM).

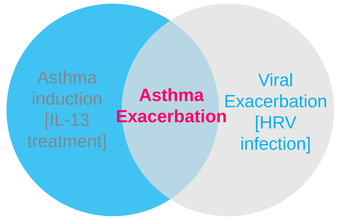
Airway Chip characterization

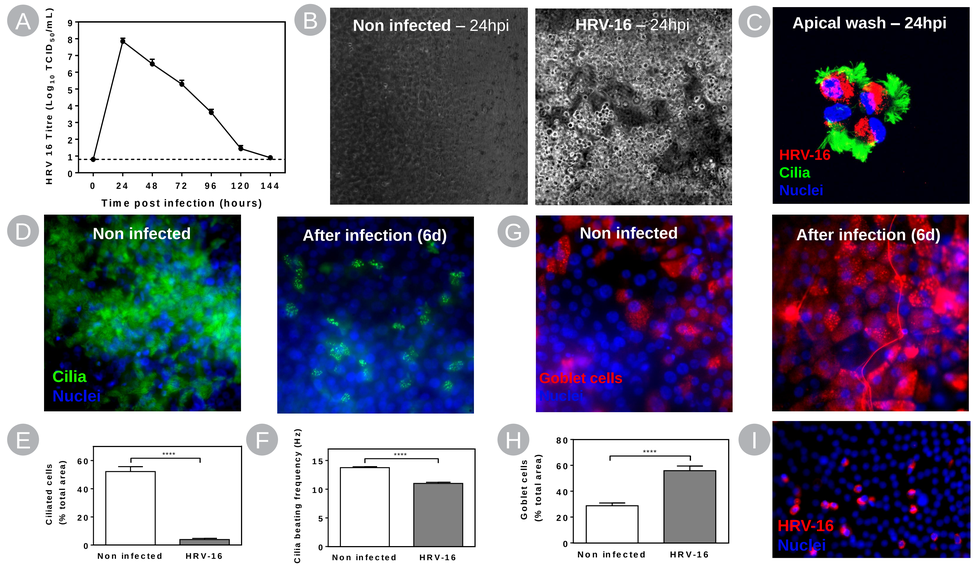


The Airway Chip recapitulates physiology and function of human airway epithelium.
A. Diagram of a cross section through the Airway Chip showing its two hollow linear channels separated by a porous membrane which supports growth and differentiation of human primary airway epithelial cells on its upper surface and human pulmonary microvascular endothelial cells underneath. **B**. 3D reconstruction showing fully differentiated, pseudostratified, airway epithelium (green, F-actin) underlined by human pulmonary endothelial cells (red, F-Actin). **C**. Scanning electron micrograph of cilia forming on the differentiated airway epithelium formed on-chip (cilia were colored in blue and non-ciliated cells in brown. **D.** The differentiated human airway epithelium exhibits continuous tight junctional connections on-chip, as evidenced by ZO1 staining and **E**. mucus producing cells as evidenced by MUC5AC staining (green) and DAPI counterstaining (blue).

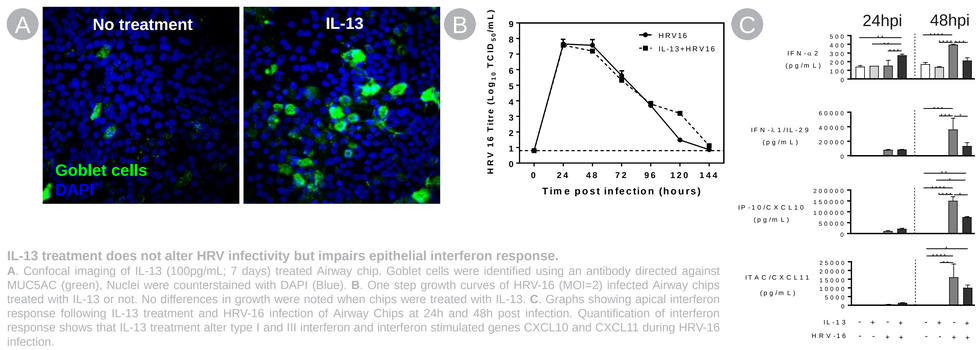
Asthma Exacerbation-on-Chip

An on-Chip model of Asthma exacerbation was generated by infecting IL-13-treated (100pg/mL for 7 days) Airway Chip with HRV. Infection of human primary airway epithelial cells cultured inside the Airway Chip with HRV-16 (MOI=2) showed obvious cytopathic effects. IL-13 treatment did not modify HRV infectivity but altered cytokines/chemokines secretion profile, including interferon response (IFN-α2, IFN-λ, CXCL10, CXCL11) and pro-inflammatory mediators (IL-8, IL-6, IL-1α).





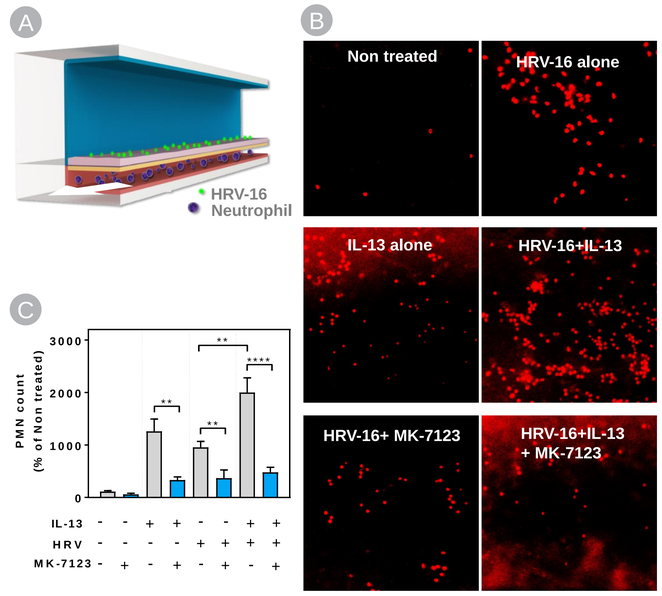
HRV-16 infects the Airway Chip and induces cytopathic effects.
A. One step growth curve of HRV-16 (MOI=2) showing that HRV-16 infects and replicates in the Airway Chip. **B**. Phase contrast micrographs of Non infected and HRV-Infected Airway Chip at 24h post infection. Infected chips display high apical cell sloughing. **C**. Confocal imaging of detached apical cells showing that most detached cells are HRV-infected ciliated cells, suggesting that HRV primarily infects multiciliated cells. **D**. Immunofluorescence staining of non infected and HRV-infected epithelium grown inside Airway Chips. Six days post infection most of ciliated cells have disappeared (quantification is presented in **E**). **F**. Decreased cilia beating frequency following HRV infection. G. Immunofluorescence staining highlight HRV-induced goblet cells hyperplasia (quantification is presented in **H**). **I**. Immunofluorescence staining showing chromatin condensation in nuclei of HRV-infected cells.



IL-13 treatment does not alter HRV infectivity but impairs epithelial interferon response.
A. Confocal imaging of IL-13 (100pg/mL; 7 days) treated Airway chip. Goblet cells were identified using an antibody directed against MUC5AC (green), Nuclei were counterstained with DAPI (Blue). **B**. One step growth curves of HRV-16 (MOI=2) infected Airway chips treated with IL-13 or not. No differences in growth were noted when chips were treated with IL-13. **C**. Graphs showing apical interferon response following IL-13 treatment and HRV-16 infection of Airway Chips at 24h and 48h post infection. Quantification of interferon response shows that IL-13 treatment alter type I and III interferon and interferon stimulated genes CXCL10 and CXCL11 during HRV-16 infection.

Pharmacological modulation of neutrophil recruitment

Neutrophils are the predominant innate inflammatory cell recruited to the lungs in virus-associated asthma exacerbations[2,3]. Here we show that freshly isolated human neutrophil perfused through the vascular microchannel are recruited to the endothelium following HRV infection and IL-13 treatment. This response can be modulated by a selective CXCR2 antagonist MK-7123.



Neutrophil recruitment following exacerbation with HRV can be reduced by a CXCR2 antagonist MK-7123
A. Schematic diagram showing the HRV-infected Airway Chip during perfusion in the vascular channel of freshly isolated human neutrophil. **B**. Fluorescence imaging of stained neutrophil (red) recruited to the endothelium. Non stimulated chips are showing limited neutrophil recruitment while HRV infected and IL-13-treated chips show increased neutrophil recruitment. Interestingly, IL-13 + HRV induce an additive increase in neutrophil recruitment, while treatment with a CXCR2 antagonist MK-7123 (10 µM) significantly reduced neutrophil recruitment under all three stimulation conditions . **C.** Quantification of neutrophil recruitment (** p<0.01; **** p<0.001).

Conclusions

The Airway Chip may provide unique opportunities to explore asthma exacerbation mechanisms in a human-relevant model in physiological 3D airway tissue structure and function. Additionally the microfluidics allow for the study of immune cell migration and interaction with endothelial and airway cells and such models may be beneficial in identifying new therapeutic targets and testing novel pharmacological agents.

1- Nat Methods. 2016 Feb;13(2):151-7

2- J Allergy Clin Immunol 1995;95:843–85

3- J Allergy Clin Immunol 2000;105:1169–1177

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