Targeting epithelial initiation of rhinovirus induced asthma exacerbations: development of a potent, neutralizing antibody to IL-25

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Background

Viral respiratory infections are the most important trigger for acute asthma flares, with asthmatics susceptible to more serious effects from viruses that would usually only cause the symptoms of the common cold – most prominently - rhinovirus (RV). Viral replication in airway epithelium leads to production of inflammatory mediators that can trigger the immune cascade that underpins an asthma exacerbation. The airway epithelium is the site of RV infection and is now the focus of research seeking to develop new treatments to suppress infection and associated triggering of inflammatory responses that drive asthma exacerbations. Several studies have reported that asthmatic airway epithelium responds abnormally to infection. In this context, we and others have provided key evidence showing that asthmatic epithelium can exhibit abnormal immune responses to RV infection including deficient anti-viral immunity and exaggerated expression of mediators such as IL-25. We have reported that undifferentiated asthmatic bronchial epithelial cells (BECs) exhibit up-regulated expression of the type2 inflammatory cytokine IL-25 during rhinovirus (RV) infection identifying this molecule as a target for mAb-based therapy for asthma exacerbations.

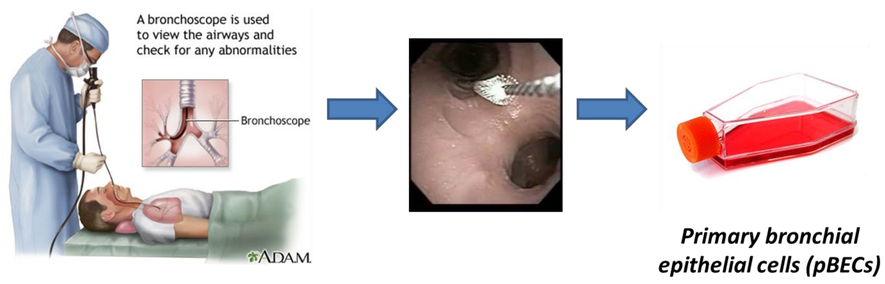
Air liquid interface culture of asthmatic bronchial epithelial cells

To investigate if the IL-25^{hi} phenotype was present in fully differentiated asthmatic primary bronchial epithelial cells (pBECs), we recruited mild, moderate and severe eosinophilic asthmatics (>3% sputum eosinophils). BECs were obtained by brushing at bronchoscopy and cultured on transwell inserts at the air-liquid interface for 28 days before low (0.1) MOI infection with minor group RV1B or major group RV43.

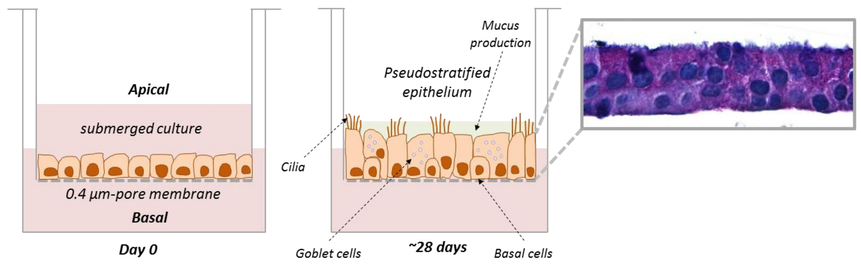
Clinical and immunological characteristics of asthmatic pBEC donors

Donor i.d.	GINA stage	Age	Sex	Exacerbation in last 12mths	OCS use	FEV1% predicted	FVC% predicted	Atopy	Sputum neuts	Sputum eos	Sputum macs	Age of Asthma Onset
AS090	Moderate Persistent	66	F	0	0	67	88	?	41.75	43.5	9.25	?
AS170	Severe Persistent	36	F	2	2	79	87	Yes	65.75	3.75	23.75	Childhood
AS131	Mild persistent	43	F	1	1	89	89	Yes	5	3.75	89.25	?
AS140	Moderate Persistent	61	F	3	0	101	98	No	77	3	20	Childhood
AS153	Mild Persistent	73	F	0	0	85	97	No	19.25	5	44.5	Adult
AS178	Mild persistent	71	M	0	0	74	75	No	11.75	5	27.25	Childhood

Sputum neutrophils (neut), eosinophils (eos) and macrophages (macs) shown as % of total sputum cells



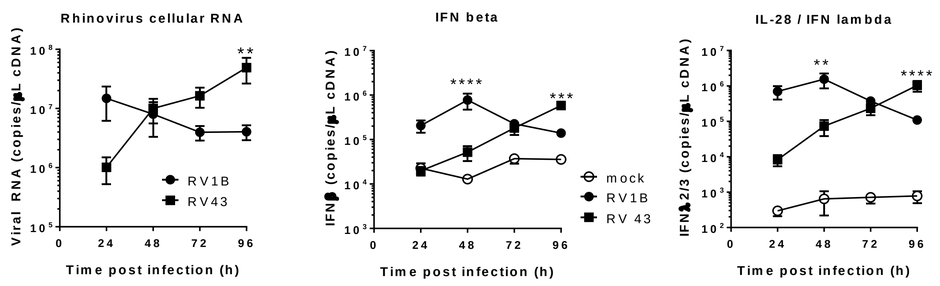
Primary bronchial epithelial cells (pBECs)



Air-liquid interface (ALI)-cultured bronchial epithelial cells

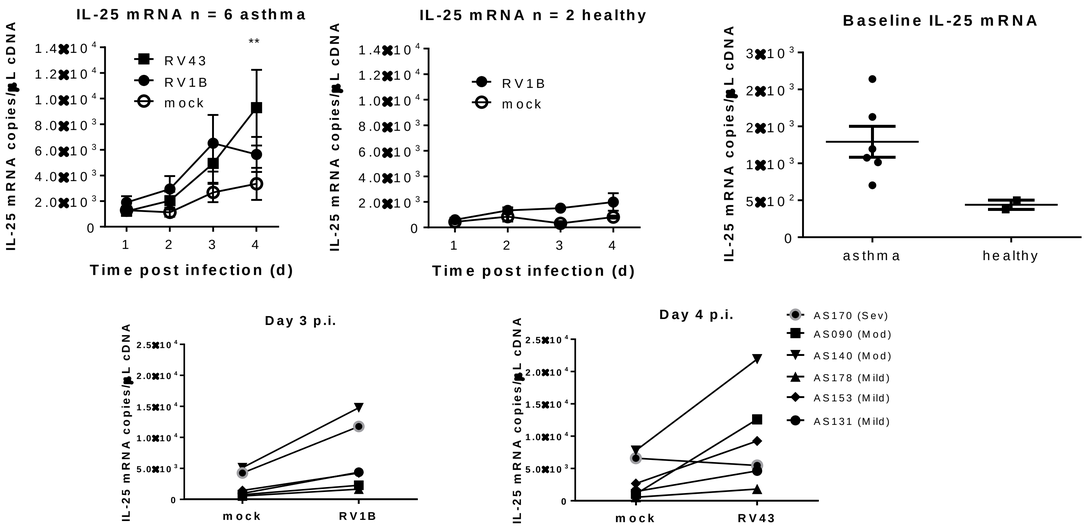
Rhinovirus infection of differentiated asthmatic epithelium

Viral replication and expression of anti-viral interferons



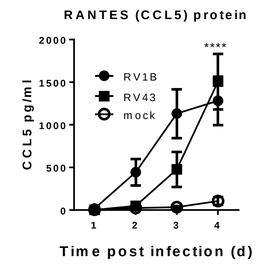
Infection of asthmatic ALI epithelium reveals different kinetics of replication and induction of interferon for minor and major group RV. Viral RNA data expressed as mean of n = 6 cultures +/- SEM. **p<0.01 increased viral RNA in 96 h RV43 infected cells; ***p<0.001, ****p<0.0001 increased expression compared to mock infected cells

IL-25 expression by differentiated epithelium is induced by rhinovirus infection and higher in asthma

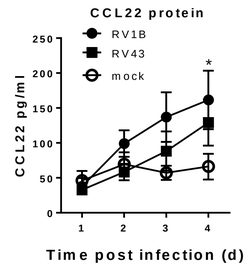


Induction of IL-25 in human epithelium during rhinovirus infection. Asthmatic ALI-differentiated epithelial cultures were infected with major group RV43 or minor group RV1B. For comparison IL-25 mRNA levels are shown in n = 2 epithelial cultures from healthy controls. IL-25 mRNA levels over four days were assessed by qPCR. In asthma more rapid replication of RV1B was associated with an earlier peak in IL-25 mRNA expression at day 3. For RV43 the peak was day 4 post-infection. Expressing baseline to peak day levels for each virus revealed that the highest expression was in cells from severe (Sev) and moderate (Mod) asthmatics. Data is expressed as mean +/- SEM **p<0.01 for RV43 infected cells compared to mock infected cells at day 4 p.i. (non-parametric t test).

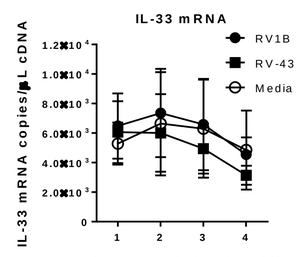
Type-2 inflammatory mediators associated with IL-25 expression



****p<0.0001 RV1B and RV43 vs mock

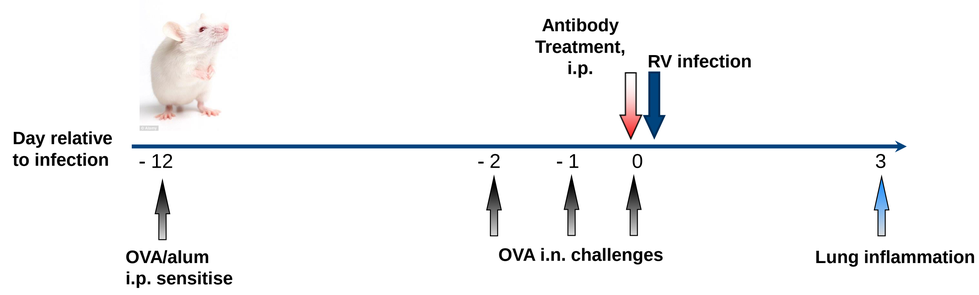


*p<0.05 RV1B vs mock



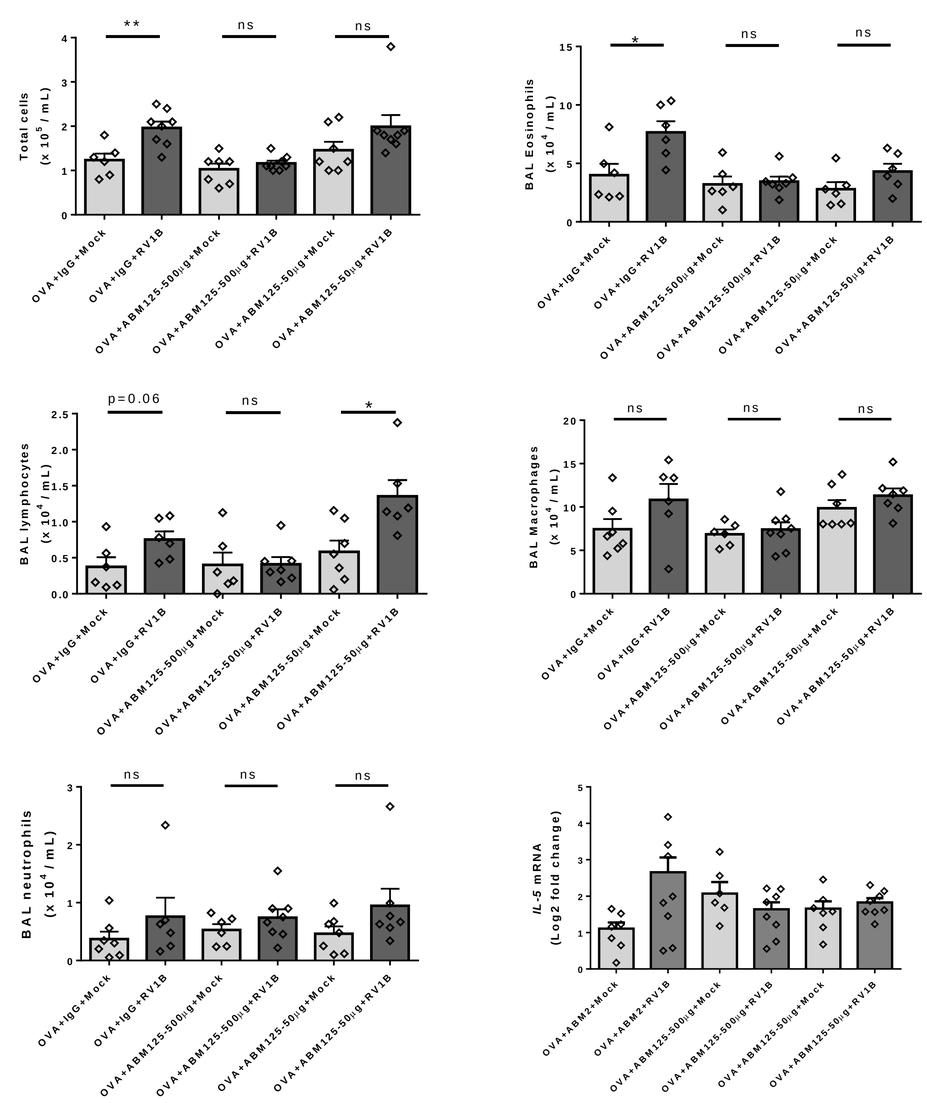
Rhinovirus infected differentiated asthmatic epithelium expresses type 2 inflammatory mediators. CCL5 and CCL22 proteins in the apical compartment of ALI-differentiated cultures were measured by ELISA. Expression of IL-33 mRNA was assessed by qPCR. Data expressed as mean +/- SEM.

Mouse model of RV-induced exacerbation of allergic airways disease with anti-IL-25 (ABM125) treatment



Mouse model of RV induced exacerbation all allergic airways disease with anti-IL-25 (ABM125) treatment. Mice were sensitized with low LPS hen egg ovalbumin (OVA 50µg in 2mg alum). Mice were then challenged with 50µg OVA intranasally (i.n.) on three consecutive days. Directly after the final OVA challenge mice were administered intraperitoneally (i.p.) ABM125 or isotype control. Four hours after mAb dosing mice were infected i.n. with 2.5 x 10^6 $TCID_{50}$ RV1B. Inflammatory responses were assessed at day three p.i.

ABM125 treatment suppresses eosinophilic airway inflammation



ABM125 treatment *in vivo* supresses RV-induced exacerbation of eosinophilic inflammation. Wild type BALB/c mice (6-8 weeks old) were sensitized and challenged with ovalbumin, treated with ABM125 or isotype control IgG, and then subsequently infected with RV1B. Cellular recruitment was then assessed in bronchoalveolar lavage (BAL) fluid and lung mRNA expression of IL-5 was assessed by qPCR with SYBR Green chemistry, expressed at Log 2 (fold change) relative to Saline/PBS controls at 3 days post-infection. Data shows mean of n = 6-8 mice +/- SEM. *p<0.05, **p<0.01 compared to relevant mock controls.

Summary

- ALI-differentiated asthmatic epithelium responds to RV infection with elevated IL-25, which was associated with more severe disease.
- Epithelial IL-25 was also associated with increased levels of the related type-2 inflammatory mediators, CCL5 and CCL22.
- Neutralizing IL-25 *in vivo* with mAb ABM125, supressed airway eosinophil recruitment in a model of RV-induced exacerbation of allergic airways disease.
- Reduced eosinophilic inflammation was associated with a trend towards reduced IL-5 mRNA expression.