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Capsule Summary: Rhinovirus (RV) infection causes a differential immune response in asthma donors during infection with RV39 that includes elevated IL25, TSLP, and IL13. This is associated with enhanced airway responsiveness to carbachol in human precision cut lung slices obtained from asthma donors.

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To the Editor:

Rhinovirus (RV) accounts for 60%-80% of viral infections in children with asthma exacerbations.^{1, 2} However, the link between RV infection and airway hyper-responsiveness (AHR) precipitating exacerbations has been difficult to establish. We utilized human precision cut lung slices (PCLS)^{3, 4} to evaluate immune responses to RV39 and AHR in tissue from donors with (n=7) and without (n=9) a history of asthma. Human PCLS retain ciliary motility and responsiveness to contractile agonists, including carbachol (CCh)—a cholinergic agonist.⁴ We hypothesized that RV infection would activate epithelial cytokine expression of IL-25 and TSLP in asthmatics, causing infection-mediated increases in AHR.

As per previous studies,^{4, 6} donor lungs were received within 12 to 36h of collection. The use of lungs from deceased donors does not constitute human subjects research as determined by the University of Arkansas for Medical Sciences' Institutional Review Board. The lungs were inflated with 1.75% low melting point agarose in PBS, hardened by cooling to 4°C, and sliced into ~15mm thick sections containing cross-sectioned airways that were identified and cored. Slices were cut using a Compresstome (Precisionary Instruments, San Jose CA), and airway viability was confirmed microscopically via ciliary motility and bronchoconstriction with CCh.

Immediately after preparation, a subset of PCLS were fixed in 4% paraformaldehyde and processed for paraffin sectioning and hematoxylin and eosin staining. Sections were reviewed by a pathologist blinded with regards to asthma diagnosis and assessed for basement membrane thickness. Serum samples were analyzed for total IgE (ImmunoCAP 100, Phadia US, Portage, MI).

PCLS airways from asthma and non-asthma donors were incubated with 3000 TCID₅₀/mL RV39 (Dr. Ron Turner, University of Virginia, Charlottesville, VA) (4 slices/time point), along with uninfected slices as controls. Infected airways were incubated at 33°C for four hours, washed with PBS, and then placed at 37 °C. Uninfected tissues were treated similarly with the obvious difference of infection. PCLS and supernatants were harvested at 10, 24, and 48h following

exposure to RV39. mRNA was isolated from the entire tissue using TriReagent (Sigma, St. Louis, MO) and quantified via PCR using primers specific for human *il25* (Qiagen, Crawley, UK), *il33* (Qiagen, Crawley, UK), *tslp*, and *il13*, as well as *ifn λ* , and *il15*. Gene expression was normalized to *β actin* and for the cytokine of interest in uninfected tissues at the same time points. Measurements of viral load were made using a quantitative PCR approach previously described^{7,8}. Secreted IL-33, TSLP, and IL-13 were measured in tissue culture supernatants from 0 and 48h using a bead array assay (EMD Millipore Milliplex, Billerica, Ma) and IL-25 by single analyte ELISA (Assay Biotech, Sunnyvale, Ca).

Contractile responses in naïve and RV-infected PCLS airways from each asthma or non-asthma donor lung were compared. Airway cross-sectional areas (CSA) were measured in photomicrographs collected before and after treatment for 15 min with 2 μ M CCh using Image J⁹ to calculate percent airway occlusion. A subset of the PCLS were then infected with RV39, with uninfected PCLS providing controls. Because PCLS airways frequently exhibit basal tone (data not shown), maximal CSA was defined as the largest measured under either basal conditions or following treatment with 4 μ M forskolin. Mock and post-infection airway occlusion induced by CCh were measured and expressed as percent-change from baseline airway occlusion.

Differences in cytokine gene expression were determined via Mann-Whitney U test. A linear mixed effects model using a random effect for lung to account for multiple slices on each lung was used to compare mean contractility between asthma and non-asthma donors. All tests were two-sided assuming a significance level of 5%.

Table 1 provides demographic information for each group. Asthma donors were significantly younger than non-asthma donors. There were no other significant differences in demographics between asthma and non-asthma donors. Histopathologic analysis found increased basement membrane thickness in asthma donor derived tissue, and total IgE levels in asthma donor sera

were also higher than non-asthma donors (Table 1). Asthma donors also had evidence of smooth muscle hypertrophy in 5/7 tissues, while 0/9 normal donors had this finding.

There was a significant increase in gene expression of *il25*, *tslp*, and *il13* for all asthma donors as compared to non-asthma at all time points measured following RV39 infection (Figure 1A, B, C). By contrast, there was no difference between asthma and non-asthma donors in the gene expression of *il15*, *il33*, or *ifn λ* at any time point (Figure 1D, E, F). TSLP protein release was higher than that of non-asthma donors at 48h (Asthma, mean (SD), 13.57 pg/mL (2.64); Non-asthma, mean (SD), below limit of detection; $p < 0.05$). IL-33 protein release was increased at 48h following RV39 infection for all subjects and was equivalent between asthma and non-asthma donors (asthma, mean (SD), 23.43 (7.39) pg/mL; non-asthma, mean (SD) 22.22 (10.57) pg/mL; $p = \text{NS}$). We were unable to detect IL-25 or IL-13 protein release in the presence or absence of RV39 infection. qPCR analysis of PCLS detected no differences in viral load at 48h between asthma (mean (CI), 18910.0 (7220.0, 87800.0) virions/200 μ L sample) and non-asthma (mean (CI), 23200.0 (7580.0, 119200.0) virions/200 μ L sample) donors at peak infection (Figure 1F).

There were no differences at baseline in mean airway occlusion to CCh between asthma donors prior to infection with RV39 (Table 1). However, after infection, CCh-induced constriction was significantly increased in asthmatic airways, with a fold change from baseline of 35% (SEM 11.96%) compared to non-asthmatic airways (17.69% (SEM 5.6%)) (Figure 1G).

While RV has clearly been associated with asthma exacerbations, understanding mechanisms underlying how infection modulates changes in contractility and inflammation within the lung has been difficult to study *in vivo*. We found in a human *ex vivo* airway explant platform that tissue from asthma donors showed enhanced gene expression of *il25*, *tslp*, and *il13* as compared to non-asthma tissue following RV39 stimulation. Further, we determined that asthma PCLS exhibited AHR to CCh after infection with RV39. These differences in immune

response and contractility were not secondary to increased viral loads in asthma PCLS. Rather, our data support an altered immune response in asthmatic airways that results in AHR following RV infection. We speculate that enhanced epithelial generation of Th2-biasing cytokines leads to AHR and could account for asthma exacerbations *in vivo*. While our study is limited by an inability to confirm the diagnosis of asthma, increased airway basement membrane thickness and elevated serum total IgE increase the probability of a correct diagnosis. Further, there was a significant age difference between asthma and normal donor PCLS. Despite these differences in age, we describe similar findings as seen in other studies that were age-matched with regard to elevations in airway-derived Th2-biasing cytokines in asthma subjects.^{s10, s11} Taken together, our studies suggest that AHR during RV-induced asthma exacerbations may be related to an altered immune response to RV39 in asthmatic airways resulting in increased gene expression of *IL25* and overproduction of TSLP during infection (Figure 2).

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159 **Table 1: Donor Demographics and Tissue Characterization**

Variable	Non-asthma (n=9)	Asthma (n=7)	All (n=16)	P
Age Mean (SD)	42 (16)	22 (8)	33 (17)	0.02
BMI Median (Q1, Q3)	29.2 (24.1, 34.3)	25.3 (22.4, 28)	26.7 (23.5, 33.4)	0.34
Race				1
Asian n (%)	1 (11.1%)	0 (0%)	1 (6.3%)	
Black n (%)	1 (11.1%)	1 (14.3%)	2 (12.5%)	
Caucasian n (%)	5 (55.6%)	4 (57.1%)	9 (56.3%)	
Hispanic n (%)	2 (22.2%)	2 (28.6%)	4 (25%)	
Male Gender n (%)	5 (55.6%)	4 (57.1%)	9 (56.3%)	1
COD				0.32
Anoxia	0 (0%)	2 (28.6%)	2 (12.5%)	
CVA	6 (67.6%)	1 (14.3%)	7 (43.75%)	
Head Trauma	3 (33.3%)	2 (28.6%)	5 (31.3%)	
Respiratory Arrest	0 (0%)	1 (14.3%)	1 (6.3%)	
GSWH	0 (0%)	1 (14.3%)	1 (6.3%)	
IGE (IU/mL) Geometric Mean (95% CI)	68.13 (30.42, 152.6)	492.4 IU/mL (184.1, 1317)		0.002
Basement membrane thickness (μm) Mean (SD)	1.11 (3.33)	35.71 (15.12)		<0.0001
Average Contraction to CCh prior to infection (%) Mean (SEM)	47.7 (6.0)	35.4 (7.5)		0.31

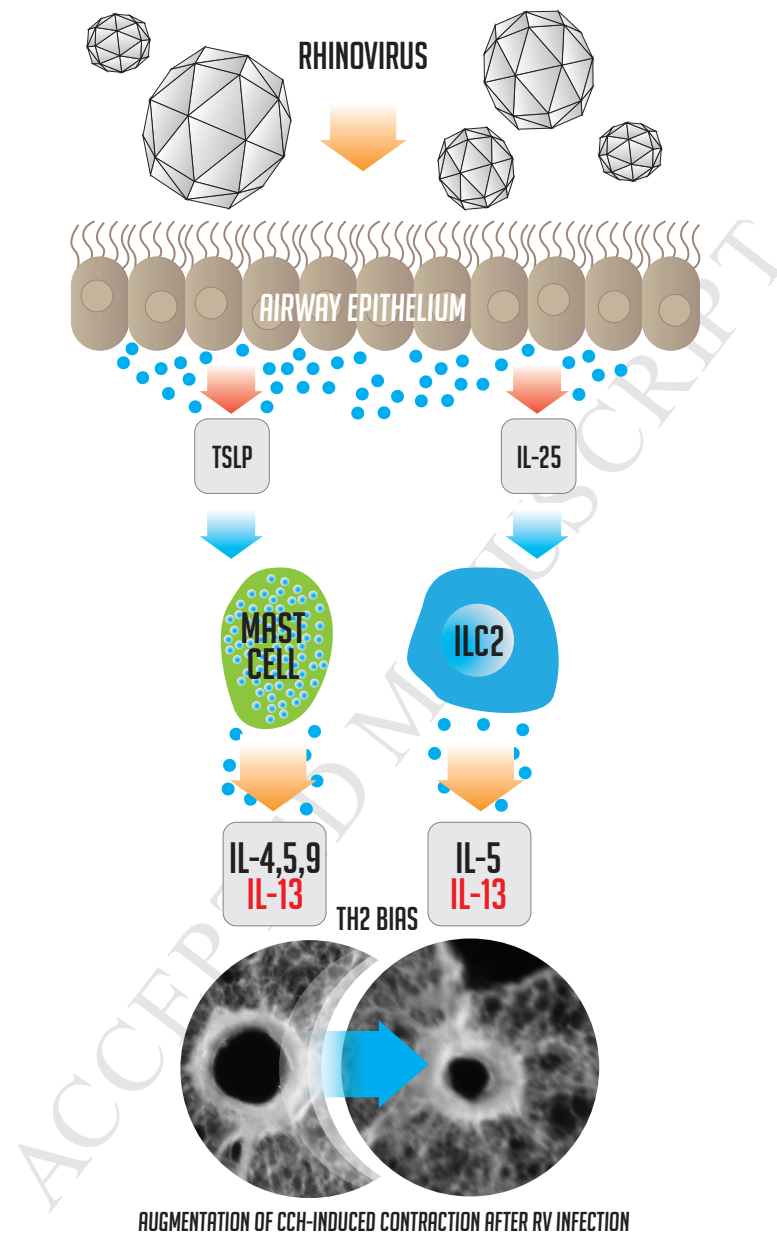
FIGURE LEGEND

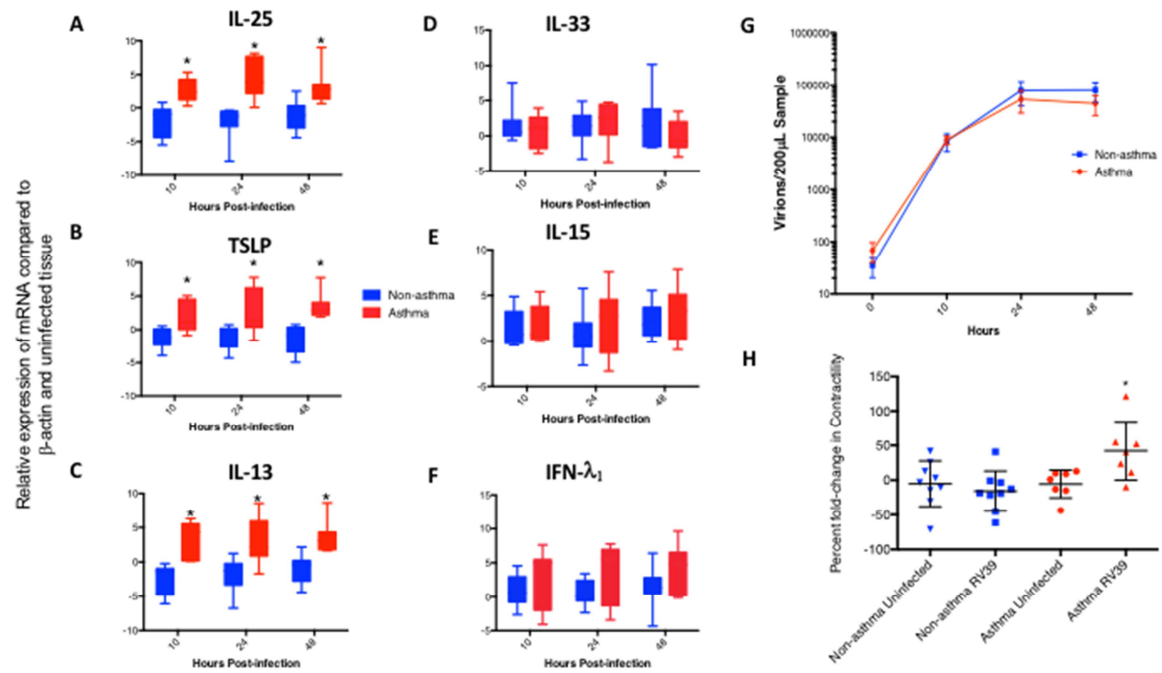
Figure 1: (A, B, C) *IL25*, *TSLP*, and *IL13* expression from RV39 infected PCLS. (D, E, F) *IL33*, *IL15*, *IFN γ ₁* expression from RV39 infected PCLS. (G) RV39 replication in PCLS derived from asthma (red) or non-asthma (blue) donors over 48 h. (H) Comparison of CCh-induced airway occlusion in donors with and without asthma before and after infection with RV39.

Figure 2: Proposed Mechanism for the Augmentation of Carbachol (CCh)-induced airway narrowing by RV infection in Precision Cut Lung Slices Derived from Asthma Donors. RV infection of the airway epithelium leads to production of IL-25 and TSLP in predisposed asthmatics. Airway mast cells and innate lymphoid cells type 2 (ILC2) are potential targets for these cytokines, leading to the generation of type 2 cytokines, including IL-13. We suggest that the generation of IL-13 causes airway hyper-responsiveness to Cch after infection with RV39.

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Online Repository

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