Secretory Inflammasome profiling by multiplex analysis in airway goblet cell culture

T. Tanabe, S. R. Webb-Parker, E. Tokita and B. K. Rubin

VCU School of Medicine and Children's Hospital of Richmond at VCU, VA

Background

IL-13, a Th2 cytokine, plays an important role in the pathogenesis of bronchial asthma and goblet cell development. It has not been shown if inflammatory mediators secreted by goblet cells contribute to airway inflammation in asthma.

Objectives

The aim of this study was to perform a multiplex bead immunoassay to examine the secreted inflammatory mediators from cultured human airway goblet cells

Methods

Cell culture model

NHBE cells were grown for 14 days at air-liquid interface (ALI) with PBS to produce a ciliated cell phenotype or with IL-13 to produce a goblet cell phenotype.

Histochemical analysis

Histology was performed using H&E and periodic acid-Schiff (PAS) stains, and immunostaining for mucins.

Multiplex bead assay

Multiplex bead assay of 25 inflammatory mediators was performed in the apical supernatants and basal culture medium of IL-13 exposed goblet cells or unexposed ciliated cells.

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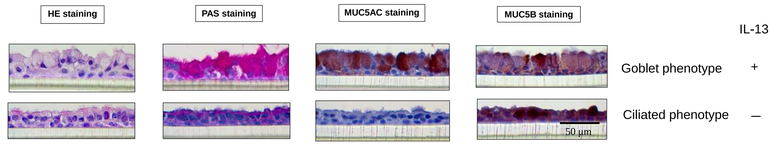
Results

Histology



Th1 cytokines & chemokines

	basolateral media			apical supernatant		
	ciliated	goblet	p value	ciliated	goblet	p value
IL-2	**53.225**	40.225	p<0.02	3.8625	0	p=0.40
IL-12	107.65	120.33	p=0.090	39.1375	51.6375	p=0.094
IFN-γ	4.95	7.4	p<0.0001	2.7375	11.8875	p<0.0001
IP-10	192.1	1391.4	p<0.0001	473.6375	1554.538	p<0.0001
RANTES	17.9	433.85	p<0.05	212.7625	750.8875	p<0.01

Other pro-inflammatory cytokines

	basolateral media			apical supernatant		
	ciliated	goblet	p value	ciliated	goblet	p value
TNF-α	5.775	11.075	p<0.0001	6.9125	27.225	p<0.01
IL-1β	3.95	7.275	p<0.05	3.775	16.0875	p<0.0001
MIP-1α	14.525	18.275	p<0.05	3.7	17.95	p<0.0001
MIP-1β	24.575	48.4	p<0.05	12.575	55.45	p<0.01
MCP-1	260.15	393.65	p<0.05	326.5	843.1125	p<0.0001
IL-7	28.7	27.975	p=0.70	10.1375	24.7875	p<0.0001
IL-15	101.075	79.9	p<0.02	13.3125	0	p=0.10

Th2 cytokines

	basolateral media			apical supernatant		
	ciliated	goblet	p value	ciliated	goblet	p value
IL-4	7.85	13.95	p<0.001	6.1875	13.4125	p<0.0001
IL-5	11.35	9.825	p=0.14	2.95	8.7375	p<0.0001
IL-9	85.225	145.275	p<0.0001	45.125	73.6375	p<0.01
IL-13	53.375	4527.6	p<0.01	20.4125	84.05	p=0.55

Anti-inflammatory cytokines

	basolateral media			apical supernatant		
	ciliated	goblet	p value	ciliated	goblet	p value
IL-1 RA	22.45	27.525	p=0.49	89.0125	113.475	p<0.01
IL-10	99.575	111.95	p=0.064	35.425	44.9625	p=0.14

Th17 & Neutrophil activating cytokines

	basolateral media			apical supernatant		
	ciliated	goblet	p value	ciliated	goblet	p value
IL-17	9.95	15.15	p=0.071	8.9125	10.45	p=0.57
IL-8	5919.775	9677.025	p<0.0001	3296.463	4094.25	p<0.05
IL-6	123.525	116.025	p=0.88	248.7625	285.0125	p=0.47
G-CSF	45.65	52.775	p=0.69	79.225	64.76667	p=0.46

Other growth factors

	basolateral media			apical supernatant		
	ciliated	goblet	p value	ciliated	goblet	p value
FGF	320.125	380.1	p<0.0001	8.9125	10.45	p=0.071
PDGF-BB	12.9	22.1	p=0.68	248.7625	285.0125	p<0.0001
VEGF	2793.775	3455.4	p<0.001	79.225	64.76667	p=0.23

Key: Blue indicates greater in ciliated cells; red indicates greater **in** goblet cells

Discussion

<u>Th1 cytokines & chemokines;</u>

IFN-γ and related chemokines released from goblet cells may attenuate Th2 inflammation

However

<u>Th2 cytokines;</u>

Th2 cytokines released from goblet cells may act in an autocrine manner to enhance Th2 inflammation, and contribute to eosinophil migration.

<u>Th17 & neutrophil activating cytokines;</u>

IL-8 released from goblet cells may enhance neutrophil migration.

<u>Other growth factors;</u>

Growth factors released from goblet cells may contribute to airway remodeling.

In most cases the above results were dose dependent and for the most part, these cytokines, chemokines and growth factors were secreted in a polarized fashion favoring the airside (apical side)

Conclusions

Inflammatory mediators released from goblet cells may act in an autocrine manner to enhance Th2 inflammation, eosinophil & neutrophil migration, and airway remodeling, which in turn contributes to the severity of asthma and other chronic airway diseases

Secretion of these mediators is primarily directed to the airside, suggesting an inflammatory response favoring local action.