Inflammasome Profiling in Airway Goblet Cells Cultured with Azithromycin or Dexamethasone

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Background

IL-13 transforms cultured normal human bronchial epithelial (NHBE) cells into goblet cells that secrete mucus, leukotrienes and inflammatory mediators.

We have shown that clarithromycin, but not dexamethasone, can inhibit IL-13 goblet cell transformation of NHBE cells.

Objectives

We hypothesized that azithromycin and dexamethasone would decrease the production of immunomodulatory mediators in goblet cells and we evaluated inflammatory mediator production by multiplex ELISA.

Methods

Cell culture model

NHBE cells were grown for 14 days at air-liquid interface (ALI) with PBS vehicle or IL-13 5 ng/mL as well as azithromycin 1 μg/mL (AZ), dexamethasone 1 μg/mL (Dex), or DMSO vehicle.

Histochemical analysis

Histology was performed using H&E and periodic acid-Schiff (PAS) stains, and immunofluorescence for MUC5AC & acetylated α-tubulin for cilia.

Multiplex bead assay

Multiplex bead assay of 25 inflammatory mediators was performed in the apical supernatants and basal culture medium from these cultured cells.

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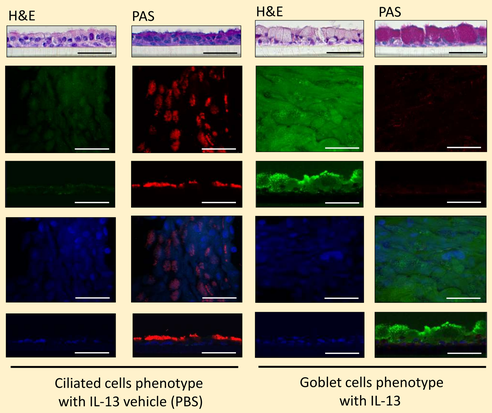
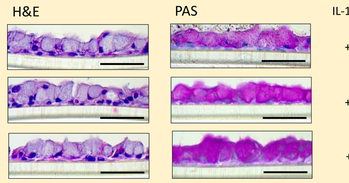
Results

Histology



Ciliated cells are weakly stained with MUC5AC and strongly stained with acetylated α tubulin at the surface of epithelial layers, whereas goblet cells with secretory granules strongly stained with MUC5AC , but there was no acetylated α-tubulin seen.

IF
green; MUC5AC
red; acetylated α-tubulin
blue; DAPI
Bar = 50 μm



Bar = 50 μm

Multiplex assay

Th1 cytokines & chemokines

Th1cytokines & chemokines (pg/mL), n=4

	basolateral media					apical supernatant				
	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value
IL-2	40.23	32.08	p=0.21	35.03	p=0.38	undetect.	undetect.		undetect.	
IL-12	120.33	128.38	p=0.28	119.35	p=0.89	51.63	43.14	p=0.25	44.96	p=0.36
IFN-γ	7.40	4.08	↓p<0.01	4.63	↓p<0.02	11.89	9.29	↓p<0.03	13.01	p=0.32
IP-10	1391.4	560.85	↓↓p<0.001	783.90	↓p<0.01	1554.5	1666.9	p=0.55	1428.3	p=0.51
RANTES	433.85	232.08	p=0.19	293.00	p=0.34	750.89	532.58	p=0.14	495.93	p=0.09

Th2 cytokines

Th2 cytokines (pg/mL), n=4

	basolateral media					apical supernatant				
	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value
IL-4	13.95	8.28	↓↓p<0.0001	8.70	↓↓p<0.0001	13.41	9.14	↓↓p<0.001	11.60	p=0.095
IL-5	9.83	8.20	p=0.062	8.03	↓p<0.05	8.74	7.23	p=0.14	8.14	p=0.48
IL-9	145.28	118.08	↓↓p<0.001	138.98	p=0.36	73.64	60.45	p=0.064	58.60	↓p<0.04
IL-13	427.60	312.98	↓p<0.02	414.20	p=0.24	84.05	55.83	↓p<0.01	73.99	p=0.27

Th17 & Neutrophil activating cytokines

Th17 &Neutrophil activating cytokines (pg/mL), n=4

	basolateral media					apical supernatant				
	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value
IL-17	15.15	4.05	↓↓p<0.0001	7.83	↓p<0.01	10.45	3.85	↓p<0.02	8.48	p=0.44
IL-8	9677.0	9217.2	p=0.18	9544.7	p=0.70	4094.4	4101.1	p=0.98	4029.4	p=0.85
IL-6	116.03	86.45	p=0.41	67.03	p=0.18	285.01	181.24	↓p<0.01	142.08	↓↓p<0.001
G-CSF	52.78	44.28	p=0.62	29.08	p=0.18	79.23	94.13	p=0.15	33.98	p=0.11

Other pro-inflammatory cytokines

Other inflammatory cytokines (pg/mL), n=4

	basolateral media					apical supernatant				
	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value
TNF-α	11.08	8.58	p=0.25	8.25	p=0.20	27.23	21.70	↓p<0.02	18.50	↓↓p<0.001
IL-1β	7.28	5.88	p=0.31	5.65	p=0.24	16.09	14.80	p=0.35	13.10	↓p<0.04
MIP-1α	18.28	15.28	p=0.08	14.28	↓p<0.03	17.95	11.85	↓↓p<0.01	12.93	↓p<0.01
MIP-1β	48.40	46.45	p=0.83	35.78	p=0.18	55.45	60.85	p=0.87	42.73	p=0.18
MCP-1	393.65	339.20	p=0.30	169.03	↓↓p<0.001	843.11	828.80	p=0.78	410.41	↓↓p<0.0001
IL-7	27.98	27.40	p=0.75	27.58	p=0.82	24.79	20.76	↓p<0.04	24.23	p=0.75
IL-15	79.90	67.95	p=0.49	72.10	p=0.65	undetect.	undetect.		undetect.	

Anti-inflammatory cytokines

Anti-inflammatory cytokines (pg/mL), n=4

	basolateral media					apical supernatant				
	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value
IL-1 RA	27.53	26.85	p=0.73	27.35	p=0.95	113.48	64.23	↓↓p<0.001	127.00	p=0.31
IL-10	111.95	121.70	p=0.31	133.20	↑p<0.04	44.96	38.93	p=0.52	39.64	p=0.57

Other growth factors

Other growth factors (pg/mL), n=4

	basolateral media					apical supernatant				
	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value	Goblet cells	AZ1ug/mL	p value	Dex1ug/mL	p value
bFGF	380.10	367.43	p=0.30	363.15	p=0.15	18.85	12.18	↓↓p<0.0001	16.74	p=0.32
PDGF-BB	22.10	18.20	p=0.83	15.88	p=0.72	187.55	190.48	p=0.87	147.35	↓p<0.04
VEGF	3455.4	3409.6	p=0.70	3160.5	↓p<0.03	1072.9	969.69	p=0.39	882.35	p=0.12

Discussion

Histology;

Neither AZ or Dex inhibited goblet cell hyperplasia.

Th1 cytokines & chemokines;

IFN-γ and related chemokines were inhibited by both AZ and Dex.

Th2 cytokines;

IL-4 and IL-9 were inhibited by both AZ and Dex. IL-13 was inhibited by AZ but not by Dex.

Th17 & Neutrophil activating cytokines;

IL-17 and IL-6 were inhibited both by AZ and Dex.

Other inflammatory cytokines;

TNF-α and MIP-1α were inhibited by both AZ and Dex.

IL-1β and MCP-1 were inhibited by Dex and apical IL-7 was inhibited by AZ.

Other growth factors;

Growth factors, basolateral FGF, PDGF and VEGF, which may contribute to airway remodeling were not inhibited by AZ, however PDGF was inhibited by Dex .

Conclusions

Inflammasome profiling suggests that the airway goblet cell is an inflammatory effector cell capable of producing proinflammatory cytokines and chemokines.

Although both AZ and Dex showed selective anti-inflammatory effects, AZ more effectively inhibited Th2 cytokines than Dex. AZ does not appear to have an effect on mediators associated with airway remodeling.