

## 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

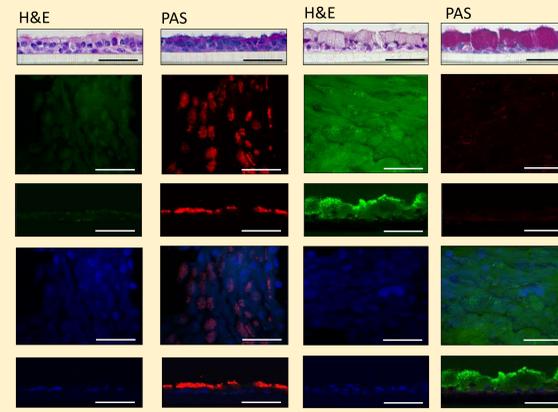
Histology was performed using H&E and periodic acid-Schiff (PAS) stains, and immunofluorescence for MUC5AC & acetylated α-tubulin for cilia.

### 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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## Histology



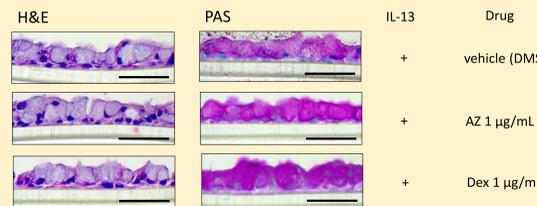
Ciliated cells phenotype with IL-13 vehicle (PBS)

Goblet cells phenotype with IL-13

## Results

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



## Multiplex assay

### Th1 cytokines & chemokines

| Th1 cytokines & chemokines (pg/mL), n=4 | basolateral media |          |         |           | apical supernatant |          |          |           |        |        |
|---|-------------------|----------|---------|-----------|--------------------|----------|----------|-----------|--------|--------|
|   | Goblet cells      | AZ1µg/mL | p value | Dex1µg/mL | Goblet cells       | AZ1µg/mL | p value  | Dex1µg/mL |        |        |
| IL-2                                    | 40.23             | 32.06    | p=0.21  | 35.03     | undetect           | undetect | undetect | undetect  |        |        |
| IL-12                                   | 120.33            | 120.38   | p=0.28  | 110.35    | p=0.89             | 51.63    | 43.14    | p=0.25    | 44.96  | p=0.38 |
| IFN-γ                                   | 7.40              | 4.08     | p<0.001 | 4.63      | p<0.02             | 11.89    | 9.29     | p<0.03    | 13.01  | p=0.32 |
| IP-10                                   | 1391.4            | 960.82   | p<0.001 | 793.90    | p<0.01             | 1554.3   | 1666.9   | p=0.55    | 1429.3 | p=0.51 |
| RANTES                                  | 433.85            | 232.08   | p=0.19  | 292.00    | 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      | AZ1µg/mL | p value  | Dex1µg/mL | Goblet cells       | AZ1µg/mL | p value | Dex1µg/mL |       |         |
| 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=0.082  | 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  | 138.88    | p=0.36             | 73.84    | 60.45   | p=0.064   | 58.00 | 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      | AZ1µg/mL | p value | Dex1µg/mL | Goblet cells       | AZ1µg/mL | p value | Dex1µg/mL |        |         |
| IL-17   | 15.15             | 4.05     | p<0.001 | 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=0.88    | 4029.4 | p=0.85  |
| IL-6  | 116.03            | 89.45    | 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      | AZ1µg/mL | p value | Dex1µg/mL | Goblet cells       | AZ1µg/mL | p value  | Dex1µg/mL |          |          |
| 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             | 24.78    | 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  | undetect | undetect |

### Anti-inflammatory cytokines

| Anti-inflammatory cytokines (pg/mL), n=4 | basolateral media |          |         |           | apical supernatant |          |         |           |        |        |
|--|-------------------|----------|---------|-----------|--------------------|----------|---------|-----------|--------|--------|
|  | Goblet cells      | AZ1µg/mL | p value | Dex1µg/mL | Goblet cells       | AZ1µg/mL | p value | Dex1µg/mL |        |        |
| IL-1 RA                                  | 27.53             | 26.85    | p=0.73  | 27.35     | p=0.95             | 113.48   | 64.25   | 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    | 39.84  | p=0.57 |

### Other growth factors

| Other growth factors (pg/mL), n=4 | basolateral media |          |         |           | apical supernatant |          |         |           |        |        |
|-----------------------------------|-------------------|----------|---------|-----------|--------------------|----------|---------|-----------|--------|--------|
|                                   | Goblet cells      | AZ1µg/mL | p value | Dex1µg/mL | Goblet cells       | AZ1µg/mL | p value | Dex1µg/mL |        |        |
| bFGF                              | 380.10            | 387.43   | p=0.30  | 383.15    | p=0.15             | 18.89    | 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   | 969.88  | 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.