

# DISTRIBUTION OF SA $\alpha$ 2,6Gal AND SA $\alpha$ 2,3Gal LINKED SURFACE RECEPTORS IN HUMAN RESPIRATORY TRACT AND ASSOCIATION WITH INFLUENZA VIRUS REPLICATION

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## BACKGROUND

Influenza virus remains the most important respiratory pathogen in the population. Its ability to evade host immune system can lead to severe winter outbreak and excess mortality, especially in those with chronic airway diseases such as asthma. This immune evasion ability is mainly due to the constant mutations in the viral haemagglutinin (HA) and neuraminidase (NA). These glycoproteins are critical in the entry and exit of influenza virus into human bronchial epithelial cells. Influenza binds to common membrane sialic acid (SA) sugar molecules. Human influenza viruses bind preferentially to SA $\alpha$ 2,6Gal linked receptors predominantly found in the upper airway, whereas the avian influenza viruses bind to SA $\alpha$ 2,3Gal linked receptors mainly in the distal respiratory tract.

Studies have shown that subjects with asthma and chronic obstructive pulmonary disease (COPD) are more susceptible to influenza infection, but patterns of expression of these sialic acid residues have not been studied in these individuals. It is also not known if bronchial epithelial cells (BECs) from these subjects are more susceptible to infection with influenza.

## AIM

To investigate the level of SA $\alpha$ 2,6Gal and  $\alpha$ 2,3Gal linked receptors on human bronchial epithelial cells from subjects with asthma, COPD and compare this to healthy control subjects. The infectivity of influenza viruses in these BECs among these subject groups in terms of virus replication are assessed.

## METHODS

### Influenza viruses

- **Human** H3N2 A / Wellington / 1 / 2004
  - **Avian** H11N9 A / Sharp-tailed Sandpiper / Australia / 6 / 2004
- Avian strains source: Carrington Wetlands, Newcastle, NSW

### Human bronchial epithelial cells

- Proximal airway cell line, Calu-3
- Distal airway cell line, A549
- Primary bronchial epithelial cells (pBECs), obtained by endobronchial brushings from healthy, asthmatics, and COPD volunteers.

### Receptor staining on epithelial cells

Epithelial cells were stained with Sambucus nigra lectin (Vector Lab) that binds SA $\alpha$ 2,6Gal receptor, and Maackia amurensis lectin II (Vector Lab) that binds to SA $\alpha$ 2,3Gal. The cells were analysed by flow cytometry and expressed as mean fluorescence intensity (MFI).

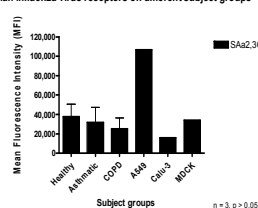
### Influenza infection

Human bronchial epithelial cells were infected at an M.O.I. of 0.005, and supernatants were harvested at 48hr post infection. Virus replication was measured in supernatants by plaque assay in MDCK cells.

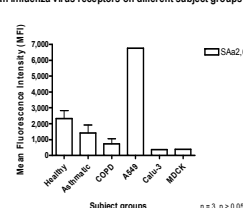
## RESULTS

### Receptor staining on epithelial cells

Human influenza virus receptors on different subject groups



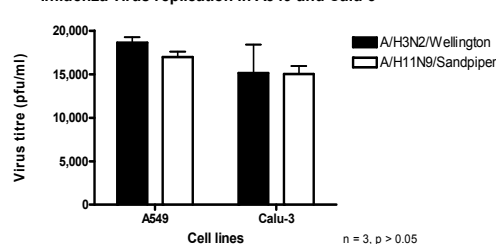
Avian influenza virus receptors on different subject groups



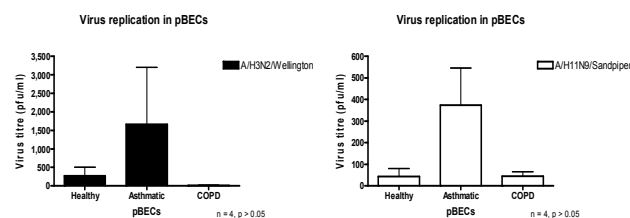
- All epithelial cells showed higher levels of SA $\alpha$ 2,6Gal receptor compared to SA $\alpha$ 2,3Gal receptor expression on the surface.
- Cell line A549 showed higher receptors level than Calu-3 and MDCK.
- There was no significant difference in expression of either SA $\alpha$ 2,6Gal or SA $\alpha$ 2,3Gal receptors in the pBECs from the three subject groups.

### Influenza virus replication

Influenza virus replication in A549 and Calu-3



- Influenza H3N2 and H11N9 virus showed good replication in cell line A549 and Calu-3, up to 20,000 pfu at 48hr post infection.
- No difference in virus replication in Calu3 and A549 despite the difference in receptor expression



- In comparison to cell lines, limited viral replication was observed in pBECs of all groups at 48hr post infection.
- There is no significant difference in viral replication between pBECs groups

## CONCLUSIONS

- The distal airway cell line A549 showed similar high viral replication to the proximal airway Calu-3 cell, despite the observed difference in SA $\alpha$ 2,6Gal and SA $\alpha$ 2,3Gal receptor expression. This may suggest that a low receptor level is required for infection, or other receptors are involved in influenza binding.
- All pBECs groups showed incomparable level of both SA $\alpha$ 2,6Gal and SA $\alpha$ 2,3Gal receptor, and demonstrated limited viral replication.

## FUTURE DIRECTIONS

- More pBECs will be obtained from subjects with asthma and COPD to assess the difference in viral replication compared to healthy subjects.
- Low viral replication observed in pBECs will be further investigated.

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