

**Modulation of responses in allergic airways disease by**  
***Haemophilus influenzae* infection**

**Ama-Tawiah Essilfie**

**B. Biomedical Science (Hons)**

Discipline of Immunology and Microbiology

School of Biomedical Science and Pharmacy

Faculty of Health

The University of Newcastle

Newcastle, NSW, Australia

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Ama-Tawiah Essilfie

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## Synopsis

Asthma is a common chronic inflammatory disease of the airways that affects over 2.2 million people in Australia. Asthma is a heterogeneous inflammatory disease typically characterised by T helper lymphocyte type 2 (Th2)-mediated eosinophilic inflammation, exaggerated responses to innocuous stimuli, mucus hypersecretion leading to airways obstruction and airway remodelling. These physiological changes result in wheezing, chest tightness, and breathing difficulties. However, it has been established that eosinophilic inflammation is only present in 50% of asthmatic patients. Around 30% of non-eosinophilic asthmatics have neutrophilic rather than eosinophilic inflammation, which is a key feature of neutrophilic asthma.

Non-typeable *Haemophilus influenzae* (NTHi) is a Gram-negative bacterium that is commonly found in the upper respiratory tract of about 75% of healthy individuals. It is normally asymptotically carried in people, however it may cause otitis media and is a common cause of community-acquired pneumonia. NTHi has also been linked to a number of chronic airway diseases. It has been detected in patients with bronchiectasis, chronic bronchitis and is commonly associated with chronic obstructive pulmonary disease (COPD) exacerbations. It has also recently been associated with neutrophilic asthma, however, the role of NTHi in neutrophilic asthma has not been investigated.

Using murine models of NTHi infection and allergic airways disease (AAD), we investigated the relationship between infection and AAD. We showed that NTHi infection induced features of neutrophilic asthma; reduced Th2-mediated eosinophilic inflammation, reduced airways hyper-responsiveness (AHR) compared to eosinophilic AAD, and importantly, significantly increased Th17 responses and neutrophilic inflammation. In the first study it was demonstrated that the combination of infection and AAD reduced the

expression of MHC II and CD86 on dendritic cells (DCs), suggesting that infection induced changes in presentation of antigen to naïve T-cells and subsequent adaptive responses. Infection also induced Interleukin (IL)-17 production from innate cells and Th17 cells. Critically, we show that inhibiting IL-17 significantly reduced neutrophilic inflammation in the airways. This highlights the crucial role of IL-17 in infection-induced neutrophilic AAD.

The second study showed that the induction of AAD during infection delayed bacterial clearance from the lungs compared to infection alone controls. In contrast to Th2-mediated eosinophilic inflammation, this model of infection-induced neutrophilic AAD was resistant to dexamethasone treatment. All features of infection-induced neutrophilic AAD, including eosinophil and neutrophil influx, antigen-specific IL-5, IL-13 and Interferon (IFN)- $\gamma$ , NTHi-specific IL-17, and AHR were unchanged with steroid treatment. This study also demonstrated that neutrophil and macrophage activation and function was inhibited in neutrophilic AAD. This lack of innate immune response may enable chronic bacterial infection.

The final study investigated clarithromycin, a macrolide, and combination therapy with dexamethasone, as possible treatment strategies for neutrophilic asthmatics. This study demonstrated that clarithromycin alone significantly reduced neutrophil influx and IL-17 responses, but increased Th2-mediated eosinophilic inflammation. However, the combination of clarithromycin and dexamethasone suppressed all key features of AAD, including eosinophilic and neutrophilic inflammation, ovalbumin (OVA)-specific IL-5, IL-13, and IFN- $\gamma$ , NTHi-induced IL-17, and AHR.

These novel findings further the understanding of the potential role of NTHi in the development of neutrophilic asthma. We have identified some mechanisms of how infection

may lead to features observed in neutrophilic asthma, and importantly, possible treatment strategies for neutrophilic asthmatics, and perhaps, other neutrophilic airway diseases with evidence of infection.

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## Abbreviations

<b>AAD</b>	Allergic airways disease	<b>MHC</b>	Major Histocompatibility Complex
<b>AHR</b>	Airways hyper-responsiveness	<b>MLN</b>	Mediastinal lymph node
<b>APC</b>	Antigen presenting cell	<b>MMP-9</b>	Matrix metalloproteinase-9
<b>BALF</b>	Bronchoalveolar lavage fluid	<b>MSC</b>	Mucus secreting cell
<b>COPD</b>	Chronic obstructive pulmonary disease	<b>MyD88</b>	Myeloid Differentiation factor 88
<b>DC</b>	Dendritic cell	<b>NE</b>	Neutrophil elastase
<b>ECP</b>	Eosinophil cationic protein	<b>NF-<math>\kappa</math>B</b>	Nuclear Factor $\kappa$ B
<b>FCS</b>	Foetal calf serum	<b>NK</b>	Natural Killer
<b>FEV<sub>1</sub></b>	Forced expiratory volume	<b>NTHi</b>	Non-typeable <i>Haemophilus influenzae</i>
<b>GM-CSF</b>	Granulocyte Macrophage Colony Stimulating Factor	<b>OVA</b>	Ovalbumin
<b>HBSS</b>	Hanks buffered salt solution	<b>PAMP</b>	Pathogen-associated molecular patterns
<b>IFN</b>	Interferon	<b>PBS</b>	Phosphate buffered saline
<b>Ig</b>	Immunoglobulin	<b>pDC</b>	Plasmacytoid dendritic cell
<b>IL</b>	Interleukin	<b>PMN</b>	Polymorphonuclear cell
<b>IN</b>	Intranasal	<b>PRR</b>	Pattern recognition receptor
<b>IP</b>	Intraperitoneal	<b>RBC</b>	Red blood cell
<b>KC</b>	Keratinocyte chemokine	<b>SEM</b>	Standard error of the mean
<b>LPS</b>	Lipopolysaccharide	<b>TCR</b>	T-cell receptor
<b>mDC</b>	Myeloid dendritic cell		

**TGF** Transforming growth factor  
**Th** T helper lymphocyte  
**Th1** Type 1 helper T lymphocyte  
**Th2** Type 2 helper T lymphocyte  
**Th17** Type 17 helper T lymphocyte  
**TLR** Toll-like receptor  
**TNF** Tumour necrosis factor  
**Treg** T regulatory cell