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

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Submitted in the fulfilment of the requirements for the award of a PhD degree

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February 2012

Acknowledgements

Firstly I would like to thank my primary supervisor, Prof. Philip Hansbro, whose encouragement, support and supervision made this work possible. Thank you also to my co-supervisors; Dr Jodie Simpson, A/Prof Margaret Dunkley, and Prof. Paul Foster. Your expertise, knowledge and advice has proven invaluable during my studies. Special thanks to The Rebecca Cooper Medical Research Foundation who provided a scholarship that supported a substantial part of my project.

Thank you also to all the staff and students in the Microbiology, Asthma and Airways Research Group who have provided their assistance and friendship throughout the last few years. Special thanks go to Dr Jay Horvat, Richard Kim, Malcolm Starkey, Caedyn Stinson, and Emma Beckett.

I would very much like to thank my family. My parents, especially my mum Violet, PapaEwusie, AbaNyanba, and NanaBaffo, who inspired, encouraged, and supported me throughout my study. Also my dearest and closest friends; Boitumelo and Kebitsaone Kelaeng, Moetapele ‘Coach’ Gasemotse and Kabo Matlho, you guys have been the best friends anyone could ever hope or pray for, thank you. Thank you also to Brian C. Kamota, uri moyo wangu.

And last, and most importantly, I want to give all praise, all glory and all honour to my Heavenly Father, without Whom none of this would have been possible.

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Chapter 5: Discussion and conclusions

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 asymptomatically 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)-γ, 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-γ, 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

AAD Allergic airways disease

AHR Airways hyper-responsiveness

APC Antigen presenting cell

BALF Bronchoalveolar lavage fluid

COPD Chronic obstructive pulmonary disease

DC Dendritic cell

ECP Eosinophil cationic protein

FCS Foetal calf serum

FEV_1 Forced expiratory volume

GM-CSF Granulocyte Macrophage Colony Stimulating Factor

HBSS Hanks buffered salt solution

IFN Interferon

Ig Immunoglobulin

IL Interleukin

IN Intranasal

IP Intraperitoneal

KC Keratinocyte chemokine

LPS Lipopolysaccharide

mDC Myeloid dendritic cell

MHC Major Histocompatibility Complex

MLN Mediastinal lymph node

MMP-9 Matrix metalloproteinase-9

MSC Mucus secreting cell

MyD88 Myeloid Differentiation factor 88

NE Neutrophil elastase

NF-κB Nuclear Factor κB

NK Natural Killer

NTHi Non-typeable *Haemophilus influenzae*

OVA Ovalbumin

PAMP Pathogen-associated molecular patterns

PBS Phosphate buffered saline

pDC Plasmacytoid dendritic cell

PMN Polymorphonuclear cell

PRR Pattern recognition receptor

RBC Red blood cell

SEM Standard error of the mean

TCR T-cell receptor

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