The role of rhinovirus and novel molecular mechanisms in allergic airways disease

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B Biomed Sci (Hons)

Thesis submitted in fulfilment of the requirements for obtaining the degree of Doctor of Philosophy in Immunology and Microbiology

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University of Newcastle

February 2016

Statement of originality

I hereby certify that to the best of my knowledge that this thesis is my own written work and contains no material previously published or written by another person except where due references and acknowledgements are made. It contains no material that has been previously submitted by me for the award of any other degree or diploma in any university or other tertiary institution.

Jason Girkin

Thesis by publication

I hereby certify that this thesis is in the form of four separate papers. I have included as part of the thesis a written statement from each co-author, endorsed in writing by the Faculty Assistant Dean (Research Training), attesting to my contribution to any jointly authored papers.

Acknowledgements

This thesis is a collection of publications achieved as a product of collaborative research efforts. For this reason, I thank all my co-authors, but especially Luke Hatchwell who worked closely with me on these projects, helped me develop many of the techniques I use today and who was my first port of call for questions and feedback early in my candidature. I also owe special gratitude to my supervisors and mentors, Professor Joerg Mattes, Laureate Professor Paul Foster and Dr Adam Collison for their guidance, their feedback, and indeed, their criticism. The research environment they have built made for an enthusiastic, helpful and collegial workplace.

Along the way, I have been lucky enough to have the understanding and support of my family, my friends and my wife. To my wife Sarah Barnes, who has been with me for the entire duration of my university education, who supported me through the lowest of times, who celebrated the best of times with me, who has helped pick up the slack where (financially, socially and logistically) I may have fallen short, who has waited for so many things, who has made sacrifices along the way and who still married me anyway, for you, my gratitude is immeasurable in a way that words will forever be inadequate. Your love, dedication and support are invaluable. Together, we forged a very solid foundation upon which we can build a long and happy future.

My mother Nicola Barrett has always been the first to say she is proud of my achievements. No matter how small the milestone, I have always had the encouragement and love of my mother. I am the man I am today because of how she raised me to be and no doubt, her unconditional pride and encouragement has driven my successes, big and small to date. Throughout my studies, I saw her less and less and

although she missed me at times, she still encouraged me the whole way. I owe her thanks for many things and this is just one of them.

I am lucky to have lots of siblings, all with different perspectives and personalities who have all shaped me in different ways and helped me understand the world from different perspectives and who have kept me grounded to reality, maintaining my sanity. I thank Stephanie, Gavin, Whitney and Timothy for every little thing they have done to make my candidature easier. I give special thanks to my eldest sister, Kerry Barrett and her family, Rodney, Adrian, Tahya and Grace. Kerry has been instrumental in a lot of lessons and milestones I have encountered throughout my life. She has been a moral compass and an anchor of understanding, like-mindedness and support, my whole life. I have always looked to her for advice. She has the most generous and helpful family, always first to offer their assistance, their time and their love.

To my growing list of friends, old and new alike I thank all of you, but I would like to give special thanks to my closest friends, Leon Sokulsky and Young Lam. You have both played a big role during my university education, providing much needed fun and distraction when required, as well as a great pair of people to study and revise with, prior to exams. It has been a great privilege to have Leon as a colleague, working alongside me in the lab the whole way through my PhD.

Finally, I thank all those who have provided me technical support. Namely, Dr Ana Pereira de Siqueira, Jane Grehan, Matthew Morton, Heather Macdonald and everyone else in the Experimental and Translational Respiratory Medicine group. I thank all who contributed to discussion and everyone within the Hunter Medical Research Institute and Bioresources who helped in any way great and small.

List of publications

Hatchwell L.*, Collison, A.*, **Girkin, J**., Parsons, K., Li, J., Zhang, J., Phipps, S., Knight, D., Bartlett, N. W., Johnston, S. L., Foster, P. S., Wark, P. A. B. & Mattes, J. Toll-like receptor 7 governs interferon and inflammatory responses to rhinovirus and is suppressed by IL-5-induced lung eosinophilia. *Thorax* **70**(9), 854-861 (2015).

Girkin, J., Hatchwell, L., Foster, P., Johnston, S. L., Bartlett, N. W., Collison, A. & Mattes, J. CCL7 and IRF7 mediate hallmark inflammatory and interferon responses following rhinovirus 1B infection. *J immunol* **194**(10), 4924-4930 (2015).

Girkin, J.,* Hatchwell, L.,* Collison, A., Yagita, H., Foster P., & Mattes J. TRAIL signalling is pro-inflammatory and pro-viral in a murine model of rhinovirus 1B infection. *AJP Lung* **312**(1), L89-L99 (2016)

Hatchwell, L., **Girkin, J.,** Dun, M. D., Morten, M., Verrills, N., Toop, H. D., Morris, J. C., Johnston, S. L., Foster, P. S., Collison, A., Mattes, J. Salmeterol attenuates chemotactic responses in rhinovirus-induced exacerbation of allergic airways disease by modulating protein phosphatase 2A. *J Allergy Clin Immunol* **133**(6), 1720-1727 (2014)

* Both authors contributed equally.

List of abbreviations

AAD	-Allergic airways disease
AAL(s)	-2-amino-4-(4-(heptyloxy)phenyl)-2-methylbutan-1-ol
Ab	-Antibody
AHR	-Airways hyperreactivity / Airway hyperresponsiveness
APC	-Allophycocyanin
BAL	-Bronchoalveolar lavage
BALF	-Bronchoalveolar lavage fluid
BEAS-2B	-Human immortalised epithelial cell line
BEGM	-Bronchial epithelial cell growth medium
CCL	-CC-motif ligand
CD	-Cluster of differentiation
CR	-Chromotrope-hematoxylin
c-FLIP	-Intracellular FLICE inhibitory protein
CXCL	-C-X-C-motif ligand
DAMP	-Danger associated molecular pattern
DC	-Dendritic cell
DMSO	- Dimethyl sulfoxide
Dpi	-Days post infection
dsRNA	-Double stranded RNA
EBV	-Epstein-Barr virus

EC	-Epithelial cell
ELISA	-Enzyme-linked immunosorbent assay
FACS	-Flourescence activated cell sorting / Flow cytometry
FCS	-Feotal calf serum
FEV-1	-Forced expiratory volume in one second
Fig	-Figure
FITC	-Fluorescein isothiocyanate
FSC	-Forward scatter area
G-CSF	-Granulocyte colony-stimulating factor
GM-CSF	-Granulocyte macrophage colony-stimulating factor
GR	-Glucocorticoid receptor
HBSS	-Hank's balanced salt solution
HDM	-House dust mite
HEPES	-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	-Human immunodeficiency virus
HRV	-Human rhinovirus
ICAM	-Intracellular adhesion molecule
ICS	-Inhaled corticosteroids
IFN	-Interferon
Ig	-Immunoglubulin
IL	-Interleukin

IP-10	-Interferon induced protein 10
IRF	-Interferon regulatory factor
LABA	-Long acting β2-agonists
LPS	-Lipopolysaccharide
MAPK	-Mitogen-activated protein kinase
MCP	-Macrophage chemotactic protein
MDA	-Melanoma differentiation-associated gene
mDC	-Myeloid dendritic cell
MHC	-Major histocompatibility class
MID-1	-Midline-1
MyD88	-Myeloid differentiation primary response gene 88
NF-κB	-Nuclear factor κB
NK	-Natural killer cell
NKT	-Natural killer T cell
OCD	-Oral corticosteroids
OVA	-Ovalbumin
PAMP	-Pathogen associated molecular pattern
PAS	-Periodic acid-Schiff
PBMC	-Peripheral blood mononuclear cells
PBS	-Phospho-buffered saline
PE	- Phycoerythrin

Pen/Strep	-Penicillin and Streptomycin
PerCP	-Peridinin-chlorophyll protein
p-ERK	-Phosphorylated Extracellular signal-regulated kinase
Phos	-Phosphorylated
p-JNK	-Phosphorylated c-Jun N-terminal kinase
PP2A	-Protein phosphatase 2A
RANTES	-Regulated on activation normal and T cell expressed and secreted cytokine
rTRAIL	-Recombinant TRAIL protein
PRR	-Pattern recognition receptor
pDC	-Plasmacytoid dendritic cell
qPCR	-Quantitative polymerase chain reaction
ssRNA	-Single stranded RNA
RIG	-Retinoic acid-inducible protein
RPMI	- Roswell Park Memorial Institute medium
RSV	-Respiratory syncytial virus
RT	-Reverse transcription
RV	-Rhinovirus
SAL	-Sterile saline
SSC	-Side scatter area
STAT	-Signal transducer and activator of transcription

$TCID_{50}$	-Tissue culture infected dose in 50% culture
TCR	-T cell receptor
Th	-T helper
TIR	-Toll-Interleukin-1-receptor domain
TLR	-Toll-like recpetor
TNF	-Tumour necrosis factor
Tnfsf10	-Tumour necrosis factor superfamily member 10
TRAIL	-Tumour necrosis factor-related apoptosis-inducing ligand
Treg	-Regulatory T cell
TSLP	-Thymic stromal lymphopoietin
TUNEL	-Terminal dUTP nick end labelling
UV	-Ultraviolet inactivated rhinovirus
VP	-Viral protein
WT	-Wild type

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Thesis abstract

Rhinovirus (RV) infections are common ailments and are the most common precipitant of asthma exacerbations. This thesis contains investigations of novel mechanisms in RV infections and RV-induced exacerbations of house dust mite (HDM) driven allergic airways disease (AAD). The role of TLR7, CCL7, IRF-7, TRAIL and the off-target effect of long-acting β_2-agonists (LABAs) on PP2A are characterised in the host response to RV with a combination of *in vivo* and *in vitro* approaches.

In chapter 2, TLR7 signalling is identified as crucial for antiviral responses to RV and for dampening allergic Th2 responses, protecting against RV-induced exacerbations of allergic airways disease. Likewise, high levels of allergic signalling through IL-5 suppresses TLR7 mediated antiviral responses. In chapter 3, results from the mouse lung transcriptome response to RV infection guided the investigation of two of the most up-regulated genes, CC-motif ligand 7 (CCL7) and interferon regulatory factor 7 (IRF-7). By inhibiting CCL7 or IRF-7 in naïve mice, the antiviral response and inflammation was suppressed following RV infection. Inhibiting CCL7 during infection of allergic mice also reduced inflammation. In chapter 3, tumour necrosis factor related apoptosis-inducing ligand (TRAIL) is shown to be pro-inflammatory and pro-viral during RV infection. TRAIL is up-regulated in the lung during the course of RV infection. TRAIL-deficient mice were protected against inflammation and airways hyperresponsiveness (AHR). RV-titre was reduced in TRAIL-deficient mice and manipulation of TRAIL *in vitro* had direct effects on viral titre. In chapter 4, the LABA Salmeterol demonstrated anti-inflammatory effects by directly activating PP2A, and suppressing AHR independently of β-2 adrenoreceptors.

In summary, I have taken multiple approaches to identify novel mechanisms of the host response to RV infection and RV-induced exacerbations of allergic airways disease to identify novel therapeutic targets that may treat the underlying inflammatory mechanisms of asthma exacerbations.