Novel therapeutic approaches for the treatment of allergic airways disease

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B Biomed Sci (Hons)

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Statement of originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

Adam Collison
Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of my thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publications.

Adam Collison
Contribution to Papers:

Chapter 3:

Joerg Mattes and Paul Foster initially conceptualised and with Adam Collison designed experiments. Joerg Mattes and Adam Collison co-ordinated mouse models and collected samples with Max Plank.

Figure 3.1: *Adam Collison* conducted miRNA specific qPCR *A and B*.

Figure 3.2: *Adam Collison* performed airways resistance measurement and with Joerg Mattes analysed data *A, B and C*. Together they collected and analysed BALF *D, E and F*.

Figure 3.3: *Adam Collison* ran mouse models and with Joerg Mattes collected an analysed data from qPCR *A*, airways reactivity *B*, BALF *C*, and histology *D,E and F*.

Figure 3.4: *Adam Collison* with Joerg Mattes collected samples then analysed cytokines by ELISA *A*. *Adam Collison* conducted FACS analysis to determine cellular profile. Joerg Mattes analysed array data identifying OBF and *Adam Collison* ran confirmatory qPCR *D*.

Joerg Mattes
Chapter 4:

Joerg Mattes and Paul Foster initially conceptualised and with Adam Collison designed experiments. Adam Collison co-ordinated mouse models and collected samples with Joerg Mattes and Max Plank.

Figure 4.1: Adam Collison performed miRNA specific qPCR B, C and D.

Figure 4.2: Adam Collison performed histological analysis A, B, C, D, E, F, and G.

Figure 4.3: Adam Collison conducted airways resistance analysis A, B and C.

Figure 4.4: Adam Collison performed cytokine measurement by ELISA A, B and C and qPCR D and E.

Supplementary Figure 4.1: Adam Collison performed miRNA specific qPCR.

Supplementary Figure 4.2: Adam Collison performed BALF analysis A and B.

Supplementary Figure 4.3: Adam Collison performed histological A and B and resistance analysis C.

Joerg Mattes
Chapter 5:

Chronic aerosolised ovalbumin induced allergic airways disease models were run in the laboratory of Rakesh Kumar at the University of New South Wales. Adam Collison ran and analysed miRNA microarrays with samples obtained from these experiments, designed and tested the antagomir against identified targets. Phenotypic effects of antagomir were quantified by Cristan Herbert.

Table 5.1: Adam Collison performed and analysed miRNA microarray.

Figure 5.1: Adam Collison conducted miRNA specific qPCR to confirm array targets.

Figure 5.2: Phenotypic measures post antagomir treatment were conducted by Cristan Herbert A, B, C and D.

Figure 5.3: qPCR of miRNA target was conducted by Cristan Herbert.

Joerg Mattes
Chapter 6:

Adam Collison ran the mouse model, extracted RNA and with Joerg Mattes analysed the microarray data that identified Midline 1 as a TRAIL regulated protein. Together they designed the allergic mouse experiments and with Luke Hatchwell extended this work into Rhinovirus models. Together Adam Collison and Luke Hatchwell conducted mouse studies as detailed below.

Peter Wark and Melinda Tooze performed and supervised studies on healthy and asthmatic subjects and performed associated cell culture experiments. Nicole Verrils and Helen Carpenter performed and analysed PP2Ac measurements and immunoprecipitation and contributed to experiment design. Anthony Don synthesised the AAL(s). Nives Zimmerman and Marc Rothenberg conducted the initial transcriptome microarray. Nathan Bartlett and Sebastian Johnson assisted in the design of Rhinovirus experiments and provided RV1B for further propagation. Ana Pereira de Siqueira and Paul Foster assisted in the coordination and supervision of mouse and human studies. Joerg Mattes conceptualized and supervised the studies.

Figure 6.1: Together Adam Collison and Luke Hatchwell ran mouse models conducted qPCR and immunohistochemistry analysis A, C, D and I. Adam Collison conducted resistance analysis E. Luke Hatchwell conducted PP2A activity assays B and H, histological F and cytokine analysis G.

Figure 6.2: Luke Hatchwell conducted PP2a activity assays A, BALF and cytokine analysis D, E and F. Adam Collison conducted resistance analysis C and with Luke Hatchwell co-ordinated and analysed qPCR data B.
Figure 6.3: Adam Collison performed resistance measurement and analysis A and F. Luke Hatchwell performed BALF analysis B and G, PP2a activity assays C and H. Together Adam Collison and Luke Hatchwell co-ordinated and analysed qPCR D and conducted multiplex cytokine analysis E and I.

Figure 6.4: A B C D E

Supplementary figure 6.1: A Adam Collison performed airways resistance measurement and analysis. Luke Hatchwell conducted B BALF analysis C histology analysis and cytokine analysis D and E.

Supplementary figure 6.2: Luke Hatchwell and Helen Carpenter conducted Western Blotts

Supplementary figure 6.3: Luke Hatchwell and Stuart Reeves conducted qPCRs in A, B, C and D

Joerg Mattes
Acknowledgments

This thesis is the culmination of the most significant body of work I have yet undertaken. Ultimately it represents the long awaited end to my formal education and the beginning of my independent research career. I have only been able to arrive here with the assistance of many others, who have seen fit to reach into my life with something of their own.

Firstly I would like to thank Alice, specifically thankyou for all the times you have so patiently allowed me to put my work ahead of our life. For the weekends and late nights that you have been put off, thankyou – I could never have continued down this path without your honest and freely given support, despite what it costs you.

To my Mum and Dad – Mark and Sandra Collison. Thanks for the love and support you have offered me since I can remember. Thanks for encouraging me to pursue my interests even when it was at a significant time and financial burden to yourselves. To JJ and Rachel likewise – thanks for the support and prayers over the years – it has allowed me to get here...

My extended family have also been fundamental, the active interest and support offered to me through my life to date has been of greater value to me than you know. Auntie Linda and Wim, Bestmate, Grandma and Grandpa thanks.

I have been fortunate to be part of a vibrant research group who were happy to help with advice and assistance at many many points along the way. Joerg has been a great supervisor through both my honours and PhD, thankyou for the keen interest you have shown in our research projects, your consistent enthusiasm has been a driving force in the completion of this work and it is much valued. I look forward to being part of your research team into the future. Paul also has my thanks, not only for his advice as a supervisor for both my honours and PhD but for creating the environment in his lab with the friendly and co-operative mindset that I have enjoyed for the past 5 years.

Others have been invaluable to me at the coalface. I would particularly like to thank Luke and Max for the various help offered along the way – particularly providing an extra set of skilled hands on lengthy sacrifice days. Thanks to Ana for helping me wade through red tape more often than we would have liked – your support was more valuable than you believe. To Dicky as well for helpful advice and open experience along the way. Also Stuart, Shannon and Fiona for their various technical assistance, particularly with the running of arrays.

The pre-eminent gratitude that I would hope to express in both my life and work is to my Saviour and Lord Christ Jesus. This world is His and everything in it, I hope that my usage of the abilities He has gifted me would always point to and glorify His great name.
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Abstract of thesis

This thesis presents studies on novel therapeutic interventions for the treatment of allergic asthma providing proof of concept through extensive investigations in mouse models of human disease. The current increase in the incidence of asthma worldwide along with inability of current medications to treat the primary causes of the disease indicates that novel therapeutic approaches are required. This will improve the quality of life and disease burden concerns of the community. I investigated two alternate therapeutic approaches in an effort to identify new candidate targets with significant therapeutic potential.

The first research chapter (see chapter 3) presents an initial study on the role of miRNA in the development of allergic airways disease (AAD). This study also demonstrates proof-of-concept for the use of modified, cholesterol conjugated complementary sequences termed antagonirs to specifically inhibit the expression of miR-126 in the airways and to alleviate AAD.

The second research chapter (see chapter 4) presents a comparative study in which treatment of allergic mice with an antagonir suppressing miR-145 is compared to mice treated with the current gold standard therapy, systemic glucocorticoids. Here it is demonstrated that the novel therapeutic approach of selectively inhibiting the upregulation of miR-145 in the airway wall is as potent as treatment with systemic dexamethasone to alleviate AAD.

The third research chapter (see chapter 5) presents a study where miR-126 was inhibited in a chronic model of AAD. The findings of this study confirm an important role of miR-126 in the regulation of allergic airways inflammation but suggest that in this model miR-126-independent mechanisms promote the
development of tissue remodelling, hallmark features of chronic asthma. These results suggest that targeting a single miRNA may not be sufficient to reduce all aspects of AAD.

The fourth research chapter (see chapter 6) presents a study of the tumour necrosis factor - related apoptosis - inducing ligand (TRAIL) induced signalling pathway in AAD and rhinovirus (RV) -induced exacerbation of AAD. Here I identify a novel role for TRAIL induced Midline-1 (Mid1) driven polyubiquination and silencing of the Protein Phosphatase 2a (PP2a). Furthermore, blocking this signalling pathway through either the silencing of Mid1 with siRNA or the synthetic reactivation of PP2a using the small molecule AAL(S) was capable of alleviating AAD and RV-induced exacerbation. This study provides proof-of-concept that modulation of the TRAIL induced signalling pathway may provide therapeutic benefit in the treatment of AAD.

Together these studies have investigated novel and relevant targets for therapeutic intervention in AAD. By targeting immuno-regulatory systems such as miRNAs and TRAIL regulated signalling cascades at the initial site of allergen exposure – the airway surface, these approaches have the potential to successfully modulate the complex aberrant immune response that initiates and underpins allergic asthma.
Publications

The following publications have arisen from data presented in the current thesis:

Research publications:


The E3 ubiquitin ligase Midline-1 links allergen and rhinovirus exposure to asthma via targeting PP2A **Adam Collison***, Luke Hatchwell*, Nicole Verrills, Peter AB Wark, Ana Pereira de Siqueira, Melinda Tooze, Helen Carpenter, Anthony Don, Nives Zimmerman, Nathan W Bartlett, Sebastian L Johnston, Marc E Rothenberg, Paul S Foster, Joerg Mattes. Accepted Pending Revision Nature Medicine 2011

*authors contributed equally to this paper

Review publications:

The emerging role of TRAIL as key regulator of inflammatory responses. **Collison A, Foster PS, Mattes J.** Clin Exp Pharmacol Physiol 2009

Emerging role of microRNAs in disease pathogenesis and strategies for therapeutic modulation. **Mattes J, Collison A, Foster PS.** Curr Opin Mol Ther. 2008
Conference publications:

2011 American Thoracic Society International Conference
“The development of House Dust Mite induced allergic airways disease is regulated by a novel E3 ubiquitin ligase-dependent deactivation of a protein phosphatase”


2011 Thoracic Society of Australia and New Zealand Annual Scientific Meeting
“The development of House Dust Mite induced allergic airways disease is regulated by a Midline-1 dependent deactivation of PP2a”

Adam Collison

2010 AusBiotech Annual Scientific Meeting
“Inhibition of a novel ubiquitin ligase using siRNA as a novel therapeutic approach for asthma”
Adam Collison

2010 Australasian Society of Immunology Annual meeting
“The development of House Dust Mite induced allergic airways disease is dependent on the ubiquitin ligase Midline-1”

Adam Collison, Luke Hatchwell, Ana Pereira de Siqueira, Nicole Verrills, Paul Foster, Joerg Mattes

2009 Australasian Society of Immunology Annual meeting
“The role of MicroRNA 145 in Allergic Airways Disease”
A Collison, J Mattes, M Plank, PS Foster

2009 Australasian Society of Immunology Annual meeting
"Inhibition of MicroRNA-126 suppresses TH2 cell effector function and the development of allergic airways disease"
A Collison, J Mattes, M Plank, PS Foster.

2009 American Thoracic Society International Conference
“The Identification of TRAIL as a Mediator of Airways Remodelling in a Chronic Model of Murine Allergic Airways Disease”
A Collison, M Plank, A Pereira de Siqueira, S Reeves, S Phipps, PS Foster, J Mattes

2009 American Thoracic Society International Conference
“MicroRNAs Are Crucial in the Development of Airways Hyperreactivity”
A Collison, J Mattes, M Plank, PS Foster

2008 Australasian Society of Immunology Annual meeting
“TRAIL is a crucial regulator of airways remodelling in a mouse model of chronic allergic airways disease”
A Collison, M Plank, A Pereira de Siqueira, S Reeves, S Phipps, PS Foster, J Mattes
List of abbreviations

AAD – allergic airways disease
AEC – airway epithelial cell
AHR – airways hyperreactivity
AP – activator protein
CCL – chemokine ligand
CxCL – chemokine (c-X-c motif) ligand
CMV - cytomegalovirus
DC – dendritic cell
DGCR8 - DiGeorge syndrome critical region gene 8
DISC - death-inducing signalling complex
Drosha - ribonuclease 3
ERK- extra- cellular signal-regulated kinase
FADD - fas-associated death domain
FasL - CD95 / fas ligand / Apo1 ligand
FEV\textsubscript{1} – forced expiratory volume in one second
FLICE - FADD-like interleukin-1b-converting enzyme
FLIP – FLICE-inhibitory protein
FOXp3 - forkhead box P3
GATA3 – GATA binding protein 3
HDM - house dust mite
HIV - human immunodeficiency virus
HPF – high power field
HSUR- Herpes virus saimiri noncoding uridine-rich RNA
HPA - hypothalamic-pituitary-adrenal axis
ICS- inhaled corticosteroids
IFN - interferon
Ig – immunoglobulin
IKK - IκB kinase
IL - interleukin
i.n. - intranasal
IPF - idiopathic pulmonary fibrosis
IRS - insulin receptor substrate
JAM - junction adhesion molecule
KLF - Krueppel-like factor
KSHV - Kaposi's sarcoma-associated herpes virus
Let-7 - lethal 7
LPS - lipopolysaccharide
MAPK - mitogen-activated protein kinase
Mid1 - midline 1
miRNA - micro ribonucleic acid
MMP - matrix metalloproteinase
MyD88 - myeloid differentiation primary response gene (88)
NF - nuclear factor
NK - natural killer
OCT - octamer-binding transcription factor
OVA - ovalbumin
PKB - protein kinase B
PMN - polymorphonuclear neutrophils
Pri-miRNA - primary microRNA transcripts
PP - protein phosphatase
PTEN - phosphatase and tensin homologue
RASGRP1 - RAS guanyl releasing protein 1
RBCC - ring-finger B-box coiled coil domain protein
RhoA - ras homologue gene family, member A
RIP - receptor-interacting protein
SHIP1 - polyphosphate-5-phosphatase
siRNA – short interfering ribonucleic acid
SOCS1 – suppressor of cytokine signalling 1
SOX - sex determining region Y –box
STAT - signal transducer and activator of transcription
TAB2 - mitogen-activated protein kinase kinase kinase 7 binding protein 2
TCR - T-cell receptor
Th - T helper
TLR - toll like receptor
TNF- tumour necrosis factor
TRAIL - tumour necrosis factor – related apoptosis - inducing ligand
TRIF - TRI-domain containing adapter-inducing interferon-β
T-regs - regulatory T-cells