THE ROLE OF TRAIL-REGULATED SIGNALLING PATHWAYS AND TLR7 IN RHINOVIRUS-INDUCED EXACERBATION OF ALLERGIC AIRWAYS DISEASE

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B Biomed Sci (Hons)

Thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
(Immunology & Microbiology)

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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 other 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 the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

Luke Hatchwell
Statement 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 publication/s/scholarly work.

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Thesis by Publication

I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the jointly authored publications.

Luke Hatchwell
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List of publications included as part of thesis

Publication 1:

THE E3 UBIQUITIN LIGASE MIDLINE 1 PROMOTES ALLERGEN AND RHINOVIRUS-INDUCED ASTHMA BY INHIBITING PROTEIN PHOSPHATASE 2A ACTIVITY

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Publication 2:

TOLL-LIKE RECEPTOR 7 GOVERNS INTERFERON AND INFLAMMATORY RESPONSES TO RHINOVIRUS AND IS SUPPRESSED BY IL-5-INDUCED LUNG EOSINOPHILIA

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Publication 3:

SALMETEROL ATTENUATES CHEMOTACTIC RESPONSES IN RHINOVIRUS-INDUCED EXACERBATION OF ALLERGIC AIRWAYS DISEASE BY MODULATING PROTEIN PHOSPHATASE 2A

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Contribution to Publications

Publication 1:

Adam Collison and Luke Hatchwell designed and performed mouse and cell culture experiments, analysed data, generated figures and edited the manuscript. Nicole Verrills and Helen Carpenter performed and analysed PP2Ac quantification and immunoprecipitation and designed in-vitro experiments. Nicole Verrills also edited the manuscript. Peter Wark and Melinda Tooze performed and supervised studies on clinical samples collected from healthy subjects and subjects with asthma and performed cell culture experiments. Ana Pereira de Siqueira coordinated and supervised mouse and human studies. Anthony Don and Jonathan Morris synthesized AAL$_{(S)}$ for use as an activator of PP2A and developed the dosing regiment. Nives Zimmermann and Marc Rothenberg coordinated and assisted in microarray array analysis. Nathan Bartlett and Sebastian Johnston assisted in design of experiments, provided stocks of RV1B for further propagation and cDNA standards and edited the manuscript. Paul Foster supervised mouse studies, interpreted data and edited the manuscript. Joerg Mattes conceptualized, coordinated, designed and supervised mouse and human studies, interpreted and analysed data, and drafted and edited the manuscript. All authors contributed to data discussion and revised the manuscript during the resubmission period.

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Publication 2:

Luke Hatchwell and Adam Collison designed and performed mouse and cell culture experiments, analysed data, generated figures and edited the manuscript. Jason Girkin and Junyao Li performed experiments and analysed data. Jie Zhang assisted in supervision. Peter Wark and Kristy Parsons performed and supervised studies on healthy subjects and subjects with asthma, collected and processed biopsies, and performed cell culture experiments. Simon Phipps assisted in the design and conceptualization of some mouse experiments. Darryl Knight supervised and interpreted cell culture experiments. Nathan Bartlett and Sebastian Johnston assisted in design of mouse experiments, provided RV1B for further propagation and cDNA standards. Paul Foster assisted in design, supervision and interpretation of mouse studies. Joerg Mattes conceptualized, coordinated, designed and supervised mouse and human studies, interpreted and analysed data, and drafted and edited the manuscript. All authors contributed to data discussion and revised the manuscript during the resubmission period.

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Publication 3:

Luke Hatchwell designed and performed mouse and cell culture experiments, analysed data, generated figures, drafted and edited the manuscript. Jason Girkin and Matthew Morten performed experiments and analysed data. Matthew Dun and Nicole Verrills designed experiments and performed and analysed PP2Ac measurements and immunoprecipitations. Nicole Verrills also edited the manuscript. Hamish Toop and Jonathan Morris synthesized AAL(S) for use as an activator of PP2A and developed the dosing regimen. Sebastian Johnston assisted in design of mouse experiments, provided RV1B for further propagation and cDNA standards. Paul Foster assisted in design, supervision and interpretation of mouse studies. Adam Collison designed and performed mouse and cell culture experiments, analysed and interpreted data, and edited the manuscript. Joerg Mattes conceptualized, coordinated, designed and supervised mouse and cell culture studies, analysed and interpreted data, and edited the manuscript. All authors contributed to data discussion and revised the manuscript during the resubmission period.

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Statement of Contribution of Others

The co-authors listed below attest that Research Higher Degree candidate Luke Michael Hatchwell was the primary contributor (first or co-first author) to the following papers/publications:

1)  The E3 ubiquitin ligase midline 1 promotes allergen and rhinovirus-induced asthma by inhibiting protein phosphatase 2A activity.
Collison, A.*, Hatchwell, L.*, Verrills, N., Wark, PA., de Siqueira, AP., Tooze, M., Carpenter, H., Don, AS., Morris, JC., Zimmermann, N., Bartlett, NW., Rothenberg, ME., Johnston, SL., Foster, PS., Mattes, J.

*These authors contributed equally to this work.

2)  Toll-like receptor 7 governs interferon and inflammatory responses to rhinovirus and is suppressed by IL-5-induced lung eosinophilia.
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3)  Salmeterol attenuates chemotactic responses in rhinovirus-induced exacerbation of allergic airways disease by modulating protein phosphatase 2A.
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Abstract of Thesis

Asthma is a chronic inflammatory disease of the airways, associated with debilitating reversible airflow obstruction. The majority of healthcare costs from asthma-related hospitalisations are attributed to exacerbations by respiratory viruses, with rhinoviruses (RV) being the most commonly detected. This thesis presents original research papers detailing investigations to elucidate the mechanisms underlying RV-induced exacerbations of allergic airways disease (AAD).

The first manuscript (see Chapter 2) details the elucidation of a novel TRAIL signalling pathway where the TRAIL-regulated gene product Midline-1 (MID1), which inhibits protein phosphatase 2A (PP2A), was found to promote AAD through increased homing of myeloid dendritic cells (mDCs) to the airway via CCL20 release. Notably, inhibition of MID1 or reactivation of PP2A abolished airway hyperresponsiveness (AHR) and attenuated airways inflammation and mucus hypersecretion in mouse models of AAD and RV-induced exacerbation.

The second manuscript (see Chapter 3) investigates the importance of Toll-like receptor (TLR) 7-elicited interferon (IFN) responses during RV infection in an asthmatic setting. We show that following exposure to house dust mite (HDM), mice deficient in TLR7 display exaggerated eosinophilic inflammation and attenuated anti-viral responses when challenged with RV. TLR7 expression in the lungs of mice was found to be suppressed by interleukin-(IL)-5-induced eosinophilia, while human asthmatics with eosinophilic but not neutrophilic airways inflammation also showed reduced TLR7 and IFN expression.

The third manuscript (see Chapter 4) revisits established therapeutic agents, long-acting β2 agonists (LABAs), in light of recently described interactions with PP2A. This study extends those findings by reporting that administration of salmeterol, or other β2 agonists, protected mice against HDM- and RV-induced lung inflammation as effectively as the corticosteroid dexamethasone. Salmeterol but not dexamethasone mediated this via increased PP2A activity, the inflammatory phenotype recapitulated when PP2A was targeted by siRNA.

Taken together, these studies have identified new targets for the therapeutic intervention of asthma and RV-induced exacerbation.