Investigation of Pathogenesis of Chronic Obstructive Pulmonary Disease

Tatt Jhong Haw

BSc Biomed Sci (Hons)

Discipline of Immunology and Microbiology and Priority Research Centre for Healthy Lungs

School of Biomedical Science and Pharmacy

Faculty of Health and Medicine

University of Newcastle and Hunter Medical Research Institute

Newcastle, NSW, Australia

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The 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.

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers, or carried out in other institutions. I have included as part of the thesis a statement in the cover page of Chapter 2, 3 and 4, which clearly outlines the extent of collaboration, with whom and under what auspices.

I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included these publications as part of the thesis as an appendix, endorsed by my supervisor, attesting to my contribution to the joint publications.

Tatt Jhong Haw

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Acknowledgements

First and foremost, I would like to express my sincerest gratitude and thanks to my primary supervisor Prof. Philip Hansbro for giving me this fantastic opportunity of pursuing a PhD in Australia. His patience, encouragement, support and supervision made this work possible. I would like to thank my co-supervisors, Dr. Malcolm Starkey, Laureate Prof. Paul Foster and Dr. Andrew Jarnicki for their expertise, knowledge and support during my studies.

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Synopsis

Chronic Obstructive Pulmonary Disease (COPD) affects more than 64 million people globally and is primarily caused by cigarette smoke (CS) exposure. It is the third leading cause of morbidity and mortality worldwide and imposes significant socioeconomic burden worldwide. COPD is a chronic lung disease characterised by chronic pulmonary inflammation, airway remodelling and emphysema. These pathologies consequently culminate in progressive lung function decline and airflow limitation. Current therapies for the management of COPD are largely ineffective. They provided symptomatic relief to patients and do not target the underlying causal factors of COPD. Hence, there is a lack of effective treatments and an urgent need for research into the identification and development of therapeutic strategies in treating COPD.

The lack of effective treatments is due to the poor understanding of immunological processes and mechanisms that underpin the pathogenesis of COPD. Our laboratory has recently established a murine experimental model of COPD by exposing mice to nose-only inhalation of tightly regulated dose of CS. Importantly, our CS-induced model of COPD recapitulates the hallmark features of human disease in a relatively short period of time. Thus, this allows us to investigate and examine the immunological processes and mechanisms that underpin the pathogenesis of COPD. The aims of the studies described in this thesis were to identify and elucidate immunological processes that underpin the pathogenesis of COPD.

The first study identified a novel role for tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in promoting CS-induced COPD. TRAIL and its receptors were increased by CS exposure in mice and in lung samples from human COPD patients. TRAIL-deficient mice or wild-type (WT) mice treated with neutralising

TRAIL monoclonal antibodies had significantly reduced CS-induced pulmonary inflammation, expression of pro-inflammatory mediators, emphysema-like alveolar enlargement and improved lung function.

The second study investigated the role of Toll-like receptor (TLR)2 and TLR4 in CS-induced pathogenesis of COPD. CS-induced pulmonary inflammation was largely unaltered in the absence of TLR2 or TLR4. TLR2-deficient mice had CS-induced emphysema-like alveolar enlargement, apoptosis and impaired lung function compared to normal air-exposed mice that was equivalent to CS-exposed WT mice, whilst small airway remodelling was not altered. By contrast, TLR4-deficient mice had reduced CS-induced emphysema-like alveolar enlargement, apoptosis and impaired lung function compared to WT mice. Interestingly, CS-induced small airway fibrosis, characterised by increased collagen deposition around small airways, was ablated in TLR4-deficient mice.

The third study identified a previously unrecognised role for TLR7 in the pathogenesis of COPD. In the absence of TLR7, CS-induced pulmonary inflammation was not altered compared to CS-exposed WT controls. CS-induced small airway epithelial cell thickening was reduced whilst collagen deposition increased in the absence of TLR7. Importantly, CS-induced emphysema-like alveolar enlargement and apoptosis were reduced in TLR7-deficient mice. Administration of the TLR7 agonist imiquimod synergistically increased CS-induced emphysema and apoptosis. Interestingly, imiquimod-induced emphysema and apoptosis may occur through the activity of mast cell-specific proteases, in particular mouse mast cell protease-6 (mMCP-6). Crucially, antibody-mediated neutralisation of TLR7 also reduced CS-induced emphysema and apoptosis in the lungs in experimental COPD.

Our novel findings indicate that TRAIL and TLRs, in particularly TLR2, TLR4 and TLR7, have critical roles in CS-induced development of COPD. TRAIL promotes CS-induced pulmonary inflammation, emphysema-alveolar enlargement and lung function impairment. TLRs have little or minor role in CS-induced pulmonary inflammation. TLR2 may protect against CS-induced emphysema and lung function impairment, whilst TLR4 and TLR7 induce these disease features of COPD. TLR4 promotes CS-induced airway fibrosis whilst TLR2 and TLR7 regulate collagen deposition around small airways. Collectively, our studies significantly advance the understanding of the immunological mechanisms that underpin the pathogenesis of COPD and may facilitate the development of novel treatments for COPD in the future.

Publication arising from this thesis

Publications included in this thesis

1. **Tatt Jhong Haw***, Malcolm R Starkey*, Prema M Nair, Stelios Pavlidis, Gang Liu, Duc H Nguyen, Alan C-Y Hsu, Irwan Hanish, Richard Y Kim, Adam M Collison, Mark D Inman, Peter A Wark, Paul S Foster, Darryl A Knight, Joerg Mattes, Hideo Yagita, Ian M Adcock, Jay C Horvat, Philip M Hansbro. Tumour necrosis factor-related apoptosis-inducing ligand promotes cigarette smoke-induced experimental COPD. *Mucosal Immunol* 2016;9:859–872
2. **Tatt Jhong Haw***, Malcolm R Starkey*, Stelios Pavlidis, Prema M Nair, Gang Liu, Irwan Hanish, Richard Y Kim, Paul S Foster, Ian M Adcock, Jay C Horvat and Philip M Hansbro. TLR2 and TLR4 have Opposing Roles in the Pathogenesis of Cigarette Smoke-induced COPD. *Am J Respir Cell Mol Biol*, in revision 2016
3. **Tatt Jhong Haw***, Malcolm R Starkey*, Stelios Pavlidis, Prema M Nair, Gang Liu, Irwan Hanish, Richard Y Kim, Kensuke Miyake, Richard L Stevens, Paul S Foster, Ian M Adcock, Jay C Horvat and Philip M Hansbro. TLR7 is promotes cigarette smoke-induced emphysema in Chronic Obstructive Pulmonary Disease. *Nat Med*, in preparation for submission, 2016.

Other paper publications

4. Gang Liu, Marion A Cooley, Andrew G Jarnicki, Alan C-Y Hsu, Prema Mono Nair, **Tatt Jhong Haw**, Michael Fricker, Shaan L Gellatly, Richard Y Kim, Mark D Inman, Gavin Tjin, Peter A B Wark, Jay C Horvat, Brian G Oliver, William S Argraves, Darryl A Knight, Janette K Burgess & Philip M Hansbro. Fibulin-1 regulates the pathogenesis of respiratory diseases. *JCI Insight* 2016;1:52–67

5. Alan CY Hsu, Malcolm R Starkey, Irwan Hanish, Kristy Parsons, **Tatt Jhong Haw**, Linda J Howland, Ian Barr, James B Mohany, Paul S Foster, Darryl A Knight, Peter A Wark and Philip Hansbro. Targeting PI3K-p110α Suppresses Influenza Viral Infection in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2015;191:1012–1023
6. Richard Kim, Jay Horvat, James Pinkerton, Malcolm Starkey, Ama-Taiwah Essilfie, Jemma Mayall, Bernadette Jones, **Tatt Jhong Haw**, Simon Keely, Joerg Mattes, Ian Adcock, Paul Foster and Philip Hansbro. MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying PI3K-mediated suppression of HDAC2. *J Allergy Clin Immunol*, in press 2016
7. Alan CY Hsu, Kamal Dua, Malcolm Starkey, **Tatt Jhong Haw**, Prema Nair, Kristy Parsons, Nathan Zammit, Shane Grey, Katherine Baines, Paul Foster, Philip Hansbro, and Peter Wark. miRNA-125a/b Inhibits A20 and MAVS to Promote Inflammation and Impair Antiviral Response in Chronic Obstructive Pulmonary Disease. *J Exp Med*, in submission 2016

Abstract publication

8. Philip Hansbro, **Tatt Jhong Haw***, Prema Nair, Irwan Hanish, Duc Nguyen, Gang Liu, Mark Inman, Richard Kim, Adam Collison, Darryl Knight, Hideo Yagita, Joerg Mattes, Jay Horvat and Malcolm Starkey*. Tumour necrosis factor-related apoptosis inducing ligand promotes the development of experimental chronic obstructive pulmonary disease (MUC1P.905). *J Immunol*, 1st May 2015, vol.194 (1 Supplement) 64.6

National and international conference presentations:

9. **Tatt Jhong Haw***, Malcolm Starkey*, Stelios Pavlidis, Prema Mono Nair, Gang Liu, Irwan Hanish, Richard Kim, Kensuke Miyake, Ian Adcock, Paul Foster, Jay Horvat and Philip Hansbro (2016, April). Toll-like receptor 7 promotes cigarette-smoke induced emphysema-like alveolar enlargement in chronic obstructive pulmonary disease. **Oral** presentation presented by Prof. Philip Hansbro at the TSANZ 2016, Perth, Australia, April 2016
10. **Tatt Jhong Haw***, Malcolm Starkey*, Prema Mono Nair, Irwan Hanish, Gang Liu, Richard Kim, Jay Horvat and Philip Hansbro (2015, Oct). The role of toll-like receptors in the pathogenesis of chronic obstructive pulmonary disease. **Poster** presentation presented by Prof. Philip Hansbro at TOLL 2015 Congress, Marbella, Spain, October 2015.
11. **Tatt Jhong Haw***, Malcolm Starkey*, Prema Mono Nair, Irwan Hanish, Duc Nguyen, Gang Liu, Mark Inman, Richard Kim, Adam Collison, Jay Horvat, Paul Foster Hideo Yagita, Joerg Mattes and Philip Hansbro (2015, Sep). Tumour necrosis factor-related apoptosis inducing ligand promotes the development of experimental chronic obstructive pulmonary disease. **Poster** presentation presented by Dr. Malcolm Starkey at 4th European Congress of Immunology Vienna September 2015.
12. **Tatt Jhong Haw***, Malcolm Starkey*, Prema Mono Nair, Irwan Hanish, Duc Nguyen, Gang Liu, Mark Inman, Richard Kim, Adam Collison, Jay Horvat, Paul Foster, Hideo Yagita, Joerg Mattes and Philip Hansbro (2015, May). Tumour necrosis factor-related apoptosis inducing ligand promotes the development of experimental chronic obstructive pulmonary disease. **Oral and poster** presentation

presented by Prof. Philip Hansbro at the American Association of Immunologists Annual Meeting 2015.

13. **Tatt Jhong Haw***, Malcolm Starkey*, Prema Mono Nair, Irwan Hanish, Duc Nguyen, Gang Liu, Mark Inman, Richard Kim, Adam Collison, Jay Horvat, Paul Foster, Hideo Yagita, Joerg Mattes and Philip Hansbro (2014, December). Tumour necrosis factor-related apoptosis inducing ligand promotes the development of experimental chronic obstructive pulmonary disease. **Poster** presentation presented at the 44th Australasian Society for Immunology Annual Meeting 2014 meeting.
14. **Tatt Jhong Haw***, Malcolm Starkey*, Prema Mono Nair, Irwan Hanish, Duc Nguyen, Gang Liu, Mark Inman, Richard Kim, Adam Collison, Jay Horvat, Paul Foster, Hideo Yagita, Joerg Mattes and Philip Hansbro (2014, November). Tumour necrosis factor-related apoptosis inducing ligand promotes the development of experimental chronic obstructive pulmonary disease. **Poster** presentation presented at the TSANZ NSW Branch Annual Scientific Meeting 2014.
15. **Tatt Jhong Haw***, Malcolm Starkey*, Prema Mono Nair, Irwan Hanish, Duc Nguyen, Gang Liu, Mark Inman, Richard Kim, Adam Collison, Jay Horvat, Paul Foster, Hideo Yagita, Joerg Mattes and Philip Hansbro (2014, October). Tumour necrosis factor-related apoptosis inducing ligand promotes the development of experimental chronic obstructive pulmonary disease. **Oral** presentation presented at the 10th Annual Newcastle Asthma Meeting 2014.

denotes co-first author

Other presentations:

16. Prema Mono Nair, Malcolm Starkey, **Tatt Jhong Haw**, Roland Ruscher, Muralidhara Rao Maradana, Ranjeny Thomas, Brendan O'Sullivan and Philip Hansbro (2014, December). RelB deficiency promotes allergic airway inflammation in mice. **Oral** presentation presented by Dr. Malcolm Starkey at 4th European Congress of Immunology Vienna September 2015.
17. Richard Kim, Jay Horvat, James Pinkerton, Malcolm Starkey, Ama-Taiwah Essilfie, Jemma Mayall, Bernadette Jones, **Tatt Jhong Haw**, Simon Kelly, Joerg Mattes, Ian Adcock, Paul Foster and Philip Hansbro (March, 2015). Infection-induced microRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying PI3K-mediated suppression of HDAC2. **Oral** presentation presented by Dr. Richard Kim at the Annual Scientific Meeting for Leaders in Lung Health & Respiratory Science 2015.
18. Prema Mono Nair, Malcolm Starkey, **Tatt Jhong Haw**, Roland Ruscher, Muralidhara Rao Maradana, Ranjeny Thomas, Brendan O'Sullivan and Philip Hansbro (2014, December). RelB deficiency promotes allergic airway inflammation in mice. **Oral and poster** presentation presented by Miss Prema Mono Nair at the 44th Australasian Society for Immunology Annual Meeting 2014 meeting.
19. Gang Liu, Andrew Jarnicki, Prema Mono Nair, **Tatt Jhong Haw**, Michael Fricker, Shaan Gellatly, Richard Kim, Mark Inman, Gavin Tjin, Jay Horvat, Brian Oliver, Darryl Knight, Janette Burgess and Philip Hansbro (2014, December). Fibulin-1 plays a critical role in the pathogenesis of chronic obstructive pulmonary disease (COPD). **Oral and poster** presentation presented by Mr. Gang Liu at the 44th Australasian Society for Immunology Annual Meeting 2014 meeting.

20. Richard Kim, Jay Horvat, James Pinkerton, Malcolm Starkey, Ama-taiwah Essilfie, Jemma Mayall, Bernadette Jones, **Tatt Jhong Haw**, Simon Kelly, Joerg Mattes, Ian Adcock, Paul Foster and Philip Hansbro (2014, December). MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying PI3K-mediated suppression of HDAC2. **Oral and poster** presentation presented by Dr. Richard Kim at the 44th Australasian Society for Immunology Annual Meeting 2014 meeting.
21. Prema Mono Nair, Malcolm Starkey, **Tatt Jhong Haw**, Roland Ruscher, Muralidhara Rao Maradana, Ranjeny Thomas, Brendan O'Sullivan and Philip Hansbro (2014, December). RelB deficiency promotes allergic airway inflammation in mice. **Poster** presentation presented by Miss Prema Mono Nair at the TSANZ NSW Branch Annual Scientific Meeting 2014.
22. Gang Liu, Andrew Jarnicki, Prema Mono Nair, **Tatt Jhong Haw**, Michael Fricker, Shaan Gellatly, Richard Kim, Mark Inman, Gavin Tjin, Jay Horvat, Brian Oliver, Darryl Knight, Janette Burgess and Philip Hansbro (2014, December). Fibulin-1 plays a critical role in the pathogenesis of chronic obstructive pulmonary disease (COPD). **Oral** presentation presented by Mr. Gang Liu at the TSANZ NSW Branch Annual Scientific Meeting 2014.
23. Malcolm Starkey, Irwan Hanish, Kamal Dua, Prema Nair, **Tatt Jhong Haw**, Alan Hsu, Paul Foster, Darryl Knight, Jay Horvat, Peter Wark and Philip Hansbro (2014, September). Interleukin-13 predisposes mice to more severe influenza infection by suppressing interferon responses and activating microRNA-21/PI3K. **Poster** presentation presented by Dr. Malcolm Starkey at the International Cytokine and Interferon Society meeting 2014.

Awards

1. My first author paper published in Mucosal Immunology entitled “Tumour necrosis factor-related apoptosis-inducing ligand promotes cigarette smoke-induced experimental COPD” was awarded best publication by the School of Biomedical Sciences & Pharmacy, University of Newcastle, in September 2015.
2. Top 50 nominee for best poster at 4th European Congress of Immunology Vienna September 2015.
3. Part of my research was used to apply the Rebecca L. Cooper Medical Research Foundation in 2014 by Dr. Malcolm Starkey, who won the Leo Dintenfass Memorial Award for most interesting or innovative research grant recipient of the Rebecca L. Cooper Medical Research Foundation in 2014.

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Abbreviations

A549: Human alveolar basal epithelial cells

AAD: Allergic airway disease

AHR: Airway hyperresponsiveness

AP: Activating protein

Apaf: ATP-dependent proteolysis factor

Bak: Bcl-2 homologous antagonist/killer

BALF: Bronchoalveolar lavage fluid

Bax: Bcl-2-associated X protein

BM: Basement membrane

CCL: Chemokine (C-C motif) ligand

CD: Cluster of differentiation

COPD: Chronic Obstructive Pulmonary Disease

CS: Cigarette smoke

CXCL: Chemokine (C-X-C motif) ligand

DC: Dendritic cell

DcR: Decoy receptor

DISC: Death inducing signalling complex

DNA: Deoxyribonucleic acid

DR: Death receptor

ECRHS: European Community Respiratory Health Survey

ELISA: Enzyme-linked immunosorbent assay

FADD: Fas-associated death domain

FDR: False discovery rate

FEV_1: Forced expiratory volume in one second

FLIP: FADD-like interleukin-1 beta-converting enzyme inhibitory protein

FVC: Forced vital capacity

γδT: Gamma delta T

GOLD: Global Initiative for Chronic Obstructive Lung Disease

H441: Alveolar club cell

HBE: Human bronchial epithelial cells

HDM: House dust mite

H&E: Hematoxylin and eosin

HeLa S3: Human epithelial cell lines

HMGB1: High mobility group box 1

HPRT: Hypoxanthine–guanine phosphoribosyltransferase

HSP: Heat shock protein

i.n: Intranasal

i.p: Intraperitoneal

i.t: Intratracheal

i.v: Intravenous

IAP: Inhibitor of apoptosis protein

IAV: Influenza A virus

IFN: Interferon

IFNAR1: Interferon receptor 1

IKK: IκB kinase

IKKi: Inducible IKK

IL: Interleukin

IPF: Idiopathic pulmonary fibrosis

IRAK: Interleukin-1 receptor-associated protein kinase

IRF: Interferon regulatory factor

LABA: Long-acting β_2-agonist

LAMA: Long-acting muscarinic antagonist

LPS: Lipopolysaccharide

LY96: Lymphocyte antigen 96

Mal: MyD88 adaptor-like

MAPK: Mitogen-activated protein kinase

MCP: Monocyte chemotactic protein

mDC: Myeloid dendritic cell

MID1: Midline-1

MIP: Macrophage inflammatory protein

MLE-15: Mouse lung epithelial cell line

mMCP-6: Mouse mast cell-specific protease-6

MMP: Matrix metalloproteinase

mRNA: Messenger RNA

MyD88: Myeloid differentiation primary response gene 88

NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells

NK: Natural killer

NKT: Natural killer T

OVA: Ovalbumin

PAS: Periodic acid-Schiff

pDC: Plasmacytoid dendritic cell

PP2A: Protein phosphatase 2A

qPCR: Real-time quantitative polymerase chain reaction

RIP: Receptor-interacting protein

RNA: Ribonucleic acid

RSV: Respiratory syncytial virus

RV: Residual volume

SAA3: Serum amyloid A3

SABA: Short-acting β_2-agonist

SAMA: Short-acting muscarinic antagonist

SAPALDIA: Swiss study on Air Pollution and Lung Disease in adults cohort study

SEM: Standard error of means

SERPINE2: Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2

SHIP-1: Src homology 2 domain–containing inositol-5-phosphatase 1

siRNA: Small interfering ribonucleic acid

SNP: Single nucleotide polymorphism

ssRNA: Single-stranded RNA

TAB: TAK1-binding protein

TAK1: TGF-β-activated kinase 1

tBid: Truncated Bid

TBK1: TRAF-family-member-associated NF-κB activator-binding kinase 1

TGF: Transforming growth factor

Th: T helper

THP-1: Human macrophage-like cell line

TIR: Toll–interleukin 1 receptor

TLC: Total lung capacity

TLR: Toll-like receptor

Tlr2$^{-/-}$: TLR2-deficient

***Tlr4*$^{-/-}$**: TLR4-deficient

***Tlr7*$^{-/-}$**: TLR7-deficient

TNF: Tumour necrosis factor

***Tnfsf10*$^{-/-}$**: TRAIL-deficient

TNFSF10: Tumour necrosis factor superfamily member 10

TORCH: Towards a Revolution in COPD Health cohort study

TRADD: TNFRSF1A-associated death domain

TRAF: Tumour necrosis factor receptor-associated factor

TRAIL: Tumour necrosis factor-related apoptosis-inducing ligand

TRIF: TIR-domain-containing adapter-inducing interferon-β

TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labelling

UPLIFT: Understanding Potential Long-Term Impacts on Function with Tiotropium

WT: Wild-type