Investigation of the mechanisms of respiratory infection-induced lung disease

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Statement of Originality

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Richard Yong Hoon Kim

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Synopsis

Asthma is a chronic allergic inflammatory disease of the airways that affects over 300 million people worldwide. The disease is driven predominantly by aberrant immune responses to normally harmless environmental stimuli. Upon encountering these stimuli, numerous immune and structural cells within the airways of the asthmatic lung release a wide range of inflammatory mediators that cause injury to the airway mucosa and surrounding tissues and leads to mucosal swelling, mucous secreting cell (MSC) hyperplasia and metaplasia and oedema. These inflammatory processes are also accompanied by an increase in bronchial smooth muscle tone in response to non-specific stimuli, a key pathological feature of asthma referred to as airways hyper-responsiveness (AHR), which results in bronchoconstriction. Together, these processes result in widespread but variable airflow obstruction in the asthmatic lung that give rise to the characteristic features of the disease, including difficulty breathing, wheezing, chest tightness and cough. In severe cases, the airflow obstruction can be so extreme that it can result in death via asphyxiation.

The majority of asthmatics can effectively control their disease through the use of bronchodilators (β2 adrenergic receptor agonists) and inhaled corticosteroids (ICS). However, these treatments do not cure disease and, importantly, a significant proportion of moderate to severe asthmatics exhibit persistent airflow obstruction and frequent exacerbations of disease despite high dose long-acting β2 agonist (LABAs) and ICS treatment. These treatment-refractory asthmatics represent a significant health burden and urgently require improved therapeutic options. An increased understanding of the mechanisms that underpin the development of asthma, particularly the pathogenesis of severe, treatment-refractory forms of the disease, may
inform novel targets for the development of improved therapeutic strategies for preventing the development of asthma and/or improving treatment outcomes.

Numerous clinical studies have demonstrated a link between certain respiratory infections and the development of asthma. Significantly, increasing clinical and experimental evidence has shown an association between a number of respiratory infections and the development of more severe, steroid-insensitive forms of asthma. In particular, a large body of evidence associates *Chlamydia* respiratory infection with the development and exacerbation of asthma, particularly severe forms of disease, in both children and adults. However, the mechanisms that underpin the association remain unknown. Our laboratory has developed a research program to investigate the link between *Chlamydia* infection and asthma using murine models of disease. We have shown that a prior neonatal and infant, but not adult, *Chlamydia* respiratory infection results in persistent AHR, emphysema-like alveolar enlargement and increased severity of allergic airways disease (AAD) in later life. We have also shown that ongoing adult *Chlamydia* respiratory infection during AAD suppresses Type 2 T helper (T\(_{H2}\)) lymphocyte and eosinophilic responses but drives a T\(_{H1}/T_{H17}\) and neutrophil-dominated form of disease that recapitulates many of the features of severe forms of asthma in humans. In this Thesis I have extended these findings by identifying key factors and signalling pathways that play important roles in neonatal *Chlamydia* respiratory infection-induced chronic lung disease and severe asthma in later life, and adult *Chlamydia* respiratory infection-induced, severe, steroid-insensitive asthma. The studies described hereafter have made important and novel observations that demonstrate roles of key microRNAs (miRNAs) and immune factors and signalling pathways that underpin *Chlamydia*-induced, severe asthma.
Initially, I used microarray analyses as a discovery tool to identify key miRNAs and genes that are altered by early life and adult *Chlamydia* respiratory infections (Chapter 2). I then conceived and designed novel studies to identify the functional roles of combinations of these factors in neonatal *Chlamydia* respiratory infection-induced severe lung disease in later life (Chapter 3) and adult respiratory infection-induced, severe, steroid-insensitive asthma (Chapters 4 and 5).

I demonstrate that 5 miRNAs (miR-155, miR-21, miR-223, miR-146b and miR-203) induced during neonatal *Chlamydia* respiratory infection promote infection-induced lung inflammation and histopathology, and drive reduced lung function, emphysema-like alveolar enlargement and increased severity of asthma in later life.

I also demonstrate that *Chlamydia* respiratory infection in established AAD induces: 1) a miR-21/phosphoinositide-3-kinase (PI3K)/phosphorylated Akt (pAkt)/histone deacetylase (HDAC)2 signalling axis, and 2) a NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome/Caspase-1/interleukin (IL)-1β signalling axis, to promote severe, neutrophilic, steroid-insensitive AAD. Additionally, I show that miR-21 and the NLRP3 inflammasome/IL-1β signalling axis also drive *Haemophilus* respiratory infection-induced, severe, neutrophilic, steroid-insensitive AAD in order to demonstrate that these factors/signalling pathways may be broadly applicable to infection-induced severe asthma.

These studies have identified potential mechanisms that drive respiratory infection-induced severe asthma. Importantly, these studies demonstrate that therapeutically targeting key respiratory infection-induced factors in the lung, including miRNAs and factors involved in key immune signalling pathways, may be effective for the prevention and/or treatment of severe forms of asthma.
Publications arising from this Thesis

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Publications that I have contributed to during my PhD:


* denotes equal contribution to manuscript (i.e. co-first author)
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Oral presentation in the Vaccines, Infection, Viruses & Asthma (VIVA) Seminar Series, Hunter Medical Research Institute (HMRI) 2013. Title: MicroRNAs in *Chlamydia* lung infection-induced pathologies

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*Awards:*

Australian Post Graduate Award (APA) PhD Scholarship. February 2007

Cooperative Research Centre for Asthma and Airways (CRCAA) Travel Award. January 2008

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Thoracic Society of Australia & New Zealand (TSANZ) Travel Grant
Abbreviations

AAD: Allergic airways disease

adu: Adult

AHR: Airways hyper-responsiveness

AIM2: Absent in melanoma 2 (also termed PYHIN4)

Akt: Protein kinase B

alum: aluminium hydroxide

AP-1: Activator protein

APC: Antigen presenting cell

ASC: Apoptosis-associated speck-like protein containing a CARD

ATP: Adenosine triphosphate

α-IL-1β: Anti-IL-1β neutralising antibody

BAL: Bronchoalveolar lavage

BALF: BAL fluid

BCG: Bacillus Calmette-Guerin

BSA: Bovine serum albumin

cAMP: Cyclic adenosine monophosphate

CARD: Caspase-recruitment domain

CBP: cAMP response element-binding (CREB)-binding protein

CCL: Chemokine (C-C motif) ligand

CEBPB: CCAAT/enhancer-binding protein-β

COPD: Chronic obstructive pulmonary disease

COX-2: Cyclooxygenase-2

Cmu: C. muridarum

cRNA: Complementary RNA

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

C. muridarum: Chlamydia muridarum

C. pneumoniae: Chlamydia pneumoniae

C. trachomatis: Chlamydia trachomatis

DAMP: Damage-associated molecular pattern

DAVID: Database for Annotation, Visualization and Integrated Discovery

DC: Dendritic cell

DC-SIGN: DC-specific Intercellular adhesion molecule-3-Grabbing Non-integrin

DGCR8: DiGeorge syndrome critical region gene 8
DEX: Dexamethasone
DMSO: Dimethyl sulfoxide
dpi: Days post infection
DR: Death receptor
dsDNA: Double-stranded DNA
EAE: Experimental autoimmune encephalomyelitis
EB: Elementary body
ELISA: Enzyme linked immunosorbent assay
FADD: Fas-associated death domain protein
FccRI: High affinity IgE receptors
FEV₁: Forced expiratory volume in one second
FGF: Fibroblast growth factor
F. novicida: Francisella novicida
geomean: Geometric mean
GR: Glucocorticoid receptor
GRE: Glucocorticoid response element
HDAC: Histone deacetylase
HDM: House dust mite
hASMC: Human airway smooth muscle cell
Hinf: H. influenzae
HPRT: Hypoxanthine-guanine phosphoribosyl transferase
H. influenzae: Haemophilus influenzae
H. pylori: Helicobacter pylori
H&E: Hematoxylin and eosin
HRP: Horseradish peroxidase
IBD: Inflammatory bowel disease
IC: Inspiratory Capacity
ICS: Inhaled corticosteroids
IFN: Interferon
IFU: Inclusion-forming units
Ig: Immunoglobulin
IL-1R: IL-1 receptor
inf: Infant
Iso: Isotype control antibodies
i.n.: Intranasally
i.p.: Intraperitoneally
i.t.: Intratracheally
IKKε: IkappaB kinase epsilon
IL-13R: IL-13 receptor
iNOS: Inducible nitric oxide synthase
IL: Interleukin
**IRAK:** Interleukin-1 receptor-associated kinase

**JNK:** c-Jun N-terminal kinase

**KGF:** Keratinocyte growth factor

**LABA:** Long-acting β2 agonist

**LM:** Mean linear intercept

**LPS:** Lipopolysaccharide

**LRR:** Leucine-rich repeat

**LY29:** LY294002

**L. monocytogenes:** Listeria monocytogenes

**MAPK:** Mitogen-activated protein kinase

**MCC950:** Novel NLRP3 inhibitor

**Mech:** Methacholine

**MDDC:** Monocyte-derived DC

**miRNA:** MicroRNA

**MSC:** Mucous secreting cell

**MyD88:** Myeloid differentiation primary response gene 88

**NBD:** NACHT nucleotide-binding domain

**ncRNA:** Non-coding RNA

**neo:** Neonatal

**NF-κB:** Nuclear factor κB

**NLR:** NOD leucine-rich repeat-containing receptor

**NLRP:** NOD-like receptor family, pyrin domain containing

**NOD:** nucleotide-binding oligomerisation domain

**nt:** nucleotide

**NTHi:** Non-typeable *H. influenzae*

**Ova:** Ovalbumin

**pAkt:** Phosphorylated Akt

**PAMP:** Pathogen-associated molecular pattern

**PB:** Persistent body

**PBMC:** Peripheral blood mononuclear cell

**PBS:** Phosphate-buffered saline

**PBS-T:** PBS and Tween 20

**PDK:** Phosphoinositide-dependent kinase

**PGE2:** Prostaglandin E2

**PH:** Pleckstrin-homology domain

**PIP2:** Phosphatidylinositol 4,5-bisphosphate

**PIP3:** Phosphatidylinositol 3,4,5-bisphosphate
**PI3K:** Phosphoinositide-3-kinase

**pol II:** Polymerase II

**poly(I:C):** Polyriboinosinic:polyriboctidylic acid

**Pre-miRNA:** Precursor-miRNA

**Pri-miRNA:** Primary miRNA

**PRR:** Pattern recognition receptors

**PTEN:** Phosphatase and tensin homologue

**PVDF:** Polyvinylidene difluoride

**PYD:** Pyrin domain

**P2X7R:** Purinergic receptor P2X, ligand-gated ion channel, 7

**qPCR:** Quantitative PCR

**RB:** Reticulate body

**RIG-I:** Retinoic acid-inducible gene 1

**RIN:** RNA integrity number

**RIPK:** Receptor-interacting serine-threonine kinase

**RISC:** RNA-induced-silencing-complex

**Rn:** Airways resistance

**RSV:** Respiratory syncytial virus

**RT:** Room temperature

**RNAi:** RNA interference

**SAA3:** Serum amyloid A 3

**Sal:** Saline (sham-sensitised, non-allergic)

**Scram:** Scrambled antagonir

**SERPINE:** Serpin peptidase inhibitor, clade E

**SH:** Src-homology

**SHIP:** SH2 domain containing inositol phosphatase

**snRNA:** Small nuclear RNA

**snoRNA:** Small nucleolar RNA

**SOCS:** Suppressor of cytokine signalling

**SPG:** Sucrose phosphate glutamate buffer

**siRNA:** Short-interfering RNA

**STAT:** Signal transducer and activator of transcription

**S. typhimurium:** Salmonella typhimurium

**TAR:** Trans-activating response

**TBP:** TATA binding protein

**TBS:** Tris-buffered saline

**TBS-T:** TBS and Tween 20

**TGF:** Transforming growth factor
\( T_H \): T helper lymphocyte

**TLR:** Toll-like receptor

**TNF:** Tumour necrosis factor

**TRAIL:** Tumour necrosis factor-related apoptosis-inducing ligand

**TRAF:** TNF receptor-associated factor

**TRBP:** Trans-activating response (TAR) RNA-binding protein

**UTR:** Untranslated region

**WT:** Wild-type

**YVAD:** Ac-YVAD-cho

**ZVAD:** z-VAD-fmk
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