Innate Immune Mechanisms of Chronic Airways Disease

A thesis submitted for the degree of
Doctor of Philosophy

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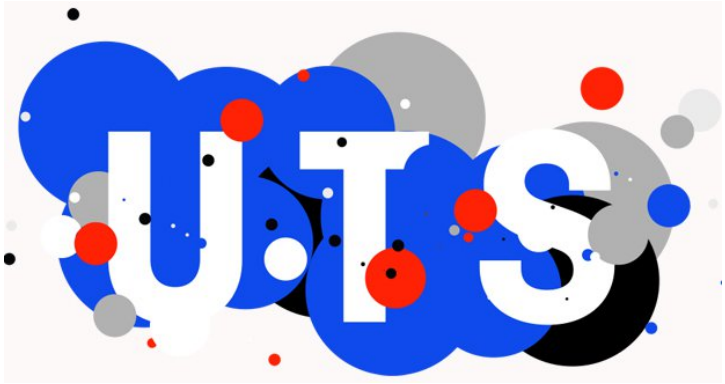

March 2019

CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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PUBLICATIONS AND PRESENTATIONS DURING CANDIDATURE

Published journal manuscripts

The parasitic 68-mer peptide FhHDM-1 inhibits mixed granulocytic inflammation and airway hyperreactivity in experimental asthma
Tanaka A, **Allam VSRR**, Simpson J, Tiberti N, Shiels J, To J, Lund M, Combes V, Weldon S, Taggart C, Dalton JP, Phipps S, Sukkar MB, Donnelly S.
The Journal of allergy and clinical immunology (2018)141: 2316-2319.

Proteomic Analysis of Extracellular HMGB1 Identifies Binding Partners and Exposes Its Potential Role in Airway Epithelial Cell Homeostasis
Wong SL, To J, Santos J, **Allam VSRR**, Dalton JP, Djordjevic SP, Donnelly S, Padula MP, Sukkar MB
Journal of Proteome Research (2018)17: 33-45.

Presentations

MIF antagonism restores corticosteroid sensitivity in a murine model of severe asthma
Allam VSRR, Adcock I, Chung KF, Morand E, Harris J, Sukkar MB
European Respiratory Journal 2018, Vol 52, Issue S62, PA979
DOI: 10.1183/13993003.congress-2018.PA979

A novel peptide from the liver fluke Fasciola hepatica inhibits the mixed granulocytic airway inflammation in a mouse model of allergic asthma.
Allam VSRR, Tanaka A, Dalton JP, Sukkar MB, Donnelly S.
Respirology 2017, pp. 81-81.

Role Of Pattern Recognition Receptors In The Regulation Of Intrapulmonary Airway Contraction Following Short-Term Cigarette Smoke Exposure In Mice.
Allam VSRR, Lam M, Sukkar MB, Bourke JE
American Journal of Respiratory and Critical Care Medicine 2017; 195:A6937

Role of RAGE and TLR4 in the regulation of intrapulmonary airway contraction following short-term cigarette smoke exposure in mice
Allam VSRR, Lam M, Sukkar MB, Bourke JE
Respirology 2017, TP-140-140

A novel peptide from the liver fluke Fasciola hepatica inhibits the mixed granulocytic airway inflammation in a mouse model of allergic asthma.
Horizons Conference 2017

A novel peptide from the liver fluke Fasciola hepatica inhibits the mixed granulocytic airway inflammation in a mouse model of allergic asthma.
Woolcock Institute of Medical Research Symposium 2018

University of Technology Sydney 3 Minute Thesis (3MT) 2016

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Awards linked to abstracts

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LIST OF ABBREVIATIONS

AGE	Advanced glycation end products
AHR	Airway hyperresponsiveness
ANOVA	Analysis of variance
APCs	Antigen-presenting cells
BALF	Broncoalveolar lavage fluid
BMDM	Bone marrow-derived macrophages
CCL3	Chemokine (C-C motif) ligand 3
CCR1	C-C chemokine receptor type 1
CSE	Cigarette smoke extract
DOCK2	Dedicator of cytokinesis 2
ELISA	Enzyme linked immunosorbent assay
ERK	Extracellular signal-regulated kinase
Ers	Total elastance
ES	Excretory-secretory
FEV1	Forced expiratory volume in one second
FhHDM-1	Fasciola hepatica helminth defense molecule-1
FVC	Forced vital capacity
G	Tissue damping
GC	Glucocorticoids
GEFs	Guanine-nucleotide exchange factors
GILZ	GC-induced leucine zipper
GINA	Global Initiative for Asthma
GO	Gene ontology
GWAS	Genome-wide association studies
H	Tissue elastance
H & E	Hematoxylin and eosin
HBSS	Hanks Balanced Salt Solution
HDM	House dust mite
IFN-γ	Interferon gamma
IL-17	Interleukin 17
IL-1α	Interleukin 1 alpha

IL-1β	Interleukin 1 beta
IL-22	Interleukin 22
IL-25	Interleukin 25
IL-33	Interleukin 33
IL-6	Interleukin 6
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LPS	Lipopolysacchride
MCh	Methacholine
MIF	Macrophage migration inhibitory factor
MMPs	Matrix metalloproteinases
MMPs	Matrix metalloproteinases
NF-kB	Nuclear factor-kappa B
NLRP3	NACHT, LRR and PYD domains-containing protein 3
PAMPs	Pathogen-associated molecular patterns
PAS	Periodic Acid Schiff
PBST	Phosphate Buffered Saline containing Tween
PCLS	Precision cut lung slices
PEEP	Positive end-expiratory pressure
RAGE	Receptor for advanced glycation endproducts
Rn	Newtonian resistance
ROS	Reactive oxygen species
Rrs	Total Resistance
SARP	Severe Asthma Research Program
SEM	Standord error mean
SNP	Single nucleotide polymorphism
TACs	Transcriptomic-associated clusters
TBST	Tris-buffered saline containing Tween
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha
TRIF	TIR-domain-containing adapter-inducing interferon-β
TSLP	Thymic stromal lymphopoietin

ABSTRACT

The human respiratory tract is exposed to environmental irritants on a daily basis. The innate immune system is composed of different cellular components including the resident airway epithelium and macrophages and acts as the first line of defense to protect the lung against inhaled irritants. Activation of innate immune pathways is associated with the release of different mediators like cytokines, chemokines, lipid mediators and complement factors to mediate the recruitment of different immune cells into the airway lumen. The role of the innate immune system in chronic airways disease is currently a major area of research in the field and the focus of this thesis.

RAGE and TLR4 are two major innate immune receptors implicated in the pathogenesis of asthma and COPD. We used TLR4, RAGE and TLR4/RAGE deficient mice to study the individual and combined role of these receptors in the airway response to acute cigarette-smoke exposure. We found that RAGE but not TLR4 deficiency protected against cigarette-smoke induced neutrophilic airway inflammation, mediator release and airway hyperreactivity (AHR). Interestingly, TLR4 deficiency exacerbated AHR. Together these findings, suggest that RAGE rather than TLR4 should be pursued as a therapeutic target in COPD.

In contrast to our findings above however, we found that dual inhibition of TLR4/RAGE signaling, but not individual inhibition of these receptor pathways, protects against corticosteroid-resistant airway neutrophilia and AHR in an experimental model of severe asthma. Also, by performing a global

phosphoproteomics analysis of lung tissue samples, we identified novel signaling pathways activated down-stream of TLR4/RAGE ligation in severe experimental asthma.

We also investigated the role of macrophage migration inhibitory factor (MIF) in severe asthma. We demonstrated increased expression of MIF, S100A8/A9 and TLR4, and reduced expression of annexin A1 (ANXA1) in subjects with predominant airway neutrophilic inflammation. We also demonstrated that MIF inhibition protects against corticosteroid-resistant neutrophilic inflammation and airway hyperreactivity, and restores corticosteroid sensitivity in an experiental mouse model of severe asthma. Beneficial effects of MIF inhibition were associated with inhibition of S100A8 and CCL11 protein in the bronchoalveolar lavage fluid and reduced proteolytic cleavage of ANXA1. While ISO-1 had no effect on the secretion of pro-neutrophilic mediators, including IL-1 family cytokines, it did render these pathways sensitive to inhibition by dexamethasone.

Finally, we identified a role for FhHDM-1, an immunomodulatory peptide derived from liver fluke *Fasciola hepatica* as a novel therapeutic treatment for asthma. Administration of FhHDM-1 protected against eosinophilic and neutrophilic inflammation, mucus secretion and AHR in a mouse model of house-dust mite induced asthma.

In summary, the studies in this thesis have uncovered new molecular mechanisms of innate immune activation associated with the inception and

progression of COPD and severe asthma, and have identified a novel helminth-based therapy for the treatment of asthma.