Understanding the Inflammatory Mechanisms That Predispose to Emphysema In Mouse Models

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B.BioMed Sc (Hons)

Submitted for the degree of

DOCTOR of PHILOSOPHY

Discipline of Immunology and Microbiology
School of Biomedical Sciences and Pharmacy
Faculty of Health
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Australia

April 2011

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 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.

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 clearly outlining the extent of collaboration, with whom and under what auspices. This statement can be found on Page III.

Eric Chuan En Lam

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April 2011

List of Collaborative Work

This section officially acknowledges the contribution from our collaborators.

Collaborator	Collaboration
A/Prof Philip Hansbro (UoN)	Supply of Non-Typeable *Haemophilus influenzae* for the investigations carried out in Chapters 3 and 4
Ms. Ama-Tawiah Essilfie (UoN)	Preparation of stock *Haemophilus influenzae* for infection of mice and bacterial recovery.
Dr. Jodie Simpson (HMRI)	Determining chemerin levels in sputum (comparing between Health and COPD subjects) and anaylsying results for Figure 4-23 in Chapter 4.

Acknowledgements

I would like to thank my supervisors Dr. Simon Phipps and Prof. Paul Foster for their guidance and support over the last 5 years, as well as the opportunity to undertake this PhD. I would also like to extend my sincere gratitude to Dr. Nicole Hansbro for proofreading the thesis. Special thanks to Adeline for the many great discussions into some of the experimental techniques and great lab mate all round. I would also like to thank all past and present members from the Foster laboratory, who had assisted in one way or another, making this journey a memorable one.

I would like to thank Anne Prins (ANU) and all staff members from the Animal Services Unit (UoN) for the support over the years. I would also like to thank our collaborators A/Prof. Philip Hansbro (UoN) and Ama-Tawiah Essilfie (UoN) for providing the *Haemophilus influenzae* and bacterial recovery work respectively, as well as Dr. Jodie Simpson (HMRI) for supplying the human chemerin data.

I would also like to acknowledge funding support from the University of Newcastle and Cooperative Research Centre for Asthma and Airways for the project.

Last but not least, a huge thank you to my parents, especially my dad for the huge support, encouragement, and funding to see me through this journey. Thank you!

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Abstract

Chronic obstructive pulmonary disease (COPD) is a growing global health problem, and this disorder is projected to rank fifth by 2020 as a worldwide burden of disease (Murray and Lopez., 1996). Remarkably, little is known about the pathogenesis of COPD and current pharmacologic agents fail to halt disease progression. Emphysema is a major inflammatory disorder that falls under the clinical description of COPD. Emphysema can be induced by smoking but can also occur in non-smokers. Emerging data suggests that the loss of alveolar tissue which characterises emphysema may result from increased cell death (apoptosis) of alveolar epithelial cells mediated by the sphingolipid mediator ceramide (Petrache *et al.*, 2005). The cause of COPD exacerbations are commonly bacterial or viral respiratory infections. Under certain conditions, immunity from infection is mediated through the initiation of apoptotic pathways by infected cells to prevent the pathogen from replicating within the host. Toll-like receptors (TLRs) recognise molecular patterns expressed by pathogens such as bacteria and viruses to initiate innate immune responses. Notably, significant amounts of the bacterial wall component lipopolysaccharide (LPS) are found in cigarette smoke. LPS is a TLR4 ligand that increases the level of the apoptotic mediator ceramide and production of proinflammatory cytokines (such as tumour necrosis factor (TNF)–α, interleukin (IL)-1β, and IL-6) implicated in the pathogenesis of emphysema. We hypothesise that chronic inhalation of LPS leads to the dysregulation of TLR4 signalling pathways that increases susceptibility to

respiratory infection, and uncontrolled inflammation that promotes alveolar cell apoptosis and emphysematous-like lesions. We developed mouse models of LPS- and bacterial-induced emphysema to determine if attenuating inflammation can prevent the development of emphysema.

Our results demonstrate that exposure to LPS or infection with Non-typeable *Haemophilus influenzae* (NTHi) (often found in patients with emphysema) can induce hallmark features of emphysema, such as alveolar enlargement (determined by mean linear intercept and percentage alveolar airspace measurements) and inflammation dominated by neutrophils and macrophages. We demonstrated that alveolar enlargement was due to the loss of alveolar parenchyma (from apoptosis), is dependent on TLR4 and myeloid differentiation factor-88 (MyD88), increased proinflammatory cytokines, chemokines, and inflammatory cells (neutrophils and macrophages) in the lung. Prophylactic administration of synthesised chemerin-derived peptide (C15) attenuated LPS- or NTHi-induced inflammation, which resulted in inhibition of the development of emphysematous-like lesions. Notably, specific depletion of alveolar macrophages protects mice from LPS- or NTHi-induced emphysema.

Collectively, we demonstrate that blocking inflammation during the development of emphysema is critical for preventing or attenuating the progression of the disease.

List of Abbreviations

AA	Amino acid
ALF	Australian Lung Foundation
APAAP	Mouse Alkaline-Phosphatase anti-Alkaline-Phosphatase
Apaf-1	Apoptotic protease activating factor-1
APCs	Antigen presenting cells
ASmase	Acid Sphingomyelinase
CAD	Caspase-activated DNAse
CCRL2	Chemokine (CC motif) receptor-like 2
cDNA	complementary DNA
CKRX	Chemokine Receptor X
CMKLR1	Chemokine-like receptor 1
COPD	Chronic obstructive pulmonary disease
2CA	2-Chloroadenosine
ECM	Extracellular matrix
E.coli	Escherichia coli
EtOH	Ethanol
FCS	Fetal Calf Serum
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GOLD	Global initiative for Chronic Obstructive Lung Disease
GPCR	Protein-coupled receptors
H.influenzae	*Haemophilus influenzae*
HKR	Human chemokine receptor
HMGB-1	High-mobility group protein B1
HMRI	Hunter Medical and Research Institute
IRFs	Interferon regulatory factors
IVC	Individually ventilated cages
LASS	Dihydroceramid Synthases
L-CCR	LPS-inducible C-C chemokine receptor related gene
LOS	Lipooligosaccharides
LPS	Lipopolysaccharide
MAL	MyD88 adaptor-like
MiP-1α	Macrophage inflammatory Protein – 1 -alpha
MLI	Mean linear intercepts
MMP-9	Matrix metallopeptidase 9
MMP-12	Macrophage Elastase
mRNA	messenger RNA
$MyD88^{-/-}$	MyD88-deficient
MyD88	Myeloid-Differentiating factor 88
NADPH	reduced Nicotinamide adenine dinucleotide phosphate
NCdase	Neutral Ceramidase
NGS	Normal Goat Serum
NHLBI	National Heart, Lung, and Blood Institute

NLRs	NOD-like receptors
Nox-3	NADPH oxidase 3
NTHi	Non-typeable Haemophilus influenzae
PAMPs	Pathogen-associated molecular patterns
PBS	Phosphate Buffered Saline
PFA	Paraformaldehyde
% alveolar airspace (%AA)	percentage alveolar airspace
qPCR	quantitative real-time PCR
RARRES2	Retinoic acid receptor responder 2
RSV	Respiratory Syncytial Virus
SARM	Sterile α and armadillo motif-containing protein
SMases	Sphingomyelinases
SMS	Sphingomyelinase Synthase
SP	Scrambled Peptide
S. pneumoniae	*Streptococus pneumoniae*
S1-P	Sphingosine-1-phosphate
Tg	Transgenic
TIG2	tazzarotene induced gene 2
TIR	Toll-IL-1 receptor
TLR	Toll-like receptor
TLR4$^{-/-}$	TLR4 deficient
TNF-α	Tumour necrosis factor-alpha
TRAIL	TNF-related apoptosis inducing ligand
TRAM	TRIF-related adaptor molecule
TRIF	TIR domain-containing adaptor inducing interferon-β
TSANZ	The Thoracic Society of Australia and New Zealand
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organisation
WT	Wild type