Investigating Aberrant Inflammatory Signalling in Asthma

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I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, 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. 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 and any approved embargo.

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I hereby certify that this thesis is in the form of a series of papers. I have included as part of the thesis a written declaration from each co-author, endorsed in writing by the Faculty Assistant Dean (Research Training), attesting to my contribution to any jointly authored papers.

Natalie Niessen

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PUBLICATIONS INCLUDED IN THIS THESIS

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Niessen, N., Gibson, P., Simpson, J., Scott, H., Baines, K., Fricker, M. (2021). Airway monocyte modulation relates to TNF dysregulation in neutrophilic asthma. *Submitted to European Respiratory Journal – Open Research*

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OTHER RESEARCH ARTICLES & CONFERENCE ABSTRACTS RELATED TO THIS THESIS

Research articles

Fricker, M., Qin, L., **Niessen, N.**, Baines, K., McDonald, V., Scott, H., Simpson, J., Gibson, P. (2020). Relationship of sputum mast cells with clinical and inflammatory characteristics of asthma. *Clinical and Experimental Allergy*, 50(6), 696–707. DOI: 10.1111/cea.13609.

Conferences

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LIST OF ABBREVIATIONS

ACQ	Asthma control questionnaire
ACTRN	Australian clinical trials registration number
ADAM	A disintegrin and metalloproteinase
AHR	Airway hyperresponsiveness
AMAZES	Asthma and Macrolides: The Azithromycin Efficacy and Safety study
ANOVA	Analysis of variance
APC	Antigen-presenting cell
ATP	Adenosine triphosphate
ATS	American Thoracic Society
AZM	Azithromycin
BAL(F)	Bronchoalveolar lavage (fluid)
BD	Bronchodilator
BDP	Beclomethasone dipropionate
β_2-AR	Beta-2 adrenergic receptor
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CBD	CREB-binding protein
CD	Cluster of differentiation
CM	Classical monocytes
COPD	Chronic obstructive pulmonary disease
CV	Coefficient of variability
DAMP	Danger-associated molecular pattern
DC	Dendritic cells
DTT	Dithiothreitol

EA	Eosinophilic asthma
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
Eos	Eosinophils
ERS	European Respiratory Society
FACS	Fluorescence-activated cell sorting
FCS	Fetal calf serum
FEV_1	Forced expiratory volume in one second
FMO	Fluorescence minus one
FSC	Forward scatter
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GR	Glucocorticoid receptor
GRE	GR response element
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HLA-DR	Human leukocyte antigen-DR
HMRI	Hunter Medical Research Institute
HREC	Human Research Ethics Committee
ICS	Inhaled corticosteroids
ICU	Intensive care unit
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
IKK	IκB-Kinase

IL	Interleukin
ILC	Innate lymphoid cell
IM	Intermediate monocytes
IQR	Interquartile range
LABA	Long-acting beta-agonist
LPS	Lipopolysaccharide
LTRA	Leukotriene receptor agonist
LUBAC	Linear ubiquitin chain assembly complex
Macs	Macrophages
MAPK	Mitogen-activated protein kinase
MCL	Myosin light chains
MFI	Median fluorescence intensity
MGA	Mixed granulocytic asthma
MHC	Major histocompatibility complex
MLCK	Myosin light chains kinase
MMP	Matrix metalloproteinase-9
Monos	Monocytes
mTNF(R)	Membrane-bound tumor necrosis factor (receptor)
NA	Neutrophilic asthma
NCM	Non-classical monocytes
NEMO	NF-κB essential modulator
NETs	Neutrophilic extracellular traps
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHMRC	National Health and Medical Research Council

NLRP3	Nucleotide-binding oligomerisation domain, Leucine rich Repeat and Pyrin domain containing Proteins
OCS	Oral corticosteroids
PAMP	Pathogen-associated pattern
PBS	Phosphate-buffered saline
PCAF	p300/CBP-activating factor
PD	Provocative dose
PGA	Paucigranulocytic asthma
PKA	Protein kinase A
PBMCs	Peripheral blood monocytes
pred	Predicted
PRR	Pathogen recognition receptor
RCT	Randomized controlled trial
RIPK	Receptor-interacting serine/threonine-protein kinase
RT	Room temperature
RV	Rhinovirus
SABA	Short-acting beta-agonists
SD	Standard deviation
SSC	Side scatter
sTNF(R)	Soluble tumor necrosis factor (receptor)
Tab	TGFβ-activated kinase 1 and MAP3K7-binding protein
TACE	TNF alpha converting enzyme
TAK	TGFβ-activated kinase
TCC	Total cell count
Th	T helper cell

TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TRADD	TNFR1-associated death domain
TRAF	TNFR-associated factor
Treg	Regulatory T cells
TSLP	Thymic stromal lymphopoietin

LIST OF TABLES

LIST OF FIGURES

TABLE OF CONTENTS

THESIS ABSTRACT

Asthma is a chronic obstructive airway disease that is estimated to effect 340 million people worldwide. The underlying inflammation of the airways is heterogeneous and different asthma inflammatory phenotypes have been identified, that are associated with varying responses to treatment. Approximately 15 % of those with asthma feature neutrophilic airway inflammation, defined by elevated sputum neutrophils, that is associated with corticosteroid resistance and more severe disease. As yet, suitable treatment for neutrophilic asthma is lacking and a better understanding of the pathophysiological changes underlying this inflammatory phenotype is necessary in order to identify novel therapeutic targets.

This thesis reveals novel aspects of neutrophilic asthma, namely, altered airway immune cell trafficking and dysregulation of the TNF signalling pathway. In **chapter 3** I demonstrate that neutrophilic asthma is associated with increased recruitment of monocytes to the airways, while airway macrophages appear to be reduced. Dysregulation of the monocyte/macrophage lineage could relate to an altered inflammatory response, as these two cell types may execute distinct functions in tissue homeostasis and inflammation. **Chapter 4** investigates the relative abundance of the inflammatory markers TNF, TNFR1 and TNFR2 in the circulation and the airways. I demonstrate that neutrophilic asthma is associated with increased soluble receptor levels in the airways, whereas membrane-bound TNF markers do not differ across asthma inflammatory phenotypes or in comparison to non-asthma controls. These alterations could relate to aberrant inflammatory signalling and/or impaired inflammatory resolution and thus contribute to airway inflammation in neutrophilic asthma. In **chapter 5**, I show that increased soluble TNF receptors in both circulation and the airways are associated with clinical features of asthma, such as reduced lung function, more frequent exacerbation and more severe asthma, suggesting that dysregulated TNF signalling contributes to worse asthma outcomes. I further demonstrate that long-term low-dose administration of azithromycin significantly reduces soluble TNF marker levels. My results imply that the mechanisms

of action of AZM could be a combination of both general and specific mechanisms and potentially involve anti-inflammatory and anti-bacterial properties of the macrolide.

My observations in neutrophilic asthma prompt new hypotheses that require further investigation and validation in mechanistic studies.