NON-INVASIVE ASSESSMENT OF AIRWAY INFLAMMATION IN ASTHMA

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Abstract

Inflammation is a central feature in current definitions of asthma. Despite this, airway inflammation remains infrequently assessed in either population-based studies or clinical practice. In this thesis, conventional and novel non-invasive methods (based on exhaled nitric oxide (FENO) measurement and sputum induction) were used to assess airway inflammation and examine the presence, characteristics and stability of inflammatory asthma phenotypes in a general population sample, which included very young and very old individuals.

It was shown that FENO measurement could be easily and cost-effectively conducted, and that flow cytometric analysis of sputum leukocyte populations is a feasible alternative to conventional manual cell counts. In particular, flow cytometric analysis was shown to be well suited to the detection of rare cell populations, and provided data suggesting that airway invariant natural killer T cells may not be a key player in asthma pathophysiology and that basophils may be a useful indicator of allergic airway inflammation in asthma.

When examining inflammatory asthma phenotypes, it was shown that less than 50% of asthmatics (both children and adults) had evidence of eosinophilic inflammation, although in one small study, altered treatment resulted in phenotype changes in more than 50% of asthmatics studied. Neutrophilic airway inflammation was rare, and was statistically significantly associated with age. Approximately half of all the asthmatics studied had no detectable evidence of airway inflammation at the time of assessment.

In conclusion, the methods developed and validated for the non-invasive assessment of airway inflammation allow more detailed investigations of asthma aetiology in population-based studies. However, a single assessment of airway inflammation may not be adequate for valid identification of inflammatory asthma phenotypes. The results of the studies described in this thesis suggest that 50% of asthmatics may have eosinophilic airway inflammation, with the remainder having no airway inflammation. Further investigations of non-inflammatory mechanisms are therefore warranted, as a better understanding of the mechanisms and the associated environmental exposures involved may guide the development of more effective therapies and control measures for this common phenotype.

"*If you know the enemy and know yourself, you need not fear the result of a hundred battles. If you know yourself but not the enemy, for every victory gained you will also suffer a defeat. If you know neither the enemy nor yourself, you will succumb in every battle*"

— Sun Tzu, the Art of War

"*If we knew what it was we were doing, it would not be called research, would it?*"

— Albert Einstein

Author's declaration

This thesis was produced according to Massey University's 'Thesis-by-Paper' Requirements. That is, it is largely based on research that is published, in-press, submitted for publication, or is in final preparation for submission. Each individual chapter is set out in the style of the journal to which it has been submitted. Consequently, some of the submitted chapters are relatively succinct, there is some repetition (particularly in the Methods sections) and there are small stylistic differences between chapters. To supplement the relative brevity of some of the chapters, the appropriate sections of the literature review have been extended.

The submitted manuscripts include other authors, including my PhD supervisors and in some cases, collaborators in different institutes in New Zealand and Australia. However, for each chapter, my input was greatest. I was the lead investigator for all the studies described (with the exception of chapter 7), involved in oversight of study design, recruitment, work co-ordination and data collection, data analysis and preparation of the manuscripts. In some cases, I was also involved in preparation of the grant applications and ethics applications prior to the study. I was, however, assisted and supported by my co-authors for all the studies herein.

In regard to chapter 7, the clinical assessments and data compilation had been conducted prior to my involvement. I was, however, heavily involved in the data analysis and responsible for interpretation of the findings of the study, and I wrote the manuscript, with some input provided by the co-authors.

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α-GalCer	alpha-galactosylceramide
AAAAI	American Academy of Allergy, Asthma & Immunology
ACQ	asthma control questionnaire
ACT	asthma control test
AHR	airway hyperreactivity/hyperresponsiveness
ANG	angiopoeitin
aOR	adjusted odds ratio
APC	allophycocyanin
APC	antigen-presenting cell
ASM	airway smooth muscle
ATS	American Thoracic Society
BAL	broncho-alveolar lavage
BCG	Bacille Calmette-Guerin
BDNF	brain-derived neutrophic factor
BDR	bronchodilator reversibility / response
BHR	bronchial hyperreactivity/hyperresponsiveness
BTS	British Thoracic Society
CCL	CC chemokine ligand
CD	cluster of differentiation
CI	confidence interval
CLCA1	calcium-activated chloride channel regulator 1
COPD	chronic obstructive pulmonary disease
CT	computerised topography
CXC	CXC-subfamily chemokine
CysLTs	cysteinyl leukotrienes
CysLTR	cysteinyl leukotriene receptor
DAPI	4’,6-diamidino-2-phenylindole
DC	dendritic cell

DCC	differential cell count
Der P1	*Dermatophagoides pteronyssinus* group 1
DMSO	dimethyl sulphoxide
DTT	dithiothreitol
DTE	dithioerythritol
EAACI	European Academy of Allergy and Clinical Immunology
EA	eosinophilic asthma
EBC	exhaled breath condensate
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
ECP	eosinophil cationic protein
ECRHS	European Community Respiratory Health Study
EDN	eosinophil-derived neurotoxin
EGF	epithelial growth factor
ELISA	enzyme linked immunosorbent assay
EMTU	epithelial-mesenchymal trophic unit
EOA	early-onset asthma
FcεR1	high-affinity immunoglobulin E receptor 1
FCS	foetal calf serum
FEV1	forced expiratory volume in one second
FENO	fraction of exhaled nitric oxide/fractional exhaled nitric oxide
FITC	fluorescein isothiocyanate
fMLP	formyl-methionyl-leucyl-phenylalanine
FoxP3	forkhead box protein 3
FSC	forward scatter
FVC	forced vital capacity
GF	growth factor
GINA	Global Initiative for Asthma
GM-CSF	granulocyte-macrophage colony stimulating factor

GWAS	genome-wide association study
HDM	house dust mite
HLA	human leukocyte antigen
HPA	hypothalamic-pituitary axis
HRCT	high resolution computerised topography
ICS	inhaled corticosteroids
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
ILC	innate lymphocyte
JAM	junction adhesion molecule
iNKT	invariant natural killer T cell
IS	induced sputum
ISAAC	International Study of Asthma and Allergies in Childhood
LABA	long acting β_2-agonist
LBP	lipopolysaccharide-binding protein
LOA	late-onset asthma
LPS	lipopolysaccharide
LTB4	leukotriene B4
LTC4	leukotriene C4
LTRA	leukotriene receptor antagonist
mAb	monoclonal antibody
MBP	major basic protein
MCP	monocyte chemotactic protein
MGA	mixed granulocytic asthma
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
mRNA	memory ribonucleic acid
MMP	matrix metalloprotease/metalloproteinase

moDC	monocyte-derived dendritic cell
MPO	myeloperoxidase
NA	neutrophilic asthma
NE	neutrophil elastase
NEA	non-eosinophilic asthma
NF-κ-B	nuclear factor kappa B
NGF	nerve growth factor
NK	natural killer
NOS	nitric oxide synthase
OR	odds ratio
ORMDL3/GSDMB	orosomucoid-like 3/gasdermin B
OVA	ovalbumin
PAF	population-attributable fraction
PAF	platelet-activating factor
PAMP	pathogen-associated molecular pattern
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PD15	provocative dose leading to a 15% reduction in FEV_1
PE	phycoerythrin
PEF	peak expiratory flow
PerCP	peridinin-chlorophyll protein complex
PGA	paucigranulocytic asthma
PMA	phorbol 12-myristate 13-acetate
PPV	positive predictive value
PRR	pathogen recognition receptor
qPCR	quantitative polymerase chain reaction
RANTES	regulated on activation, normal T cell-expressed and secreted protein
RNA	ribonucleic acid

ROC	receiver-operator characteristics
ROR	Receptor tyrosine kinase-like orphan receptor
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute medium
RSV	respiratory syncytial virus
SABA	short acting β_2-agonist
SCF	stem cell factor
SCG	sodium chromoglycate
SD	standard deviation
SELDI-TOF	surface-enhanced laser desorption/ionisation-time-of-flight
SPF	specific pathogen free
SPT	skin prick test
SSC	side scatter
TCR	T cell receptor
TGF-β	transforming growth factor beta
TH_1	T helper 1
TH_2	T helper 2
TIMP-1	Tissue inhibitor of metalloproteinase 1
TLR	Toll-like receptor
TNF-α	tumour necrosis factor alpha
Treg	regulatory T cell
TSLP	thymic stromal lymphopoietin
VEGF	vascular endothelial growth factor
YKL	chitinase-3-like protein 1