The Innate Immune Mechanisms Underlying the Interplay Between Respiratory Infections and Asthma

By

Gerard Emil Kaiko BBiomedical Sci (Hons)

Research Centre for Asthma and Respiratory Diseases,
Discipline of Immunology and Microbiology,
Faculty of Health,
The University of Newcastle
NSW, Australia

A dissertation submitted as fulfilment of the requirements for the award of a PhD degree (Immunology and Microbiology).

June, 2011
THESIS STATEMENTS

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.

Gerard Kaiko
24/6/2011

THESIS BY PUBLICATION STATEMENTS

I hereby certify that this thesis is submitted in the form of a series of publications of which I am first author. I have included as part of the thesis a written statement from each co-author, and endorsed by the Deputy Head of Faculty (Research), attesting to my contribution to the joint publications.

For all three publications my work includes conceptualising the studies, designing the studies, performing the studies, and writing the manuscripts. For all three publications Professor Paul Foster, Dr. Simon Phipps, and Professor Kenneth Beagley were involved in conceptualising and designing the studies, and editing the drafts of the manuscripts. All other co-authors provided either a critical reagent, or mouse strain.

This thesis consists of a comprehensive introduction to the topic encapsulating the three papers and the rationale for the work. Followed by the three individual manuscripts with specific introduction, methods, results and discussion sections, the first published in 2008, the second published in 2010, and the third in submission. These publications have been re-formatted for consistent styling, as their original format was journal-specific. Please note: In the case of the third publication the introduction, methods, results, and discussion sections have also been lengthened to provide further detail beyond the word restrictions of the journal. The thesis is concluded with an overall discussion of the three publications to place them in the context of an established body of knowledge.

Gerard Kaiko
24/6/2011
I would like to give great thanks to my supervisors Professor Paul Foster and Dr Simon Phipps for their wonderful mentoring and advice throughout my PhD studies. This has proved invaluable not only in the research presented herein but also in a broader scientific context moving towards a future career in medical research. Special thanks to Professor Paul Foster for his great assistance in the transition period from PhD student to postdoctoral researcher and for providing me with the resources to conduct a diverse array of research throughout my PhD, some of which does not make it into this thesis due to its focus on unrelated topics.

Many thanks also to the CRC for Asthma and Airways (Australia) for their support during my PhD studies.
LIST OF PUBLICATIONS

PUBLICATION #1


PUBLICATION #2


PUBLICATION #3


LIST OF ADDITIONAL PUBLICATIONS WITH RELEVANCE TO THIS THESIS

Appendix 1:

# TABLE OF CONTENTS

ABSTRACT.......................................................................................................................................................1

CHAPTER I: INTRODUCTION .................................................................................................................................2

I.1 Overview of Asthmatic Disease ........................................................................................................................3
I.2 Pathology and Immunology of Asthma .............................................................................................................4
I.3 Etiology of Asthma: Environmental Factors Influencing the Origin of Asthma ...................................................13
    I.3.1 Diet .........................................................................................................................................................13
    I.3.2 Increased Exposure to Indoor Allergens ..............................................................................................14
    I.3.3 Smoking and Air Pollution ................................................................................................................15
    I.3.4 The ‘Hygiene Hypothesis’ ....................................................................................................................15
    I.3.5 Early-life Respiratory Infections ........................................................................................................17

I.4 Chlamydophila pneumoniae ............................................................................................................................18
    I.4.1 Association of Chlamydophila pneumoniae with Acute Exacerbations of Pre-existing Asthma ...............20

I.5 Respiratory Syncytial Virus (RSV) ................................................................................................................22
    I.5.1 RSV and Disease ................................................................................................................................24
    I.5.2 RSV and Asthma ................................................................................................................................25

I.6 RSV and CD4 T Helper (Th) Responses ........................................................................................................31
    I.6.1 The CD4 T Helper Cell Response to RSV in Humans .........................................................................31
    I.6.2 RSV and the IgE Response in Humans .................................................................................................35
    I.6.3 RSV, Eosinophils and Mast Cells in Humans .......................................................................................36
    I.6.4 Mouse Models of RSV Vaccine-Enhanced Disease ...........................................................................38
    I.6.5 RSV and Mouse Models of Allergy ......................................................................................................40
    I.6.6 Mouse Models of Natural RSV Infection ............................................................................................41
I.7 Pneumonia Virus of Mice (PVM) ................................................................. 45
  I.7.1 PVM as a Model of Severe RSV Infection ............................................. 47

I.8 Innate Immune Responses to RSV ............................................................. 49

I.9 Broad Overview of the Innate Immune System ........................................ 52
  I.9.1 The Innate Immune System and Th2 Immunity ................................. 53
  I.9.2 Th2-inducing Cytokines ...................................................................... 53
  I.9.3 Cells Involved in the Induction of Th2 Responses ............................... 59
  I.9.4 Newly Discovered Innate Immune Cells as Key Effectors of Th2 immunity 63
  I.9.5 Hematopoietic Progenitor Cells as Effector Cells of Th2 Immunity ....... 65
  I.9.6 Dendritic Cells and Re-activation of Th2 cells in Asthma .................. 68

I.10 Role of Toll-like Receptors (TLRs) in Asthma ......................................... 69

I.11 SUMMARY AND RATIONALE ................................................................. 71

I.12 SPECIFIC AIMS OF THESIS ................................................................. 73

CHAPTER II: PUBLICATION #1 ................................................................. 74

II.1 ABSTRACT ................................................................................................ 75

II.2 INTRODUCTION ..................................................................................... 76

II.3 MATERIALS AND METHODS ............................................................... 78

II.4 RESULTS ................................................................................................ 83

II.5 DISCUSSION ......................................................................................... 97

CHAPTER III: PUBLICATION #2 .............................................................. 102

III.1 ABSTRACT ............................................................................................ 103

III.2 INTRODUCTION ................................................................................... 104

III.3 MATERIALS AND METHODS ............................................................ 107

III.4 RESULTS ............................................................................................. 113

III.5 SUPPLEMENTARY DATA ..................................................................... 138
III.6 DISCUSSION ...........................................................................................................148

CHAPTER IV: PUBLICATION #3 ..................................................................................154

IV.1 ABSTRACT ...........................................................................................................155
IV.2 INTRODUCTION .....................................................................................................156
IV.3 MATERIALS AND METHODS ..............................................................................161
IV.4 RESULTS ...............................................................................................................166
IV.5 SUPPLEMENTARY DATA ......................................................................................191
IV.6 DISCUSSION ..........................................................................................................197

CHAPTER V: DISCUSSION AND CONCLUSIONS ......................................................202

REFERENCES CITED IN THIS THESIS ........................................................................213
LIST OF FIGURES

Chapter I: Introduction
Figure 1: Pathways Involved in the Creation of T helper cells.................................6
Figure 2: Factors Involved in T cell polarisation to Th1, Th2, and Th17......................7
Figure 3: Schematic Diagram of the Development Cycle of Chlamydia......................19
Figure 4: Diagram of the structure of Respiratory Syncytial Virus (RSV)................23
Figure 5: Two-hit hypothesis of asthma......................................................................29
Figure 6: Viral genomic structure.............................................................................46
Figure 7: The innate immune pathways involved in the induction of Th2 cells........66

Chapter II: Publication#1
Figure 1. Chlamydia muridarum forms a viable infection within murine dendritic cells .........................................................................................................................84
Figure 2. Chlamydia infection of BMDC prevents up-regulation of MHC class II and CD80 but increases CD86 expression.................................................................87
Figure 3. Chlamydia infection of BMDC increases the secretion of a diverse array of cytokines including Th2-inducers..............................................................89
Figure 4. Co-culture of antigen-specific T cells with Chlamydia-infected BMDC creates a highly proliferative Th2 cell. .................................................................92
Figure 5. Adoptive transfer of Chlamydia-infected BMDC leads to DC-derived IL-13 in the BALF and increased AHR.................................................................96

Chapter III: Publication#2
Figure 1. NK Cells Protect Against Viral-mediated Th2 Responses.........................115
Figure 2. Th2 responses persist long-term.................................................................118
Figure 3. NK Cell Depletion During RSV Infection Induces Th2 Responses to OVA. ..............................................................................................................................121
Figure 4. The RSV-induced Th2 Phenotype is Reversible by Intranasal IFN-γ.........125
Figure 5. RSV Induces IL-25 Expression in the Respiratory Epithelium...............129
Figure 6. RSV-induced Th2 Responses are IL-25-dependent....................................132
Figure 7. RSV-induced Th2 Differentiation is Dependent on DC Jagged1.............136
Supplementary Figure S1. NK cell numbers in the lungs and lymph nodes........138
Supplementary Figure S2. NK cells producing IFN-γ in the lungs ..........................139
Supplementary Figure S3. Micrographs of airway mucus secreting cells and mucus plugging. .......................................................................................................................141
Supplementary Figure S4. RSV viral titre during late stages of infection ..........142
Supplementary Figure S5. Mast cells in lung tissue ..............................................143
Supplementary Figure S6. IL-4 and IL-5 protein produced by lymph node cells in culture. .................................................................................................................................144
Supplementary Figure S7. RSV viral titre early during T cell differentiation ......145
Supplementary Figure S8. Presence of basophils in the lymph nodes ..............146
Supplementary Figure S9. Changes in lung DC numbers but not phenotype .......147

Chapter IV: Publication#3

Figure 1. Reduced viral clearance and enhanced pathology occurs in the absence of TLR7 .................................................................169
Figure 2. Diminished or delayed antiviral Type I and II IFN production occurs in the absence of TLR7 .................................................................................................172
Figure 3. Outgrowth of a mixed Th response after neonatal Pneumoviral infection in the absence of TLR7 .........................................................................................176
Figure 4. Secondary protective memory responses are generated independently of TLR7 ..............................................................................178
Figure 5. Re-infection with virus in later-life in the absence of TLR7 leads to Th2 responses, airway remodelling, and AHR .........................................................182
Figure 6. Mast cell numbers and IgE increase with each subsequent viral infection.185
Figure 7. Long term airway inflammation and reactivity generated in the absence of TLR7 is dependent on memory CD4 T cells .................................187
Figure 8. Sputum cells from human asthmatics express lower levels of TLR7 .......190
Supplementary Figure S1. Neutrophil influx after PVM infection .......................191
Supplementary Figure S2. Hypersecretion of mucous following PVM infection....192
Supplementary Figure S3. Tertiary infection in the absence of TLR7 leads to exacerbation of Th2 responses and AHR .................................................................194
Supplementary Figure S4. Depletion CD4+ cells and re-generation of antigen-naïve CD4 T cell population ..............................................................196
Chapter IV: DISCUSSION AND CONCLUSIONS

Figure 1: Proposed mechanism of Th2 induction by RSV. ........................................207
Figure 2: Proposed mechanism underlying development of Th2 memory response in TLR7-deficiency. .................................................................210

LIST OF TABLES

Table 1: Summary of the clinical studies examining the association between RSV bronchiolitis and recurrent wheezing.................................................27
Table 2: Summary of human studies examining Th1 vs Th2 cytokine production in RSV infection..................................................................................34
Table 3: Major alterations in gene expression of human epithelial cells in response to RSV infection in vitro.................................................................51
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>Respiratory Syncytial Virus</td>
</tr>
<tr>
<td>PVM</td>
<td>Pneumonia Virus of Mice</td>
</tr>
<tr>
<td>Cpn</td>
<td>Chlamydophila pneumoniae</td>
</tr>
<tr>
<td>Cmu</td>
<td>Chlamydia muridarum</td>
</tr>
<tr>
<td>LRT</td>
<td>lower respiratory tract</td>
</tr>
<tr>
<td>URT</td>
<td>upper respiratory tract</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility</td>
</tr>
<tr>
<td>TCR</td>
<td>T cell receptor</td>
</tr>
<tr>
<td>PFU</td>
<td>plaque forming units</td>
</tr>
<tr>
<td>IFU</td>
<td>infection forming units</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus-activated kinase</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducer and activator of transcription</td>
</tr>
<tr>
<td>HDM</td>
<td>house dust mite</td>
</tr>
<tr>
<td>TNF</td>
<td>tumour necrosis factor</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>CXCL</td>
<td>chemokine ligand</td>
</tr>
<tr>
<td>CXCR</td>
<td>chemokine receptor</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>APC</td>
<td>antigen presenting cell</td>
</tr>
<tr>
<td>GATA-3</td>
<td>GATA binding protein 3</td>
</tr>
<tr>
<td>T-box</td>
<td>T-box expressed in T cells</td>
</tr>
<tr>
<td>ROR-γt</td>
<td>retinoic acid receptor-related orphan receptor γ-t</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>PRR</td>
<td>pathogen recognition receptor</td>
</tr>
<tr>
<td>PAMP</td>
<td>pathogen associated molecular pattern</td>
</tr>
<tr>
<td>DAMP</td>
<td>damage associated molecular pattern</td>
</tr>
<tr>
<td>OVA</td>
<td>ovalbumin</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>i.n.</td>
<td>intranasal</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>MyD88</td>
<td>myeloid differentiation factor 88</td>
</tr>
<tr>
<td>Th</td>
<td>CD4 T helper cell</td>
</tr>
<tr>
<td>Th1</td>
<td>CD4 T helper cell type 1</td>
</tr>
<tr>
<td>Th2</td>
<td>CD4 T helper cell type 2</td>
</tr>
<tr>
<td>Th17</td>
<td>CD4 T helper cell type 17</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T cell</td>
</tr>
<tr>
<td>TGF</td>
<td>transforming growth factor</td>
</tr>
<tr>
<td>NHC</td>
<td>natural helper cell</td>
</tr>
<tr>
<td>MPPtype2</td>
<td>multipotent progenitor cell type 2</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>BAL</td>
<td>bronchoalvelar lavage</td>
</tr>
<tr>
<td>AHR</td>
<td>airways hyper-reactivity</td>
</tr>
<tr>
<td>MSC</td>
<td>mucous secreting cell</td>
</tr>
<tr>
<td>PAS</td>
<td>periodic-acid Schiff</td>
</tr>
<tr>
<td>BM</td>
<td>basement membrane</td>
</tr>
<tr>
<td>BMDC</td>
<td>bone marrow-derived dendritic cell</td>
</tr>
</tbody>
</table>
NOD; Nucleotide-binding oligomerisation domain-containing protein
DC-SIGN; Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
NS protein; non-structural protein
MIP; macrophage inflammatory protein
MCP; monocyte chemotactic protein
RANTES; regulated upon activation, normal T cell expressed and secreted
ICAM; inter-cellular adhesion molecule
VCAM; vascular cell adhesion molecule
VEGF; vascular endothelial growth factor
GM-CSF; granulocyte-macrophage colony stimulating factor
Df; *Dermatophagoides farinae*
RIG-I; retinoic acid-inducible gene-I
LT; leukotriene
Ag; antigen
GWAS; genome wide association study
SNP; single nucleotide polymorphism
DEP; diesel exhaust particles
ETS; environmental tobacco smoke
EB; elementary body
RB; reticulate body
PB; persistent body
ECP; eosinophil cationic protein
FI; formalin-inactivated
TSLP; thymic stromal lymphopoietin
HPC; hematopoietic progenitor cell
PAR; protease activated receptor
pDC; plasmacytoid dendritic cell
mDC; myeloid dendritic cell
TARC; thymus and *activation-regulated* chemokine
LIST OF APPENDICES

Appendix 1:

ABSTRACT

Asthma is a complex heterogenous disease, which may involve a dynamic interplay between multiple gene and environmental factors. There is a growing need to develop better therapies for the treatment and prevention of asthma. In order for this to occur an improved understanding of disease origins and pathogenesis is required. This thesis utilises experimental animal modelling and in vitro culture systems in order to dissect the pathways and mechanisms underlying the interaction between respiratory infections, the innate immune system, and asthma. This thesis consists of three publications. The first publication examines the role of immune evasion of Chlamydophila pneumoniae in its association with acute exacerbations of asthma, and how immune deviation of dendritic cells may be central to both outcomes. The second publication investigates the association between Respiratory Syncytial Virus (RSV) and asthma. This work defines a mechanism by which the innate immune system may induce a viral-specific Th2 response. The third publication attempts to delineate a possible 2-hit hypothesis of asthma pathogenesis. This involves mouse modelling of a specific genetic susceptibility (identified in humans) and an early-life viral infection acting as an environmental insult to the respiratory tract. This work evaluates whether an interplay between these two factors and the innate immune system could potentially predispose to the hallmark features of asthma in later-life.