A *Streptococcus pneumoniae*-based immunoregulatory therapy for asthma

*Submitted in total fulfilment of the requirements of the degree of*

Doctor of Philosophy.

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July, 2010

Discipline of Immunology and Microbiology
School of Biomedical Sciences and Pharmacy
The University of Newcastle
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DECLARATION

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 this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis preface a written statement signed by each co-author, endorsed by the Deputy Head of Faculty Research, attesting to my contribution to the joint publications.

............................................

Alison Thorburn

30/07/10
ACKNOWLEDGEMENTS

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Thank you to all the staff and students in the Discipline of Immunology and Microbiology for their friendship, assistance and exchange of ideas. I am also grateful for the support from the Asthma CRC, Asthma NSW, Khancoban community, Australian Society of Immunology and University of Newcastle.

Last but not least, I would like to thank my family and Scott for their support and enabling me to pursue my goals.
### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>DECLARATION</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>II</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>III</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>XIII</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>XVII</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>XVIII</td>
</tr>
<tr>
<td>PREFACE</td>
<td>XX</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>XXIV</td>
</tr>
</tbody>
</table>

### CHAPTER 1

#### INTRODUCTION

1

1.1 **ASTHMA**

1.1.1 Asthma: An overview 2

1.2 **PATHOGENESIS OF ASTHMA**

1.2.1 Asthma pathogenesis: An overview 3

1.2.2 Early phase response 3

1.2.3 Late phase response 4

1.3 **IMMUNE RESPONSES IN ASTHMA**

1.3.1 Immune response 4

1.3.2 Effector T cells and their roles in asthma 5
1.3.2.1 Th1 cells 5
1.3.2.2 Th2 cells 9
1.3.2.3 Th17 cells 9
1.3.3 Natural killer T (NKT) cells and their roles in asthma 10
1.3.4 Antigen presenting cells and their roles in asthma 11
1.3.5 PRRs 12
1.3.6 Eosinophils 13

1.4 **REGULATORY T CELLS (TREGS)** 14
1.4.1 Tregs and asthma 14
1.4.2 Characterisation of Tregs 15
1.4.3 Mechanisms of suppression 15
1.4.4 Treg-mediated suppression in asthma 20

1.5 **MICROBIAL AGENTS AND ASTHMA** 22
1.5.1 Microbial agents and asthma: An overview 22
1.5.2 Microbial agents that increase asthma risk 23
1.5.3 Microbial agents that reduce asthma risk 23

1.6 **S. PNEUMONIAE** 24
1.6.1 *S. pneumoniae* epidemiology 24
1.6.2 *S. pneumoniae* characteristics and components 26
   1.6.2.1 Capsule 26
   1.6.2.2 Cell wall 26
   1.6.2.3 Pneumolysin 30
1.6.3 Immune response to *S. pneumoniae* 30
1.6.4  *S. pneumoniae* vaccines  
1.6.4.1 Polysaccharide vaccine  
1.6.4.2 Conjugate vaccine  
1.6.4.3 New vaccines  

1.7  *S. pneumoniae* and asthma  
1.7.1  Clinical associations  
1.7.2  Animal models  

1.8  Therapeutics for asthma  
1.8.1  Current therapeutics  
1.8.2  Potential therapeutics  
1.8.2.1  Allergen-specific immunotherapy  
1.8.2.2  Tregs as novel therapeutic  
1.8.3  Route of administration  

1.9  Mouse models to test novel therapeutics  

1.10  Hypothesis and aims  

**Chapter 2**  

**Pneumococcal Conjugate Vaccine-Induced Regulatory T Cells Suppress the Development of Allergic Airways**  

**Disease**  

2.1  Abstract  
2.2  Introduction
2.3 METHODS

2.3.1 Mice 43
2.3.2 AAD model 43
2.3.3 Treatment 44
2.3.4 Assessment of cellular inflammation 44
2.3.5 T cell cytokine release 45
2.3.6 Airways hyperresponsiveness 45
2.3.7 Serum antibodies 46
2.3.8 Lung histology 46
2.3.9 Flow cytometry 46
2.3.10 Cell isolation 47
2.3.11 Cell suppression assay 47
2.3.12 Data analysis 47

2.4 RESULTS 48

2.4.1 Treatment with the conjugate but not polysaccharide vaccine suppresses the development of hallmark features of AAD 48
2.4.2 Conjugate vaccine treatment before or after sensitisation suppresses the development of AAD 51
2.4.3 Conjugate vaccine treatment suppresses established AAD 51
2.4.4 Intramuscular administration of the conjugate vaccine had limited effects on the development of AAD 57
2.4.5 Conjugate vaccine treatment suppresses the development of systemic Th2 responses 57
2.4.6 Conjugate vaccine treatment induces Tregs

2.5.7 Conjugate vaccine treatment induces Tregs that have a greater suppressive capacity

2.4.8 CD25 inactivation restores AAD following conjugate vaccine treatment

2.5 DISCUSSION

CHAPTER 3

PNEUMOCOCCAL-BASED THERAPY SUPPRESSES NATURAL KILLER T CELLS AND ALLERGIC AIRWAYS DISEASE BY INDUCING REGULATORY T CELLS

3.1 ABSTRACT

3.2 INTRODUCTION

3.3 METHODS

3.3.1 Animals

3.3.2 Models of AAD

3.3.3 S. pneumoniae and components

3.3.4 Assessment of cellular inflammation

3.3.5 Cell preparation

3.3.6 T cell cytokine release

3.3.7 Airways hyperresponsiveness

3.3.8 Lung histology
3.3.9 Flow cytometry  
3.3.10 Adoptive transfer of NKT cells  
3.3.11 Data analysis  

3.4 RESULTS  

3.4.1 Identification of *S. pneumoniae* immunoregulatory components  
3.4.2 Identification of *S. pneumoniae* components that suppress AHR  
3.4.3 T3P+Ply immunoregulatory therapy suppresses the development of additional features of AAD  
3.4.4 T3P+Ply immunoregulatory therapy suppresses AAD when administered during established disease  
3.4.5 T3P+Ply immunoregulatory therapy suppresses the induction of HDM-induced AAD  
3.4.6 T3P+Ply immunoregulatory therapy suppresses the accumulation of NKT cells in the lungs and NKT cell-induced AHR  
3.4.7 T3P+Ply-induced Tregs are required for the suppression of AAD  
3.4.8 T3P+Ply-induced Tregs are required to suppress the induction of NKT cells  

3.5 DISCUSSION  

VIII
CHAPTER 4

PNEUMOCOCCAL COMPONENTS INDUCE REGULATORY T CELLS
THAT MEDIATE IMMUNE DEVIATION AND SUPPRESSION TO
ATTENUATE THE DEVELOPMENT OF ALLERGIC AIRWAYS

DISEASE

4.1 ABSTRACT 107

4.2 INTRODUCTION 108

4.3 METHODS 109

4.3.1 Animals 109
4.3.2 AAD 110
4.3.3 Immunoregulatory therapy 110
4.3.4 Cell preparation 110
4.3.5 Flow cytometry 111
4.3.6 Assessment of cellular inflammation 111
4.3.7 T cell cytokine release 111
4.3.8 Airways hyperresponsiveness 112
4.3.9 Real-time PCR 112
4.3.10 In vitro culture 113
4.3.11 Data analysis 113

4.4 RESULTS 113

4.4.1 T3P+Ply induces an early expansion of Tregs in the
MLNs and a late increase in Tregs in the lung

4.4.2 Anti-CD25 antibody depletes the number of CD4+ cells that are CD25+FoxP3+ in the MLNs and lungs

4.4.3 T3P+Ply induction of Tregs in the early phase induction is CD25-dependent and required for suppression of Th2 responses and AAD

4.4.4 T3P+Ply induced IL-2/IL-2R interactions may contribute to the suppression of AAD

4.4.5 T3P+Ply-induced TGF-β mediates the attenuation of Th2 responses and contributes to the suppression of AAD.

4.4.6 T3P+Ply suppresses the establishment of Th17 effector cell responses

4.4.7 T3P+Ply suppresses the establishment of Th2 and Th17 effector cell responses in vitro

4.4.8 T3P+Ply-induced Tregs in the lungs have a highly suppressive phenotype

4.4.9 T3P+Ply suppressed CD86 expression on mDCs and the number of DCs carrying OVA

4.5 DISCUSSION

CHAPTER 5

DISCUSSION
5.1 **Significant Outcomes**

5.2 *S. pneumoniae Vaccines and the Suppression of AAD*  
5.2.1 Conjugate vaccine-mediated suppression of AAD  
5.2.2 Conjugate vaccine: Route of administration and dose

5.3 *S. pneumoniae Components and the Suppression of AAD*  
5.3.1 T3P+Ply-mediated suppression of AAD  
5.3.2 T3P+Ply-mediated suppression in different models of AAD

5.4 **Mechanism of Suppression of AAD by S. pneumoniae Immunoregulatory Therapy**

5.4.1 *S. pneumoniae* immunoregulatory therapy-induced Tregs  
5.4.1.1 Depletion of Tregs  
5.4.1.2 Induction of Tregs  
5.4.1.3 Mechanism of Treg-mediated suppression

5.4.2 Suppression of NKT cells

5.4.3 Suppression of Th1, Th2 and Th17 effector T cells

5.4.4 Effects on APCs

5.5 *S. pneumoniae Immunoregulatory Therapy for Asthma*  
5.5.1 Conjugate vaccine versus T3P+Ply  
5.5.2 Routes of administration  
5.5.3 Dosing strategies and requirements

5.5.4 Harnessing Tregs for therapeutic use
5.6 **FUTURE DIRECTIONS**

5.6.1. Suppressive effects of *S. pneumoniae* immunoregulatory therapy on chronic features of AAD 150

5.6.2 Further investigation of the role of Tregs 150

5.6.2.1 Suppression of NKT cells 150

5.6.2.2 Suppression of Th cells 151

5.6.2.3 Suppressive mechanisms 151

5.6.2.4 Utilisation of new methods 153

5.6.3 Further investigation of the role of APCs 153

5.6.3.1 APC uptake of *S. pneumoniae* components 153

5.6.3.2 PRRs 154

5.6.3.3 Nuclear factor-(NF)-κB 155

5.6.3.4 Activation of a suppressive DC phenotype? 155

5.6.4 Chemokines and migration pathways 156

5.6.5 Potential use of *S. pneumoniae* immunoregulatory therapy for other inflammatory conditions 157

5.6.6 Assessing the effect of *S. pneumoniae* immunoregulatory therapy using clinical samples 157

5.6.7. Translation of *S. pneumoniae* immunoregulatory therapy into the clinic: Clinical trials 158

**REFERENCES** 160

**APPENDIX** 186
# LIST OF FIGURES

## CHAPTER 1

**INTRODUCTION**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1.</td>
<td>T cell subtypes</td>
<td>6</td>
</tr>
<tr>
<td>Figure 1.2.</td>
<td>The role of Tregs in asthma</td>
<td>21</td>
</tr>
<tr>
<td>Figure 1.3.</td>
<td>Pneumococcal structure</td>
<td>27</td>
</tr>
</tbody>
</table>

## CHAPTER 2

**PNEUMOCOCCAL CONJUGATE VACCINE-INDUCED REGULATORY T CELLS SUPPRESS THE DEVELOPMENT OF ALLERGIC AIRWAYS**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>The effect of pneumococcal vaccines on AAD</td>
<td>49</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>The effect of CJ and PS vaccines with or without CpG-ODN on AHR</td>
<td>52</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>The effect of CJ treatment when administered before or after sensitisation</td>
<td>53</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>The effect of CJ backbone components AlPO₄+CRM₁₉₇</td>
<td>54</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>The effect of CJ treatment on established AAD</td>
<td>55</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>The effect of intramuscular administration of CJ on AAD</td>
<td>58</td>
</tr>
</tbody>
</table>
FIGURE 2.7 The effect of CJ treatment on additional features of AAD 60

FIGURE 2.8 The effect of CJ treatment on Tregs 61

FIGURE 2.9 The effect of CJ treatment on Treg phenotype and suppressive capacity 64

FIGURE 2.10 Representative flow cytometric data used in Treg phenotyping 65

FIGURE 2.11 The effect of anti-CD25 antibody depletion of Tregs on CJ-induced suppression of AAD 67

CHAPTER 3

PNEUMOCOCCAL-BASED THERAPY SUPPRESSES NATURAL KILLER T CELLS AND ALLERGIC AIRWAYS DISEASE BY INDUCING REGULATORY T CELLS 74

FIGURE 3.1 Identification of S. pneumoniae components that suppress AAD 83

FIGURE 3.2 Selected S. pneumoniae components suppress AHR 86

FIGURE 3.3 T3P+Ply suppress the development of additional features of AAD. 89

FIGURE 3.4 T3P+Ply attenuate AAD when administered during established disease 91

FIGURE 3.5 T3P+Ply suppress the development of HDM-induced...
AAD 93

FIGURE 3.6 T3P+Ply suppress NKT cells in AAD 94

FIGURE 3.7 T3P+Ply induces Tregs that are required for the suppression of AAD 97

FIGURE 3.8 T3P+Ply-induced Tregs are required for the suppression of NKT cells 100

CHAPTER 4

PNEUMOCOCCAL COMPONENTS INDUCE REGULATORY T CELLS THAT MEDIATE IMMUNE DEVIATION AND Suppression TO ATTENUATE THE DEVELOPMENT OF ALLERGIC AIRWAYS DISEASE 106

FIGURE 4.1 Time course analysis of T3P+Ply-induced Tregs in the MLNs and lung 111

FIGURE 4.2 The effects of anti-CD25 antibody (αCD25) administration on Treg populations in the MLNs and lung when administered day -3 (-3) or day 9 (+9) 114

FIGURE 4.3 The effects of anti-CD25 antibody administration on T3P+Ply-mediated suppression of AAD 117

FIGURE 4.4 The effect of T3P+Ply on Treg-inducing factors and Th17 cells 119

FIGURE 4.5 The effects of T3P+Ply treatment in vitro 122
Figure 4.6  The effects of T3P+Ply treatment on Treg functional markers  

Figure 4.7  The effects of T3P+Ply treatment DCs
List of Tables

Chapter 1

Introduction 1

Table 1.1. Cytokines and their function in asthma 7

Table 1.2. Treg population origins and Treg subtype characteristics 16

Table 1.3. Treg markers and their association 17

Table 1.4. Mechanisms of Treg-induced suppression 18

Table 1.5. Microbial agents and mechanisms that have been associated with reduced asthma risk 25

Table 1.6. The role of S. pneumoniae virulence factors 28
Asthma is a chronic inflammatory disease of the airways that affects over 300 million people worldwide. The disease is characterised by episodes of breathlessness, coughing, wheezing and airway hyperresponsiveness (AHR). Asthma results from a dysregulation in immunity that is underpinned by a cohort of effector T cell populations including T helper (Th)1, Th2, Th17 and natural killer T (NKT) cells. These effector T cells produce numerous inflammatory cytokines and chemokines that induce eosinophil influx, mucus hypersecretion and AHR. Antigen presenting cells play key roles in priming these responses. Regulatory T cells (Tregs) are essential for suppression of aberrant immune responses and maintenance of immune homeostasis. Both the number and function of Tregs is impaired in asthmatics, compared to healthy individuals. This reinforces the importance of Tregs in regulating a balanced immune response.

Microbial agents have been associated with increased or decreased risk of asthma. Microbial agents that have been associated with decreased asthma risk are under intense investigation for their potential utilisation in therapeutic strategies for asthma. *Streptococcus pneumoniae* vaccination has been associated with decreased asthma-related hospitalisations in children and the elderly. Furthermore, early mouse studies observed that *S. pneumoniae* infection attenuated blood eosinophilia during parasitic infection. More recent studies have shown that both live and ethanol killed *S. pneumoniae* suppress the development of allergic airways disease (AAD) in mice, including eosinophil recruitment to the lungs, Th2 cytokine release, mucus hypersecretion and AHR. Therefore *S. pneumoniae* has the potential for development into a novel immunotherapy for asthma.
To examine this concept we first investigated the capacity of human *S. pneumoniae* vaccines, which were developed to prevent *S. pneumoniae* infection, to suppress AAD in mouse models (Chapter 2). In the next study, and in order to determine which components were required for *S. pneumoniae*-mediated suppression of AAD, *S. pneumoniae* components were tested for their capacity to suppress AAD (Chapter 3). Two potential *S. pneumoniae*-based immunotherapies were identified: the conjugate vaccine and the combination of type 3 capsular polysaccharide and pneumolysin (T3P+Ply). These *S. pneumoniae* immunotherapies suppressed the development of AAD when administered before, during and after sensitisation. Importantly, *S. pneumoniae* immunotherapy also attenuated established AAD. This demonstrated that *S. pneumoniae* immunotherapy has potential for therapeutic use in the prevention and/or treatment of asthma.

To determine the mechanisms involved in *S. pneumoniae*-mediated suppression of AAD a number of investigations were performed. Tregs were shown to be induced by *S. pneumoniae* immunotherapy. Furthermore, anti-CD25 antibody-mediated depletion of Tregs reversed the effect of immunotherapy. Hence, Tregs were required for immunotherapy-mediated suppression of AAD. In the third study, Tregs were shown to be induced in a biphasic manner to suppress immune responses and AAD through a broad range of mechanisms (Chapter 4). Together, these studies have identified potential and novel *S. pneumoniae* immunotherapies for asthma and determined the mechanism of action that underpins suppression of AAD.
Peer reviewed publications

These publications are not presented in this thesis but included in the Appendix.


Publications prepared for submission

These publications form the basis of this thesis.


• Thorburn, A.N., Foster, P.S., Gibson, P.G., Hansbro, P.M. Pneumococcal components induce regulatory T cells that mediate immune deviation and suppression to attenuate the development of allergic airways disease. Prepared for submission to J. Immunol. Presented in Chapter 4 of this thesis.

Published abstracts


• Thorburn, A.N., Foster, P.S., Gibson, P.G., Hansbro, P.M. Streptococcus pneumoniae vaccine, Prevenar, utilizes Tregs to suppress asthma. J. Immunol. 2009;182:140.2.


Research Higher Degree candidate Alison Thorburn contributed wholly to the publications/prepared manuscripts that form the basis of this thesis, as listed in the preface. This contribution involved the development of the projects, generation and analysis of experimental data and completion of the manuscript in collaboration with the other authors.

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Professor Ranjeny Thomas Date:

Professor Rakesh Kumar Date:

Professor Paul Foster Date:

Professor Peter Gibson Date:

Associate Professor Philip Hansbro Date:

Endorsed by:

Professor John Rostas (Deputy Head of Faculty Research) Date:
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAD</td>
<td>Allergic airways disease</td>
</tr>
<tr>
<td>AHR</td>
<td>Airways hyperresponsiveness</td>
</tr>
<tr>
<td>ALI</td>
<td>Acute lung injury</td>
</tr>
<tr>
<td>AlPO₄</td>
<td>Aluminium phosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen presenting cell</td>
</tr>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>AUD</td>
<td>Australian dollar</td>
</tr>
<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacillus calmette-guerin</td>
</tr>
<tr>
<td>CbpA</td>
<td>Choline binding protein A</td>
</tr>
<tr>
<td>CCL</td>
<td>Chemokine ligand</td>
</tr>
<tr>
<td>CCR</td>
<td>Chemokine receptor</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>cfu</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>CJ</td>
<td>Conjugate</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CpG-ODN</td>
<td>CpG-oligodinucleotide</td>
</tr>
<tr>
<td>CREB</td>
<td>Cyclic adenosine monophosphate response element-binding</td>
</tr>
<tr>
<td>CRM₁⁹⁷</td>
<td>Cross-reactive material 197</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Cytotoxic T lymphocyte antigen-4</td>
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<td>CW</td>
<td>Cell walls</td>
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<td>DC</td>
<td>Dendritic cell</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DPPE-PEG</td>
<td>di-palmitoyl-phosphatidyl-ethanolamine polyethylene glycol</td>
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<td>Ebi3</td>
<td>Epstein-barr virus induced gene 3</td>
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<td>ECP</td>
<td>Eosinophil cationic protein</td>
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<td>EDN</td>
<td>Eosinophil-derived neurotoxin</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>EPO</td>
<td>Eosinophil peroxidise</td>
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<td>FACS</td>
<td>Fluorescent-activated cell sorting</td>
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<td>Fragment crystallisable</td>
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<td>Fibrinogen-like protein 2</td>
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<td>FITC</td>
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<tr>
<td>FoxP3</td>
<td>Fork-head box protein 3</td>
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<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
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<tr>
<td>GITR</td>
<td>Glucocorticoid-induced TNFR-related protein</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage colony stimulating factor</td>
</tr>
<tr>
<td>GPR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HBSS</td>
<td>HANKS balanced salt solution</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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</tr>
<tr>
<td>HDM</td>
<td>House dust mite</td>
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<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HO</td>
<td>Heme oxygenase</td>
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<tr>
<td>i.m.</td>
<td>Intramuscular</td>
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<td>i.n.</td>
<td>Intranasal</td>
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<td>i.v.</td>
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<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule-1</td>
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<td>ICOS</td>
<td>Inducible T cell co-stimulator</td>
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<td>IDO</td>
<td>Indolamine 2,3-dioxygenase</td>
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<td>IFN</td>
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<td>Immunoglobulin</td>
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<td>Keratinocyte chemoattractant</td>
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<td>LAG</td>
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</tr>
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</tr>
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<tr>
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</tr>
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<tr>
<td>MCP-1</td>
<td>Monocyte chemotactic protein-1</td>
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<tr>
<td>mDC</td>
<td>Myeloid DC</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<td>min</td>
<td>Minute</td>
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<td>MIP2</td>
<td>Macrophage inflammatory protein 2</td>
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<tr>
<td>MLN</td>
<td>Mediastinal lymph node</td>
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<tr>
<td>mM</td>
<td>Milimolar</td>
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<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
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<tr>
<td>MyD88</td>
<td>Myeloid differentiation factor 88</td>
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<tr>
<td>n.s.</td>
<td>Not significant</td>
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<tr>
<td>NFAT</td>
<td>Nuclear factor of activated T cells</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor κB</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NKT</td>
<td>Natural killer T</td>
</tr>
<tr>
<td>Nrp</td>
<td>Neuropilin</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees celcius</td>
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<tr>
<td>OVA</td>
<td>Ovalbumin</td>
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<td>OVAp</td>
<td>OVA peptide</td>
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<tr>
<td>PAMP</td>
<td>Pattern associated molecular pattern</td>
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<tr>
<td>PBMCs</td>
<td>Peripheral blood mononuclear cell</td>
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<tr>
<td>PD-1</td>
<td>Programmed death 1</td>
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</table>
pDC  Plasmacytoid
PDGF  Platelet-derived growth factor
pg    Picogram
Ply   Pneumolysoid
PMA   Phorbol myristate acetate
PPR   Pattern recognition receptor
PS    Polysaccharide
PsaA  Pneumococcal surface adhesin A
PspA  Pneumococcal surface protein A
R     Receptor
RA    Rheumatoid arthritis
RANTES Regulated upon activation, normal T cell expressed and secreted
Rel   Reticuloendotheliosis
RNA   Ribonucleic acid
Rory-t RAR-related orphan receptor gamma-thymus
RT-PCR Reverse transcriptase-polymerase chain reaction
RV    Rhinovirus
SEM   Standard error of the mean
Smad  Mother against decapentaplegic homolog
SpsA  *Streptococcus* secretory IgA binding protein
STAT  Signal transducer and activator of transcription
T3P   Type 3 polysaccharide
TARC    Thymus and activation-regulated chemokine
T-bet    T-box expressed in T cells
TCR     T cell receptor
TGF     Transforming growth factor
Th      T-helper
TIR     Toll/IL-1R
TLR     Toll-like receptor
TNF     Tumour necrosis factor
TNFRS   Tumour necrosis factor receptor superfamily
Tr1     Treg 1 subtype
Tr3     Treg 3 subtype
TRAF    TNF receptor associated factor
TRAM    TRIF-related adaptor molecule
Treg    T regulatory cell
TRIF    TIR-domain-containing adapter-inducing interferon-β
USD     United States dollar
VEGF    Vascular endothelial growth factor
xg      Times g-force
αCD25   Anti-CD25 antibody
αGal-Cer α-galactosylceramide
αTGF-β  Anti-TGF-β antibody
µg      Microgram
µl      Microlitre