

**MECHANISMS BY WHICH INTERLEUKIN-4
SUPPRESSES INFLAMMATORY
CYTOKINE PRODUCTION BY ACTIVATED
HUMAN MONOCYTES**

by

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This thesis is dedicate to my father,

John Woodward.

(1944 to 2009)

*Who always insisted I would finish. His lifetime of dedication
and passion will never stop inspiring me.*

Statement of Candidate

Contribution

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

.....

Eleanor Anne Woodward

In instances where work has been performed by other parties, permission has been granted from these parties to include this work in this thesis.

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Prof Prue Hart

Abstract

Improving understanding of how inflammatory responses by monocytes and macrophages are regulated may aid in the development of more targeted therapies for chronic inflammatory disease. In this thesis the mechanisms by which the cytokine, IL-4, can suppress inflammatory cytokine production by LPS-stimulated human monocytes have been examined.

IL-4 suppressed LPS-induced TNF α transcription, without inhibiting LPS signalling through I κ B, the mitogen-activated protein kinase (MAPK) pathways, or LPS-mediated activation of the transcription factor NF- κ B. Histone acetylation regulated LPS-induced cytokine production but not the suppression of these cytokines by IL-4. IL-4 induced three molecules with potential anti-inflammatory properties, suppressor of cytokine signalling-1 (SOCS1), peroxisome proliferator-activated receptor gamma (PPAR γ) and triggering receptors expressed on myeloid cells-2 (TREM-2), but suppressed LPS-induced TNF α production independently of these molecules.

Targeted gene arrays for Toll-like receptor (TLR) signalling pathways revealed that IL-4 down-regulated mRNA levels of LPS-induced inflammatory cytokines and chemokines, without altering other NF- κ B-dependent genes or mRNA levels of TLR-related signalling molecules. Instead, the anti-inflammatory actions of IL-4 may be mediated by up-regulation of an unknown signalling molecule or transcriptional regulator. In LPS-treated monocytes, IL-4 up-regulated mRNA levels for IL-10, receptor-interacting serine-threonine kinase 2 (RIPK2), RP105 and c-Maf. However, the anti-inflammatory actions of IL-4 did not require IL-10 or the kinase activity of

RIPK2. While the TLR-homolog, RP105, is likely to negatively regulate LPS responses by monocytes, IL-4 had no effect on cell surface expression of RP105. Additional studies may determine whether c-Maf, a transcription factor which induces IL-10, also regulates the suppression inflammatory cytokine production by IL-4.

This study identified novel candidates induced by IL-4 in LPS-stimulated human monocytes. However, the molecules involved in the regulation by IL-4 of LPS-induced TNF α production were not definitively identified. Further studies may identify the mechanisms by which IL-4 is anti-inflammatory. Ultimately, this research will contribute towards the development of novel therapies for inflammatory disease.

Table of Contents

PREFACE

Abstract	i
Table of Contents	iii
List of Figures	xii
List of Tables	xvii
Abbreviations	xix
Acknowledgements	xxv
Publications Arising from this Thesis	xxvii

CHAPTER ONE **1**

INTRODUCTION AND REVIEW OF THE LITERATURE

1.1	Immunity and Inflammation	2
1.2	Monocytes and Macrophages	3
1.3	Toll-like Receptor Activation of Monocytes and Macrophages	5
	<i>1.3.1 LPS Activation of Monocytes and Macrophages as a Model for Inflammation</i>	<i>7</i>
1.4	Cytokines and Regulation of the Immune Responses	8
1.5	Pro-inflammatory Cytokines	9
	<i>1.5.1 TNFα</i>	<i>10</i>
	<i>1.5.2 Regulation of LPS-induced TNFα Production by Monocytes</i>	<i>10</i>
	<i>1.5.2 Other Pro-inflammatory Agents</i>	<i>11</i>
1.6	Anti-inflammatory Cytokines	12
1.7	Chronic Inflammatory Disease	14

1.8	Rheumatoid Arthritis	14
	<i>1.8.1 Pathogenesis of Rheumatoid Arthritis</i>	14
	<i>1.8.2 Current Treatments for Rheumatoid Arthritis</i>	16
	<i>1.8.3 Biological Therapies in the Treatment of Rheumatoid Arthritis</i>	18
1.9	Signalling Through the Interleukin-4 Receptor	20
	<i>1.9.1 IL-4 Activation of STAT6 Signalling</i>	21
	<i>1.9.2 IL-4 Activation of IRS-1/2 Signalling</i>	23
	<i>1.9.3 IL-4 Activation of Ras/MAPK Signalling</i>	23
	<i>1.9.4 Regulation of IL-4 Signalling</i>	25
1.10	Anti-inflammatory Actions of IL-4 in Monocytes and Macrophages	25
	<i>1.10.1 Alternative Activation of Macrophages by IL-4</i>	26
1.11	Potential of IL-4 in the Treatment of Rheumatoid Arthritis	28
	<i>1.11.1 Use of IL-4 in Animal Models</i>	29
	<i>1.11.2 Potential Inflammatory Actions of IL-4</i>	30
	<i>1.11.3 Use of IL-4 in Human Studies</i>	31
1.12	Mechanisms of the Anti-inflammatory Effects of IL-4	33
	<i>1.12.1 Role of STAT6 in Mediating the Anti-inflammatory Effects of IL-4</i>	34
	<i>1.12.2 Transcriptional Regulation by IL-4</i>	35
	<i>1.12.3 Post-transcriptional Regulation by IL-4</i>	35
	<i>1.12.4 Differences in IL-4 Signalling Between Monocytes and Macrophages</i>	37
1.14	Summary and Aims of this Thesis	40

CHAPTER TWO **41**

MATERIALS AND METHODS

2.1	Ethics Approval	42
2.2	Isolation and Culture of Human Cells	42
	<i>2.2.1 Isolation of Human Peripheral Blood Mononuclear Cells (PBMCs)</i>	42

2.2.2	<i>Isolation of Human Monocytes</i>	43
2.2.3	<i>Isolation of Synovial Fluid Cells</i>	44
2.2.4	<i>Culture of Human Monocytes and PBMCs</i>	46
2.3	Isolation and Culture of Murine Cells	46
2.3.1	<i>Generation and Maintenance of Socs1^{-/-}Ifnγ^{-/-}, Ifnγ^{-/-} and IL10^{-/-} Mice</i>	46
2.3.2	<i>Isolation and Culture of Murine Bone-Marrow Derived Macrophages</i>	47
2.4	Assessment of Functional Responses to IL-4	47
2.4.1	<i>Inhibition in vitro of LPS- and IL-4-mediated Pathways</i>	49
2.5	Detection of Human and Murine Cytokines in Tissue Culture Supernatants	49
2.5.1	<i>Detection of Prostaglandin E₂ in Supernatants</i>	50
2.6	Detection of Gene Expression Using Real-time PCR	51
2.6.1	<i>RNA Extractions</i>	51
2.6.2	<i>Reverse Transcription of RNA</i>	52
2.6.3	<i>Real-time PCR</i>	53
2.7	PCR Array Analysis of TLR Related Gene Expression in LPS- and IL-4-treated Human Monocytes	55
2.8	Western Blotting	56
2.9	Nuclear Extraction, NF-κB and STAT6 EMSAs	58
2.10	Flow Cytometric Analysis of CD14, TLR4 and RP105 Surface Expression and Intracellular RP105	60
2.10.1	<i>Antibodies</i>	60
2.10.2	<i>Cell Surface Staining</i>	60
2.10.3	<i>Intracellular Staining of RP105</i>	61
2.10.4	<i>Flow cytometric analysis</i>	62
2.11	Statistical Analysis	62

CHAPTER THREE

63

DIFFERENCES IN THE SUPPRESSION OF INFLAMMATORY CYTOKINE PRODUCTION BY IL-4 IN FRESHLY ISOLATED MONOCYTES AND MONOCYTES AFTER OVERNIGHT CULTURE

3.1	Introduction	64
3.2	Results	67
3.2.1	<i>Changes in Cellular Morphology as Human Monocytes Differentiate in vitro</i>	67
3.2.2	<i>Suppression of LPS-induced TNFα, IL-1β and IL-10 Production by IL-4</i>	68
3.2.3	<i>Effect of the Timing of IL-4 Addition on the Suppression of LPS-induced TNFα Production by IL-4</i>	70
3.2.4	<i>Suppression of LPS-induced TNFα mRNA by IL-4</i>	72
3.2.4	<i>Effect of IL-4 on TNFα mRNA Stability</i>	72
3.2.5	<i>Effect of IL-4 on STAT6 Phosphorylation and STAT6 DNA Binding Activity</i>	74
3.2.5	<i>Summary of the Differences between Freshly Isolated Monocytes and Monocytes after Overnight Culture in M-CSF</i>	74
3.3	Discussion	76

CHAPTER FOUR

81

EFFECT OF IL-4 ON LPS-ACTIVATION OF TOLL-LIKE RECEPTOR SIGNALLING COMPONENTS AND TRANSCRIPTION FACTORS

4.1	Introduction	82
4.2	Results	86
4.2.1	<i>IL-4 has no Effect on LPS-induced Phosphorylation and Degradation of IκB</i>	86
4.2.2	<i>IL-4 has No Effect on LPS-activation of NF-κB</i>	86

4.2.3	<i>IL-4 Enhances LPS-activation of ERK MAPK but has no Effect on LPS-activation of p38 and JNK MAPK</i>	88
4.2.4	<i>IL-4 Weakly Activates ERK and p38 MAPK in the Absence of LPS</i>	92
4.2.5	<i>Blocking MAP Kinase Pathways and Proteosomal Degradation does not Prevent IL-4 Suppression of LPS-induced TNFα Production by Human Monocytes</i>	93
4.3	Discussion	95

CHAPTER FIVE **99**

ROLE OF HISTONE ACETYLATION IN IL-4 REGULATION OF LPS-INDUCED TNF α , PGE₂ AND IL-10 PRODUCTION BY HUMAN MONOCYTES

5.1	Introduction	100
5.2	Results	104
5.2.1	<i>HDAC Inhibitors have Biphasic Effects on LPS-induced TNFα Production but do not Prevent IL-4 Suppression of LPS-induced TNFα Production</i>	104
5.2.2	<i>HDAC Inhibitors Enhance LPS-induced PGE₂ Production but do not Prevent IL-4 Suppression of LPS-induced PGE₂ Production</i>	106
5.2.3	<i>Broad Spectrum, but not Class I HDAC inhibitors, Suppress LPS-induced IL-10 Production, but have No Effect on IL-4 Suppression of IL-10</i>	106
5.3.4	<i>Summary of Effects of HDAC Inhibitors</i>	109
5.3	Discussion	110

CHAPTER SIX

117

ROLE OF NEGATIVE REGULATORS OF CYTOKINE SIGNALLING IN THE SUPPRESSION OF LPS-INDUCED INFLAMMATORY CYTOKINE PRODUCTION BY IL-4 IN HUMAN MONOCYTES

6.1	Introduction	118
6.2	Results	124
6.2.1	<i>IL-4 Induces SOCS1 and CIS mRNA in Human Monocytes</i>	124
6.2.2	<i>LPS and IL-4 Induction of CIS, SOCS1, SOCS3, SOCS4 and SOCS5 mRNAs in Freshly Isolated Monocytes is Similar to that in Monocytes Cultured Overnight in M-CSF</i>	126
6.2.3	<i>LPS and IL-4 do not Modulate Expression of SHP, SHIP and PIAS mRNAs in Human Monocytes</i>	126
6.2.4	<i>Expression of SOCS mRNA in Synovial Fluid Mononuclear Cells from Patients with Inflammatory Arthritis</i>	128
6.2.5	<i>Kinetics of Induction of SOCS1 mRNA and Protein by IL-4, IFNγ and LPS</i>	130
6.2.6	<i>Effect of IL-4 on LPS-induced TNFα Production by Murine Socs1^{-/-}Ifnγ^{-/-} Bone Marrow Derived Macrophages</i>	132
6.3	Discussion	133

CHAPTER SEVEN

139

THE ROLE OF PPAR γ AND TREM-2 IN THE SUPPRESSION OF LPS-INDUCED TNF α PRODUCTION BY IL-4 IN HUMAN MONOCYTES

7.1	Introduction	140
7.2	Results	145
7.2.1	<i>TREM-2 and PPARγ Expression is Up-regulated by IL-4-induced (M2) Polarisation and Down-regulated by IFNγ-induced (M1) Polarisation</i>	145

7.2.2	<i>IL-4 Causes an Early Up-regulation of PPARγ mRNA which is Stronger in Freshly Isolated Monocytes than in Monocytes Cultured Overnight in M-CSF</i>	146
7.2.3	<i>IL-4 Induces TREM-2 mRNA in Monocytes after Overnight Culture in M-CSF, but Freshly Isolated Monocytes do not Express TREM-2</i>	146
7.2.4	<i>Expression of PPARγ and TREM-2 in Synovial Fluid Mononuclear Cells from Patients with Inflammatory Arthritis</i>	149
7.2.5	<i>Summary of PPARγ and TREM-2 Expression in Human Monocytes and Macrophages</i>	149
7.3	Discussion	153

CHAPTER EIGHT

159

IDENTIFICATION OF LPS-INDUCED GENES THAT ARE REGULATED BY IL-4 IN HUMAN MONOCYTES USING GENE ARRAYS

8.1	Introduction	160
8.2	Results	163
8.2.1	<i>Effect of LPS on TLR-related Gene Expression</i>	163
8.2.2	<i>IL-4 Down-regulates LPS-induced Inflammatory Cytokines and Chemokines, Without Altering mRNA Levels of TLRs, Signalling Molecules or Transcription Factors</i>	163
8.2.3	<i>IL-4 Causes an Early Up-regulation of IL-10, RIPK2, RP105 and c-Maf mRNA in LPS-treated Human Monocytes</i>	167
8.2.4	<i>The Early Up-regulation of LPS-induced IL-10 Secretion by IL-4 Correlates with the Early Suppression of LPS-induced TNFα Production by IL-4</i>	171
8.2.5	<i>The Suppression of LPS-induced TNFα Production by IL-4 Occurs Independently of IL-10</i>	173

8.2.6	<i>The Up-regulation of RIPK2 and RP105 by IL-4 in LPS-treated Monocytes Occurs Independently of IL-10</i>	175
8.2.7	<i>RIPK2 Kinase Inhibitors do not Prevent the Suppression of LPS-induced TNFα Production or Enhancement of LPS-induced IL-10 production by IL-4</i>	177
8.3	Discussion	179

CHAPTER NINE **185**

**ROLE OF RP105 IN THE REGULATION OF MONOCYTE
RESPONSES TO LPS AND IL-4**

9.1	Introduction	186
9.2	Results	189
9.2.1	<i>Effect of LPS and IL-4 on mRNA levels of RP105, TLR4 and CD14 in Human Monocytes</i>	189
9.2.2	<i>LPS Causes a Transient Loss of Cell Surface CD14 Expression in Human Monocytes which is Coupled with a Loss of RP105 and TLR4 Expression</i>	191
9.2.3	<i>LPS, but not IL-4, Alters Cell Surface TLR4 and RP105 Expression in Human Monocytes</i>	194
9.2.4	<i>Intracellular Expression of RP105 in Human Monocytes</i>	196
9.2.5	<i>IL-4 Suppresses both TLR4- and TLR2-driven TNFα Production</i>	196
9.2.6	<i>Summary of RP105, TLR4 and CD14 Expression in LPS- and IL-4-treated Human Monocytes</i>	199
9.3	Discussion	200

<u>CHAPTER TEN</u>	207
GENERAL DISCUSSION AND CONCLUSIONS	
10.1 Aims of this Thesis and Summary of Findings	208
10.1.1 Aim 1: <i>To Compare the Mechanisms by which IL-4 Suppresses Inflammatory Cytokine Production by Freshly Isolated Monocytes and Monocytes After Overnight Culture.</i>	208
10.1.2 Aim 2: <i>To Identify the TLR-signalling Components that are Targeted by IL-4</i>	214
10.1.3 Aim 3: <i>To Identify IL-4-induced Proteins that may Mediate the Anti-inflammatory Actions of IL-4</i>	217
10.2 Significance of Findings and Future Directions	220
10.3 Concluding Remarks	222
<u>REFERENCES</u>	225

List of Figures

Figure 1.1:	Differentiation of macrophages <i>in vivo</i> .	5
Figure 1.2:	LPS signalling through TLR4 in monocytes and macrophages.	7
Figure 1.3:	Summary of Th1/Th2 induction	9
Figure 1.4:	Comparison of the histology of a normal joint (A) and an arthritic joint (B) in a murine model of bovine serum albumin (BSA)-induced arthritis.	15
Figure 1.5:	Cytokine networks in rheumatoid arthritis.	17
Figure 1.6:	The IL-4 receptor.	21
Figure 1.7:	The STAT6 activation pathway.	22
Figure 1.8:	Activation of signalling pathways through the I4R motif of the IL-4R α .	24
Figure 1.9:	Key properties and functions of classically activated (M1) and alternatively activated (M2) macrophages.	27
Figure 1.10:	Protective effect of local treatment of IL-4 in murine collagen-induced arthritis.	30
Figure 2.1:	Cellular composition of isolated human cells.	45
Figure 2.2:	Gating of monocytes in the PBMC population.	62
Figure 3.1:	Changes in cellular morphology of cultured blood monocytes during M-CSF differentiation.	67
Figure 3.2:	Suppression of LPS-induced TNF α , IL-10 and IL-1 β production by IL-4 in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	69
Figure 3.3:	Effect of the timing of IL-4 addition, relative to LPS, on the suppression of LPS-induced TNF α production by IL-4.	71

Figure 3.4:	Suppression of LPS-induced TNF α mRNA by IL-4 in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	73
Figure 3.5:	Effect of IL-4 on TNF α mRNA stability in freshly isolated monocytes.	73
Figure 3.6:	Effect of IL-4 on activation of STAT6 in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	75
Figure 4.1:	LPS-activation of signalling pathways and transcription factors in monocytes.	83
Figure 4.2:	Effect of IL-4 on LPS-induced phosphorylation and degradation of I κ B in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	87
Figure 4.3:	Effect of IL-4 on LPS-activation of NF- κ B.	88
Figure 4.4:	Effect of IL-4 on LPS-induced p38 phosphorylation in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	89
Figure 4.5:	Effect of IL-4 on LPS-induced ERK phosphorylation in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	90
Figure 4.6:	Effect of IL-4 on LPS-induced JNK phosphorylation in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	91
Figure 4.7:	IL-4-activation of ERK and p38 MAPK in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	92
Figure 4.8:	Effect of MAP kinase inhibitors and proteosomal inhibitors on IL-4 suppression of TNF α production by LPS-stimulated monocytes	94
Figure 5.1:	Regulation of gene expression by HDAC and HAT enzymes.	100

Figure 5.2:	Effect of HDAC inhibitors on the suppression of LPS-induced TNF α production by IL-4.	105
Figure 5.3:	Effect of HDAC inhibition on the suppression of LPS-induced PGE ₂ production by IL-4.	107
Figure 5.4:	Effect of HDAC inhibitors on the suppression of LPS-induced IL-10 production by IL-4.	108
Figure 5.5:	HDAC regulation of TNF α production.	114
Figure 5.6:	HDAC regulation of PGE ₂ production.	115
Figure 5.7:	HDAC regulation of IL-10 production.	116
Figure 6.1:	Induction and function of negative regulators of cytokine signalling.	123
Figure 6.2:	Induction of SOCS mRNA by IL-4, IL-10 and LPS.	125
Figure 6.3:	Comparison of the induction of SOCS mRNA in freshly isolated monocytes (Fresh) and monocytes cultured overnight in M-CSF (Overnight).	127
Figure 6.4:	Induction of SHP, SHIP and PIAS mRNA by IL-4 and LPS.	128
Figure 6.5:	SOCS mRNA expression in synovial fluid mononuclear cells from rheumatoid arthritis patients.	129
Figure 6.6:	Comparison of the kinetics of induction of SOCS1 mRNA by IL-4, IFN γ and LPS.	131
Figure 6.7:	Induction of SOCS1 protein by IL-4, IFN γ and LPS.	131
Figure 6.8:	Effect of IL-4 on LPS-induced TNF α production by BMM from wildtype, <i>Ifnγ^{-/-}</i> and <i>Socs1^{-/-}Ifnγ^{-/-}</i> mice.	132
Figure 7.1:	PPAR γ signalling.	141
Figure 7.2:	TREM-2 signalling.	143
Figure 7.3:	Effect of M1 and M2 polarisation on TREM-2 and PPAR γ expression in human monocytes.	145
Figure 7.4:	Kinetics of LPS-induced down-regulation and IL-4-induced up-regulation of PPAR γ mRNA levels in human monocytes.	147

Figure 7.5:	Kinetics of LPS-induced down-regulation and IL-4-induced up-regulation of TREM-2 mRNA levels in human monocytes.	148
Figure 7.6:	TREM-2 and PPAR γ mRNA expression in synovial fluid mononuclear cells from rheumatoid arthritis patients.	150
Figure 7.7:	Effect of PPAR γ antagonists and agonists on LPS-induced TNF α production and the suppression by IL-4 in human monocytes.	152
Figure 7.8:	Summary of PPAR γ and TREM-2 expression at different stages of monocyte differentiation.	158
Figure 8.1:	IL-4 down-regulates LPS-induced mRNA levels of inflammatory cytokines and chemokines.	164
Figure 8.2:	IL-4 up-regulates mRNA levels of IL-10, RIPK2 and RP105 in LPS-treated human monocytes.	168
Figure 8.3:	Confirmation of IL-10, RIPK2 and c-Maf expression using real-time PCR.	170
Figure 8.4:	IL-4 up-regulation of LPS-induced IL-10 secretion by human monocytes and murine BMM correlates with a suppression of LPS-induced TNF α production.	172
Figure 8.5:	The suppression of LPS induced TNF α production by IL-4 is independent of the early up-regulation of LPS-induced IL-10.	174
Figure 8.6:	IL-10 and IL-4 have distinct effects on RIPK2 and RP105 expression in LPS-treated human monocytes.	175
Figure 8.7:	IL-4 up-regulation of RIPK2 and RP105 expression in LPS-treated human monocytes occurs independently of IL-10.	176
Figure 8.8:	RIPK2 kinase inhibitors do not prevent the anti-inflammatory actions of IL-4 in LPS-treated human monocytes.	178
Figure 9.1:	Negative regulation of TLR4 signalling by RP105.	188
Figure 9.2:	Effect of LPS and IL-4 on mRNA levels of RP105/ MD-1, TLR4/ MD-2 and CD14 in human monocytes.	190
Figure 9.3:	Confirmation of RP105 expression using real-time PCR.	191

Figure 9.4:	Effect of LPS and IL-4 on cell surface CD14 expression by human monocytes.	193
Figure 9.5:	Effect of LPS and IL-4 on cell surface RP105 and TLR4 expression by human monocytes.	195
Figure 9.6:	Intracellular expression of RP105 in untreated monocytes.	197
Figure 9.7:	Effect of LPS and IL-4 on intracellular RP105 expression in human monocytes.	198
Figure 9.8:	Suppression of LPS- and Pam3Cys-induced TNF α production by IL-4 in human monocytes.	198
Figure 10.1:	Summary of the mechanisms by which IL-4 exerts its anti-inflammatory actions in human monocytes identified in this thesis.	224

List of Tables

Table 1.1:	Pro-inflammatory mediators.	12
Table 1.2:	Anti-inflammatory cytokines.	13
Table 1.3:	Biological therapies currently in use for the treatment of chronic autoimmune and inflammatory conditions.	19
Table 1.4:	Mechanisms of the anti-inflammatory actions of IL-4 in human and murine monocytes and macrophages.	37
Table 1.5:	Differences in IL-4 signalling and suppression of LPS-induced cytokines by monocytes, monocyte derived macrophages (MdMacs) and synovial fluid macrophages (SF).	39
Table 2.1:	List of inhibitors used to assess functional responses to IL-4.	48
Table 2.2:	List of antibodies sets used for TRF assays.	50
Table 2.3:	List of primers used for real-time PCR.	54
Table 2.4:	Real-time PCR conditions.	55
Table 2.5:	List of antibodies used for Western blotting.	58
Table 5.1:	Histone Deacetylase (HDAC) Enzymes.	101
Table 5.2:	Therapeutic benefits of HDAC inhibitors in the treatment of inflammatory disease.	101
Table 5.3:	Summary of the effect of HDAC inhibition on TNF α , IL-10 and PGE ₂ production by human monocytes incubated with LPS \pm IL-4	109
Table 6.1:	Summary of the factors that induce SOCS family proteins and the physiological processes that are regulated by SOCS proteins.	122
Table 7.1:	Summary of TREM-2 and PPAR γ expression.	151

Table 8.1:	Fold-change (mean \pm SEM) in mRNA expression for 84 genes in human monocytes treated for 1, 2 or 3 h with LPS (500 ng/ml) with or without IL-4 (10 ng/ml).	165
Table 9.1:	Summary of the effect of LPS and IL-4 on RP105, TLR4 and CD14 expression in human monocytes.	199
Table 10.1:	Differences in responses to IL-4 by freshly isolated monocytes (Fresh) and monocytes cultured overnight (Overnight).	210

Abbreviations

°C	degrees Celsius
15d-PGJ ₂	15-deoxy- $\Delta^{12,14}$ -Prostaglandin J ₂
3'UTR	3' untranslated region
ACPA	anti-citrullinated protein antibody
AdV	adenovirus
AP-1	activating protein 1
APC	allophycocyanin
ARE	AU-rich elements
ATF2	activating transcription factor 2
BCL-6	B cell lymphoma 6
BMM	bone marrow-derived macrophages
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CBP	CREB-binding protein
ChIP	chromatin immunoprecipitation
CIA	collagen induced arthritis
CIS	cytokine-inducible SH2-containing protein
COX	cyclooxygenase
CREB	cAMP response element binding
CT	cycle threshold
DC	dendritic cell
DMARD	disease-modifying anti-rheumatic drug

DMEM	Dulbecco's modified Eagle's medium
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECM	extracellular matrix
EDTA	ethylene diamine tetraacetic acid
EGF	epidermal growth factor
EMSA	electrophoretic mobility shift assay
ERK	extracellular signal-regulated kinase
FITC	fluorescein isothiocyanate
GKN	glucose potassium sodium buffer
GKN-BSA	glucose potassium sodium buffer supplemented with 0.2% BSA
GM-CSF	granulocyte macrophage colony-stimulating factor
GTP	guanosine-5'-tri-phosphate
h	hours
HAT	histone acetyl transferase
HBSS	Hanks balanced salt solution
HDAC	histone deacetylase
HEPES	N-2-hydroxyethyl piperazine-N-ethane sulfonic acid
HLA	human leukocyte antigen
HRP	horseradish peroxidase
I4R	insulin IL-4 receptor
IFN	interferon
Ig	immunoglobulin
IGF	insulin-like growth factor
IKK	I κ B kinase

IL	interleukin
IL-1ra	IL-1 receptor antagonist
IRAK	interleukin-1 receptor associated kinase
IRF	interferon regulatory factor
IRS	insulin receptor substrate
JAK	Janus kinase
Jmjd3	Jumonji domain containing-3
JNK	c-Jun N-terminal kinase
LBP	lipid binding protein
LIF	leukaemia inhibitor factor
LPS	lipopolysaccharide
μ Ci	microCurie
μ g	micrograms
μ l	microlitres
μ M	micromolar
MAL	MyD88-adaptor like
MAPK	mitogen-activated protein kinase
M-CSF	macrophage colony-stimulating factor
MdMac	monocyte-derived macrophages
MFI	mean fluorescence intensity
mg	milligram
MHC	major histocompatibility complex
min	minutes
miR	micro-RNA
ml	millilitres

mM	millimolar
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
NCoR	nuclear hormone receptor co-repressor
NF- κ B	nuclear factor kappa B
NO	nitric oxide
NOD	nucleotide-binding oligomerization domain containing
NSAID	non-steroidal anti-inflammatory drug
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PCR	polymerase chain reactions
PE	phycoerythrin
pg	picograms
PGE ₂	prostaglandin E ₂
pH	potential of hydrogen
PI3K	phosphoinositide-3-kinase
PIAS	protein inhibitors of activated STATs
PKC	protein kinase C
PMA	phorbol ester myristate
PPAR	proliferator-activated receptor
PPRE	peroxisome proliferator response elements
PTB	phosphotyrosine binding
RA	rheumatoid arthritis
rh	recombinant human
RIPK2	receptor interacting serine-threonine-protein kinase 2

rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute 1640 (media)
RT	room temperature
RXR	retinoid X receptor
SAHA	suberoylanilide hydroxamic acid
SDS	sodium dodecyl sulfate
sec	seconds
SEM	standard error of the mean
SH2	src-homology 2
SHIP	SH2-containing inositol 5' phosphatase
SHP	SH2-containing phosphatase
SMRT	silencing mediator for retinoic acid receptor and thyroid hormone receptor
SOCS	suppressors of cytokine signalling
SRF	serum response factor
STAT	signal transducers and activators of transcription
TAB2	transforming growth factor β activated kinase 1 binding protein 2
TAK1	transforming growth factor β activated kinas
TBS	Tris buffered saline
TGF	transforming growth factor
Th	T helper
TIR	toll/ interleukin-1 receptor
TLR	toll-like receptor
TNF	tumour necrosis factor
TPA	tetradecanoyl phorbol acetate

TRAF6	tumour necrosis factor receptor activated factor 6
TRF	time-resolved fluorescence (assay)
TRIF	TIR-domain-containing adapter-inducing interferon- β
TREM-2	triggering receptor expressed on myeloid cells-2
TSA	trichostatin A
TZD	thiazolidinedione
UBE2D2	ubiquitin-conjugating enzyme E2D2
$\times g$	multiplied by the acceleration of gravity

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Publications Arising from this Thesis

- Woodward EA, Kolesnik TB, Nicholson SE, Prêle CM, Hart PH. The anti-inflammatory actions of IL-4 in human monocytes are not mediated by IL-10, RP105 or the kinase activity of RIPK2. Submitted to *Cytokine*. 6th Dec 2011.

Data included in Chapters 8 and 9 of this thesis

- Woodward EA, Prêle CM, Nicholson SE, Kolesnik TB, Hart PH. (2010). The anti-inflammatory effects of interleukin-4 are not mediated by suppressor of cytokine signalling-1 (SOCS1). *Immunology*. 131: 118-27.

Data included in Chapters 3, 4 and 6 of this thesis

- Prêle CM, Woodward EA, Bisley J, Keith-Magee A, Nicholson SE, Hart PH. (2008). SOCS1 regulates the IFN but not NFkappaB pathway in TLR-stimulated human monocytes and macrophages. *J Immunol*. 181: 8018-26.

Data included in Chapter 6 of this thesis

