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.

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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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Eleanor Anne Woodward

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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 TNFα transcription, without inhibiting LPS signalling through IκB, the mitogen-activated protein kinase (MAPK) pathways, or LPS-mediated activation of the transcription factor NF-κB. 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 (PPARγ) and triggering receptors expressed on myeloid cells-2 (TREM-2), but suppressed LPS-induced TNFα 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-κB-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 TNFα 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.

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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	IκB 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
µCi	microCurie
µg	micrograms
µl	microlitres
µM	micromolar
MAL	MyD88-adapter 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-κB	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
x *g*	multiplied by the acceleration of gravity

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Publications Arising from this Thesis

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- 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