MODULATION OF MACROPHAGE FUNCTION AND IMMUNE RESPONSE BY A HELMINTH-DERIVED CYSTEINE PROTEASE

A thesis submitted for the Degree of
Doctor of Philosophy

by

Stephanie Nicole Dowdell B.Sc. (Hons)

School of Medical and Molecular Biosciences
Faculty of Science
University of Technology Sydney, Australia
2013

CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that this thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

_____

Stephanie Nicole Dowdell

2013

ACKNOWLEDGEMENTS

There are a number of people that I would like to thank, who have provided support for me during the completion of my thesis. My PhD project originally focused on a different topic, however, after more than a years' worth of work, results were ultimately not reproducible. I am therefore thankful for my supervisors, Associate Professor Bronwyn O'Brien, Dr Najah Nassif and Dr Andrew Hutchinson, who established a new project for me and shared their expertise and knowledge. Thank you also to Dr Lisa Sedger for advice and critical scientific discussions, I'm forever grateful.

Thank you to Dr Nicholas Archer, who taught me so much during the early years, your support and friendship has been invaluable. My fellow postgraduate students, particularly Charmain Castel with our cups of tea each morning, have been a godsend and I know that I have made friends for life during my time at UTS. Thanks also to my best friend Helen Thompson, who provided encouragement and a listening ear when I needed it most.

To the Helmedix group, including Dr Sheila Donnelly, Dr Mark Robinson, Dr John Dalton, Joyce To, Raquel Alvarado and Maria Lund. Thank you for your expertise, your discussions and advice. It's been a pleasure working with all of you, especially Raquel and Maria, and I wish you all great success with the current research focus.

Finally and most importantly, to my parents, Rosalyn and Robert Dowdell, who have stood by me through these long years, picking me up at the low points and cheering me on during the highs. Without your love and support, this would not have been possible. This thesis is dedicated to you. I will never be able to thank you enough.

PUBLICATIONS IN PEER-REVIEWED JOURNALS

Mark W. Robinson, Raquel Alvarado, Joyce To, Andrew T. Hutchinson, **Stephanie N. Dowdell**, Maria Lund, Lynne Turnbull, Bronwyn A. O'Brien, John P. Dalton and Sheila Donnelly. (2012). 'A helminth cathelicidin-like protein suppresses antigen processing and presentation in macrophages via inhibition of lysosomal vATPase', *The FASEB Journal*, 26(11): 4614-27.

PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

Andrew T. Hutchinson & **Stephanie N. Dowdell**, Maria Lund, Mark W. Robinson, John P. Dalton, Bronwyn A. O'Brien, Sheila Donnelly. (2010). 'A helminth cysteine protease inhibits TRIF-dependent LPS activation of human macrophages.' Australasian Society of Immunology Scientific Meeting. Perth. (Oral presentation).

Stephanie N. Dowdell, Lynne Turnbull, Cynthia B. Whitchurch, Bronwyn A. O'Brien, Andrew T. Hutchinson. (2010). 'TLR4 endosomes are localised to tunnelling nanotubes in human macrophages.' Australasian Society of Immunology Scientific Meeting. Perth. (Poster presentation).

Stephanie N. Dowdell, Lynne Turnbull, Cynthia B. Whitchurch, Bronwyn A. O'Brien, Andrew T. Hutchinson. (2010). 'TLR4 endosomes are localised to tunnelling nanotubes in human macrophages.' RNSH/UTS Scientific Research Meeting. Sydney. (Oral presentation).

Stephanie N. Dowdell, Najah Nassif, Bronwyn A. O'Brien. (2008). 'Functional Studies of *SLC11A1* gene polymorphisms.' RNSH/UTS Scientific Research Meeting. Sydney. (Oral presentation).

AWARDS

Awarded the Poster Prize for the 2011 Science Research Day at the University of Technology, Sydney.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ΔCt	Change in cycle threshold
A	Absorbance
ADCC	Antibody-dependent cell-mediated cytotoxicity
AF	Alexa Fluor
AP-1	Activating protein-1
APCs	Antigen presenting cells
Arg1	Arginase 1
bp	Base pair
BSA	Bovine serum albumin
CBA	Cytokine bead array
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
C_t	Threshold cycle
CTLA	Cytotoxic T lymphocyte antigen
Cy5	Cyano 5
DAPI	4',6'-diamidino-2-phenylindole
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleoside triphosphate (or deoxyribonucleotide)
dsRNA	Double stranded ribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EEA-1	Early endosome antigen-1
ERK	Extracellular signal-related kinase
ES	Excretory/secretory
FACS	Fluorescence activated cell sorting
FBS	Foetal bovine serum
FhCL1	*Fasciola hepatica* cathepsin L1
FhES	*Fasciola hepatica* excretory/secretory products
FITC	Fluoroscein isothiocyanate
Fizz1	Resistin like alpha
Foxp3	Forkhead box p3
FSC	Forward Scatter
FSW	FACS Staining Wash
GAD	Glutamate decarboxylase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HDM-1	Helminth defence molecule 1
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IFN	Interferon
Ig	Immunoglobulin
IκBα	Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α
IKK	IκB kinase
IL	Interleukin
IMDM	Iscove's modified Dulbecco's medium

iNOS	Inducible nitric oxide synthase
IRAK	IL-1R-associated kinase
IRF	Interferon regulatory factor
JNK	c-Jun NH2-terminal kinases
kDa	Kilo dalton
KEGG	Kyoto encyclopedia of genes and genomes
LBP	LPS-binding protein
LNFPIII	Lacto-N-fucopentaose III
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MD-2	Myeloid differentiation protein-2
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
MMSCs	Mesenchymal multipotent stromal cells
mRNA	Messenger ribonucleic acid
MyD88	Myeloid differentiation primary response gene 88
NF-κB	Nuclear factor κ B
NO	Nitric oxide
NOD	Non-obese diabetic
O/N	Overnight
p	Phosphorylated
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Poly (I·C)	Polyinosinic:polycytidylic acid
Prx	Peroxiredoxin
RT-qPCR	Reverse transcriptase-quantitative polymerase chain reaction
RNA	Ribonucleic acid
RNase	Ribonuclease
RPL36AL	Ribosomal protein L36A like
RPMI	Roswell Park Memorial Institute
RT	Room temperature
RT-PCR	Reverse transcriptase-polymerase chain reaction
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEA	Soluble egg antigen
SSC	Side scatter
T1D	Type 1 diabetes
TBK	TRAF-family-member-associated NF-κB activator binding kinase
TGF	Transforming growth factor
Th	T helper
TIR	Toll-interleukin-1 receptor
TIRAP	Toll-interleukin-1 receptor domain containing adaptor protein
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TRAF	Tumour necrosis factor receptor-associated factor
TRAM	TRIF-related adaptor molecule
TRIF	Toll-interleukin-1 receptor-domain-containing adaptor-inducing interferon-β
Ym1	Chitinase 3-like 3

ABSTRACT

Helminth-derived excretory/secretory (ES) products have been demonstrated to mediate the anti-inflammatory/regulatory environment associated with helminth infection (for a review see Allen *et al.* 2011). The ES products of helminths have been exploited for therapeutic benefit in both murine and human models of autoimmune diseases (Zaccone *et al.* 2003; Zheng *et al.* 2008; Motomura *et al.* 2009; Ruyssers *et al.* 2009; Johnston *et al.* 2010; Cancado *et al.* 2011; Carranza *et al.* 2012; Kuijk *et al.* 2012). In our laboratory, the ES products of the liver fluke trematode, *Fasciola hepatica*, have been shown to prevent autoimmune type 1 diabetes in a murine model (Lund *et al.* in preparation). Disease prevention was associated with the initiation and perpetuation of anti-inflammatory/regulatory immune responses, including the generation of alternatively activated macrophages, regulatory T cells and regulatory B cell populations (Lund *et al.* in preparation). Nevertheless, the individual molecular components within the ES responsible for these phenomenon are unknown. Therefore, HPLC fractionation of the ES products of *Fasciola hepatica* was undertaken. This revealed a number of components with immune-modulatory effects. One of these *Fasciola hepatica* products is a cysteine protease, cathepsin L1 (FhCL1), and in fact it comprises a large proportion of the total ES products. In mice, FhCL1 suppresses pro-inflammatory immune responses through cleavage of toll-like receptor (TLR)-3, resulting in modulation of cell signalling in peritoneal macrophages (Donnelly *et al.* 2010).

This thesis therefore examines the effect of FhCL1 in human monocyte-derived macrophages. FhCL1 was shown to enhance expression of pro-inflammatory cytokines IL-6 and IL-8 in response to lipopolysaccharide. This was associated with the up-regulation of surface CD14, and the activation of TLR4 cell signalling via both the myeloid differentiation primary response gene 88 (MyD88)-dependent and toll-interleukin-1 receptor-domain-containing adaptor-inducing interferon-β (TRIF)-dependent signalling pathways. Furthermore, expression of IL-10 and co-stimulatory molecule CD86 was down-regulated in FhCL1-treated human monocyte-derived macrophages, and this was attributed to suppression of late endosomal TRIF-dependent signalling, with down-regulation of TRAF3. Although, FhCL1 modulated TLR

signalling in human and murine macrophages, and suppressed TRIF-dependent signalling in both human and mouse macrophages, FhCL1 enhanced pro-inflammatory cytokine expression in human monocyte-derived macrophages. Therefore, FhCL1 modulates immune responses in human monocyte-derived macrophages, albeit differently from murine peritoneal macrophages. Furthermore, while FhCL1 degraded TLR3 in murine peritoneal macrophages, FhCL1 had no effect on TLR3 or TLR4 expression or localisation in human monocyte-derived macrophages. However, treatment with FhCL1 was shown to suppress the uptake of lipopolysaccharide (LPS) by human macrophages, which appeared to correlate with altered α-tubulin localisation. Thus suppressed uptake of LPS correlates with the suppression of TRIF-dependent late endosomal signalling.

Nanotubes are cellular protrusions which connect cells and are utilised for the transport of cellular components between cells (reviewed in Gerdes *et al.* 2008; and Gurke *et al.* 2008). An incidental finding of this study was the observation of nanotubes connecting monocyte-derived macrophages in culture, and the documented trafficking of TLR4 between human macrophages, through these nanotubes. Interestingly, LPS stimulation enhanced the movement of TLR4 into nanotubes, but this was partially suppressed by FhCL1.

Taken together, the work presented in this thesis provides insight into the mechanism of action of FhCL1 in human monocyte-derived macrophages. Investigating the immune-modulatory effects of individual helminth-derived molecules is an important step in understanding the mechanisms by which helminths modulate host immune responses. Ultimately, such products may be harnessed as potential therapeutic agents in various situations, depending on their effect on the immune system.