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Characterisation of an immune-modulating peptide secreted by a helminth worm

A thesis submitted for the degree of

Doctor of Philosophy

by

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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 part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the 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.

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Publications in peer-reviewed journals arising from PhD research

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-	Negative
+	Positive/ plus
±	Plus/minus
↑	Increase
↓	Decrease
^{o}C	Degrees celsius
α	Alpha/anti
β	Beta
δ/Δ	Delta
κ	Kappa
γ	Gamma
µg	Microgram
µl	Microlitre
µm	Micrometre
µM	Micromolar
BHK	Baby hamster kidney cells
BMDM	Bone marrow derived macrophage
CD	Cluster of differentiation
CDAI	Crohn's disease activity index
cDNA	Complementary deoxyribonucleic acid
CEMA	Cationic alpha helical peptide
ChIP	Chromatin immunoprecipitation
CIA	Collagen-induced arthritis
CNS	Central nervous system
CPP	Cell-penetrating peptide
C_t	Cycle threshold value
CXCL	C-X-C-motif chemokine ligand
DAPI	4',6-Diamidine-2'-phenylindole dihydrochloride
DC	Dendritic cell
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
DNBS	Dinitrobenzene sulfonic acid

dNTP	Deoxynucleotide triphosphate
DSS	Dextran sodium sulfate
EAE	Experimental autoimmune encephalomyelitis
EC50	Half maximal effective concentration
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
ES	Excretory/Secretory (products)
FACS	Fluorescence activated cell sorting
FDR	False discovery rate
FhCL1	*Fasciola hepatica* cathepsin L1
FhES	*Fasciola hepatica* excretory/secretory (products)
FhHDM-1	*Fasciola hepatica* helminth defence molecule-1
FhPrx	*Fasciola hepatica* peroxiredoxin
FSC-A	Forward scatter profile
FSC-H	Forward scatter height profile
FSW	Flow cytometry staining wash
GM-CSF	Granulocyte macrophage colony stimulating factor
h	hours
HDP	Host defence peptide
IBD	Inflammatory bowel disease
ICD	Idiopathic chronic diarrhoea
IDR	Innate defence regulator
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMP	Investigational medicinal product
i.p.	Intraperitoneal
IPA	Ingenuity pathway analysis
kDa	Kilodalton
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
LXR	Liver X receptor
M1	Pro-inflammatory macrophage
M2	Anti-inflammatory macrophage
MCP-1	Monocyte chemotactic protein-1

M-CSF	Macrophage colony stimulating factor
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
mer	Amino acid residues
min	Minutes
MIP	Macrophage inflammatory protein
ml	Mililitre
MLDS	Multiple-low dose streptozotocin
Mphi	Macrophages
MPO	Myeloperoxidase
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
msec	Milliseconds
NEJ	Newly excysted juvenile
NET	Neutrophil extracellular trap
ng	Nanograms
NK	Natural killer cells
nm	Nanometre
NOD	Non-obese diabetic (mice)
OD	Optical density
O/N	Overnight
Ova	Ovalbumin
Ov-HDM	*Opisthorchis viverrini* helminth defence molecule
PI	Peak I
PII	Peak II
PC	Phosphorylcholine
PMA	Phorbol myristate acetate
Poly (I:C)	Polyinosinic:polycytidylic acid
PPAR	Peroxisome proliferator activated receptor
PSMβ1	Proteasome subunit, beta type1
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RA	Rhematoid arthritis
Relm-α	Resistin-like molecule alpha
RNA	Ribonucleic acid

ROS	Reactive oxygen species
RRMS	Relapsing-remitting multiple sclerosis
RT	Room temperature
RXR	Retinoid X receptor
SEA	Soluble egg antigens
sec	Seconds
SEM	Standard error of mean
SLE	Systemic lupus erythematous
SNP	Single nucleotide polymorphisms
SUMO	Small ubiquitin-like modifier proteins
SPMS	Secondary progressive multiple sclerosis
sup	Supernatant
SSC-A	Side scatter profile
TGF-β	Transforming growth factor-beta
Th1	T-helper 1
Th2	T-helper 2
Th17	T-helper 17
TID	Type 1 diabetes
TIM-3	T cell immunoglobulin domain and mucin domain-3
TLR	Toll-like receptor
TNBS	Trinitrobenzene sulfonic acid
TNF	Tumour necrosis factor
Treg	T-regulatory
uPA	urokinase-type plasminogen activator
vATPase	Vacuolar ATPase
v/v	Volume/volume
x g	Relative centrifugal force

Abstract

Parasitic worms (helminths) have evolved mechanisms to potently modulate the mammalian immune response to ensure their long-term survival, while concomitantly preventing excessive tissue pathology within the host. The outcome of this activity is a potent suppression of mammalian pro-inflammatory Th1 and Th17 immune responses. This immune-modulatory phenomenon is attributable to the molecules excreted/secreted by helminths as they migrate through their human hosts. The identification of these molecules has the potential to contribute to the development of novel therapeutics for the treatment of autoimmune diseases as these diseases are mediated by pro-inflammatory Th1 and Th17 immune responses.

It has previously been shown that the delivery of a single peptide, FhHDM-1, which is excreted/secreted from the helminth, *Fasciola hepatica*, prevented the development of murine type 1 diabetes and multiple sclerosis. The aetiological similarity between these two diseases, is the establishment of the pro-inflammatory environment by macrophages and neutrophils. Therefore, it was hypothesised that FhHDM-1 likely mediated its protective effect through anti-inflammatory mechanisms directed at these cells. In this study, it was confirmed that FhHDM-1, specifically interacted with both murine and human neutrophils and macrophages. Furthermore, it was demonstrated that FhHDM-1 modulated the activity of these cells, by inhibiting the secretion of pro-inflammatory cytokines/chemokines, which, in turn, prevented further activation of pro-inflammatory T cells and dendritic cells.

The use of transcriptional profiling revealed that, of the 41,436 genes analysed in macrophages, treatment with FhHDM-1 altered the expression levels of only 6 of these. Of these, only the expression level of *SerpinB2* was increased, and this was shown to subsequently mediate the suppression of pro-inflammatory cytokine secretion by T cells. In the context of an inflammatory stimulus, FhHDM-1 regulated the expression of numerous pro-inflammatory genes in macrophages. Pathway analysis predicted that this anti-inflammatory activity was mediated through the activation of PPAR-γ pathways. This is the first report of a PPAR-γ agonist being secreted by a helminth parasite as a mechanism of modulating mammalian innate immune responses.

Importantly, a homologous parasite peptide from a related trematode parasite demonstrated the same activity suggesting a conserved mechanism of action among parasite helminths.

Analysis of the sequence of the FhHDM-1 peptide, combined with immunological assays of peptide derivatives, supported the discovery of the minimally active sequence, FhHDM-1.C2. This peptide contained both the amphipathic C-terminus region, previously identified as functional, and a sequence of 5 amino acids (KARDR), which was newly identified as essential for the binding and internal localisation of FhHDM-1. This C2 derivative mimicked the activity of the full length peptide in every way, such as the ability to bind and be internalised by macrophages, to increase macrophage lysosomal pH, to induce the expression of *SerpinB2*, and to inhibit the production of pro-inflammatory cytokines.

In summary, this thesis has newly identified the binding and active domains of the parasite immune-modulatory peptide, FhHDM-1, and discovered the novel mechanisms by which the peptide regulates pro-inflammatory innate immune responses. Combined, these findings have significantly advanced the progress towards translation of the FhHDM-1 peptide for therapeutic use in immune-mediated diseases.