

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

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A thesis submitted for the degree of

Doctor of Philosophy

by

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## Certificate of Authorship/ Originality

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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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## List of Abbreviations

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-	Negative
+	Positive/ plus
±	Plus/minus
↑	Increase
↓	Decrease
°C	Degrees celsius
α	Alpha/anti
β	Beta
δ/Δ	Delta
κ	Kappa
γ	Gamma
μg	Microgram
μl	Microlitre
μm	Micrometre
μM	Micromolar
<b>BHK</b>	Baby hamster kidney cells
<b>BMDM</b>	Bone marrow derived macrophage
<b>CD</b>	Cluster of differentiation
<b>CDAI</b>	Crohn's disease activity index
<b>cdNA</b>	Complementary deoxyribonucleic acid
<b>CEMA</b>	Cationic alpha helical peptide
<b>ChIP</b>	Chromatin immunoprecipitation
<b>CIA</b>	Collagen-induced arthritis
<b>CNS</b>	Central nervous system
<b>CPP</b>	Cell-penetrating peptide
<b>C<sub>t</sub></b>	Cycle threshold value
<b>CXCL</b>	C-X-C-motif chemokine ligand
<b>DAPI</b>	4',6-Diamidino-2'-phenylindole dihydrochloride
<b>DC</b>	Dendritic cell
<b>DMSO</b>	Dimethyl sulfoxide
<b>DNA</b>	Deoxyribonucleic acid
<b>DNase</b>	Deoxyribonuclease
<b>DNBS</b>	Dinitrobenzene sulfonic acid

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

<b>M-CSF</b>	Macrophage colony stimulating factor
<b>MFI</b>	Mean fluorescence intensity
<b>MHC</b>	Major histocompatibility complex
<b>mer</b>	Amino acid residues
<b>min</b>	Minutes
<b>MIP</b>	Macrophage inflammatory protein
<b>ml</b>	Mililitre
<b>MLDS</b>	Multiple-low dose streptozotocin
<b>Mphi</b>	Macrophages
<b>MPO</b>	Myeloperoxidase
<b>MRI</b>	Magnetic resonance imaging
<b>mRNA</b>	Messenger ribonucleic acid
<b>MS</b>	Multiple sclerosis
<b>msec</b>	Milliseconds
<b>NEJ</b>	Newly excysted juvenile
<b>NET</b>	Neutrophil extracellular trap
<b>ng</b>	Nanograms
<b>NK</b>	Natural killer cells
<b>nm</b>	Nanometre
<b>NOD</b>	Non-obese diabetic (mice)
<b>OD</b>	Optical density
<b>O/N</b>	Overnight
<b>Ova</b>	Ovalbumin
<b>Ov-HDM</b>	<i>Opisthorchis viverrini</i> helminth defence molecule
<b>PI</b>	Peak I
<b>PII</b>	Peak II
<b>PC</b>	Phosphorylcholine
<b>PMA</b>	Phorbol myristate acetate
<b>Poly (I:C)</b>	Polyinosinic:polycytidylic acid
<b>PPAR</b>	Peroxisome proliferator activated receptor
<b>PSM<math>\beta</math>1</b>	Proteasome subunit, beta type1
<b>qRT-PCR</b>	Quantitative reverse transcriptase polymerase chain reaction
<b>RA</b>	Rhematoid arthritis
<b>Relm-<math>\alpha</math></b>	Resistin-like molecule alpha
<b>RNA</b>	Ribonucleic acid

<b>ROS</b>	Reactive oxygen species
<b>RRMS</b>	Relapsing-remitting multiple sclerosis
<b>RT</b>	Room temperature
<b>RXR</b>	Retinoid X receptor
<b>SEA</b>	Soluble egg antigens
<b>sec</b>	Seconds
<b>SEM</b>	Standard error of mean
<b>SLE</b>	Systemic lupus erythematosus
<b>SNP</b>	Single nucleotide polymorphisms
<b>SUMO</b>	Small ubiquitin-like modifier proteins
<b>SPMS</b>	Secondary progressive multiple sclerosis
<b>sup</b>	Supernatant
<b>SSC-A</b>	Side scatter profile
<b>TGF-<math>\beta</math></b>	Transforming growth factor-beta
<b>Th1</b>	T-helper 1
<b>Th2</b>	T-helper 2
<b>Th17</b>	T-helper 17
<b>T1D</b>	Type 1 diabetes
<b>TIM-3</b>	T cell immunoglobulin domain and mucin domain-3
<b>TLR</b>	Toll-like receptor
<b>TNBS</b>	Trinitrobenzene sulfonic acid
<b>TNF</b>	Tumour necrosis factor
<b>Treg</b>	T-regulatory
<b>uPA</b>	urokinase-type plasminogen activator
<b>vATPase</b>	Vacuolar ATPase
<b>v/v</b>	Volume/volume
<b>x g</b>	Relative centrifugal force

## Abstract

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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- $\gamma$  pathways. This is the first report of a PPAR- $\gamma$  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.