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

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 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.

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Stephanie Nicole Dowdell

2013

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## PUBLICATIONS IN PEER-REVIEWED JOURNALS

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## PAPERS PRESENTED AT SCIENTIFIC CONFERENCES

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## AWARDS

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 LIST OF ABBREVIATIONS

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

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

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## 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; Ruysers *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- $\beta$  (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  $\alpha$ -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 immunomodulatory 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.