Identification of host cell proteins involved in *Shigella flexneri* pathogenesis

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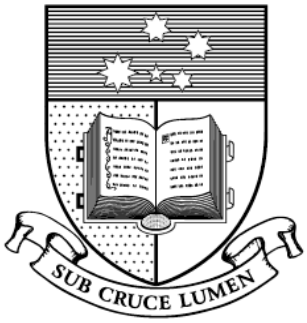

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Abstract

Shigella flexneri is the etiological agent of bacillary dysentery (shigellosis). It is transmitted via the faecal-oral route and is a significant human pathogen due to the high morbidity among children <5 years in developing countries. The key pathogenic features of *Shigella* include cell death induction in myeloid immune cells and circumventing cell death in colonic epithelial cells, the site of bacterial infection. *Shigella* also interact with host proteins to initiate *de novo* actin synthesis to facilitate its intra- and intercellular spread to disseminate in the host.

In this thesis, the role of three host proteins: myosin IIA, dynamin II, and dynamin-related protein 1 (Drp1) during *Shigella* cell-to-cell spreading was examined. The myosin IIA specific kinase, myosin like chain kinase (MLCK), was previously shown to be important for *Shigella* plaque formation. Myosin IIA and MLCK have also been implicated in septin caging of non-motile *Shigella* which are targeted for degradation. Chemical inhibition and siRNA knockdown of myosin IIA reduced *Shigella* plaque formation. Curiously HeLa cells infected with *Shigella* mutants defective in cell-to-cell spreading have significantly reduced myosin IIA levels when quantified by immunofluorescence microscopy.

Dynamin II and Drp1 are members of the dynamin superfamily. Both proteins have self-assembly driven GTPase activation. Dynamin II is important for clathrin-mediated endocytosis and pinches the budding clathrin-coated vesicle, and Drp1 is essential for mitochondrial fission. It was hypothesized that *Shigella* protrusion formation into adjacent host cells resembles endocytic and exocytic processes, and components of these processes may facilitate *Shigella* dissemination. When dynamin II GTPase was inhibited with dynasore and dynamin II was knocked down with siRNA, *Shigella* cell-to-cell spreading was significantly reduced. The *in vivo* efficacy of dynasore was tested in a murine Sereny model. No significant reduction in inflammation was observed but mice were protected against weight loss during infection. Further experimentation suggested dynasore protected mice against cytotoxic effects from the three secretion system (TTSS) effectors expressed by *Shigella* during infection.

Drp1 was investigated in this thesis as dynasore also inhibits the GTPase of this mitochondrial fission protein. Mitochondrial fission is important in maintaining mitochondrial

dynamics and also in events downstream of intrinsic apoptosis and programmed necrosis pathways activation. Loss of mitochondrial function in *Shigella*-induced epithelial cell death has been reported previously. Hence the role of Drp1 in *Shigella* plaque formation and HeLa death was examined with the Drp1-specific inhibitor, Mdivi-1, and siRNA knockdown. HeLa cell death was significantly reduced; suggesting loss of mitochondrial function observed previously may now be attributed to Drp1 and subsequent Drp1-mediated mitochondrial fission. The impairment in *Shigella* cell-to-cell spreading in the absence of Drp1 suggests maintaining an intact mitochondrial network is essential for *Shigella* lateral spread since loss of Drp1 function would result in excessive mitochondrial fusion, leading to formation of net-like or perinuclear structures.

The outcomes of this thesis highlight the importance of host proteins during different stages of *Shigella* infection. By improving our understanding on the host and bacteria interaction, future work on novel approaches to prevent *Shigella* dissemination can be developed.

Declaration

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Publications

Lum M, Attridge SR & Morona R (2013). Impact of dynasore an inhibitor of dynamin II on *Shigella flexneri* Infection. *PLoS One* 8, e84975.

Lum M & Morona R (2014). Dynamin-related protein Drp1 and mitochondria are important for *Shigella flexneri* infection. *Int J Med Microbiol* 304, 530-541.

Lum M & Morona R (2014). Myosin IIA is essential for *Shigella flexneri* cell-to-cell spread. *Pathog Dis*, DOI: 10.1111/2049-632X.12202.

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Abbreviations

~	approximately
aa	amino acids
ABM	actin-based motility
ADP	adenosine diphosphate
AJ(s)	adherens junction(s)
APC(s)	apical junctional complex(es)
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BDM	2,3-butanedione monoxime
BSE	bundle signalling element
CFU	colony forming units
D0 / D1 / D2 / D3	day 0 / day 1 / day 2 / day 3
DCCR	DharmaFECT Cell Culture Reagent
DLP(s)	dynamin-like protein(s)
DLP1	dynamin-like protein 1 (alternate name for DNM1L/Drp1)
DNM1L	dynamin-1-like protein (alternate name for DLP1/Drp1)
DNM2	dynamin II gene
DMSO	dimethyl sulfoxide
Drp1	dynamin-related protein 1 (alternate name for DLP1/DNM1L)
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GED	GTPase effector domain
GTP	guanosine triphosphate
GTPase(s)	guanosine triphosphatase(s)
h	hour(s)
IF	immunofluorescence
IP	intraperitoneal
kDa	kilodaltons
LDH	lactate dehydrogenase
Lo	low myosin IIA protein levels
LPS	lipopolysaccharide

MEFs	mouse embryonic fibroblasts
Mdivi-1	*m*itochondrial *div*ision *i*nhibitor-*1*
MYH9	myosin, heavy chain 9, non-muscle gene (myosin IIA heavy chain gene)
min	minute(s)
MLCK	myosin light chain kinase
moi	multiplicity of infection
MOMP	mitochondrial outer membrane permeabilisation
myosin IIA / B / C	non-muscle myosin IIA / B / C
N-WASP	Neural Wiskott-Aldrich syndrome protein
NF-κB	nuclear factor-κB
NMP	*N*-methyl-2-pyrrolidone
NPF(s)	nucleation promoting factor(s)
OM	outer membrane
PEG / PEG300	polyethylene glycol 300
PGN	peptidoglycan
PH	pleckstrin homology
PMN(s)	polymorphonuclear cell(s)
PRD(s)	proline-rich domain(s)
PtK2	*Potorous tridactylis* kidney epithelial (cells)
R-LPS	rough LPS
ROS	reactive oxygen species
S-LPS	smooth LPS
siRNA	small interfering RNA
STS	staurosporine
t	time
TJ(s)	tight junction(s)
TTSS	type three secretion system
VP	virulence plasmid
VP^{-} / VP-	virulence plasmid-cured
WT	wild type