HELICOBACTER PYLORI: REDUCED PHAGOCYTIC KILLING AND ALTERED PHAGOSOME MATURATION IN PRIMARY HUMAN MACROPHAGES

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ABSTRACT

Helicobacter pylori (*H. pylori*) colonises the human gastric mucosa and is the principal causative agent of gastric and duodenal ulcers. Long term infection with *H. pylori* represents a major risk for the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. It is estimated that half of the world's population is infected with *H. pylori* with rates of infection up to 90% in the developing world. Despite eliciting a vigorous and sustained immune response in the host, *H. pylori* is able to persist in the gastric mucosa for life. In this study we have developed an *in vitro* infection model to (1) investigate the ability of primary human monocytes and macrophages to effectively kill *H. pylori* and (2) examine the process of *H. pylori* phagosome maturation in infected macrophages.

Five *H. pylori* strains were selected on the basis of their clinical phenotype and characterised for the VacA (vacuolating cytotoxin), *cag*PAI (*cag* Pathogenicity Island), urease and catalase virulence factors by Western blot and PCR analysis. Each strain possessed a unique combination of virulence factors and there was only limited correlation between molecular typing results and clinical phenotype.

These five *H. pylori* strains were then used to individually infect i*n vitro* cultures of primary human monocytes and macrophages. At various time points after infection, the infected monocytes and macrophages were lysed and the remaining viable bacteria were counted to determine phagocytic killing efficacy. Primary human monocytes had a higher capacity to kill certain strains of *H. pylori* when compared to macrophages. Three of the *H. pylori*

strains were killed by monocytes after 48 hours whereas none of the *H. pylori* strains were killed by macrophages over the same time. There appeared to be no correlation between the virulence factors studied and differential killing in monocytes. The virulence factors studied were not predictive of the capacity for *H. pylori* to avoid monocyte and macrophage killing.

The process of *H. pylori* phagosome maturation was then investigated using the same *in vitro* infection model. Macrophages were infected with *H. pylori* and the amount of early endosome (Rab5 and EEA1), late endosome (Rab7 and CD63) and lysosome (LAMP-1 and LAMP-2) markers that co-localised with phagosomes was determined over a four hour time course. There was a dramatic change in the kinetics of phagosome maturation between *H. pylori* phagosomes and control *E. coli* phagosomes and it was proposed that this could contribute to the reduced killing of *H. pylori* observed in macrophages. *H. pylori* phagosomes retained the characteristics of early and late endosomes despite gaining lysosome markers. This demonstrated a fundamental change in phagosome maturation whereupon the *H. pylori* phagosome underwent normal fusion with the elements of the endocytic network, but blocked the subsequent fission part of the interaction.

Macrophages are the critical regulatory component of the innate and adaptive immune responses in the stomach. Restoring the normal process of phagosome maturation in *H. pylori* infection may realise a strategy, enabling the effective killing of *H. pylori*. Reinstating the efficacy of the immune response generated by *H. pylori* to ultimately clear an *H. pylori* infection would have enormous benefits, particularly in the developing world where *H. pylori* infection has a very high prevalence.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Glenn N. Borlace and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Glenn N. Borlace

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LIST OF ABBREVIATIONS

16S rRNA	16S component of the 30S subunit of prokaryotic ribosomal RNA		
AF 488	Alexa Fluor 488		
AGS	Human gastric epithelial cell line		
Amino Acids	A	Ala	Alanine
	D	Asp	Aspartic acid
	C	Cys	Cysteine
	E	Glu	Glutamic acid
	G	Gly	Glycine
	I	Ile	Isoleucine
	K	Lys	Lysine
	L	Leu	Leucine
	N	Asn	Asparagine
	P	Pro	Proline
	Q	Glu	Glutamine
	R	Arg	Arginine
	S	Ser	Serine
	T	Thr	Threonine
	Y	Tyr	Tyrosine
ANOVA	Analysis of variance		
ATPase	Adenosine triphosphate hydrolase		
B cell	B lymphocyte (matures in the bone marrow)		
Bak	Apoptosis regulator Bak; BCL2 homologous antagonist/killer		
Bax	Apoptosis regulator Bax; BCL2-associated X protein		
BCA	Bicinchoninic acid		
BME	Basal modified Eagle's medium		
BSA	Bovine serum albumin		
C. burnetii	*Coxiella burnetii*		
CagA	Cytotoxin associated geneA protein		
cagA	CagA structural gene		
$CagA^{P\text{-}TYR}$	Tyrosine phosphorylated CagA		
cagE1/E2	*cagE* structural gene alleles		
*cag*PAI	*cag* pathogenicity island		
CD	Cluster of differentiation		
CD206	CD antigen 206, macrophage mannose receptor		
CD63	CD antigen 63		
CFU	Colony forming units		
CIMPR	Cation-independent mannose 6 phosphate receptor		
Class II MHC	Class II major histocompatibility complex		

ClpA	ATP-binding subunit of Clp caseinolytic peptidase
ClpP	Proteolytic subunit of Clp caseinolytic peptidase
cMet	Hepatocyte growth factor receptor/scatter factor receptor
Csk	C-terminal c-Src tyrosine kinase, the physiological inhibitor of c-Src
c-Src	c-Src tyrosine kinase
Cy3	Red fluorescent cyanine dye 3
CYWHS	Children, Youth and Women's Health Service
DAPI	4', 6-diamidino-2-phenylindole
Dot/Icm	Defective organelle trafficking/Intracellular multiplication genes
DPSS laser	Diode-pumped solid state laser
EB1	Microtubule tip associating protein EB1
ECL	Enhanced chemiluminescence
EcoR1	*E. coli* restriction enzyme 1
EDTA	Ethylenediaminetetraacetic acid
EEA1	Early endosome antigen 1
EPIYA	Glu-Pro-Ile-Tyr-Ala phosphorylation motif in CagA protein
ERK	Extracellular signal-regulated kinase
E. coli	*Escherichia coli*
ESCRT	Endosomal sorting complex required for transport
Fc	Fragment, crystallisable of antibody
FITC	Fluorescein isothiocyanate
flaA	Flagella structural protein A gene of *H. pylori*
FYVE	Zinc finger named after the four proteins that it has been found in: Fab1, YOTB/ZK632.12, Vac1 and EEA1
Gapex5	GTPase activating protein exchange factor 5
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
GTPase	Guanosine triphosphate hydrolase
H. pylori	*Helicobacter pylori*
HEK293	Human renal epithelial cell line
HEp2	Human epithelial cell line
HpaA	*N*-acetylneuraminyllactose-binding haemagglutinin A
HpaII	*Haemophilus parainfluenzae* restriction enzyme II
HPNAP	*H. pylori* neutrophil activating protein
IARC	International Agency for Research on Cancer
IFNγ	Interferon gamma
IgG	Immunoglobulin G
IL-(1, 2, 6, 8, 10, 12)	Interleukin-(1, 2, 6, 8, 10, 12)
IMVS	Institute of Medical and Veterinary Science
iNOS	Inducible nitric oxide synthase
JAM	junctional adhesion molecule, F11 receptor

LAMP-1, LAMP-2	Lysosome associated membrane protein-1 and -2
LCV	Large communal vesicle (of *H. pylori*)
LBPA	Lysobisphosphatidic acid
LDRU	Lysosomal Disease Research Unit
m1, m2	Variable forms of the middle (m) region of the VacA cytotoxin
M. tuberculosis	*Mycobacterium tuberculosis*
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate oxidase
NF-κB	Nuclear factor-kappa B
NIH	National Institutes of Health
NL101, NL103	*H. pylori* clinical isolates
NL106, NL107	*H. pylori* clinical isolates
Nod-1	Nucleotide-binding oligomerisation domain containing-1
N-terminus	Amino terminus
OMP	Outer membrane protein
p	P value, calculated probability
PAMP	Pathogen associated molecular patterns
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGN	Peptidoglycan
PI 3-kinase	phosphoinositide 3-kinase
PI(3)P	Phosphatidylinositol-3-phosphate
PLCγ	phospholipase C gamma
PMN	Polymorphonuclear cell
PMSF	Phenylmethylsulphonyl fluoride
PRR	Pattern recognition receptor
pUC19	Plasmid cloning vector pUC19
PVDF	Polyvinylidene fluoride
Rab5, Rab7	Small GTPases of the Ras superfamily; Rab5 and Rab7
RILP	Rab interacting lysosomal protein
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RuvC	Holliday junction resolvase
s1a, s1b, s2	Variable forms of the signal (s) region of the VacA cytotoxin
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SH2	Src homology 2 domain
SHP-2	Src homology 2 domain containing tyrosine phosphatase
SpoT	(p)ppGpp synthetase II
SPP1	*Bacillus subtilis* bacteriophage SPP1
SS1	*H. pylori* Sydney Strain 1
T cell	T lymphocyte (matures in the thymus)

T4SS	Type IV secretion system
Taq	*Thermus aquaticus*
Th1, Th2	T helper 1 lymphocyte, T helper 2 lymphocyte
TLR4, TLR5	Toll like receptor 4 and 5
TMPD	*N,N,N',N'*-tetramethyl-*p*-phenylenediamine
TNFα	Tumour necrosis factor alpha
Treg	T regulatory lymphocyte
Tris	Tris (hydroxymethyl) amino methane
VacA	Vacuolating cytotoxin A
vacA	VacA structural gene
voxel	Volume pixel (3D pixel)
VPS34	Vacuolar protein sorting factor 34 (yeast equivalent of PI 3-kinase)
v/v	Volume per volume
w/v	Weight per volume
ZO-1	Host cell scaffolding protein zonula occludens-1