

***HELICOBACTER PYLORI*: REDUCED
PHAGOCYTTIC 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), *cagPAI* (*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 *in 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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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 |
| <i>C. burnetii</i> | <i>Coxiella burnetii</i> |
| CagA | Cytotoxin associated geneA protein |
| <i>cagA</i> | CagA structural gene |
| CagA ^{P-TYR} | Tyrosine phosphorylated CagA |
| <i>cagE1/E2</i> | <i>cagE</i> structural gene alleles |
| <i>cagPAI</i> | <i>cag</i> 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 |

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|-------------------------|--|
| 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 | <i>E. coli</i> 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 |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| ESCRT | Endosomal sorting complex required for transport |
| Fc | Fragment, crystallisable of antibody |
| FITC | Fluorescein isothiocyanate |
| <i>flaA</i> | Flagella structural protein A gene of <i>H. pylori</i> |
| 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 |
| <i>H. pylori</i> | <i>Helicobacter pylori</i> |
| HEK293 | Human renal epithelial cell line |
| HEp2 | Human epithelial cell line |
| HpaA | <i>N</i> -acetylneuraminylactose-binding haemagglutinin A |
| HpaII | <i>Haemophilus parainfluenzae</i> restriction enzyme II |
| HPNAP | <i>H. pylori</i> neutrophil activating protein |
| IARC | International Agency for Research on Cancer |
| IFN γ | 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 <i>H. pylori</i>) |
| LBPA | Lysobisphosphatidic acid |
| LDRU | Lysosomal Disease Research Unit |
| m1, m2 | Variable forms of the middle (m) region of the VacA cytotoxin |
| <i>M. tuberculosis</i> | <i>Mycobacterium tuberculosis</i> |
| NADPH oxidase | Nicotinamide adenine dinucleotide phosphate oxidase |
| NF-κB | Nuclear factor-kappa B |
| NIH | National Institutes of Health |
| NL101, NL103 | <i>H. pylori</i> clinical isolates |
| NL106, NL107 | <i>H. pylori</i> 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 | <i>Bacillus subtilis</i> bacteriophage SPP1 |
| SS1 | <i>H. pylori</i> Sydney Strain 1 |
| T cell | T lymphocyte (matures in the thymus) |

| | |
|--------------|--|
| T4SS | Type IV secretion system |
| <i>Taq</i> | <i>Thermus aquaticus</i> |
| Th1, Th2 | T helper 1 lymphocyte, T helper 2 lymphocyte |
| TLR4, TLR5 | Toll like receptor 4 and 5 |
| TMPD | <i>N,N,N',N'</i> -tetramethyl- <i>p</i> -phenylenediamine |
| TNF α | Tumour necrosis factor alpha |
| Treg | T regulatory lymphocyte |
| Tris | Tris (hydroxymethyl) amino methane |
| VacA | Vacuolating cytotoxin A |
| <i>vacA</i> | 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 |