A functional genomic-based study of the streptomycin mouse model of human *Salmonella* Typhimurium gastroenteritis

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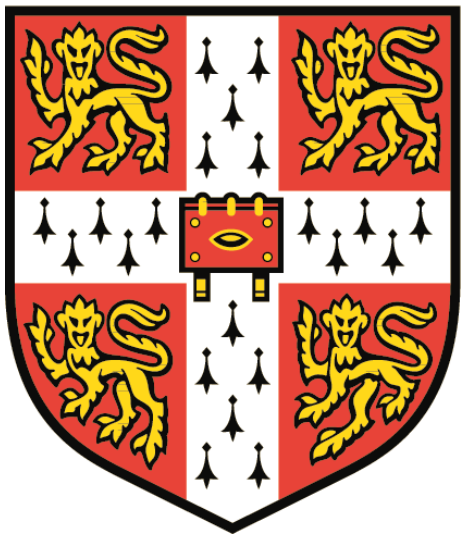

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Declaration

This thesis is the result of my own work and is unlike any work I have previously submitted for any other qualification. Work performed in collaboration is declared below and/or specified in the materials and methods section. This thesis does not exceed the word limit of 60,000 words (excluding bibliography, figures and appendices) required by the University of Cambridge School of Biological Sciences.

Cordelia Brandt, Katherine Harcourt and Leanne Kane performed cervical dislocation and cardiac puncture, and assisted with the collection of tissue from mice. George Notley weighed mice and monitored their wellbeing. David Goulding performed three-dimensional confocal imagining and took conventional confocal microscope images with my assistance. RNAseq library preparation, RNAseq and DNA sequencing were performed by the Wellcome Trust Sanger Institute (WTSI) sequencing core facility. Alignment of sequence reads with the mouse reference genome and generation of read counts was performed by the WTSI pathogen informatics facility as part of the RNAseq transcript mapping pipeline. Dr Lu Yu assisted in protocol development for extraction of protein from mouse caecum for mass spectrometry (MS), and performed MS and database searching. Dr James Wright performed analysis on MS data to produce fold changes in protein abundance and T-test p-values. Prof. Mark Arends (University of Edinburgh Division of Pathology) performed pathological scoring of mouse tissue. Dr Maria Duque performed qPCR analysis of tissues from naïve *IL22ra1*$^{tm1a/tm1a}$ mice presented in Figure 6.4B.

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Abstract

Antibiotic treatment abolishes resistance to invading microbes conferred by the natural murine microflora, creating an opportunity for *Salmonella* Typhimurium to colonise the gut. Pathological changes occurring in intestinal tissue during infection in mice mirror aspects of the inflammatory effects of *S.* Typhimurium upon the human intestinal mucosa. The streptomycin mouse model has emerged as a valuable tool to investigate both the host response to *Salmonella* in an intestinal setting, and bacterial virulence factors important for intestinal colonisation.

The Wellcome Trust Sanger Institute has established a phenotypic screening platform using novel mutant mice that incorporates a pathogen challenge component. This screen includes a systemic but not an oral *Salmonella* challenge. In this thesis I explore the potential of the murine *Salmonella* oral streptomycin treatment model as a secondary phenotyping component of such a screen. Using a combination of functional genomic approaches including RNAseq and proteomics I catalogue molecular changes which occur in caecal tissue during *S.* Typhimurium infection. Pathway analysis was used to aid interpretation of these large datasets and gain mechanistic insight into aspects of the host response. I found upregulated genes overrepresented in numerous immune-related pathways whereas downregulated genes were overrepresented in metabolic pathways; indicting infection leads to extensive disruption of local host metabolism.

Significantly overrepresented during infection at both the level of RNA and protein, the complement pathway was selected for further investigation in light of limited understanding of its role in mucosal infection. By Western blotting I demonstrated proteolytic activation of the complement protein C3 in intestinal tissue and using immunofluorescence staining showed patterns of C3 localisation in the mucosa. Using mutant mice, I identified genes with potential involvement in susceptibility to oral infection with *S.* Typhimurium and applied functional genomic approaches to describe the roles of these genes. In summary, this work explores the combination of high throughput approaches for identification of key signatures of infection with hypothesis-driven experiments in a model of *Salmonella* gastroenteritis, aiming to advance our understanding of host factors involved in the immune response to gastrointestinal infection.

Abbreviations

A/E	Attaching & effacing
APR	Acute phase response
BCR	B cell receptor
BMDM	Bone marrow-derived macrophage
cDNA	Complementary DNA
CFU	Colony forming units
CSA	Common structural antigens
DDA	Data-dependent acquisition
DE	Differentially expressed
DIA	Data-independent acquisition
DSS	Dextran sodium sulphate
EPEC	Enteropathogenic *Escherichia coli*
ES cell	Embryonic stem cell
EHEC	Enterohaemorrhagic *Escherichia coli*
FAE	Follicle-associated epithelium
FCS	Foetal calf serum
FDR	False discovery rate
GALT	Gut-associated lymphoid tissue
GEMS	Global Enteric Multicentre Study
GO	Gene ontology
GPCR	G-protein coupled receptor
GWAS	Genome-wide association study
HPLC	High pressure liquid chromatography
IBD	Inflammatory bowel disease
IEL	Intraepithelial lymphocyte
IKMC	International Knockout Mouse Consortium
ILC	Innate lymphoid cell
ILF	Innate lymphoid follicle

iNOS	Inducible nitric oxide synthase
iNTS	Invasive non-typhoidal *Salmonella*
IP	Intraperitoneal
LEE	Locus of enterocyte effacement
LPS	Lipopolysaccharide
LB	Luria Bertani
M cell	Microfold cell
MAC	Membrane attack complex
MBL	Mannose-binding lectin
MGP	Mouse genetics project
miRNA	microRNA
MLEE	Multi-locus enzyme electrophoresis
mLN	Mesenteric lymph node
MLST	Multi-locus sequence typing
mRNA	messengerRNA
MS	Mass spectrometry
NLR	Nod-like receptor
NTS	Non-typhoidal *Salmonella*
ORA	Overrepresentation analysis
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate buffered saline
PCA	Principal component analysis
PCR	Polymerase chain reaction
PFA	Paraformaldyde
PI	Post-infection
PMN	Polymorphonuclear leukocyte
PRR	Pattern recognition receptor
qPCR	Quantitative polymerase chain reaction
RNP	Ribonucleoprotein

ROS	Reactive oxygen species
rRNA	Ribosomal RNA
SCV	*Salmonella*-containing vacuole
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
sIgA	Secretory immunoglobulin A
SILT	Solitary isolated lymphoid tissue
SNP	Single nucleotide polymorphism
SPF	Specific pathogen free
SPI	Salmonella pathogenicity island
ST	Sequence type
TCR	T cell receptor
TLR	Toll-like receptor
T3SS	Type 3 secretion system
WGS	Whole genome sequencing
WHO	World Health Organisation
WTSI	Wellcome Trust Sanger Institute

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