

Table of contents:**Page number:**

Chapter 1: Introduction	1
1.1 Classification of <i>Salmonellae</i> and global burden of disease.....	1
1.2 Pathogenesis of and host response to <i>Salmonella</i> infection.....	4
1.2.1 Initial host-epithelial interactions.....	4
1.2.1.1 From ingestion to the mucosal barrier.....	4
1.2.1.2 Penetration of the intestinal mucosa.....	6
1.2.1.3 Entry into epithelial cells.....	7
1.2.1.4 Intracellular survival.....	9
1.2.1.5 Invasion factors specific to typhoidal strains.....	11
1.2.2 Innate immune response to <i>Salmonella</i> infection.....	13
1.2.3 <i>Salmonella</i> within the macrophage.....	16
1.2.4 Systemic spread of <i>Salmonella</i>	19
1.2.5 Adaptive immune response to <i>Salmonella</i>	21
1.3 Treatment and prevention of <i>Salmonella</i> infections.....	22
1.3.1 Treatment of <i>Salmonella</i> infections and concerns about MDR organisms.....	22
1.3.2 Status of vaccine development against typhoid, paratyphoid and NTS disease.....	26
1.4 Models for study of host-pathogen interactions and reasons for their use.....	31
1.4.1 Current methods of studying host-pathogen interactions for <i>S. Typhi</i> , <i>S. Paratyphi A</i> and NTS strains.....	31
1.4.2 Advantages of using the hiPSC-derived iHO model.....	35
1.5 Host defences against enteric pathogens.....	37
1.5.1 The role of the intestinal epithelium in defence against enteric pathogens....	37
1.5.2 Phagolysosomal fusion as a mechanism of pathogen destruction.....	42
1.5.2.1 Formation of the phagolysosome.....	42
1.5.2.2 Avoidance of phagolysosomal fusion.....	43
1.5.3 The Interleukin-22 (IL-22) pathway.....	46
1.5.3.1 Components of the IL-22 pathway and its mechanism of action on the intestinal epithelium.....	46

1.5.3.2 Sources of IL-22.....	49
1.6 hiPSC-derived systems for recapitulating host response to pathogens in vitro.....	50
1.6.3 Production of hiPSCs.....	50
1.6.4 Generation of macrophages from hiPSC.....	52
1.6.5 Generation of intestinal organoids from hiPSC.....	54
1.6.6 Applications of organoid technology, including host-pathogen interactions.....	57
1.7 Summary.....	62
1.8 Aims of the thesis.....	62
References.....	64

Chapter 2: Materials and methods	95
2.1 Growth and differentiation of hiPSCs into iHO.....	95
2.1.1 Culture and passage of induced pluripotent stem cells.....	95
2.1.2 Differentiation from iPSC to hindgut.....	96
2.1.3 Embedding of hindgut into Matrigel.....	97
2.1.4 Maintenance and passage of iHO.....	98
2.1.5 Phenotyping of iHO.....	100
2.2 Pre-stimulation of iHO with rhIL-22.....	104
2.3 Microinjection of iHO and intracellular invasion / intracellular survival / luminal killing assays.....	105
2.4 Electroporation of TIMER ^{bac} plasmid into alternative bacteria.....	108
2.5 Production and culture of murine organoids (iMO)	108
2.6 Microinjection of iMO and intracellular invasion assays.....	110
2.7 Single cell sequencing of iHO following rhIL-22 stimulation.....	111
2.8 Western blotting for proteins of interest in iHO.....	111
2.9 FACS for expression of proteins in iHO after stimulation / infection.....	113
2.10 Production of hiPSC lines with isogenic mutations.....	114
2.11 Growth and differentiation of hiPSC into macrophages.....	114
2.12 Intracellular infection assays using hiPSC-derived macrophages.....	116
2.13 Immunostaining of infected hiPSC-derived macrophages.....	116
2.14 TEM of infected hiPSC-derived macrophages.....	117

2.15 Luminex assays for cytokines post-infection in iHO and macrophages.....	117
2.16 Bulk RNA-Seq for infected iHO and macrophages.....	118
References.....	119

Chapter 3: Methods modification	121
3.1 Growth and differentiation of iHO.....	121
3.2 Establishing multiplicity of infection (MOI) in the iHO model.....	123
3.3 Alternative antibiotic protection assays, to study gentamicin-resistant bacteria.....	125
3.4 Use of IncuCyte for observation of progress of infection in iHO.....	126
3.5 Development of CryoEM methods for CL3 pathogens.....	127
References.....	128

Chapter 4: The role of IL-22 in restriction of *Salmonella* invasion of the intestinal epithelium **129**

4.1 Introduction.....	129
4.2 Phenotyping iHO derived from healthy volunteer cell lines to demonstrate presence of the IL-22 receptor complex.....	131
4.3 Response of IL-22-regulated genes in iHO following rhIL-22 stimulation.....	133
4.4 Effect of IL-22 stimulation on <i>S. Typhimurium</i> SL1344 infection of iHO.....	133
4.5 Effect of IL-22 stimulation on <i>S. Typhimurium</i> SL1344 infection in iMO.....	136
4.6 Establishing the mechanism of protection mediated by IL-22 treatment in the iHO model.....	138
4.7 The role of Calgranulin B in IL-22-induced phagolysosomal fusion.....	141
4.8 Single cell responses after IL-22 stimulation.	145
4.9 Discussion.....	152
References.....	155

Chapter 5: Investigation of the iHO luminal response to infection, iHO as a model for alternative pathogens, competition between bacterial strains and interactions of *Salmonella* with iHO derived from cell lines with isogenic mutations **159**

5.1 Introduction.....	159
5.2 Assessing whether luminal bacterial killing occurs in the iHO model.....	161

5.3 Reviewing the luminal contents of the iHO and their effects on other bacterial strains.....	166
5.4 Other applications for the iHO model – study of competition between bacterial strains.....	171
5.5 Other applications for the iHO model – investigating mutations of interest.....	173
5.6 Discussion.....	180
References.....	183
Chapter 6: Interactions of <i>S. Typhi</i> and <i>S. Paratyphi A</i> with the hiPSC-derived iHO epithelium and macrophages	189
6.1 Introduction.....	189
6.2 Generation and phenotyping of alternative iHO lines for use in assays.....	191
6.3 Studies on the interactions of <i>S. Typhi</i> and <i>S. Paratyphi A</i> with hiPSC-derived iHO.....	192
6.3.1 Establishing infectivity in the iHO model.....	192
6.3.2 Imaging of interactions during infection.....	195
6.3.3 Transcriptomic changes during iHO infection.....	197
6.3.3.1 Transcriptomic changes in Kolf2 iHO.....	200
6.3.3.2 Transcriptomic changes in Sojd2 iHO.....	207
6.3.3.3 Transcriptomic changes in Rayr2 iHO.....	212
6.3.3.4 Transcriptomic differences between cell lines.....	218
6.3.4 Cytokine response in iHO infected with <i>S. Typhi</i> and <i>S. Paratyphi</i>	221
6.4 Study of interactions of <i>S. Typhi</i> and <i>S. Paratyphi A</i> with hiPSC-derived macrophages.....	223
6.4.1 Assessing infectivity of <i>S. Typhi</i> and <i>S. Paratyphi A</i> in macrophages.....	223
6.4.2 Imaging of interactions during infection.....	224
6.4.3 Transcriptomic changes witnessed during macrophage infection.....	228
6.4.4 Cytokine response in macrophages infected with <i>S. Typhi</i> and <i>S. Paratyphi A</i>	239
6.5 Preliminary studies of the interactions of clinical (H58) <i>S. Typhi</i> with the iHO epithelium.....	240
6.5.1 Assessing the infectivity of H58 <i>S. Typhi</i> in the iHO model.....	240
6.5.2 Transcriptomic changes witnessed during H58 iHO infection.....	243

6.5.3 Assessing the infectivity of H58 <i>S. Typhi</i> in the macrophage model.....	245
6.5.4 Transcriptomic changes witnessed during H58 macrophage infection.....	246
6.6 Discussion.....	254
References.....	262
Chapter 7: Future directions	271
7.1 More detailed transcriptional profiling of the iHO model.....	271
7.2 Luminal studies.....	273
7.3 Alteration of iHO to closer resemble <i>in vivo</i> scenarios.....	274
7.4 Neglected pathogens.....	276
References.....	277
Appendix 1: Single cell sequencing methods	279
Appendix 2: Generation of S100A9^{-/-} hiPSC line	289
Appendix 3: Appendix 3 - Bulk RNA-Seq data analysis methods	291