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Interactions between commensal obligate anaerobes
and human intestinal cells

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

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2013
ABSTRACT

The human intestinal epithelium is formed by a single layer of epithelial cells which regulates intestinal barrier permeability. Increased permeability can result in the entry of potentially harmful compounds into the body, and is implicated in autoimmune, inflammatory and atopic diseases. The intestinal tract is inhabited by an estimated $10^{14}$ microbes and it is increasingly evident that they affect intestinal barrier function. However, over 90% of commensal intestinal bacteria are obligate anaerobes, making it difficult to co-culture them with oxygen-requiring mammalian cells in vitro.

To investigate the interactions between obligate anaerobes and epithelial cells that regulate the intestinal barrier, an apical anaerobic model of the human intestinal epithelium, which utilises a dual-environment co-culture chamber, was developed and validated. The chamber allowed for polarised monolayers of the intestinal cell line Caco-2 to be grown such that the apical (luminal) side was exposed to an anaerobic environment, while maintaining an aerobic basal side. The cell viability and barrier function of Caco-2 monolayers was unaffected by culture in the apical anaerobic model for at least 12 hours. Global gene expression analysis predicted upregulation of cell survival and proliferation in Caco-2 cells cultured in the apical anaerobic model, compared to Caco-2 cells grown under conventional conditions, suggesting an adaptation of the Caco-2 cells to a lower supply of oxygen.

The apical anaerobic model was used to co-culture the commensal obligate anaerobe Faecalibacterium prausnitzii with Caco-2 cells. The survival of F. prausnitzii was improved in the anaerobic apical environment compared to when cultured in an aerobic atmosphere. Live F. prausnitzii, but not non-viable (UV-killed) F. prausnitzii, were shown to increase permeability across Caco-2 monolayers. Furthermore, global gene expression analysis suggested that live F. prausnitzii cells have more profound effects on Caco-2 cells than non-viable F. prausnitzii, illustrating the importance of maintaining viability of obligate anaerobes in an in vitro co-culture system.

The apical anaerobic model can be used to gain insights into the mechanisms of crosstalk between commensal obligate anaerobic bacteria and intestinal cells, and new knowledge generated using this model will assist in the development of strategies to improve intestinal barrier function.
Dedicated to aththamma, seeya, and 'big' aththamma.

My greatest inspirations. Love always.
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I owe my deepest gratitude to Dr John Koolaard for his invaluable advice on most of the statistical analysis carried out in this project. I would also like to acknowledge Dr Mark McCann (cell culture, flow cytometry, qPCR), Graham Naylor (anaerobic microbiology), Dr Adrian Cookson (microbiology), Dr Jurgen Karczewski (confocal microscopy and cell viability assay), Bruce Sinclair (dissolved oxygen assays), Dr Peter van Baarlen (microarray analysis) and Catherine Lloyd-West (statistical analysis) for their valuable advice and sharing their expertise. My thanks also
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## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>BHI</td>
<td>Brain-heart infusion</td>
</tr>
<tr>
<td>BLASTN</td>
<td>NCBI nucleotide Basic Local Alignment Search</td>
</tr>
<tr>
<td>CAR</td>
<td>Coxsackie and adenovirus receptor</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>cIAP</td>
<td>Cellular inhibitor of apoptosis protein</td>
</tr>
<tr>
<td>CLA</td>
<td>Conjugated linoleic acids</td>
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<td>cAMP response element</td>
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</tr>
<tr>
<td>dNTP</td>
<td>Deoxyribonucleotide</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>DSMZ</td>
<td>Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures)</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
</tr>
<tr>
<td>EGCG</td>
<td>Epigallocatechin gallate</td>
</tr>
<tr>
<td>Abbreviation</td>
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<tr>
<td>EHEC</td>
<td>Enterohemorrhagic <em>Escherichia coli</em></td>
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<tr>
<td>EIEC</td>
<td>Enteroinvasive <em>Escherichia coli</em></td>
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<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
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<tr>
<td>EPEC</td>
<td>Enteropathogenic <em>Escherichia coli</em></td>
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<tr>
<td>ERK</td>
<td>Extracellular signal regulated kinases</td>
</tr>
<tr>
<td>FBS</td>
<td>Foetal bovine serum</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
</tr>
<tr>
<td>GLA</td>
<td>γ-linolenic acid</td>
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<tr>
<td>GO</td>
<td>Gene ontology</td>
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<tr>
<td>hCGΔ</td>
<td>Choriogonadotropin</td>
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<tr>
<td>HIF</td>
<td>Hypoxia-inducible factor</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>IEC6</td>
<td>Intestinal epithelial cell line 6</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IKK</td>
<td>IκB kinase</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IPA</td>
<td>Ingenuity Pathway Analysis</td>
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<tr>
<td>IRAK</td>
<td>IL-1-receptor-associated kinase</td>
</tr>
<tr>
<td>IRF</td>
<td>IFN-regulatory factor</td>
</tr>
<tr>
<td>IκB</td>
<td>Inhibitor of NF-κB</td>
</tr>
<tr>
<td>JAM</td>
<td>Junctional adhesion molecules</td>
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<tr>
<td>KEGG</td>
<td>Kyoto Encyclopaedia of Genes and Genomes</td>
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<tr>
<td>Lh</td>
<td>Luteinising hormone</td>
</tr>
<tr>
<td>LOESS</td>
<td>Locally weighted smoothing spline</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>M199</td>
<td>Medium 199</td>
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<tr>
<td>MAMPs</td>
<td>Microbe-associated molecular patterns</td>
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<tr>
<td>MAPK</td>
<td>Mitogen activated protein kinases</td>
</tr>
<tr>
<td>MDCK</td>
<td>Madin Darby canine kidney</td>
</tr>
<tr>
<td>MLC</td>
<td>Myosin II regulatory light chain</td>
</tr>
<tr>
<td>MLCK</td>
<td>Myosin light chain kinase</td>
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<tr>
<td>MOI</td>
<td>Multiplicity of infection</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
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<tr>
<td>MRS</td>
<td>Man, Rogosa and Sharpe</td>
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<tr>
<td>MyD88</td>
<td>Myeloid differentiation primary response gene</td>
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<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
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<tr>
<td>NEAA</td>
<td>Non-essential amino acids</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa B</td>
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<tr>
<td>NOD</td>
<td>Nucleotide-binding and oligomerisation-domain</td>
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<tr>
<td>NRF2</td>
<td>NF-E2-Related Factor 2</td>
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<tr>
<td>OD</td>
<td>Optical density</td>
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<tr>
<td>PAMPs</td>
<td>Pathogen-associated molecular patterns</td>
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<tr>
<td>pamr</td>
<td>Prediction Analysis for Microarrays</td>
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<tr>
<td>PC</td>
<td>Polycarbonate (Transwell® cell culture inserts)</td>
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<tr>
<td>PCA</td>
<td>Principal component analysis</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PDZ</td>
<td>PSD95–DlgA–ZO-1 homology</td>
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<tr>
<td>PEPT1</td>
<td>H+/di-tripeptide transporter</td>
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<td>Polyester (Transwell® cell culture inserts)</td>
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<td>PGE2</td>
<td>Prostaglandin E2</td>
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<td>Full Form</td>
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<tr>
<td>PI</td>
<td>Propidium iodide</td>
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<tr>
<td>PKC</td>
<td>Protein kinase C</td>
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<tr>
<td>PLA2</td>
<td>Group IIA phospholipase A2</td>
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<tr>
<td>PPAR</td>
<td>Peroxisome-proliferator-activated receptor</td>
</tr>
<tr>
<td>PRRs</td>
<td>Pathogen recognition receptors</td>
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<td>PTFE</td>
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<tr>
<td>q</td>
<td>False discovery rate</td>
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<tr>
<td>REML</td>
<td>Restricted maximum likelihood</td>
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<tr>
<td>RICK</td>
<td>Receptor-interacting serine/threonine kinase</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>RNase</td>
<td>Ribonuclease A</td>
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<tr>
<td>ROCK</td>
<td>Rho kinases</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
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<tr>
<td>SCFAs</td>
<td>Short chain fatty acids</td>
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<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SULTs</td>
<td>Sulphotransferases</td>
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<tr>
<td>TAK-1</td>
<td>Transforming growth factor-β-activated kinase-1</td>
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<tr>
<td>TEER</td>
<td>Transepithelial electrical resistance</td>
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<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>TJ</td>
<td>Tight junction</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
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<td>TNFR</td>
<td>Tumour necrosis factor receptor</td>
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<td>TRAF6</td>
<td>TNF-receptor-associated factor 6</td>
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<td>Abbreviation</td>
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<tr>
<td>TREM</td>
<td>Triggering receptor expressed on myeloid cells</td>
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<tr>
<td>TSLP</td>
<td>Thymic stromal lymphopoietin</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VegF</td>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>ZO</td>
<td>Zonula occludens</td>
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