Effect of *Faecalibacterium prausnitzii* on intestinal barrier function and immune homeostasis

A dissertation presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Nutritional Science

Massey University
Manawatū, New Zealand

Eva Maier

2017

Abstract

Various gastrointestinal (GI) diseases, for example inflammatory bowel disease, are linked to impaired barrier function, chronic inflammation and dysbiosis of the resident microbiota. *Faecalibacterium prausnitzii*, an abundant obligate anaerobe of the healthy human microbiota, has reduced abundance in the GI tract of people with these diseases, and has been suggested to exert beneficial effects. Only a few studies have investigated its mechanisms of action, partly due to the difficulty of co-culturing live obligate anaerobes with oxygen-requiring human cells. The novel apical anaerobic co-culture model used in this study allows this co-culture through the separation of anaerobic and aerobic compartments. This model was used to investigate the effects of live *F. prausnitzii* (strains A2-165, ATCC 27768 and HTF-F) on intestinal barrier integrity, measured by trans-epithelial electrical resistance (TEER) of the intestinal epithelial cell line Caco-2, and on immune homeostasis, specifically on Toll-like receptor (TLR) activation. Method development was required to adapt these assays to the novel model and to optimise the growth of *F. prausnitzii* co-cultured with Caco-2 cells and TLR-expressing cell lines while maintaining their viabilities. Firstly, the optimised co-culture conditions were used to determine the effect of the three *F. prausnitzii* strains on barrier integrity of healthy and tumour necrosis factor alpha (TNF-α) treated Caco-2 cells. Live and growing *F. prausnitzii* did not alter the TEER across healthy Caco-2 cells. However, under TNF-α mediated inflammatory conditions, dead *F. prausnitzii* decreased TEER, whereas live bacteria maintained TEER. Secondly, the TLR activation assay was adapted to be carried out in the novel model. Using the adapted assay conditions it was determined that live *F. prausnitzii* induced greater TLR2 and TLR2/6 activation than dead *F. prausnitzii*. Collectively, these results indicate greater immuno-stimulatory effects of live *F. prausnitzii*, via TLR2

activation, and this effect is potentially linked to its barrier maintaining properties, because previous research showed enhancement of barrier integrity induced by TLR2 signalling. This new knowledge contributes to the understanding of how *F. prausnitzii* may maintain immune homeostasis in the GI tract. Unravelling the biological mechanisms used by prevalent species of the human microbiota, such as *F. prausnitzii*, will ultimately allow better comprehension of microbial regulation of GI function.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisors associate Prof Nicole Roy and Dr Rachel Anderson for their continuous support during my PhD. Thanks a lot for your time and effort and the many helpful advice and discussions that kept me positive and motivated throughout my research. Besides my supervisors at AgResearch, I would like to thank my supervisor Prof Paul Moughan at the Riddet Institute. Furthermore, I acknowledge AgResearch and the Riddet Institute for funding this research and my PhD fellowship.

For their technical assistance, I would like to thank Kelly Armstrong, Leigh Ryan and Stacey Burton, for always taking the time to answer my questions and helping out with the cell culture in busy times. Also, thanks to Dr Mark McCann and Kelly Armstrong for their advice on stable transfections and to Dr Eric Altermann for helping me with the bioinformatics analyses. For developing the prototype II co-culture chamber and the adapter for the oxygen probe I would like to acknowledge the AgResearch engineering team, specifically Steve Gebbie and Scott Sevier. My thanks also go to associate Prof Hermie Harmsen and Dr Muhammad Tanweer Khan for providing the *F. prausnitzii* strains used in these studies. For the help with the statistical analysis I am grateful to Dr Carlos Montoya who was an immense help during the very busy times in the final stage of my PhD. In addition, I would like to thank Denise Martin for helping with the many administrative tasks along the road to completing this thesis. Furthermore, my special thanks go to Natascha Stroebinger for the help with the formatting of this thesis.

A special thanks also goes to my fellow students, it was amazing to share an office with such great people, and meeting so many people from all over the world. I never wanted to

miss our lunch breaks which were always fun and contributed a lot to keep up the positive energies and motivation.

In the end I would like to thank my parents and my brother and sister for their constant support, despite being on the other side of the world. Finally, for the constant motivation and moral support I want to thank Carlos. Your positive energies definitely helped me a lot to complete this work.

Table of contents

List of figures

List of tables

Abbreviations

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BHI	Brain-heart infusion
CD	Cluster of differentiation
CFU	Colony-forming unit
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DSM	Deutsche Sammlung von Mikroorganismen (German Collection of Microorganisms)
DSS	Dextran sodium sulphate
FBS	Foetal bovine serum
GALT	Gut-associated lymphoid tissue
GI	Gastrointestinal
HEK	Human embryonic kidney

Abbreviations

HKLM	Heat-killed *Listeria monocytogenes*
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IEC	Intestinal epithelial cell
IFN-γ	Interferon gamma
IKK	Inhibitor of kappa B kinase
IL	Interleukin
IRAK	Interleukin-1 receptor-associated kinase
IκB	Inhibitor of kappa B
LPS	Lipopolysaccharide
LSD	Least Significant Difference
M199 Std	M199 Standard medium
MAPK	Mitogen-activated protein kinase
MOI	Multiplicity of infection
MyD88	Myeloid differentiation primary response protein 88
NCBI	National Center for Biotechnology Information
NEAA	Non-Essential Amino Acid
NF-κB	Nuclear factor-kappa B
NOD	Nucleotide-binding and oligomerisation domain

OD	Optical density
ODS	Output delivery system
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PRR	Pattern recognition receptor
rRNA	Ribosomal ribonucleic acid
SCFA	Short-chain fatty acid
SEM	Standard error of the mean
SFB	Segmented filamentous bacteria
sIgA	Secretory immunoglobulin A
TEER	Trans-epithelial electrical resistance
TJ	Tight junction
TLR	Toll-like receptor
TNBS	2,4,6-Trinitrobenzenesulfonic acid
TNF-α	Tumour necrosis factor alpha
TOLLIP	Toll-interacting protein

TRAF	TNF receptor associated factor
TRIF	TIR-domain-containing adapter-inducing interferon beta
YCFAG	Yeast extract, casitone, fatty acid, glucose
ZO	Zona occludens