Identification of biomarkers of colitis to monitor effects of dietary omega-3 polyunsaturated fatty acids in the interleukin-10 gene-deficient mouse model of inflammatory bowel diseases

A thesis presented in partial fulfilment of the requirements for the

degree of

Doctor of Philosophy

in

Nutritional Science

at Massey University, Manawatū,

New Zealand.

Nadja Berger

2016

ABSTRACT

Inflammatory bowel diseases (IBD) are characterised by chronic inflammation of the gastrointestinal tract including the colon (colitis). Increased dietary intake of salmon, which is rich in eicosapentaenoic acid (EPA), was well tolerated by IBD patients, leading to a perceived decrease in symptoms. However, better knowledge of the mechanisms by which EPA-rich diets affect IBD severity, and appropriate biomarkers for assessing these effects, are needed for potential targeted nutritional interventions.

This dissertation aimed to determine the temporal effects (early (9 weeks of age) vs. established (12 weeks)) of a diet containing 3.7% EPA, and the dose-dependent effects (15% to 45%) of a salmon diet at 12 weeks of age, on the severity of colitis. Molecular responses in colon and/or liver of the interleukin-10 gene-deficient (*Il10*$^{-/-}$) mouse model of IBD and healthy mice were assessed. Caecum digesta, urine and blood were mined to identify biomarkers (microbiota, metabolites and genes) of these responses.

The EPA diet reduced the severity of colitis only in 12-week-old *Il10*$^{-/-}$ mice. This response was associated with changes in gene expression associated with lymphocyte function, eicosanoid signalling and peroxisome proliferator-activated receptor gamma signalling. The blood immune cell gene expression profile did not correlate with reduced colitis in these mice, but the urine metabolite profile was related to changes in colonic tryptophan metabolism.

The effects of the salmon diets on colitis were dose-dependent in 12-week-old *Il10*$^{-/-}$ mice. The intermediate amount of salmon (30%) reduced the severity of colitis and lymphocyte-related gene expression, while enhancing genes in metabolic pathways. Tryptophan metabolism was not affected in these mice, but the urinary metabolite profile correlated with effects on hepatic tocopherol metabolism, as shown by reduced abundance of gamma-carboxyethylhydroxychroman glucoside. The abundances of *V. akkermansia*, *Eubacterium* spp., and an unclassified *Rikenellaceae* were further affected in these mice.

This is the first report describing molecular responses in the colon and liver of *Il10*$^{-/-}$ mice fed a salmon diet associated with reduced colitis. Ultimately these responses could be validated for use in humans, and potentially enable management of IBD with diet.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my supervisors Assoc Prof Nicole Roy, Dr Emma Bermingham, Prof Warren McNabb (all AgResearch Grasslands, Palmerston North, NZ) and Dr Janine Cooney (Plant & Food Research Ruakura, Hamilton, NZ) for your guidance and support throughout this project. Thank you for your encouragement, wisdom and finally your patience. I could not possibly have reached this point without you. I also acknowledge AgResearch for funding this project within the Nutrigenomics New Zealand partnership.

I would like to thank Dr Matthew Barnett (AgResearch Grasslands) for letting me be part of the "EPA time-course experiment" after one of my own mouse experiments had failed. Your enthusiasm and positivity was much appreciated when I did not know how to continue.

I am grateful to Dr Wayne Young (AgResearch Grasslands), an expert in 'Omics' and microbiota analyses, for providing various codes in R and helping with all enquiries. Also, thank you for proof-reading parts of this dissertation and providing valuable feedback.

Proteomics work was performed at Plant & Food Research in Auckland under the guidance of Ms Di Brewster. Thank you Di for taking the time to teach the art of 2D-DIGE and your ongoing technical advice. Also, thanks to all members from this laboratory for your welcoming attitudes which made an otherwise difficult process great. I am also grateful to Dr Janine Cooney for conducting MS identification.

A number of people behind this project deserve to be both acknowledged and thanked here: Mr Shuotun Zhu (University of Auckland, Auckland, NZ) for histological analysis. Ms Kelly Armstrong for the training in histology, RNA extraction and microarray analysis. Dr John Koolaard (AgResearch Grasslands) for statistical advice. Mr Paul Maclean (AgResearch Ruakura) for assistance with microarray data processing. Mr Jason Peters and Ms Leigh Ryan for help with animal work (AgResearch Grasslands). Mr Bruce Sinclair (AgResearch Grasslands) for reading various drafts of this dissertation and lending a helping hand during experiments. Dr Mark McCann (AgResearch Grasslands) for assistance with qPCR validation. Metabolomics experts Dr Karl Fraser and Dr Jan Huege (who sadly passed away during the course of this project) for their

valuable advice and sharing their expertise (AgResearch Grasslands). Thank you all for your help and contribution!

I am grateful to Prof Chris Evelo for giving me the opportunity to conduct parts of my last research chapter in the BigCaT Bioinformatics Group at the University of Maastricht in the Netherlands and would like to thank all the members from this group for making my stay in Maastricht a great one. Special thanks to Dr Lars Eijssen for assistance with data analysis, sharing his knowledge in R and the good times biking through the streets of Maastricht on the search for a beer (or two).

I have been very lucky to go through this process with a group of people that provided moral support and most importantly their friendship. Thank you to Ms Denise Martin (AgResearch Grasslands) for your encouraging words when I had already given up. You are an incredible person and have become a true friend. A huge thanks to my fellow postgraduate students for the chats around the lunch table and the many laughs we shared during road trips to Auckland or a girls-night-out. You truly made the day more enjoyable.

Finally I would like to thank my family with all my heart. My parents Nora and Wolfgang for your ongoing love and support. My brother Gerold for watching over me since 1987. My granny Waldtraud and aunt Traudi for your generosity. To my partner Paul thank you for being absolutely amazing throughout the last few years.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha-linolenic acid
AMP	Antimicrobial protein
ANOVA	Analysis of variance
CAM	Cell adhesion molecule
CD	Crohn's disease
CEBPB, CEBPD, CEBPE	CCAAT/enhancer binding protein (alpha, beta, delta)
CEHC	Carboxyethylhydroxychroman
CFU	Colony-forming units
CIF	Complex intestinal microbiota
DHA	Docosahexaenoic acid
DIGE	Difference gel electrophoresis
DPA	Docosapentaenoic acid
DSS	Dextran Sodium Sulfate
EF	*Enterococcus faecalis* and *E. faecium*
EF×CIF	Solution for bacterial inoculation
EPA	Eicosapentaenoic acid
ESI	Electrospray ionisation
FC	Fold-change
FDR	False discovery rate
GIT	Gastrointestinal tract
GLA	Gamma-linolenic acid
GSEA	Gene Set Enrichment Analysis
GWAS	Genome-Wide Association Study
HE	Haematoxylin and eosin
HIS	Histological injury score
HMDB	Human Metabolome Database
IBD	Inflammatory bowel diseases
IEF	Isoelectric focussing
IL[number]	Interleukin [number]
Il10$^{-/-}$	Interleukin-10 gene-deficient
IPA	Ingenuity Pathway Analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
LA	Linoleic acid
LC	Long-chain
LPS	Lipopolysaccharide
LysoPC	Lysophosphatidylcholine

LysoPE	Lysophosphatidylethanolamine
m/z	Mass-per-charge ratio
MANOVA	Multivariate ANOVA
MDT	Marine-derived tocopherol
MS	Mass spectrometry
n-3 PUFA	Omega-3 polyunsaturated fatty acid
n-6 PUFA	Omega-6 polyunsaturated fatty acid
NCBI	National Center for Biotechnology Information
NKT cell	Natural killer T cell
NMR	Nuclear magnetic resonance
OA	Oleic acid
OTU	Operational taxonomic unit
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PC	Principal coordinate
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
pI	Isoelectric point
PLS-DA	Partial Least Squares-Discriminant Analysis
PPAR	Peroxisome proliferator-activated receptor
PRR	Pattern recognition receptor
QIIME	Quantitative Insights Into Microbial Ecology
qRT-PCR	Real-time reverse transcription polymerase chain reaction
REML	Restricted maximum likelihood
REST	Relative Expression Software Tool
RIN	RNA integrity number
ROS	Reactive Oxygen Species
RT	Retention time
SCFA	Short-chain fatty acid
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SNP	Single nucleotide polymorphisms
TG	Triacylglycerol
Th cell	T helper cell
TMAO	Trimethylamine N-oxide
TNBS	Trinitrobenzenesulfonic acid
Treg	Regulatory T helper cell
UC	Ulcerative colitis