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Effects of dietary sheep, cow and goat milk solids on colitis in the interleukin-10 gene deficient mouse model of Inflammatory Bowel Disease.

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

Doctor of Philosophy

in

Physiology

at Massey University, Palmerston North, New Zealand.

Anna-Lynne Elizabeth Russ

2013
Abstract

Inflammatory Bowel Disease (IBD) is a group of chronic, immunologically-mediated gastrointestinal disorders resulting from interactions between environmental influences, host genetic susceptibility, and the intestinal microbiota. Dietary factors can ameliorate symptoms, providing a rationale for using targeted nutrition to alleviate symptoms. Food components, including milk-derived oligosaccharides and conjugated linoleic acid, have shown anti-inflammatory effects in IBD patients or animal models of IBD. Additionally, some ruminant milks are perceived by some IBD patients to have more beneficial effects on their symptoms (goat, sheep) than others (cow). Soy-based milk substitutes are perceived to be more beneficial than milk. No reports describe the effects of milk solids from different species on molecular pathways in the intestine that might explain differential effects in IBD. This thesis aimed to investigate the effects of dietary intervention with milk solids on the severity of colitis (histology) and molecular pathways (microarrays and qPCR) in the interleukin-10 gene deficient (Il10⁻⁻) mouse model of IBD.

First, laser microdissection (LMD) combined with microarrays was used to analyse colon epithelium gene expression in 6 and 12 week old Il10⁻⁻ mice fed a control diet. This indicated that intact colon was an appropriate tissue in which to study global changes in gene expression when colitis is established. It also showed that studying colon epithelium during the early stages of inflammation (6 weeks old) may identify molecular changes not seen in intact colon. Secondly, analysis of DNA methylation changes (both globally, and in specific inflammation-associated genes (Ppara, Stat1 and Tap2)) in Il10⁻⁻ mouse colon showed that changes in total DNA methylation were correlated with changes in global gene expression, and changes in Stat1 methylation during inflammation correlated with Stat1 gene expression. However, these techniques had limitations for obtaining a global overview of molecular changes (DNA methylation) in response to dietary intervention in established inflammation (LMD) and therefore were not applied in the dietary intervention study. Finally, diets containing goat and cow whole milk solids (40% w/w) fed for 6 weeks had anti-inflammatory effects in the colon of 11-12 week old Il10⁻⁻ mice, shown by a reduction in colitis severity and immune-related gene expression. Further research is required to elucidate the physiological and molecular mechanisms of these anti-inflammatory effects.
Acknowledgements

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Histological scoring was performed by William Zhu (University of Auckland, Auckland, NZ) and Kelly Armstrong (AgResearch Grasslands, Palmerston North, NZ). Statistical analysis of mouse weight, intake and histology data was performed with assistance from Dr John Koolaard and Zaneta Park (AgResearch Grasslands). Microarray designs were discussed for both mouse experiments with Zaneta Park (AgResearch Grasslands), and analysis of the microarray data was performed with the...
assistance of Zaneta Park, Dr Wayne Young, and Paul Maclean (AgResearch Ruakura, Hamilton, NZ). For the laser microdissection work, preparation of slides and microdissection of cells was performed with the assistance of Jason Peters (AgResearch Grasslands) and RNA amplification, labelling and microarray analysis was performed with assistance from Kelly Armstrong. DNA methylation analysis was performed with the assistance of Kelly Armstrong (sample preparation and method development) and Bryan Treloar (HPLC analysis of samples) (AgResearch Grasslands), Leticia Castro (The University of Auckland), Cameron Maclean (AgResearch), Dr Harold Henderson (AgResearch Ruakura) and Dr Matthew Barnett (AgResearch). The qPCR work was performed with assistance from Dr Mark McCann (AgResearch Grasslands).

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May it be when darkness falls your heart will be true.
You walk a lonely road. Oh, how far you are from home!
May it be the shadow's call will fly away.
May it be your journey on to light the day.
When the night is overcome, you may rise to find the sun.

Selected lyrics from the song “May it be” by Enya.
Featured in the film “The Fellowship of the Ring”.

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<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BCM7</td>
<td>β-casomorphin-7</td>
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<tr>
<td>C57BL/6J, C3H/HeJ Bir, BALBc, 129 SvEv, 129 Ola</td>
<td>various strains of inbred mice</td>
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<td>CD</td>
<td>Crohn’s disease</td>
</tr>
<tr>
<td>CIF</td>
<td>complex intestinal flora</td>
</tr>
<tr>
<td>CLA</td>
<td>conjugated linoleic acid</td>
</tr>
<tr>
<td>DSS</td>
<td>dextran sodium sulphate</td>
</tr>
<tr>
<td>EASE</td>
<td>expression analysis systematic explorer</td>
</tr>
<tr>
<td>EF</td>
<td>E. faecalis/faecium culture</td>
</tr>
<tr>
<td>EF.CIF</td>
<td>a 1:1 mixture of EF and CIF</td>
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<tr>
<td>FC</td>
<td>fold change</td>
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<tr>
<td>FDR</td>
<td>false discovery rate</td>
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<td>general health score</td>
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<td>gene set enrichment analysis</td>
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<td>HIS</td>
<td>histological injury score</td>
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<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<td>IBD</td>
<td>inflammatory bowel disease</td>
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<tr>
<td>IEL</td>
<td>intraepithelial lymphocyte</td>
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<td>Ig</td>
<td>immunoglobulin</td>
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<td>interleukin</td>
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<td>IPA</td>
<td>Ingenuity pathway analysis</td>
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<td>Limma</td>
<td>linear models for microarray analysis</td>
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<td>LMD</td>
<td>laser microdissection</td>
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<td>LSD</td>
<td>least significant difference</td>
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<tr>
<td>MALDI-TOF</td>
<td>matrix-assisted laser desorption ionisation-time of flight (mass spectrometry)</td>
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<tr>
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</tr>
<tr>
<td>MDR1</td>
<td>multi-drug-resistant gene/protein</td>
</tr>
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<td>Mdr1a/-</td>
<td>multi-drug-resistant gene deficient (mouse)</td>
</tr>
<tr>
<td>n-3/n-6</td>
<td>omega-3/6 (fatty acid)</td>
</tr>
<tr>
<td>NOD</td>
<td>nucleotide oligomerisation domain</td>
</tr>
<tr>
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<td>polyunsaturated fatty acids</td>
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<td>qPCR</td>
<td>quantitative real-time polymerase chain reaction</td>
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<td>RIN</td>
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<td>SPF</td>
<td>specific pathogen free</td>
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<tr>
<td>TH</td>
<td>T-helper (cell)</td>
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<tr>
<td>TNBS</td>
<td>trinitrobenzenesulfonic acid</td>
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