

D1: TNF- α induces a biphasic change in the expression and distribution of tight junction proteins in human colonic enteroids

Gadeock, S.¹, Schultz, M.^{2,3}, Butt, A.G¹.

¹Department of Physiology, Otago School of Medical Sciences and ²Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ.

TNF- α has a key role in the increased intestinal permeability seen in inflammatory bowel disease patients (1). Here we have used human colonic enteroids to investigate the effects of TNF- α on the expression and distribution of tight junction proteins, as they form the main paracellular barrier that determines the permeability of the intestinal epithelium. Enteroids were grown from crypts isolated from transverse colonic biopsies from healthy patients, suspended in Matrigel[®] and overlaid with stem cell media. After 11d or 14d of growth, enteroids were exposed to TNF- α (1, 10 or 100ng/mL) for either 24h or 72h and their structure was determined by electron microscopy. Transcript levels of tight junction (TJ) proteins were determined by qPCR, and quantification and localization of TJ proteins by immunoblotting and immunofluorescent microscopy. Statistical significance was determined by unpaired Student's *t*-test. TNF- α at low doses of 1ng/mL and 10ng/mL had no effect on the on the TJs but a dose of 100ng/mL of TNF- α caused a disruption of the TJs. Exposure to TNF- α for 24h caused a redistribution of the barrier forming TJ proteins, occludin and ZO-1 from the junctions into both the cytoplasm and the basolateral membrane of the epithelial cells. In contrast, following 72h exposure to TNF- α , a significant reduction in the barrier forming TJ protein, occludin ($P < 0.05$) and a parallel increase in the pore-forming TJ protein, claudin-2 ($P < 0.05$) was observed. This was associated with a loss of occludin and ZO-1 from the TJs, cytoplasm and the basolateral membrane of the epithelial cells. This data indicates that the increase in intestinal permeability induced by pathological concentrations of TNF- α involves an initial disruption of the barrier forming TJ proteins followed by an increase in the pore-forming TJ protein.

(1) Weber, C. R. and J. R. Turner (2007). "Inflammatory bowel disease: is it really just another break in the wall?" Gut **56**(1): 6-8.

Supported by a University of Otago Research Grant and Grants from the Dean's Fund, Otago School of Medical Sciences and the Department of Physiology.

D2: Analysis of specific T cell infiltrate can predict patient prognosis in a NZ cohort of colorectal cancer patients

Ward-Hartstonge, K.¹, McCulloch, T.², Cretney, E.², Fran Munro, F.³, John McCall, J.³, Kemp, R.¹

¹Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, NZ, ²Walter and Eliza Hall of Research Institute, Melbourne, Australia, ³Department of Surgical Sciences, Dunedin School of Medicine, University of Otago, NZ.

Colorectal cancer staging in patients is currently based on tumour morphology and does not take into account the complexity of the anti-tumour immune response. Early-stage colorectal patients are usually treated with surgery alone and are not recommended additional adjuvant therapy. Up to twenty-five percent of these patients have relapse of disease, indicating that the current staging does not accurately predict disease-free survival. The Immunoscore has been proposed as a way to incorporate the T cell infiltrate in the tumour into current staging protocols to improve estimates of disease-free survival. The Immunoscore is currently being validated worldwide. We aimed to validate the Immunoscore in a New Zealand cohort of patients.

Immunofluorescence was used to analyse immune cell infiltrates in early stage (II) colorectal cancer patients and to compare those with recurrent and non-recurrent disease. Patients with a high Immunoscore (high T cell infiltrate) had increased disease-free survival than patients with a low Immunoscore (low T cell infiltrate). The ability to predict patient outcome was improved by measuring the infiltrate of CD4+FoxP3+Blimp-1+ cells (effector regulatory T cells). Patients with a low Immunoscore but high infiltrate of CD4+FoxP3+Blimp-1+ cells at the centre of the tumour had increased disease-free survival than those with a low Immunoscore and a low infiltrate of CD4+FoxP3+Blimp-1+ cells.

These results show that incorporating the complexity of the local immune response into current practice can improve prediction of patient outcome in colorectal cancer.

D3: The effect of vitamin D on gene expression in colorectal cancer and normal colon

Munro, F.M.¹, McCall, J.L.¹, Black, M.A.²

¹Department of Surgical Sciences, Otago Medical School, University of Otago, Dunedin, NZ,

²Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin, NZ.

Background: Epidemiological studies have reported an association between vitamin D status and incidence of CRC. Serum vitamin D levels are sub-optimal in NZ, with deficiency more prevalent in the winter months, particularly in Otago and Southland.

Methods: The study was a randomised, double-blind, placebo-controlled trial of a single dose of vitamin D (200,000 IU) administered to patients in the window between diagnosis and surgery for CRC at Dunedin Hospital. The aim was to examine whether vitamin D could have a measurable effect on vitamin D responsive genes in the tumour and corresponding normal tissue. RNA from resected normal and tumour tissue was profiled on microarray gene-chips. Array expression data were analysed for differentially expressed single genes and pathways in the normal and tumour tissue.

Results: There were no baseline differences between the groups. Pre-incision vitamin D concentrations were higher in the treatment than in the placebo group (mean 87 +/- 22 vs 49 +/- 19 nmol/L; $p = >0.001$). There were no significant differences in single gene expression between treatment and placebo, however differences in pathway expression were identified. In normal tissue, these include down-regulation of fatty acid, xenobiotic and vitamin metabolism pathways in the vitamin D group. In tumour, down regulation of Fatty Acid Metabolism and Fatty Acid beta-Oxidation pathways was observed in the vitamin D group.

Conclusion: Vitamin D did not alter single gene expression but was associated with down-regulation of fatty acid metabolism pathways, particularly in tumour tissue.

D4: Chronic exposure to LPS modified lineage development via the TLR4 dependent pathway In human colonic enteroids

Rodrigues, E.^{2,3}, Slobbe, L.¹, Samuels, G.¹, Gadeock, S.¹, Schultz, M.^{2,3}, Butt, A. G.¹

¹Department of Physiology, Otago School of Medical Sciences and ²Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ.

The adult colonic epithelium undergoes continuous self-renewal throughout life due to the activity of adult stem cells. These specialised cells divide to produce one stem cell and one daughter cell. The daughter cell undergoes several rounds of cell division (transiently amplifying cells) to give rise to one of three cell types: colonocytes, enteroendocrine cells and goblet cells. The regions at which the adult stem cells and transient amplifying cells reside are important sites where bacteria could modulate epithelial regeneration and homeostasis. The aim of this research was to determine if bacterial components modulate epithelial lineage development and affect the intestinal epithelium. Enteroids were grown from crypts isolated from the transverse colon of healthy individuals and transferred to Matrigel[®] and growth media for 15 days in the presence and absence of lipopolysaccharide (LPS, 20 ng/ml). Organoid structure was assessed by light microscopy and gene expression by microarray, qPCR, Western Blotting and immunohistochemistry (IHC). LPS increased the total number of goblet cells from 2.7% + 1.2 to 20%+2.3% (n=5, p<0.05). Microarray analysis showed an increase in the goblet cell associated genes *MUC2*, *ATOH1* and *CLCA1*. Western blot confirmed an increase in the goblet cell marker *MUC2*, and IHC confirmed an increase in the number of *MUC2* positive cells. Additionally, the effect of LPS was dose dependent with increasing amounts of *MUC2* expression over the range of 2-200 ng/ml LPS. The presence of LPS receptor (TLR4) was confirmed by mRNA and protein analysis and blocking of the TLR4 receptor activity (CLI-095, 1µg/ml) abrogated the LPS-driven *MUC2* increase, indicating a possible mechanism of action through the activation of the TLR4 pathway. Collectively, these data indicate that LPS affects epithelial regeneration and brings about a lineage change in the colonic epithelium via the activity of the TLR4 receptor.

D5: The expression of functional toll like receptors in human colonic organoids

Samuel, G.¹, Rodrigues, E.^{2,3}, Slobbe, L.¹, Schultz, M.^{2,3}, Butt, A.G¹.

¹Department of Physiology, Otago School of Medical Sciences and ²Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ.

Three-dimensional primary cultures of colonic epithelium, or organoids, are thought to be a suitable model to investigate the properties of human intestinal epithelium and its interaction with commensal microbiota. However, to be of use, the organoids will need to express the pattern recognition receptors found in the native intestinal epithelium and respond to appropriate microbial associated molecular patterns (MAMPS). Therefore, we have investigated the expression of toll like receptors (TLRs) by colonic organoids and their response to TLR agonists. Organoids were grown from crypts isolated from transverse colonic biopsies from healthy patients and maintained in Matrigel[®] overlaid with stem cell media (1). Expression of TLR 2, 4 & 5 transcript and protein in the enteroids were determined by qPCR & immunoblotting and compared with the expression in isolated crypts. The organoids were stimulated with TLR agonists [TLR 2 - Pam 2CSK4 & Pam3CSK4 (200ng/ml) and lipoteichoic acid (LTA, 10µg/ml), TLR4 - lipopolysaccharide (LPS, 200ng/ml), and TLR5- flagellin (100ng/ml)] for 3, 6 & 24h after 14d of culture. IL-8 and TNF-α transcript was determined by qPCR. Statistical significance was determined by unpaired Student's *t*-test. Two morphologically distinct types of organoids developed in culture, colonospheres, which consisted of thin, non-polar cells and enteroids, which consisted of a well-developed columnar epithelium. In both enteroids and colonospheres TLR 2, 4, 5 were expressed at levels comparable to that in crypts. Addition of the TLR4 and TLR 5 agonists to the culture media stimulated a significant ($P<0.05$) increase IL8 and TNF- α production. In contrast, the TLR2 agonists did not induce an inflammatory response. These data indicate that human colonic enteroids express TLRs and respond to MAMPs at physiological concentrations. The absence of a response to TLR2 agonists may reflect that they were added to the serosal side of epithelium.

(1) Sato, T., Stange, D. E., Ferrante, M., Vries, R. G. J., van Es, J. H., van den Brink, S., et al. (2011). *Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium*. *Gastroenterology*, 141(5), 1762–1772.

Supported by a University of Otago Research Grant and Grants from the Dean's Fund, Otago School of Medical Sciences and the Department of Physiology.

D6: Review: What dietary components trigger hepatic fat accumulation in Non-Alcoholic Fatty Liver Disease (NAFLD)?

Sharp, K.P.H., Coppel, K.J., Schultz, M.

¹Department of Medicine, University of Otago, Dunedin, NZ.

Non-Alcoholic Fatty Liver Disease (NAFLD) is the most prevalent form of liver disease in western countries, with the prevalence as high as 30%. NAFLD can progress to liver cirrhosis and hepatocellular cancer, and increases the risk of cardiovascular disease type 2 diabetes. Obesity is the key risk factor for NAFLD, but it is unclear if a particular dietary composition (eg high fat low carbohydrate, low fat high carbohydrate, high saturated fat) or simply excess calories, irrespective of the macronutrient composition of the diet, triggers the accumulation of excess fat in the liver. The aim of this literature review was to examine which dietary components and which dietary patterns may initiate excess hepatic fat deposition (>5% wet weight).

Databases were systematically searched with appropriate key words through November 2014. The studies were limited to those investigating humans, articles or reviews, and those published in English. The title and abstract were reviewed for relevance, and if the article or review was relevant, it was read in full.

There was limited literature. Most studies examined the diets of those with established NAFLD. There were very few experimental studies and these usually tested extreme dietary quantities. An energy mediated response is most likely, while specific dietary components may also have an effect. Excess energy intake can induce hepatic fat accumulation, but the source of the excess energy can affect this pathogenic process. Simple sugars and saturated fats appear to increase hepatic fat, while polyunsaturated fats appear to be protective. Risk of NAFLD may decrease with increased intake of omega-3 fatty acid. There is a significant gap in the understanding of the role of nutrition in the initiation and progression of NAFLD.

D7: Modulation of colonic stem cell proliferation by bacterial components

van Hout, I.¹, Rodrigues, E.^{2,3}, Slobbe, L.¹, Schultz, M.^{2,3}, Butt, A.G.¹

¹Department of Physiology Otago, School of Medical Sciences and ²Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ.

The intestine is home to a large number of commensal microbes, which are restricted to the intestinal lumen by the epithelial barrier. In the colon, which has the greatest number of commensal microbes, the epithelial barrier is maintained by turnover of the epithelium every 3-5 days by a small population of stem cells found at the base of the crypts. Here we have used human colonic enteroids to determine if the colonic bacteria modulate the proliferation of the colonic stem cells and hence the properties of the colonic epithelium. Colonic enteroids were grown from crypts isolated from healthy patients and suspended in Matrigel[®] overlaid with a specific stem cell growth media¹. The effect of removal of growth factors (e.g., Wnt 3A), induction of goblet cell differentiation by the γ secretase inhibitor DAPT (1 μ m, [N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester), or inclusion of bacterial components [lipopolysaccharide (LPS) or muramyl dipeptide (MDP) (all 20ng mL⁻¹)] in the growth media, on cell proliferation was assessed by measurement of the expression of genes associated with cell proliferation (*Ki67*, *LGR5*, *FOXM1*, *P15*, *Cyclin D* and *LTBP1*) by qPCR. Statistical significance was determined by unpaired Student's *t*-test. Consistent with its role in the maintenance of intestinal stem cell proliferation, the removal of Wnt3A (n=6) reduced proliferation as indicated by the reduced expression of *LGR5* ($P=0.0005$), *FOXM1* ($P=0.0229$) and *P15* ($P=0.0222$). Similarly, induction of goblet cell differentiation by inclusion of DAPT in the media decreased proliferation. Interestingly, MDP, and LPS, which signal via NOD2 and toll like receptor 4, respectively, both decreased proliferation, as indicated by the decrease in expression of genes associated with proliferation. While these data need to be confirmed with direct measurements of cell proliferation, they suggest that in the colon, bacteria inhibit stem cell proliferation, potentially by inducing differentiation.

1. Sato, T., Stange, D. E., Ferrante, M., Vries, R. G. J., van Es, J. H., van den Brink, S., et al. (2011). *Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium*. *Gastroenterology*, 141(5), 1762–1772.

Supported by a University of Otago Research Grant and Grants from the Dean's Fund, Otago School of Medical Sciences and the Department of Physiology.

Summary of Abstracts for the Poster Session Template

No.	Title	Presenter	Institutions
D1	TNF- α induces a biphasic change in the expression and distribution of tight junction proteins in human colonic enteroids	Gadeock, S. ¹ , Schultz, M. ^{2,3} , Butt, A.G ¹	¹ Department of Physiology, Otago School of Medical Sciences and ² Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³ Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ.
D2	Analysis of specific T cell infiltrate can predict patient prognosis in a NZ cohort of colorectal cancer patients	Ward-Hartstonge, <u>K.</u> ¹ , McCulloch, T. ² , Cretney, E. ² , Fran Munro, F. ³ , John McCall, J. ³ , Kemp, R. ¹	¹ Department of Microbiology and Immunology, Otago School of Medical Sciences, University of Otago, NZ, ² Walter and Eliza Hall of Research Institute, Melbourne, Australia, ³ Department of Surgical Sciences, Dunedin School of Medicine, University of Otago, NZ.
D3	The effect of vitamin D on gene expression in colorectal cancer and normal colon	Munro, F.M. ¹ , McCall, J.L. ¹ , Black, M.A. ²	¹ Department of Surgical Sciences, Otago Medical School, University of Otago, Dunedin, NZ, ² Department of Biochemistry, Otago School of Medical Sciences, University of Otago, Dunedin, NZ.
D4	Chronic exposure to LPS modified lineage development via the TLR4 dependent pathway In human colonic enteroids	Rodrigues, E. ^{2,3} , Slobbe, L. ¹ , Samuels, G. ¹ , Gadeock, S. ¹ , Schultz, M. ^{2,3} ,	¹ Department of Physiology, Otago School of Medical Sciences and ² Department of

		Butt, A. G. ¹	Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³ Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ
D5	The expression of functional toll like receptors in human colonic organoids	Samuel, G. ¹ , Rodrigues, E. ^{2,3} , Slobbe, L. ¹ , Schultz, M. ^{2,3} , Butt, A.G. ¹ .	¹ Department of Physiology, Otago School of Medical Sciences and ² Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³ Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ.
D6	Review: What dietary components trigger hepatic fat accumulation in Non-Alcoholic Fatty Liver Disease (NAFLD)?	Sharp, K.P.H. , Coppell, K.J. , Schultz, M.	Department of Medicine, University of Otago, Dunedin, NZ.
D7	Modulation of colonic stem cell proliferation by bacterial components	van Hout, I. ¹ , Rodrigues, E. ^{2,3} , Slobbe, L. ¹ , Schultz, M. ^{2,3} , Butt, A.G. ¹	¹ Department of Physiology, Otago School of Medical Sciences and ² Department of Medicine, Dunedin School of Medicine, University of Otago, Dunedin and ³ Gastroenterology Unit, Dunedin Hospital, Southern District Health Board, Dunedin, NZ.