2015/2016 Summer Studentship Project Application Form

Send to: Research Office, University of Otago Christchurch, PO Box 4345, Christchurch, by 5pm on **3 July 2015**

Supervisor Information (First named supervisor will be the contact):	
Supervisor's Name and Title(s): Jacqui Keenan (Co-supervisor Andrew Day)	
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Research Category (Choose one category only – to be used for judging the students' presentations):		
	Laboratory	

Project Title

Growing enteroids to study bowel disease

Project Description:

Background: Crohn's disease (CD) has become increasingly common over recent years, including in childhood, but the precise pathogenesis of this chronic disease is unknown and CD remains incurable. Over the last 20 years exclusive enteral nutrition (EEN), a liquid diet using elemental or polymeric formulae (PF), has become established as an effective, non-medicinal treatment to induce remission in active CD. Therapy involves giving this liquid diet exclusively over a prolonged period and is particularly successful in children. In addition to anti-inflammatory effects, EEN enhances mucosal healing and permits avoidance of medication-related side effects. However, while the clinical benefits of EEN are established, the mechanisms of action of this therapy are yet to be fully defined.

We have shown that PF has a dose-dependent effect on the expression of carcinoembryonic antigen cell adhesion molecule (CEACAM)6 on the surface of Caco-2 cells, which are used as an *in vitro* model of intestinal enterocytes. CEACAM6, which is up regulated in active CD, is a marker of differentiated intestinal epithelial cells that release antimicrobial peptides into the gut lumen. These peptides, which include intestinal alkaline phosphatase and cathelicidin, are shown to have antibacterial activity. In addition, they are considered to bolster the host innate immune response by acting as a "secreted barrier". We speculate that the release of antimicrobial peptides is increased in the presence of PF and we propose to use a novel 3 dimensional model of the gut wall to test our hypothesis.

Single crypts or stem cells derived from the small intestine or colon can be expanded *ex vivo* over long periods to generate epithelial structures that closely resemble the self-renewing crypt-villus architecture of the gut. Referred to as enteroids, these three-dimensional structures contain differentiated cells of all types. Accordingly, they provide a near physiological *ex vivo* model of the gut that can be monitored in real time.

Aim: To investigate the efficacy of the enteroid model to determine the mechanism(s) by which polymeric formula causes clinical improvement in Crohn's disease.

Methods: Mouse and/or human biopsies will be digested with EDTA and then fractionated to give a crypt-rich preparation that will be cultured on Matrigel in defined medium, resulting in three dimensional structures that contain multiple cell types. The enteroids will be grown to the point where they develop the distinct crypt-like and villus-regions of the gut before being exposed to PF. Cell proliferation, markers of apoptosis and evidence of intestinal phosphatase activity will be used

to assess if PF has a measurable effect of cell differentiation and therefore the rate of enteroid growth. In addition, known markers of innate immunity will be measured *in* situ in enteroid-derived cell homogenates using enzyme activity assays, western blotting, immunohistochemistry.

Significance. This project is part of a wider research theme directed at better understanding the mechanisms that underlie the host innate immune response to gut bacteria and how we might exploit these in the prevention and/or management of gastrointestinal disease.

Student Prerequisites (eg. Medical Student) if applicable: