2014/2015 Summer Studentship Project Application Form

Send to: Research Office, University of Otago Christchurch, PO Box 4345, Christchurch, by 5pm on **4 July** 2014

Supervisor Information (First named supervisor will be the contact):	
Supervisor's Name(s): Jacqui Keenan/Andrew Day	
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Mailing Address: UOC	

Research Category (Choose one category only – to be used for judging the students' presentations):		
Clinical	**Laboratory X**	**Community**

Project Title (20 words MAXIMUM):

Intestinal membrane vesicles: a novel innate defense mechanism?

Project Description:

Background. Carcinoembryonic antigen cell adhesion molecule (CEACAM)6 is a specific cell-surface marker found on intestinal epithelial cells. More specifically, CEACAM6 is a marker of differentiated intestinal epithelial cells that shed membrane vesicles (MV) from their microvilli into the gut lumen. These vesicles have antibacterial activity and are thought to bolster the host innate immune response by acting as a "secreted barrier". Although there is evidence to suggest that CEACAM6 is contained within these MV, they likely also contain other active moieties.

We have previously demonstrated that CEACAM6 is released from the surface of epithelial cells following stimulation with interferon-gamma or treatment of the cells with a polymeric formula (PF). Further, we have demonstrated that the released CEACAM6 protein is then able to bind adherent invasive *Escherichia coli* (AIEC) thereby preventing the bacteria reaching the epithelial cell. More recently, we have shown that MV are expressed from intestinal epithelial cells, and have co-localised MV with AIEC using electron microscopic techniques.

We **hypothesise** that enterocyte membrane vesicles contain various other active molecules which also contribute to innate immune defences and that CECAM6 (and other active vesicle components) interact with various bacterial species in addition to AIEC.

The **specific goals** of this project are to ascertain the broader anti-bacterial activity of MVs and to determine whether this involves CEACAM6 or other MV moieties.

Methods. The student will stimulate Caco-2 epithelial cells with PF to induce cell differentiation and MV release. Cell culture supernatants will be ultracentrifuged and the resultant pellet fractionated by sucrose density gradient centrifugation. MV–rich fractions (identified by CEACAM6 staining and negative-staining electron microscopy) will be incubated with bacteria (*S. typhimurium*, *C. jejuni* and/or *E. coli*) and anti-bacterial activity will be quantitated by determination of bacterial colony forming units over time. In contrast, the ability of MV to block bacterial binding to the gut wall will be determined by pre-incubating MV with bacteria before adding to cell cultures. After washing, remaining cell-associated bacteria will be quantitated by serial dilution and plating of lysed cells.

Significance. This novel self-contained project is part of a wider programme, directed at better understanding the mechanisms that underlie the host innate immune response to infection and how we might exploit these in the prevention and/or management of gastrointestinal disease.

The student will work closely with the supervisors but would be solely responsible for undertaking the outlined project. The initial experimental work would be completed in 10 weeks. The data arising from these endeavours will be presented at national and/or international conferences. The student will also contribute to the submission of these data to an appropriate peer-review journal for publication.