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Investigating M cell signal transduction pathways using multi-labelling of M-cells with putative markers and bacterial transport

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M cells within the gastrointestinal epithelium perform a vital role in antigen transport and presentation in the initiation of the mucosal immune system response and thus are a focus for oral vaccine development. In this study, M cell-like cells differentiated from the human adenoma carcinoma cell line (Caco-2) after co-culture with Raji B cells or media, permit investigation of M cell signal transduction pathways. This study examined direct labelling of M cell like cells for surface markers important in the translocation of bacteria.

Glycoprotein lectin galectin-9 is up-regulated on M cell-like cells (Pielage, 2007) and bacterial interactions with microbial pattern recognition receptors (such as TLR-4 and α5β1 integrin) on the surface of M cells are important in translocation of bacteria (Tyrer, 2007). We investigated Caco-2 cells, differentiated into M cell-like cells using multicolour flowcytometric labelling to examine several putative M cell-like cell markers. Co-cultures (containing differentiated M cell-like cells) exhibited increased simultaneous labelling with galectin-9, α5β1 integrin, TLR-4 or TLR-2 (conjugated to different fluorophores) than was observed in monocultures of Caco-2 cells only. These initial experiments confirm the capacity for multicolour flowcytometric examination of simultaneously labelled M cell-like cells with several markers.

To investigate M cell signal transduction pathways, *in vitro* co-cultures were established based on the models described by Gullberg (2000), Mack (2009) and Skalska (2010) with some modification. Bacterial transcytosis by M cell-like cells after co-culture was performed using *H. influenza* 289 (NTHi 289), *E. coli* (HMN075) and *B.* thailandensis and samples collected for post cell signal transduction pathway elucidation using Real Time PCR. Preliminary results demonstrated greater transport of *H. influenza* 289 (NTHi 289), *E. coli* (HMN075) and *B. thailandensis* after co-culture than by Caco-2 cells alone (mono-culture).

These technologies permit future investigations of cell surface receptors specific to M cells that mediate translocation of bacteria for targeted oral vaccine development.

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