

Macrophages regulate colonic crypt renewal

Introduction:

The colonic epithelium is constantly renewed every 4-5 days to serve as a protective barrier against food and microbes. To maintain its barrier function and maintain gut homeostasis, Lgr5+ epithelial stem cells at the base of the crypt proliferate, differentiate into absorptive enterocytes and secretory goblet, enteroendocrine and tuft cells, which proceed to migrate along the crypt axis and are then shed into the crypt lumen. This process of epithelial renewal is finely balanced and the stem cell niche can be influenced by a number of extrinsic factors. Among those, Skoczek et al, 2014 has demonstrated that monocyte-stem cell cross-talk can modulate colonic crypt renewal. Furthermore, recent studies have shown that colony-stimulating factors (CSF)-dependent macrophages may regulate epithelial differentiation and homeostasis in the small intestine *in vivo* (Sehgal et al., 2018).

During homeostasis, monocytes are continually recruited into the gut *lamina propria* from the peripheral circulation where they differentiate into macrophages with a highly tolerogenic profile, known as M2 macrophages. The cellular and molecular mechanism by which M2 macrophages affect stem cell-driven colonic crypt renewal is not well understood. Using an *in vitro* 3D co-culture model of M2 macrophages and colonic crypts the interactions between these two cell types can be studied.

Hypothesis: Homeostatic macrophages regulate colonic crypt renewal

Methods:

Murine C57BL/6 bone marrow cells were isolated and differentiated *in vitro* towards a macrophage phenotype using macrophage-colony stimulating factor (M-CSF). Macrophages were further polarised for 24 hours towards the homeostatic (M2) lineage by supplementing media with IL-13 (10ng/ml) and IL-4 (10ng/ml). Colonic crypts and macrophages were co-cultured as previously described (Skoczek et al. 2014). Colonic crypts and macrophages were co-cultured for 24 hours. Crypt proliferation studies; following 24 hours in co-culture, EdU was added overnight and EdU incorporation determined using a Click-iT kit and confocal microscopy. Crypt stem cell and enteroendocrine cell numbers were determined using a primary antibody against Lgr5 and Cro-A, respectively, followed by a corresponding species-specific fluorescently-conjugated secondary antibody, alongside an antibody to E-cadherin. A Rhodamine-labelled UEA-1 antibody was used to quantify goblet cell numbers. Immunofluorescent labelling was visualised on a Zeiss LSM510-confocal microscope (x63 NA 0.75mm oil immersion). Image stacks were taken at 1 μ m intervals (5 μ m above and below the equatorial plane) and analysed using Image J analysis software. Data are expressed as mean \pm SEM and significance was determined via two-tailed unpaired t-tests with p-values <0.05 considered significant.

