Scaffold-Free Colonic Intestinal Spheroids: Preliminary Design for IBD Disease Modelling

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Introduction

The inflammatory bowel disease (IBD) includes gastrointestinal chronic pathologies (Crohn's disease and Ulcerative colitis) characterized by prolonged inflammatory state of the gastrointestinal tract associated with a progressive impairment of intestinal barrier integrity. Aberrant trafficking of immune system and critical activation of immunocompetent cells are responsible of severe mucosal inflammation and tissue destruction in IBD. Furthermore, a defective gut barrier and microbial dysbiosis induce accumulation and local activation of immune cells determining a pro-inflammatory cytokine loop that overrides anti-inflammatory signals and causes chronic intestinal inflammation. VitroScreen ORA™ INTESTINAL is a novel engineered scaffold-free intestinal spheroids able to reproduce *in vitro* the vicious cycle of gut-immune cells crosstalk and the mutual interaction of multicompartmental pro-inflammatory mediators at the origin of IBD.

Experimental design

Primary colonic fibroblasts and human epithelial colonic cells were seeded in sequential co-culture using hanging drop technology with adapted culture media: once the spheroids were formed, they were cultivated for 10 days.
To mirror the vicious cycle of gut-immune cells crosstalk, a multistep approach was applied resulting in three different models of increasing complexity: 1>2>3 schematically represented in Fig.1.
In model 1, an acute pro-inflammatory state was induced on ORA™ healthy intestine by exposure to 10 ng/mL of IL-1ß for 24h and the inflammation was evaluated by the quantification of IL-1ß release in the culture media: readouts were performed after 24h of recovery. In the second step, the conditioned media (CM) from inflamed spheroids was added at 50% to monocytes THP-1 for 24h and the immunocompetent profile switch was monitored by the transcriptional activity of ICAM-1 (qRT-PCR). In the third step, 20% conditioned medium of immuno-competent activated THP-1, was added to healthy ORA™ INTESTINAL.
The inflammatory profile after 48h was quantified by IL-1ß release and barrier structure modification was investigated on cleared whole mount spheroids by IF for Zonulin-1 (Fig.2).

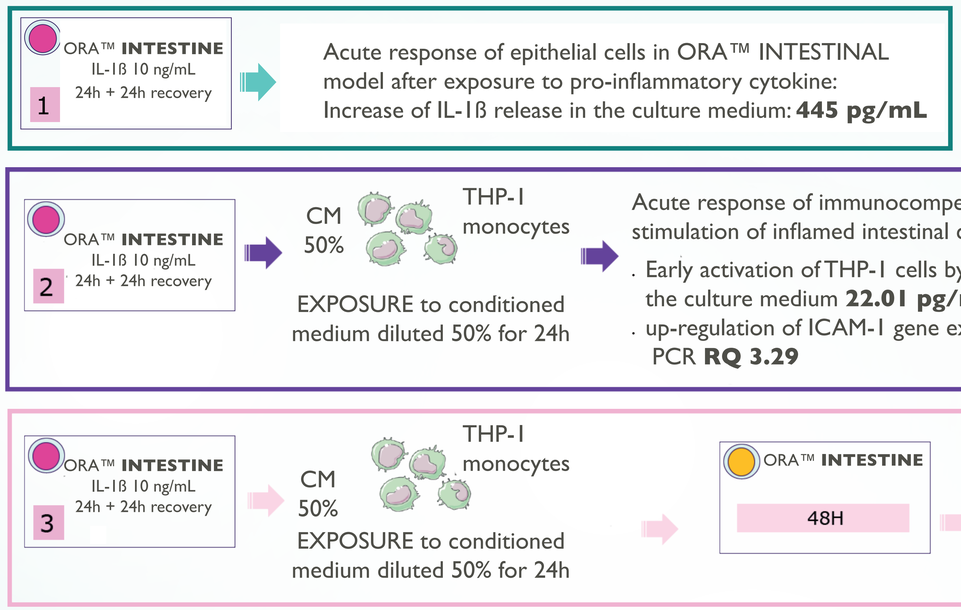

Figure 1. Schematic summary of experimental designs applied for model 1, 2 and 3 (gut induction, gut-immune system induction and gut-immune system-gut induction, respectively)

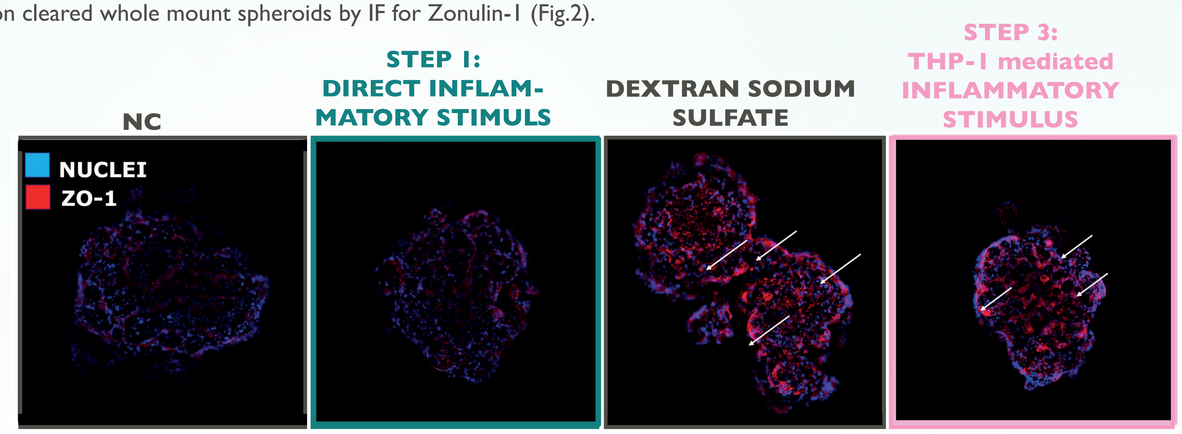

Figure 2. ZO-1 cytoplasmatic mis-localization in damaged intestinal barrier (by 3D whole mount) by IF. Acquisition performed by Leica Thunder DMi8. Mag.20X.

Results

This project is in progress and the following results for each step are underlined:
1. The direct exposure of intestinal spheroids to IL-1ß (10 ng/mL) for 24h significantly increased IL-1ß release (by ELISA) mirroring an acute pro-inflammatory status. ORA™ INTESTINAL has shown a high biological responsiveness to external stimuli as pro-inflammatory mediators.
2. Exposure of THP-1 cells to conditioned media derived from inflamed spheroids (Step1) led to the up-regulation of ICAM-1 (by qRT-PCR) and IL-1ß release was increased suggesting a phenotypical switch of THP-1 monocytes in adherent macrophagic immunocompetent cells.
3. Exposure to conditioned media from activated THP-1, led healthy intestinal spheroids to swift to a pro-inflammatory profile confirmed by high IL-1ß cytokine secretion; an impairment of barrier structure integrity was observed in this step (and it was not visible in the spheroids of Step1): dotted and fragmented ZO-1 expression was detected in whole mount samples by IF (Fig.2). The cytoplasmatic mis-localization of Zonulin-1 in gut-immune system-gut model (Step 3) was similar to ones exposed to reference dextran sodium sulfate for 72h.

Conclusion

VitroScreen ORA™ INTESTINAL spheroids alone or in conditioned with THP-1 cells are designed to mimic the complexity of human intestinal tissue, allowing the simulation of *in vitro* multi-compartmental interactions, as occurred in chronic inflammatory conditions. The aberrant vicious cycle of gut-immune system crosstalk at the origin of IBD was simulated by a direct exposure of human colonic spheroids to a pro-inflammatory cytokine and by an indirect multistep approach including THP-1 cells. The three different pro-inflammatory settings mirror the mutual interaction between pro-inflammatory mediators released by multi-compartments cross-talk.

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