Development of an *ex-vivo* Model for Microscopy-Aided Monitoring of IBD

A. Lichtenstein[1], B. Bernshtein[2], S. Jung[2] and B. Geiger[1]*
[1] Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel;
[2] Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel
benny.geiger@weizmann.ac.il

Keywords: *Inflammatory bowel disease (IBD); Intestinal macrophages; IL10Ra; Tight junctions; Focal adhesions; IL-22*

Abstract

The purpose of our study is to develop a novel approach for *ex-vivo*, microscopy-aided monitoring of intestinal epithelial dysfunctions, associated with IBD. Our strategy is based on a Multi-Compartment Cell Culture Device (MCCD), enabling 3D high-resolution monitoring of the interaction and cross talk between 3 cell populations: bacteria, intestinal epithelial cells and immune cells. Further adaptation of the MCCD for multi-well format and automated microscopy will enable the use of this system for testing and monitoring the effects of novel therapeutic IBD modalities.

A critical feature of the gut landscape is ongoing tissue renewal, with the single epithelial cell layer being replaced every five days from crypt-resident stem cells and an equally dynamic immune cell composition. Macrophages that reside in the connective tissue underlying the intestinal epithelium, the *lamina propria*, match this dynamic state with a short half-life that is unique among tissue macrophages. Here we use co-culture of normal mouse gut epithelial cells with macrophages isolated from wild type or IL10Ra deficient ($Cx3cr1_{cre}IL10Ra_{fl/fl}$) mice. These $Cx3cr1_{cre}IL10Ra_{fl/fl}$ mice were shown to develop severe spontaneous enterocolitis. We found that in case of co-culture of YAMC cells with IL10Ra-depleted macrophages activated with LPS caused severe disintegration of the tight junctions (TJ). Furthermore, we demonstrated that mutant macrophages induced TJ disruption rather than prevented their formation. Pro-inflammatory factors secreted by IL10-depleted macrophages into growth medium and induced major damage in tight junction integrity. Also, we discovered that addition of LPS, TNFα, IL1β, IFNγ, or combinations thereof to the epithelial monolayer resulted in alterations in the organization of tight junctions and cell matrix adhesions in human CaCo2 cells. These results indicate that pro-inflammatory factors affect the integrity of tight junction and the structure and number of focal adhesions.

Stimulation of the monolayer with IL-22 didn't result in noticeable changes in the integrity of tight junctions or the structure of the cytoskeleton. Still, combination of IL-22 with LPS or TNFα partially inhibited their effect on tight junction integrity.