ANTI-INFLAMMATORY EFFECT OF PROBIOTICS ON CO-CULTURE MODELS OF MACROPHAGE-LIKE AND CACO-2 CELLS

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INTRODUCTION
Commensal microbes in gastrointestinal tract play an essential role in nutrition and food digestion. These bacteria impact metabolism and regulate immune system. The use of probiotic bacteria have therapeutic effects in gastrointestinal disorders: inflammatory bowel disease (IBD) and irritable bowel syndrome. IBD has been associated with defects in the intestinal barrier and impaired immune function. Probiotics modulating signaling pathways in macrophages should have corresponding effects on mucosal immunity. One of the most important roles of the intestinal epithelium is to act as a mucosal barrier. Epithelial cells respond to intestinal luminal changes by varying their signal to immune cells within the intestine. IBDs have been characterized by an exaggerated proinflammatory immune response to the commensal intestinal microbial flora. Studies have demonstrated also that this aberrant inflammation leads to an increased permeability of the intestinal barrier, allowing toxins and microbes to reach the underlying tissues. Intestinal epithelium plays the central role in inflammatory responses, therefore the aim of this study was to establish different in vitro experimental models to assess the immunomodulation capability of human intestinal microbiota, to investigate the response of immune cells to pro-inflammatory stimuli and to provide new insight into intestinal inflammation as a screening tool for anti-inflammatory compounds.

1. In vitro immunocompetent gut using a co-culture of intestinal epithelial cells (Caco-2) and macrophages (differentiated THP-1), one of the major immune cells, which immune responses are induced by extrinsic factors such as pathogenic bacteria and food components. The immunocompetent cells underneath Caco-2 are indirectly activated by inflammatory mediators released from intestinal cells directly stimulated by commensal bacterial contact. This points to the possibility of analyzing the Caco-2-mediated activation of the host immune system by probiotics or prebiotic food that represent interesting ingredients for nutraceuticals.

2. Inflammatory intestinal mucosa model. This was achieved by stimulating intestinal epithelial cells with a proinflammatory cytokine IL-1β. While most IBDs have so far been considered as incurable, therapeutic measures are directed to treat the symptoms by anti-inflammatory drugs and to prolong the remission by various immunomodulators, especially corticosteroids. For this reason, Dexamethasone Sodium Phosphate 0.15% has been used as a reference molecule to study the outcome of the inflamed status of the mucosa. The intestinal anti-inflammatory activity of different compounds has been evaluated in a static assay using the down-regulation of cell adhesion of THP-1 on the Caco-2 cells as an endpoint parameter. In both models the following product’s efficacy has been assessed: 

- Malus domestica Borkh. and Bifidobacterium Bifidum/Lactobacillus Acidophilus
- and the treatment of Caco-2 cells has been performed during 24h.

EXPERIMENTAL MODELS

IMMUNOCOMPETENT GUT MODEL - Co-culture of intestinal and immunity cells

Caco-2 cells form a continuous monolayer epithelium on a microporous filter, which constitutes the bottom of the Transwell insert. The monolayer of Caco-2 cells is continuous and thus separates the chamber into an upper chamber (A) and a lower chamber (B) containing THP-1 cells at the bottom of well. THP-1 cells for co-culture experiments have been differentiated to macrophage-like cells by treatment with phorbol myristate acetate (Sigma) for 72 hr before the co-culture experiments.

INFLAMMATORY MODEL - Monocyte-epithelial adhesion assay

Caco-2 cells were pretreated with IL-1β, the pro-inflammatory cytokine, overnight before adhesion assay to create the inflamed mucosa. Washed mononuclears were then treated for 24h with probiotics, prebiotics or drugs. Labeled THP-1 cells were added over the Caco-2 per well. THP-1 was labeled with the fluorescent dyes chloromethyl fluorescein diacetate (CMFDA), Invitrogen). After incubation, the mononuclears were gently washed to remove non adherent THP-1 cells. Fluorescent-labeled adherent cells were visualized with fluorescent microscope.

RESULTS

- **mRNA on THP-1**
  - THP-1 increased the expression of CD14, IL-8 and IL10 compared to non differentiated THP-1 confirming the interaction between the 2 compartments.
  - Malus domestica Borkh induced over expression of CD14, IL8 and IL10.
  - Lactobacilli induced a decrease of CD14 compared to controls; IL-10 was reduced compared to the THP-1 differentiated.

- **mRNA on CACO-2**
  - Malus domestica Borkh and Lactobacilli have both induced a significant over expression of IL8 and IL10.

IONS PARACELLAR PASSAGE

- A marked increase of paracellular passage has been induced by lactobacilli treatment of Caco-2.
- Malus domestica Borkh reduced barrier properties increasing its uptake and stimulating the immunomodulatory response.

PARAMETERS

- **Biomarkers relevant and predictive of immunomodulatory pathways have been evaluated by gene expression by Real Time PCR with Taqman assay technology**
- **IL8: pro-inflammatory marker**
- **IL-10: anti-inflammatory inhibits the generation of IL8-mediated immune responses**
- **CD14: macrophage differentiation marker**
- **Epithelial barrier integrity and fence properties have been evaluated by Trans-Epithelial Electrical Resistance (TEER)**

- **Anti-inflammatory activity has been evaluated by Monocyte-epithelial adhesion assay**
- **Dexamethasone Sodium Phosphate 0.15% correlates with an ANTI-INFLAMMATORY EFFECT**
- **Epithelial barrier integrity and fence properties have been evaluated using Trans-Epithelial Electrical Resistance (TEER)**

- **Stimulation with IL1β: increase the IL-8 and TNF-α mRNA production in Caco-2 cells**
- **Dexamethasone, the reference control, as expected, induced a down regulation of both inflammatory mediators**
- **Lactobacilli induced a decrease of both inflammation biomarkers**
- **Malus domestica Borkh induced a marked decrease in TNF-α expression**

- **FENCE PROPERTIES**
  - After pre-treatment by adding IL-1β to the apical compartment, the TEER value of Caco-2 decreased compared non stimulated control values confirming the establishment of the immunocompetent model.
  - After Dexamethasone and Malus domestica Borkh the barrier integrity was recovered.
  - Lactobacilli induced a drastic barrier impairment.