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

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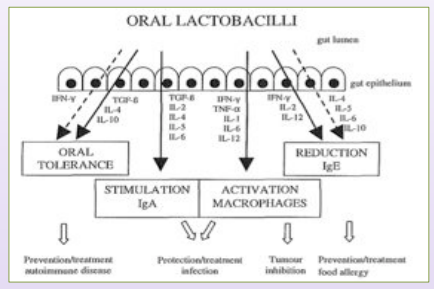
INTRODUCTION

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 epithelial barrier, allowing toxins and microbes to reach the underlying tissues. Intestinal epithelium plays the central role in inflammatory response, therefore, the aim of this study was to establish **2 different in vitro experimental models** to assess the **immunomodulation capability of human intestinal microflora,** 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 IL1β. 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 overcome of the inflammed 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 products'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**.

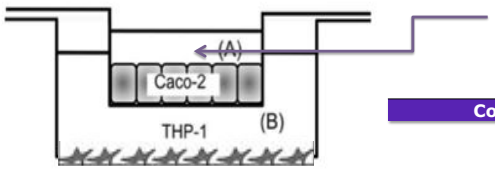


Modulation of cytokines by oral administration of lactobacilli

Lactobacilli have been shown to activate monocytes and macrophages, which play a pivotal role in antigen processing, presentation and activation of antigen-specific immunity and to stimulate IgA immunity. In particular, these cells together with dendritic and T regulatory cells are essential in the deviation of immune response to the so called type 1 response with cytotoxic effector cells or towards type 2 response characterized by antibody response.

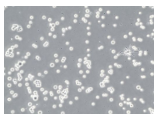
EXPERIMENTAL MODELS

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



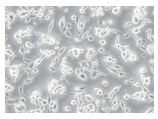
Co-culture MODEL INDUCTION

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.



THP-1= Human acute monocytic leukemia cell line

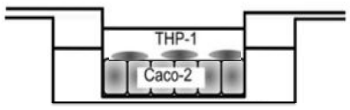
Activation by PMA (72h)



THP-1= Mature macrophages

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 cell mediated immune responses **CD14:** macrophage differentiation marker •**Epithelial barrier integrity** and fence properties have been evaluated by Trans-Epithelial Electrical Resistance (TEER)

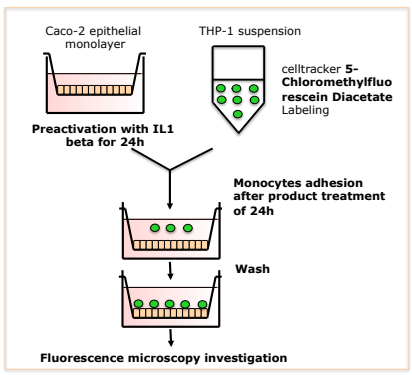
INFLAMMATORY MODEL - *Monocyte-epithelial adhesion assay*



INFLAMMATORY MODEL INDUCTION

Treatment of Caco-2 cells overnight with with **IL-1β**

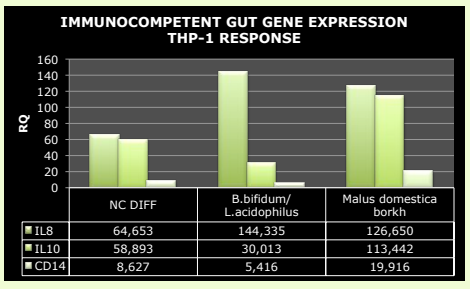
Caco-2 cells were pretreated with IL-1β, the pro-inflammatory cytokine, overnight before adhesion assay to create the inflammed mucosa. Washed monolayers were then treated for 24h with prebiotics, probiotics or drugs. Labeled THP-1 cells were added over the Caco-2 per well. THP-1 was labeled with the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



PARAMETERS	
	•**Biomarkers related to inflammatory and immunomodulatory pathways** have been evaluated by gene expression by Real Time PCR with TaqMan gene assay technology •**Anti-inflammatory activity** has been evaluated by Monocyte-epithelial adhesion assay > a DOWN-REGULATION OF ADHESION correlates to an ANTI-INFLAMMATORY EFFECT •**Epithelial barrier integrity** and fence properties have been evaluated by Trans-Epithelial Electrical Resistance **(TEER)**

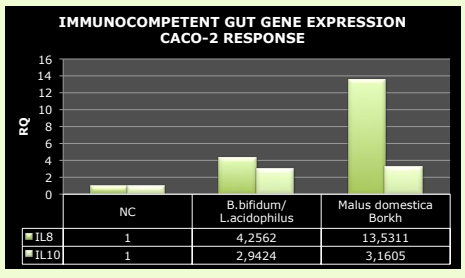
RESULTS

IMMUNOCOMPETENT GUT MODEL



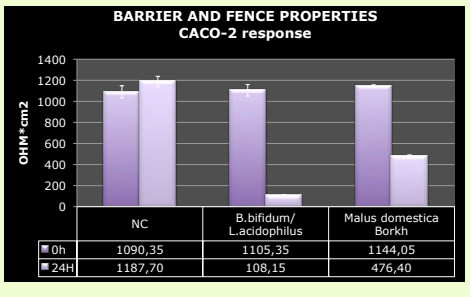
mRNA on THP-1

- THP-1 increased the expression of CD14 / IL8 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 control; IL-10 was reduced compared to the THP-1 differentiated.



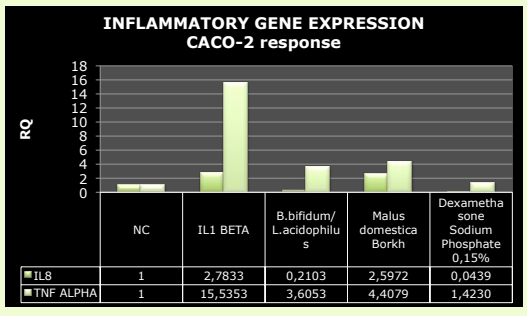
mRNA on CACO-2

- Malus domestica Borkh and Lactobacilli have both induced a significant over expression of IL8 and IL10

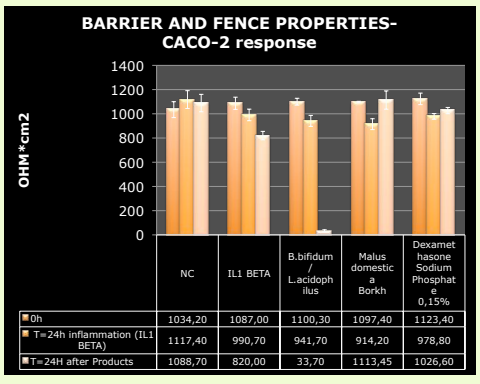


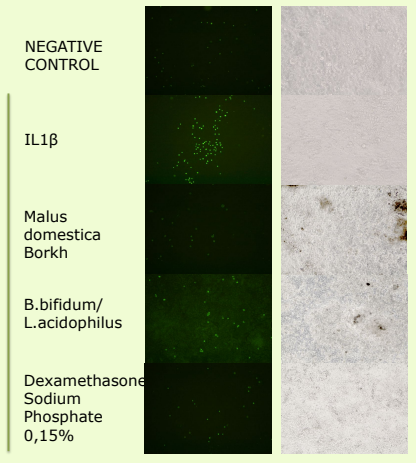
IONS PARACELLULAR PASSAGE

- A significant increase of ions paracellular passage has been induced by Lactobacilli treatment of Caco-2
- Malus domestica Borkh reduced barrier properties increasing its uptake and stimulating the immuno-modulatory response.



- 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 genes expression
- Lactobacilli induced a decrease of both inflammation biomarkers
- Malus domestica Borkh induced a marked decrease in TNF-α expression





Weak labelling suggests anti-inflammatory action

ANTI-INFLAMMATORY ACTIVITY

- The inflammatory model has been achieved by the overnight addition of IL1β: increase of THP-1 adhesion after treatment
- Very low amount of THP-1 were adherent to the Caco-2 epithelium
- Dexamethasone, the reference control, induced a down regulation of cell-adhesion.
- Anti-inflammatory activity of Malus domestica Borkh
- Increase of THP-1 adhesion with lactobacilli

FENCE PROPERTIES

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