PROBIOTICS
Wednesday, July 8

W.55. Probiotic Fermented Milk as Mucosal and Systemic Adyuvant in Malnutrition

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Background: In protein-energy malnutrition (PEM) there is a significant impairment of the immunological response. We evaluated the adjuvant effect of probiotic fermented milk (PFM) in a PEM non-severe experimental model on the local and systemic-immunity, to demonstrate its effectiveness in risk groups to reinforce the vaccine effect. Methods: mice were divided in 4 groups. G1 normal control with balanced food ad-libitum and water. G2 control PEM with restriction of 25% of food. G3 after PEM continued with restriction diet plus milk. G4: after PEM continued with restriction diet plus PFM during 5 days. After re-nutrition period studied thymus and small intestine (SI) histology, number of IgA+, cytokine+ and T cells. For systemic-immunity after of re-nutrition animals were immunized with ovoalbumin. Specific-IgG-anti-ovo in serum by ELISA was determined. Results: The thymus and SI histology was altered in G2. G4 showed a histological improvement in both tissues. IgA+ cells also increased significantly in G4. Significant increase of specific-IgG-anti-ovo in serum of mice re-nourished with PFM prior-immunization was observed. Conclusions: PFM had better adjuvant effect than milk in the recovery histological and immune parameters as well as on systemic-immunity with an increased in specific-IgG-anti-ovo showing it effectiveness in sub-nourished groups previous to vaccination.

W.56. Antigen Presenting Cells Exposed to *Lactobacillus Acidophilus* NCFM™, *Bifidobacterium Bifidum* BI-98 and BI-504 Reduce Regulatory T Cell Activity

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The *in vitro* effect of six probiotic strains was studied on splenic enteroantigen-presenting cells (APC) and $CD4^+CD25^+$ T regulatory cells (Tregs) in APC-T cell proliferation assays. Exposure of APCs to enteroantigens and the probiotics did not influence the stimulatory capacity of APCs on enteroantigen-specific T cells. However, exposure to three of the six probiotics (*B. bifidum* BI-98, *L. acidophilus* NCFM™ and *B. bifidum* BI-504) consistently reduced the suppressive activity of Tregs added to the T cell proliferation assay. The suppressive activity was analyzed using fractionated components of the *B. bifidum* BI-98 strain, and indicated that bacterial cell-wall and not the nucleus or cytoplasm is responsible for the decreased Treg activity. The probiotic-induced suppression of Treg is not mediated by changes in APC-secretion of the inflammatory cytokines (interleukin) IL-6 or IL-1β. We conclude that reduced Treg activity in the enteroantigen-specific T cell proliferation assay is caused by a direct APC-to-Treg cell contact. The APC-mediated suppressive effect on Tregs of certain probiotics may oppose the anti-inflammatory activity often desired from probiotic therapy. This unexpected function of certain probiotic strains should be considered when designing adjuvant therapies with these bacteria, or when probiotics are selected for improvement of gut-associated inflammation like IBD.

W.57. Transcriptional Investigation of Murine Dendritic Cells upon Stimulation with the Probiotic *Bacterium Lactobacillus* Acidophilus NCFM

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Probiotic bacteria have the ability to influence our general health by interacting with immune cells located in the gut. Dendritic cells (DC) are of special relevance as they function as central gatekeepers and regulators of the immune response, invoked by orally ingested antigens, including the gut microbiota. With the aim to elucidate the molecular signaling pathways in DC upon their activation through stimulation with the probiotic bacterial strain *Lactobacillus acidophilus* NCFM, we investigated the expression profile of murine bone marrow derived DC *in vitro* stimulated with *Lactobacillus acidophilus* NCFM by genome wide microarray analysis. Upon stimulation, DC were harvested at different time points (0h, 4h, 10h and 18h) to generate a comprehensive view of the expression profile. Data were statistically analyzed by ANOVA, and the differentially expressed genes were categorized into early response genes, middle response genes and late response genes. Preliminary biological data analysis shows an early upregulation of clusters of immunologically related genes, whereas middle to late upregulated genes are mainly metabolic. Interestingly, the gene encoding toll-like receptor 3 (TLR3), a key player involved in viral defense, along with other virus-defense related genes, was strongly induced after 4h of stimulation with *Lactobacillus acidophilus* NCFM.

W.58. Bifidobacteria Soluble Factors Shut Down Phosphorylation Involved in Epithelial Secretion During Intestinal Inflammation

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Background: The mechanisms by which Bifidobacterium breve C50 (Bb) and its conditioned medium (Bb-CM) can modulate cytokine/chemokine production and chloride secretion in epithelial cells were analyzed and compared with other Gram(+)

bacteria. Methods: Carbachol-induced chloride secretion in HT29-19A cells was recorded in Ussing chambers as the increase in short-circuit current after treatment with Bb or Bb-CM. TNFα-induced chemokine (CXCL8) secretion and alteration of NF-κB and AP-1 signalling pathways by Bb were studied by EMSA, confocal microscopy and western blotting. Anti-inflammatory capacity was tested in a model of colitis in mice. Results: Carbachol-induced chloride secretion was dose-dependently and specifically inhibited by Bb and Bb-CM. Inhibition occurred downstream of epithelial Ca2+ mobilisation and did not involve CFTR expression or phosphatase activity, but was linked to inhibition of the kinase PKC. Bb and Bb-CM also induced an inhibition of CXCL8 secretion by epithelial cells through AP-1 and NF-κB pathways, driven by decreased phosphorylation of p38-MAPK and IκB-α molecules. In colitis in mice, Bb-CM decreased inflammation, essentially through dendritic cell conditioning. Conclusions: Soluble factors released by the probiotic Bb target intestinal chloride secretion and chemokine production. The results suggest that Bb might down regulate inflammation by inhibiting various serine/threonine kinases involved in inflammatory processes.

W.60. *Lactobacillus Acidophilus* NCFM Induces a Fast and Early Maturation of T Cells in Mono-associated Mouse Pups

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In vitro studies previously performed in our lab on the widely used probiotic *Lactobacillus acidophilus* NCFM (NCFM) have shown a very strong Th1 skewing potential for this strain. In the present project, the immune modulating properties of NCFM as a first colonizer are tested *in vivo* by the use of mono-associated mouse pups as an *in vivo* model. We aim to clarify the impact of the first bacterial colonizer on the infant immune system. By the use of flow cytometry it is investigated how an NCFM mono-colonization in the newborn mice, compared to pups with a conventional flora and germ free pups, modulates the early development of immune cell populations in spleen and mesenteric lymph nodes. Preliminary results indicate that NCFM serves as a strong modulator of early immune maturation, as a significantly faster and earlier development of CD4+ and CD8+ T-cells is seen in the mono-associated pups. Ongoing experiments are focused on intestinal epithelial integrity and bacterial translocation to organs. Fluorescent *in situ* hybridization and gene expression studies on gut sections will be used for elucidation of the mechanisms behind the NCFM immune maturation potential.