Molecular and Cellular Analysis of the Commensal Bacteria-Host Interactions in Intestinal and Peripheral Sites: Toll-Like Receptors and Related Intracellular Signalling Cascade

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The original idea about probiotic bacteria has always been to change the composition of the intestinal microflora by reducing the number of potentially harmful microorganisms and by increasing a microflora that would be beneficial for the host. Until now, it is scientifically accepted that probiotic strains influence a broad range of functions related to the host`s immune system. Immunomodulating effects have been shown *in vitro*, in animal models and in humans. However, the underlying mechanisms have not been fully elucidated yet because of the complex interactions between the host`s own microflora, the ingested microorganisms, and the host`s immune system.

In the past years, new families of so-called pattern recognition receptors (PRRs) have been discovered. Immune cells and intestinal epithelial cells, as an element of immune system, recognize bacteria via PRRs. There are three classes of PRRs: Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). At least one of these, the toll-like receptors, seem to play a crucial role im mediating immunomodulatory effects triggered by probiotic bacteria and their components such as DNA. The alleviation or prevention of different diseases by probiotic bacteria and their components such as DNA has been shown. These were primarily gastrointestinal diseases. But other studies have also shown effects outside the gastrointestinal tract, suggesting that the effects of probiotics on the host immune response may be based on more than the manipulation of intestinal microflora alone and may represent a systemic modulation of inflammatory processes.

In study I, we investigated the influence of *Lactobacillus rhamnosus GG, Lactobacillus gasseri* PA16/8*, Bifidobacterium bifidum* MF20/5**,** *Bifidobacterium longum* SP07/3, and their genomic DNA on Th1/Th2 cytokine response by peripheral blood mononuclear cells (PBMC) from healthy and Dermatophagoides pteronyssinus (Dpt)-allergic subjects in the presence or absence of Staphylococcal enterotoxin A (SEA) superantigen or Dpt and determined IL-4, IL-5, and IFN-γ secretion.

In study II, we tested the influence of DNA from *Lactobacillus rhamnosus GG* and *Bifidobacterium longum* on gene and protein expression profiling of TLR9 and related intracellular signalling by polarized intestinal epithelial cells (IECs) using semi-quantitative RT-PCR, TaqMan qRT-PCR, ELISA, Western blot, EMSA, NF-*κ*B-dependent luciferase reporter gene assays, and gene silencing technology. Also, transepithelial resistance (TER) was monitored as an indication of tight junction formation in the polarized epithelial monolayers using an EVOM epithelial voltohmmeter and electrode. Further, transmonolayer movement of natural commensal-origin DNA across monolayers was monitored using qRT-PCR and nested PCR based on bacterial 16S rRNA genes.

In study I, live probiotic bacteria and their genomic DNA inhibited IL-4 and IL-5 secretion by SEA- and Dpt stimulated PBMCs from healthy and Dpt-allergic subjects. In contrast, live probiotic bacteria and their genomic DNA induced SEA-and Dpt stimulated IFN-γ secretion by PBMCs from healthy and Dpt-allergic subjects.

In study II, apical treatment of TLR9 with DNA isolated from LGG and B.l up-regulated TLR9 expression in a specific manner, which is subsequently associated with attenuation of TNF-α-induced NF-κB activation and NF-*k*B-mediated IL-8 expression by polarized IECs. Also, DNA from LGG diminished TNF-α-induced NF-*k*B activation by reducing I*k*Bα degradation and p38 MAP kinase phosphorylation, which are two key factors for the activation of NF-*k*B. Further, LGG DNA diminished subsequent translocation of NF-*k*B into the nucleus, where it regulates various genes. LGG DNA did not decrease the transepithelial resistance (TER) but rather diminished the TNF-α-induced TER reduction. Translocation of natural commensal-origin DNA into basolateral compartments did not occur under tested conditions. This implies that regulation of TLR9 signalling induced by DNA in these experiments was based on apical DNA exposition and mediated exclusively by the apical TLR9. TLR9 signalling may mediate, at least in part, the anti-inflammatory effects of DNA from probiotic bacteria on the gut, since TLR9 silencing abolished the inhibitory effect of DNA from probiotic bacteria on TNF-α-induced IL-8 secretion. Our results and previous data imply that invasiveness of bacteria, nature of DNA, polarity of cells, surface-specific expression of TLR9, and tight junction (TJ) integrity have to be taken into account to predict the outcome of TLR9-related signalling pathway.

Together, these findings indicate that probiotic bacteria and their components such as DNA modulate immune response to antigens and inflammatory processes dose dependently. Although in vitro models indicate beneficial effects and provide a rationale for the preventive and therapeutics use of probiotics, the beneficial health effects of probiotics have to be verified by double-blind placebo-controlled trials.