

Cross-talk of human gut with bifidobacteria

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The gut constitutes a prominent part of the immune system. Its commensal microflora plays an important role in defense and in tolerance to diet allergens. Disturbances in immune regulations may lead to food allergy. Among commensal bacteria, bifidobacteria are able to induce mechanisms of immune tolerance. Comprehension of their mutual cross-talk with the host is necessary for understanding their role in the diet and in food supplements.

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INTRODUCTION

Bifidobacterium spp. comprise a large group of bacteria, which are natural inhabitants of the gastrointestinal tract (particularly of the colon), mouth, and vagina. They are found in humans and other mammals but also in invertebrates such as bees. They are responsible for suppressing pathogenic microorganisms and are rarely pathogenic themselves.¹ Infections caused by some species of *Bifidobacterium* include obstetric and gynecological sepsis, peritonitis, pulmonary infections, urinary tract infections, bacteremia, and endocarditis. These gram-positive, non-motile anaerobic rods were first isolated by Henry Tissier from the Pasteur Institute in 1899 from the feces of breastfed infants.² They were found also in breast milk (mainly *B. longum* followed by *B. animalis*, *B. bifidum*, and *B. catenulatum*).^{1,3} Although predominating in newborns and constituting up to 95% of intestinal microbes in breastfed infants,⁴ they form a mere 3% of adult gut microflora, which includes over a thousand bacterial species.⁵ In contrast with current opinion, a study performed by Palmer et al.⁶ could not confirm the predominance of bifidobacteria in the intestinal microflora of babies.

Bifidobacteria belong to *Actinobacteria* and are not related to other lactic bacteria such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, or *Weissella*, which are *Firmicutes*. Bifidobacteria are about one hundred times more numerous in the colon than lactobacilli.⁷ Both

genera share several metabolic properties and form a group known as lactic acid bacteria (LAB). The LAB are used as live bacteria in numerous food preparations, e.g., as starter cultures, food supplements, or probiotics (microorganisms with a favorable effect on human health). Among about 30 human species of bifidobacteria, *B. bifidum*, *B. breve*, *B. longum* subsp. *infantis*, *B. longum*, *B. animalis* subsp. *lactis*, and *B. adolescentis* are considered as probiotics.⁸

Bifidobacterium spp. are used as a form of probiotic that is thought to have health-promoting properties in humans. They favorably alter the intestinal microflora balance,⁹ inhibit the growth of harmful bacteria,¹⁰ promote good digestion,¹¹ relieve children's diarrhea¹² or constipation,¹³ boost immune function, and increase resistance to infection.¹⁴ It has been claimed that colonization of the mammalian gut with bifidobacteria supports the shaping of appropriate gut microflora and induction of oral tolerance and decreases the frequency of allergic disorders.¹ Allergic infants were shown to have fewer bifidobacteria and more clostridia and, in addition, to have adult-type bifidobacteria.¹⁵ The relation of bifidobacteria to allergy is not, however, unequivocal, because colonization of infants with these bacteria is not associated with a reduced risk of allergies.¹⁶ Some beneficial effects of probiotic bifidobacteria are documented, the mode of their action on the modulation of the host immune system is, however, largely unknown.

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RECOGNITION OF BACTERIA

It is no wonder that the gut, which contains about 10^{14} bacteria and many other microorganisms (about 1 kg in total mass) is protected by the largest immune structure of the body and possesses powerful mechanisms of natural immunity.¹⁷ Not only has this structure to resist pathogens (they form only a minute fraction of all intestinal microorganisms – probably less than 1 ppm), it also has to prevent non-virulent microbes and opportunistic pathogens from translocating into the internal environment, which could lead to the establishment of oral tolerance to them, and it must process the mass of food containing several tons of antigens in the course of human life.^{9,14,18,19}

To be able to precisely differentiate between commensals and pathogens, multicellular organisms possess a sophisticated cognitive system tolerating the former and eliminating the latter. During the course of co-evolution, the hosts and commensal microbes developed numerous mechanisms to coexist, which can be traced even in the host genome.²⁰ It is believed that restricted recognition of microbes antedates the adaptive immune system and formed the basis of natural immunity.

All microbes contain distinct structures or microbe-associated molecular patterns (MAMPs), which are recognized by the host.²¹ The original name of these structures was PAMPs or pathogen-associated molecular patterns. However, this is not completely accurate since the same structures can be found on non-pathogenic bacteria as well as on pathogens. Bacterial MAMPs are repetitive, frequent, unexpendable structures such as unmethylated CpG DNA motifs, lipopolysaccharide, muramyl dipeptide, flagellin, or others.^{22,23} The MAMPs are recognized in the host by pattern recognition receptors (PRRs).^{24,25}

The decisive steps in the differentiation of commensals from parasites are host cell damage and liberation of substances that are sensed as danger-associated molecular patterns (DAMPs). These were predicted by the guard hypothesis or danger model of Polly Matzinger.²⁶ A plethora of molecules has recently been proposed as DAMPs,²⁷ including the following examples: high mobility group box-1 protein, uric acid/monosodium urate, extracellular adenosine triphosphate, galectin, DNA, heat shock proteins,²⁸ and some antimicrobial peptides (defensins, cathelicidins, S100).

The PRRs are membrane or cytoplasmic molecules that serve to recognize microbes and induce innate immunity responses. The PRRs are numerous in immune cells, but they are also found in other cells of the organism. They belong to phylogenetically old proteins containing 20–30 recognition leucine-rich repeat (LRR) motifs. The LRR proteins are also found in defense

systems of plants or invertebrates and in protein-protein interactions of bacteria.^{23,24}

An important class of PRRs is Toll-like receptors (TLRs). The TLRs TLR1–TLR10^{29,30} were discovered as homologues of *Drosophila* Toll proteins, playing an important role in differentiation and defense. Each TLR recognizes a different MAMP. Membrane heterodimers TLR2 + TLR1 (or TLR6, respectively) recognize lipids of microorganisms – lipopeptides of gram-negative bacteria, lipoteichoic acid of gram-positive bacteria, lipooligosaccharides of mycobacteria or zymosan of fungi. TLR4 senses lipopolysaccharide, TLR5 flagellin, whereas TLR3, TLR7, TLR8, and TLR9 are cytosolic receptors for nucleic acids.^{23,24}

Another significant group of PRRs is the NOD-like receptor (NLR) group^{31,32} residing in the cytoplasm. The recent discovery of NLR solved the persisting problem of innate recognition of intracellular parasites (the intracellular MHC proteins known so far present antigenic peptides to T cells as receptors of adaptive immunity). NOD1 and NOD2 are NLRs that recognize microbial peptidoglycans. Other NLRs (NALPs, NAIP, and IPAF) form part of the inflammasome, a cytosolic protein complex that induces inflammatory response. Thus, NALP3 recognizes bacterial peptidoglycans and “danger” molecules signaling damage of cells, e.g., extracellular adenosine triphosphate or monosodium urate, and IPAF is a sensor of bacterial flagellin (e.g., intracellular parasite *Salmonella*).^{33–36}

Endocytic PRRs enable endocytosis of microbes by phagocytes. They include dectin-1 recognizing β -glucans of yeast, and scavenger receptors and mannose receptors recognizing bacteria.^{37–39}

The intestinal epithelium and leukocytes of gut mucosa, especially dendritic cells, play a major role in the recognition of microbes in the gut. The dendritic cells are spidery mononuclear leukocytes that transfer nature immune responses to specific ones. They are on patrol in mucosae and organs; upon recognizing antigens by their PRRs, they migrate to the lymph nodes, where they present the antigenic peptides to T lymphocytes and help B cells start specific immune responses, e.g., production of antibodies. Zeuthen et al.⁴⁰ demonstrated the importance of TLR2 heterocomplexes on the membrane of dendritic cells for the recognition of lipoproteins in bifidobacteria. This signal inhibits activation of cytoplasmic NOD2 by the peptidoglycans of these bacteria and is thus responsible for the development of the tolerogenic phenotype of dendritic cells. This process ensures that recognition of bifidobacteria by host cells does not lead to inflammation. Instead, it is interpreted as a symbiotic signal.

Pattern recognition receptors induce transcription factors such as nuclear factor-kappaB (NF- κ B) and

transcription of genes coding for pro- and anti-inflammatory cytokines. Several inflammatory cytokines produced in the gut by immune and epithelial cells (enterocytes, dendritic cells of lamina propria, neutrophils, macrophages, lymphocytes, mast cells, and eosinophils) orchestrate the resultant natural immune response involved in the specific immunity of T and B lymphocytes. Thus, bifidobacteria could also modulate the adaptive immune response. At an early age, infants delivered by cesarean section have been found to harbor fewer bifidobacteria than vaginally delivered infants; they also mounted a stronger humoral immune response.^{41,42}

ANTIMICROBIAL SUBSTANCES

Antimicrobial substances and genes responsible for resistance to them have their origins in the prokaryotic world.⁴³ Bifidobacteria synthesize their own antimicrobial peptides⁴⁴ that inhibit the growth of *Helicobacter pylori*. Antimicrobial substances are also found in all hosts of bacteria, from protists to vertebrates. Mammalian antimicrobial peptides and proteins (AMPPs) are mostly small substances that insert into microbial membranes and disrupt them, bind vital substances (e.g., iron and zinc), or digest microbial structures.^{45–47} To face undesirable microorganisms, the gut contains the largest amount of AMPPs in the body – several grams of these “animal antibiotics”. The intestinal epithelium (enterocytes and Paneth cells) produces defensins and cathelicidins, cryptdin-related sequence peptides, bactericidal/permeability increasing protein (BPI), lysozyme, and phospholipases A₂. Intestinal leukocytes contain calprotectin (S100A8/A9), NK-lysin, BPI, lactoferrin, lysozyme, phospholipases A₂, serprocidins, and other AMPPs. Some AMPPs are sensed as danger molecules and induce cytokines in the enterocytes or professional immune cells in the lamina propria. This leads to enhanced clearing of bacteria and presenting of their substances to relevant T cells.^{45,48–52}

Wang et al.⁵³ demonstrated that *Bifidobacterium* cell-wall proteins induce expression of human defensin 2 mRNA in human intestinal epithelial cells. The induction of AMPPs was also observed in some other probiotics.⁵⁴

Commensal bacteria, such as bifidobacteria (with the exception of *Bifidobacterium dentium*, which is recognized as a human pathogen),⁵⁵ have intrinsic ability to induce tolerogenic mechanisms in the host and alleviate the resulting immune response. The successfully colonized neonatal gut shows marks of immune maturation without pathological inflammation. The absence of inflammation in the presence of myriad microbes is ensured by mutualistic interplay between commensals and the host and involves numerous alternative mecha-

nisms of downregulation triggered by autochthonous microflora and supported by host cells. The inhibition of I-kappaB ubiquitination in epithelial cells and the resulting inhibition of induction of NF-kappaB is the major tolerogenic mechanism. High expression of Toll-interacting protein, nuclear-cytoplasmic shuttling of peroxisome proliferator-activated receptor gamma-related protein (PPAR gamma), and zinc finger protein A20 are key inhibitors of TLR4 mediated inhibition of NF-kappaB.³⁰ Decreased surface expression of TLRs in the intestinal epithelium supports decreased signaling, but there are other mechanisms. These include inhibition of IL-1 receptor-associated kinase M (IRAKM), modulation of single immunoglobulin domain containing IL-1R-related protein (SIGIRR), or suppressive pathways of IL-10 and TGF- β -signaling cascades, leading to the activation of regulatory T cells, which in turn inhibit the immune response and induce mucosal tolerance. Some commensals and probiotics upregulate the expression of suppressors of cytokine signaling.^{56–60}

CYTOKINE RESPONSES

Due to their beneficial probiotic effect, it was presumed that bifidobacteria could downregulate proinflammatory cytokine responses. The first results were encouraging. Niers et al.⁶¹ found the induction of IL-10 in peripheral blood mononuclear cells cultivated with bifidobacteria. On the other hand, Kim et al.⁶² observed that bifidobacteria obtained from monogastric animals were able to induce nitric oxide and the pro-inflammatory cytokines IL-1 β , IL-6, IL-12, and TNF- α in murine macrophages, even after proteolytic pepsin treatment. Studies on dendritic cells isolated from human mesenteric lymph nodes have shown a different cytokine response against commensal *Bifidobacterium longum* subsp. *infantis* or pathogenic *Salmonella enterica* ser. Typhimurium, with the former inducing IL-10 and TGF- β and the latter inducing IL-6, IL-12, and TNF- α .⁶³

A recent in vitro study revealed dendritic cells exhibiting a different response against lactic bacteria.⁴⁰ Whereas IL-10 predominated in the response against bifidobacteria, high levels of IL-12 and TNF- α response were found in dendritic cells stimulated by lactic bacteria. This difference was caused by different types of recognition in PRRs, i.e., peptidoglycan of lactobacilli was recognized by cytoplasmic receptor NOD2, whereas lipoprotein of bifidobacteria interacted with TLR2 in a complex with TLR1 or TLR6, which induced an inhibitory signal, preventing NOD2 activation and inducing regulatory T cells.

Intestinal epithelium is thoroughly engaged in recognition of bacteria and is also an important producer of inflammatory cytokines IL-1 β , IL-6, IL-8, IL-18, and TNF- α . Human enterocyte line Caco-2 can be stimulated

by bacterial commensals to produce IL-8, thymic stromal lymphopoietin TSLP, and TGF- β , which can induce differentiation of tolerogenic dendritic cells.⁴⁰ Thus, together with local immune cells, it is the intestinal epithelium that decides about the induction of oral tolerance or inflammation.

Wong and Ustunol⁶⁴ found that heat-inactivated bifidobacteria induced the production of IL-6 and IL-8 in Caco-2 cells, whereas irradiation-inactivated bacteria attenuated the production of both cytokines. These results showed that probiotic effect can be changed completely by the conditions of processing in food industry.

All in vitro models are simplified and neglect the effect of cell-cell interactions in a complex organic microenvironment, which completely changes the resulting cytokine response. Experiments on gnotobiotic mice showed that different strains of bifidobacteria isolated from fecal microflora of infants had either no effect or induced TH1 cytokines (TNF and IFN- γ), TH2 cytokines (IL-4 and IL-10), or both.⁶⁵ Thus, bifidobacteria did not unequivocally cause an induction of some common cytokine pattern, and their effect depended on bacterial strain.

CONCLUSION

The effect of bifidobacteria in the gut is favorable and probably more anti-inflammatory, tolerogenic, and antimicrobial than immunostimulating. O'Hara et al.⁵⁸ showed that bifidobacteria downregulated an inflammatory response of the human intestinal cell line against *Salmonella*. Recently, Ouwehand¹⁵ found an anti-inflammatory effect (a decrease of serum levels of TNF- α and IL-10 and an increase of TGF- β) in elderly persons who received high doses of probiotic bifidobacteria.

Several studies have demonstrated the beneficial effect of bifidobacteria in subjects with diarrhea or dysmicrobia: however, that is not typical for these species only.⁸ The finding of this effect led to an unprecedented expansion of commercial dairy products (fermented milk, cheese, yoghurts) containing viable bifidobacteria. It should be noted, however, that the number of probiotic bacteria in food products is three orders of magnitude less (10^7 – 10^8 /g at the time of production) than the number of colony forming units used in significant medical studies, and it decreases with storage. Bifidobacteria could also be used in food preservation. For example, *Bifidobacterium bifidum* exhibited synergy with thymol as a biopreservative, thereby extending the shelf life of fresh meat.⁶⁶

The favorable but ambiguous effect of bifidobacteria seems to predetermine them for use as food supplements, whereas justification for their use in medicine is rather feeble. Treatment with bifidobacteria can be advanta-

geous in the elderly, whose immune systems are adversely affected by age, or in individuals with dysmicrobia caused by antibiotic therapy.

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