

# Beneficial Effects of Probiotic Consumption on the Immune System

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## Keywords

Probiotics · Immune system · Mechanisms · Probiotic fermented milk

## Abstract

**Background:** The gastrointestinal tract is one of the most microbiologically active ecosystems that plays a crucial role in the working of the mucosal immune system (MIS). In this ecosystem, the consumed probiotics stimulate the immune system and induce a network of signals mediated by the whole bacteria or their cell wall structure. This review is aimed at describing the immunological mechanisms of probiotics and their beneficial effects on the host. **Summary:** Once administered, oral probiotic bacteria interact with the intestinal epithelial cells (IECs) or immune cells associated with the lamina propria, through Toll-like receptors, and induce the production of different cytokines or chemokines. Macrophage chemoattractant protein 1, produced by the IECs, sends signals to other immune cells leading to the activation of the MIS, characterized by an increase in immunoglobulin A<sup>+</sup> cells of the intestine, bronchus and mammary glands, and the activation of T cells. Specifically, probiotics activate regulatory T cells that release IL-10. Interestingly,

probiotics reinforce the intestinal barrier by an increase of the mucins, the tight junction proteins and the Goblet and Paneth cells. Another proposed mechanism of probiotics is the modulation of intestinal microbiota by maintaining the balance and suppressing the growth of potential pathogenic bacteria in the gut. Furthermore, it has been demonstrated that long-term probiotics consumption does not affect the intestinal homeostasis. The viability of probiotics is crucial in the interaction with IECs and macrophages favoring, mainly, the innate immune response. Macrophages and Dendritic cells (DCs) play an important role in this immune response without inducing an inflammatory pattern, just a slight increase in the cellularity of the lamina propria. Besides, as part of the machinery that probiotics activate to protect against different pathogens, an increase in the microbicidal activity of peritoneal and spleen macrophages has been reported. In malnutrition models, such as undernourishment and obesity, probiotic was able to increase the intestinal and systemic immune response. Furthermore, probiotics contribute to recover the histology of both the intestine and the thymus damaged in these conditions. Probiotic bacteria are emerging as a safe and natural strategy for allergy prevention and treatment. Different mechanisms such as the generation of cytokines from activated pro-T-helper type 1, which favor

the production of IgG instead of IgE, have been proposed. **Key Messages:** Probiotic bacteria, their cell walls or probiotic fermented milk have significant effects on the functionality of the mucosal and systemic immune systems through the activation of multiple immune mechanisms.

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## Introduction

The gastrointestinal tract (GT) is one of the most microbiologically active ecosystems containing a mass of bacteria crucial for the maturation of immune cells. In the gut, a large number of bacteria from the microbiota and those that reach the intestine through food intake, coexist with each other and with the immune cells associated with the lamina propria of the villi. This intestinal microbiota does not interact directly with the epithelial cells; however, the microbiota stimulates the maturation and functionality of the immune cells through their metabolites [1].

There is a group of beneficial bacteria called probiotics. Initially they were defined as “Live microbial feed supplements which beneficially affect the host, improving its intestinal microbial balance” [2]. This definition was revised, and currently probiotics are defined as “Live microorganisms that when being administered in appropriate doses, confer a benefit to the health of the host” [3]. Many probiotic bacteria are members of the intestinal microbiota, some of which have been increasingly incorporated into foods to improve the gut health by maintaining the gastrointestinal microbial balance.

The most common microorganisms used as probiotics are lactic acid bacteria (LAB), particularly the genus: *Lactobacilli*, *Streptococci*, *Pediococcus*, *Enterococcus*, *Bifidobacteria*, and some yeast like *Saccharomyces boulardii* [4]. However, not all the bacteria can be probiotic, as they need to be strain-specific.

The beneficial effects of probiotics have been extensively used in improving the host health and for treating different infectious and non-infectious pathologies in animal models. Namely, protection against infections [5–7], relief of irritable bowel symptoms [8], inhibition of *Helicobacter pylori* growth [9], prevention of cancer [10–12], decrease in gut inflammatory response [13], and prevention of allergies [14, 15]. In humans, although probiotics have shown encouraging results in several health conditions like diabetes, multi-drug resistant pathogens, irritable bowel syndrome [16–18], exhaustive research is still required to incorporate probiotics into human health, nutrition, and regulation of different abnormalities.

## Mechanisms Induced by Probiotics to Stimulate the Immune System

To exhibit beneficial health impact, probiotic microbes should be able to survive in harsh conditions of the stomach and GI tract of humans. This claim may include the ability of the probiotics to withstand the gastric juice and bile salt, survive passage through the upper GT, multiply, colonize, and function in the gut [19]. Many microbes claimed as probiotics could not survive the acidity level of gastric juices and bile salt.

One of the ways probiotics promote human health is by inhibiting the growth of pathogenic bacteria. Probiotics compete for nutrients for growth and proliferation that would otherwise be utilized by pathogens. Several studies demonstrated that probiotics such as *Lactobacillus rhamnosus* strain GG and *L. plantarum* showed the ability to inhibit attachment of enteropathogenic *Escherichia coli* in the GI tract [20].

Additionally, one of the most important properties required for a potential probiotic strain is the capacity of sticking to the epithelial cells. In this regard, Galdeano et al. [21] demonstrated using electronic microscopy that 2 probiotic microorganisms, *L. casei* CRL 431 and *L. paracasei* CNCM I-1518, adhere to the intestinal epithelial cells (IECs) through the Toll-like receptors (TLRs) and mediate immune stimulation. Following this interaction, an increase in the cytokines production such as IL-6 and macrophage chemoattractant protein 1 from the IECs occurred, without altering the intestinal barrier; only a slight increase in the mononuclear cell infiltration of small intestine was observed.

The authors also demonstrated that only fragments of the probiotic bacteria, and not the whole bacteria, were internalized inside the IECs. As a consequence, the IECs initiate a complex network of signals that stimulate the immune cells associated with the lamina propria and activate mainly the innate response and the cytokines released by T cells [21].

The intestinal epithelium exhibits numerous physical adaptations to separate the host connective tissue from the external environment. This physical barrier includes a single layer of epithelial cells, their intercellular tight junctions, and the mucus that covers the epithelial surface [22]. Additionally, this physical barrier is reinforced by numerous biochemical adaptations such as a glycocalyx formed by the secretion and apical attachment of a heavily glycosylated mucin-rich layer by Goblet cells. Together, these form a viscous and relatively impermeable sheet on the apical surface of the epithelium [23]. In view of this, pro-

biotics have been shown to strengthen the intestinal barrier by increasing the number of Goblet cells which reinforce the mucus layer [24]. Moreover, several *Lactobacillus* species have been shown to increase mucin expression in human intestinal cell lines [25, 26]. VSL#3, which contains some *Lactobacillus* species, increases the expression of MUC2, MUC3, and MUC5AC in HT29 cells [27]. Moreover, *L. acidophilus* A4 cell extract increased the MUC2 expression in HT29 cells, and this effect was independent of probiotic adhesion to the cell monolayer [28].

One of the ways probiotics promote human health is by inhibiting the growth of pathogenic bacteria through the synthesis of low molecular weight compounds such as organic acid and large molecular weight antimicrobial compounds termed bacteriocins [29]. Organic acids are acetic and lactic acids. These compounds have been proven to exhibit strong inhibitory effects against pathogenic gram-negative bacteria such as *H. pylori* [30]. Some bacteriocins produced by probiotics are lactacin B from *L. acidophilus*, bifidocin B produced by *Bifidobacterium bifidum* NCFB, plantaricin from *L. plantarum*, and nisin from *Lactococcus lactis* [31].

Paneth cells, characteristic epithelial cells of the small intestine located at the bottom of the intestinal crypts, are responsible for the secretion of diverse antimicrobial peptides like lysozyme, secretory phospholipase A2, defensins, defensin-like peptides (elafin and SLPI), and cathelicidins [32]. *B. longum* and a prebiotic (Synergy 1) treatment in patients with active UC provoked the release of defensins from epithelial cells [33]. In addition, the unidirectional peristaltic movements of the intestine also aid in preventing the entry of microbes from the dense distal gut to the small intestine.

Besides, several studies have indicated that probiotics are able to reinforce intestinal barrier integrity through increased gene expression in tight junction signaling. *S. thermophilus* and *L. acidophilus* limited adhesion and invasion of enteroinvasive *E. coli* in HT29 and Caco-2 cells by the maintenance (actin, ZO-1) or enhancement (actinin, occludin) of cytoskeletal and tight junctional protein phosphorylation [34]. Dai et al. [35] showed that VSL#3 probiotics protected the epithelial barrier and increased the tight junction protein expression in vitro and in vivo by activating the p38 and ERK signaling pathways.

Recently, Cazorla et al. [36] observed an increase in Paneth cells at the base of the small intestinal Lieberkühn crypt by the oral administration of probiotics. Accordingly, an increase in the antimicrobial activity of the intestinal fluids that lead to a breakdown of the bacteria was observed by using electronic microscopy. Habil et al. [37]

concluded that probiotic strains differentially regulate human beta 2 defensin mRNA expression and protein secretion. These modulations were guided by inflammatory stimulus and cytokine environment.

Few studies reported the bactericidal effect of *E. faecium* supernatant against an enteroaggregative *E. coli*, inducing membrane damage and cell lysis [38]. This bacterium has the ability to produce enterocins, which in turn can be applied as food biopreservatives [39, 40].

Antimicrobial peptides could be considered in the future as a new class of therapeutics to induce lesser resistance and have a selective antimicrobial activity to protect the host.

Probiotics modulate the composition of gut microbial species by maintaining the balance and suppressing the growth of potential pathogenic bacteria in the gut. It has been reported that *L. acidophilus* or *L. casei* increased LAB with a concomitant decrease of fecal coliforms and anaerobes [41, 42]. In addition, a study by Li et al. [43] found that probiotics caused shifts in the gut microbiota composition toward specific beneficial bacteria, for example, Prevotella and Oscillibacter. These bacteria are known to produce anti-inflammatory metabolites, which subsequently decreased the Th17 polarization and favored the differentiation of anti-inflammatory Treg/Type 1 regulatory T (Tr1) cells in the gut.

A widespread requirement of some probiotic effects is their viability, which means that they must be resistant to acid and bile secretions. In light of this, is the probiotic effect on the gut epithelial cells mediated by particles or by the whole LAB? Do the probiotics have to be viable for immune stimulation? It was demonstrated that only the viable bacteria are able to interact with IECs, and the probiotic cellular fragments are phagocytosed by macrophages and dendritic cells (DCs) associated with the Peyer's patches (PPs) or the lamina propria of the villi. By contrast, non-viable bacteria are cleared fast from the intestinal lumen [21].

How long must these bacteria or their fragments be in contact with the immune cells for their stimulation? To address this question, Galdeano et al. [21] performed an assay using fluorescent probiotic bacteria and analyzed the presence of fluorescence inside the immune cells from PPs, small intestine villi, and lymph nodes of the large intestine. They found that probiotic particles remain until 72 h inside the immune cells, in a similar manner to any particulate antigen. As a consequence of this interaction, probiotics induce an increase in the expression of the receptors TLR2 and mannose (CD206) on the surface of macrophages and DCs. These results reinforce the idea

that the main activation induced by probiotics is on the innate immune response [44]. This fact is a key for the later stimulation of an adaptive immune response.

Probiotics confer protection against pathogen colonization by inducing their direct killing, competing with nutrients, and enhancing the response of the gut-associated immune repertoire [45–50].

More important, the probiotic oral administration protects against infection in gut distant mucosae like bronchi and urogenital mucosae [51–53]. A study involving 54 women reported that daily probiotic consumption for 6 months enhanced the clearance of human papillomavirus, which is known to cause cervical cancer [54]. In animal models, oral probiotic administration protects against *Salmonella typhimurium* infection by activating the phagocytic and microbicidal activity of peritoneal and spleen macrophages [55]. Probiotic lactobacilli can also significantly reduce the risk of antibiotic-associated diarrhea in children and adults [56].

The gut barrier plays a crucial role by spatially compartmentalizing bacteria to the lumen through the production of mucus and secretory immunoglobulin A (sIgA). The IgA antibody is a major functional component of the humoral adaptive immune system, specifically at mucosal sites. The antibodies are predominantly produced by plasma cells localized in the intestinal lamina propria as dimers linked by the connecting chain. The binding of dimeric IgA to the polymeric immunoglobulin receptor contributes to its transportation through IECs and secretion into the intestinal lumen [57]. The secretory component ensures the binding of sIgA to the mucus layer site, where this immunoglobulin leads to the immune exclusion of mucosal antigens [58]. The sIgA has an important role, not only in the gut lumen, but also in the underlying tissue, translocating via M cells, to PP, to preserve the local homeostasis [59–61]. In the intestine, sIgA antibodies bind to commensal and pathogenic bacteria, and toxins, blocking them through a non-inflammatory process commonly known as “immune exclusion” [62, 63]. Additionally, sIgA antibodies facilitate the sampling of intestinal environments by DCs in the subepithelial dome region of the PPs. Major efforts are underway to understand the generation, distribution, and maintenance of IgA antibody-secreting plasma cells in intestinal tissues. In this regard, oral administration of probiotics increased the number of IgA<sup>+</sup> cells in the lamina propria of the intestine [64] and also in bronchus and mammary glands [13, 65]. These studies demonstrated that probiotics induce the IgA cycle, reinforce, and maintain the immune surveillance in mucosal sites distant from the gut.

T lymphocytes also play an important role in protecting against pathogenic microorganisms in the digestive system, and in regulating the responses against food and commensal antigens. Besides, the adaptive immune system is profoundly shaped by the presence of the commensal intestinal microbiota. This includes increases in the size and number of germinal centers in PPs, IgA-secreting plasma cell numbers, lamina propria CD4<sup>+</sup> T cells, and  $\alpha\beta$  T cell receptor-expressing intraepithelial CD8 $\alpha\beta$ <sup>+</sup> T cells [66]. In healthy mice and humans, the presence of commensal microorganisms in the intestine is tolerated without an acute neutrophil infiltrate. CD4<sup>+</sup> regulatory T (Treg) cells are an essential component of this mutualism.

DCs are immune cells with characteristic projections (dendrites), acquired during development, and are specialized for antigen presentation to B and T cells. CD4<sup>+</sup> T cells will then differentiate in response to cytokine to different subsets: TH1, TH2, TH17, and regulatory T cells. Probiotic bacteria regulate mucosal immune responses through the induction of different cytokines. This effect is dependent on the probiotic strain itself [67–69]. After oral probiotic administration, cytokines produced by T cells in the lamina propria of the small intestine were secreted in slightly higher levels than those observed in the presence of commensal bacteria; specifically IFN- $\gamma$  and TNF- $\alpha$  cytokines [70–73]. Through the production of cytokines, probiotics trigger the stimulation of an adaptive immune response and establish a network of signals among the different immune cells. Some probiotics may alter cytokine production by modulating cellular signal transduction. They can either block the degradation of the inhibitor I- $\kappa$ B and interfere with proteasome function, or promote nuclear export of NF- $\kappa$ B subunit RelA, through a PPAR- $\gamma$ -dependent pathway [74, 75]. IL-10 produced by Th2 lymphocytes and macrophages has been reported to be the main immunomodulator cytokine induced by *L. casei* CRL 431 to maintain the gut homeostasis [55, 76].

In recent years, there has been an increasing interest in probiotic fermented milk (PFM). Fermentation may improve the digestibility and nutritional quality of food through the enrichment of food substrates like vitamins, proteins, essential amino acids, and essential fatty acids. In this sense, using fermented milk containing probiotic bacteria (PFM), Maldonado Galdeano et al. [77], analyzed the role of the cytokine released by probiotics on immune cells distant from the intestine. The administration of PFM increases the phagocytic and microbicidal activity of the peritoneal and spleen macrophages. Inter-

estingly, probiotics also stimulate the systemic immune response, with an increase in specific antibody production. These antibodies have been shown to play a critical role in decreasing the spread of pathogenic bacteria to the liver and the spleen after a challenge with *S. typhimurium*. This effect has shown to be more remarkable in an undernourishment model [78].

Malnutrition is a systemic alteration caused by an imbalance between the nutrient intake and energy requirements. It affects the immune response, causing a significant decrease in the defense mechanisms and making the host more susceptible to infections. Hence, malnutrition becomes a good model to study the probiotic impact on the host's health. On an undernourishment mice model, the administration of PFM as a re-nutrition diet reconstituted the intestinal mucosa architecture and stimulated local and systemic immunity [78]. Considering the fact that malnutrition causes a significant impairment of the immune system, and the thymus being one of the most affected organs, thymus histology restoration by probiotic consumption becomes relevant. The authors also observed a decrease in the cellular apoptosis of this organ and a recovery of the CD4<sup>+</sup> and CD8<sup>+</sup> single-positive thymocytes. Besides, an increase in different cytokines in the thymus of the mice fed with PFM was also reported [78].

Although information about the minimum effective concentration is still controversial, it is generally accepted that probiotic products should have a minimum concentration of 10<sup>6</sup> CFU/mL or gram and that a total of 10<sup>8</sup>–10<sup>9</sup> probiotic microorganisms should be consumed daily [79]. Importantly, the long-term consumption of PFM has been proved to exert immunomodulatory effects to maintain the intestinal homeostasis without secondary effects. The gut immunity balance was preserved and down-regulated by cytokines such as IL-10, avoiding gut inflammatory immune response [80].

The beneficial effect of probiotics in allergy processes is well described [81–83]. The IgE increase is one of the most relevant signs that characterize this process. Probiotics have been shown to be efficient in decreasing this immunoglobulin, as well as in alleviating symptoms.

**Fig. 1.** Immunomodulatory mechanisms exerted by probiotic bacteria in the gut mucosa. Probiotic bacteria adhere to IECs and activate them through the pattern recognition receptors. In this scenario, IECs release cytokines and chemokines that create a micro-environment in the gut lamina propria, bronchi, and mammary glands, allowing the clonal expansion of B cells to produce IgA. At the same time, cytokines stimulated by probiotic bacteria lead to the expression of Treg cells (Foxp3<sup>+</sup>) that maintain the immune homeostasis in the gut mucosa (unpublished data). PPs macro-

phages release cytokines after probiotic bacteria stimulation. However, they maintain a characteristic state of hyporesponse to commensal microbiota. Besides, after probiotic stimulation, macrophages distant from the GT such as peritoneum and spleen, increase their functionality (cytokines production, phagocytic and microbicidal activity) reinforcing the innate immune response. Probiotic bacteria administration primes a Th1 profile response, with high levels of IL-10 and IFN- $\gamma$  that play an important role in the immunomodulation. PP, peyer patch; Treg, T regulatory.

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Furthermore, in vivo studies showed that the administration of probiotics is effective in improving lipid profiles, including the reduction of adipose tissue, serum/plasma total cholesterol, LDL-cholesterol and triglycerides, and increasing the HDL-cholesterol [84, 85]. Clinical trials confirmed that probiotics reduce blood glucose and insulin levels in patients with diabetes. They can also improve Hb1Ac and insulin resistance. Mechanisms for these obesity-related effects include regulation of immune differentiation and insulin sensitivity, inhibition of pathogenic bacteria adhesion to the intestine and translocation to adipose tissue, and improvement of intestinal barrier function [86].

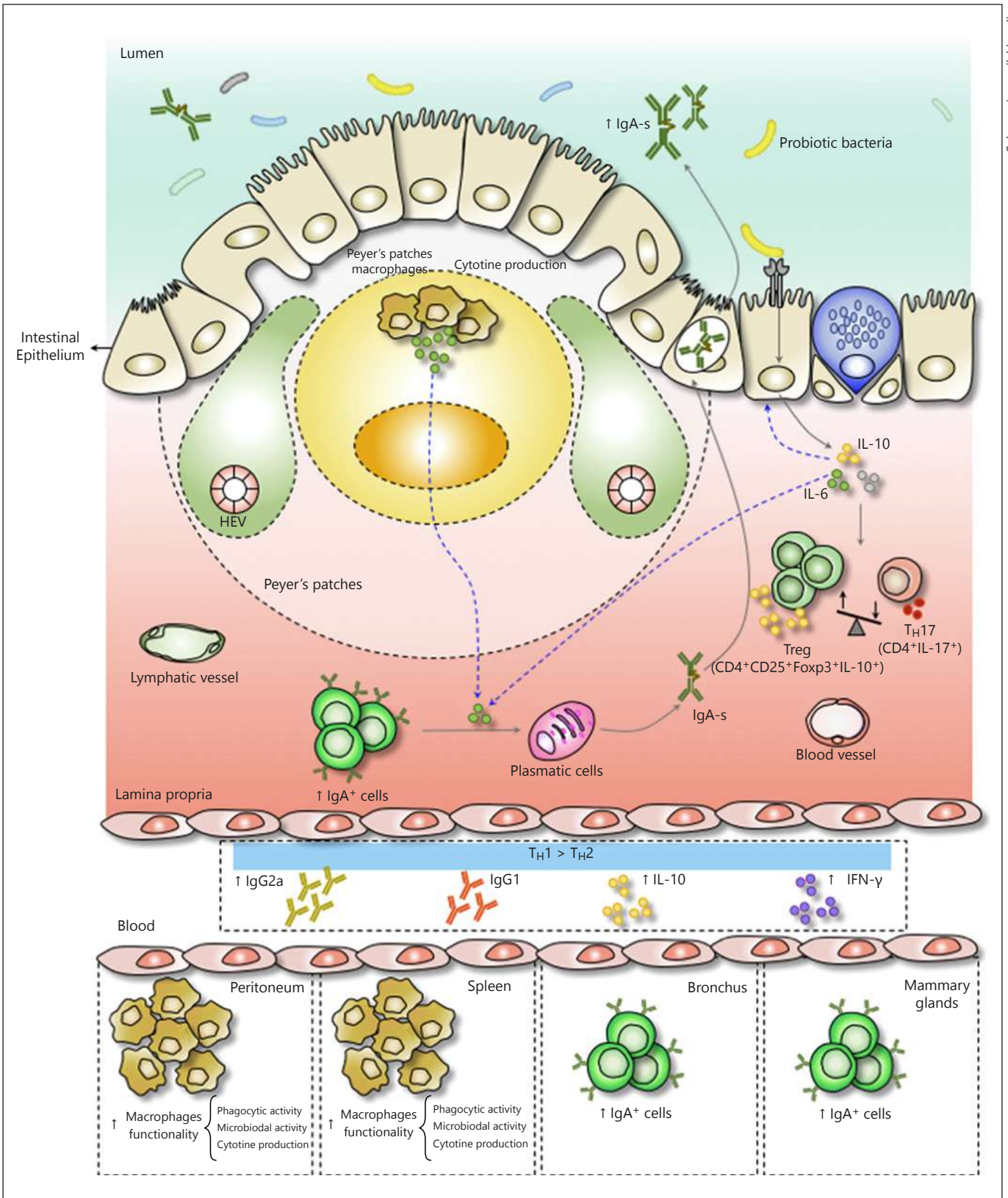
The unquestionable effect of probiotics as anti-cancer agents seems to be due to a combination of multiple mechanisms. Probiotics change the composition and metabolites of the intestinal flora, reduce the number of harmful bacteria, display anti-genotoxic and anti-gene mutation function, and inhibit enzymes in the colon. Besides, through the interaction with colonic cells probiotics regulate the immune system [87]. Probiotics may prevent neoplastic transformation by protecting the mucosal and GT barrier stability, competing with pathogenic bacteria, reducing anti-inflammatory reactions, degrading potential carcinogens, affecting cell proliferation and polyamine metabolism at gastric mucosa [88].

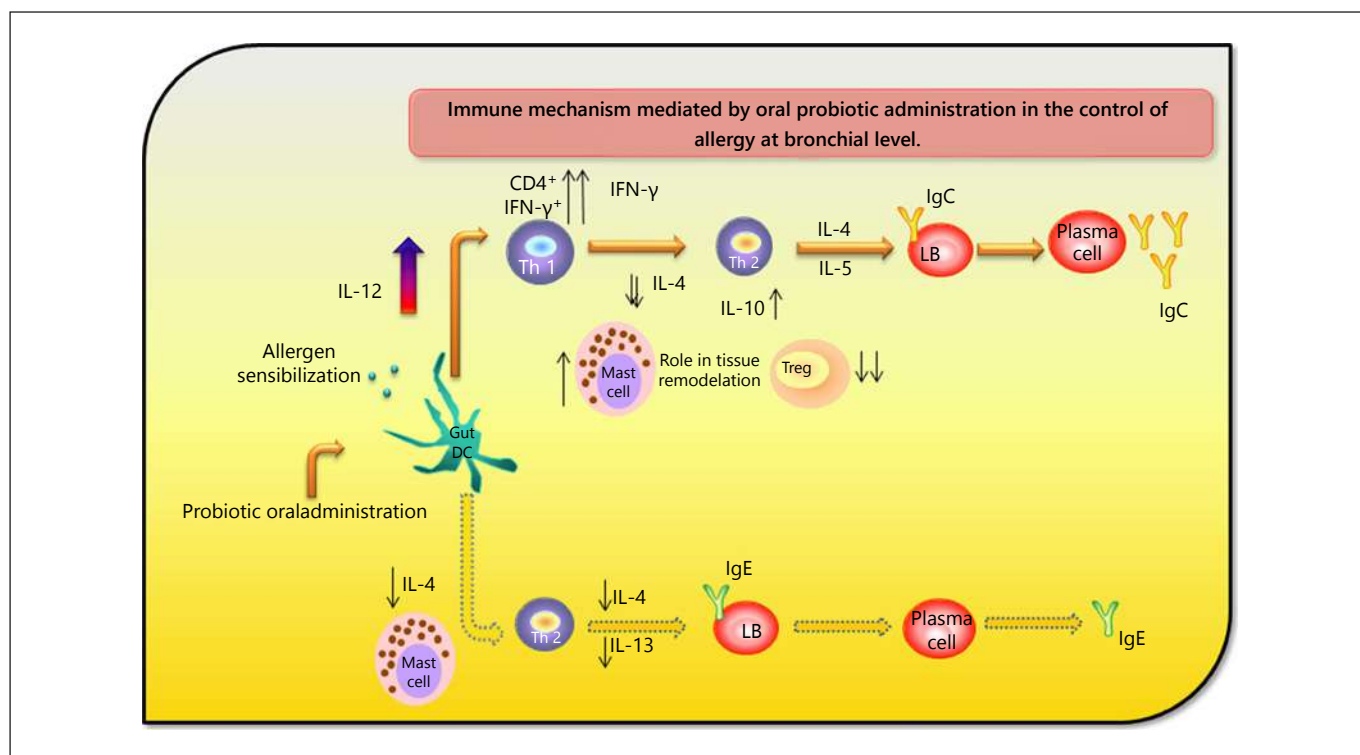
## Conclusion

Based on the results obtained, the immune mechanisms elicited by the probiotics are summarized in Figure 1.

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(For figure see next page.)





**Fig. 2.** Immune mechanisms mediated by oral probiotic administration to control allergy at bronchial level. The probiotic oral administration induces activation of DCs at the gut level, with an IL-12 release that balances the adaptive response to a Th1 profile at the bronchus. The increase in the expression of CD4 and IFN- $\gamma$  on Th1 cells leads to an enhancement on IgG production instead

of IgE. Treg cells did not increase, so the regulatory effect exerted by the probiotic seemed to be mediated by IL-10, produced by Th1 and Th2 cells. Mast cells are also increased to mediate the tissue repair. In parallel, the Th2 response was significantly diminished with a decrease in the IL-4, IL-13, and IgE production. DC, dendritic cell; Treg, T regulatory.

– Probiotics interact with IECs. Due to the privileged position of those cells in the GT, they act as active sensors, setting a dialogue between the host and the external environment. Probiotic bacterial fragments can be internalized into the IECs and produce the subsequent activation of immune cells associated with the gut. This result led to infer that the cell wall of the probiotic bacteria activates the immune system, an activation mediated by TLRs. New studies have been performed with this bacterial structure to confirm this hypothesis.

– Other important cells that play a pivotal role in the epithelial barrier are the Paneth cells. Probiotics have important effects on these cells, increasing their number in the intestinal crypts with the aim of reinforcing the epithelial barrier.

– The time of permanence of the probiotic bacteria in the intestinal lumen (72 h) is enough to induce changes in the gut immune cells, increasing the number of macrophages and DCs of the lamina propria, and en-

hancing their functionality, reflected in cytokines production.

– Importantly, the activation of immune cells does not alter intestinal homeostasis, probably by the regulatory cells activation that maintains a tolerogenic environment. These facts ensure the safety of probiotics consumption for long periods of time without adverse effects. The cytokine microenvironment generated by immune cells in response to probiotics favors an increase in the gut IgA<sup>+</sup> cells. Besides, the cytokines induce locally, influence the activity of immune cells distant from the gut-like macrophages from spleen and peritoneum, and also other mucosa sites such as bronchi and mammary glands.

– In malnutrition processes, the probiotic administration contributes to restore the thymus histology and stimulates the adaptive immune response.

– Probiotics induce a clear balance to a Th1 profile that is essential for the control of an allergy process (Fig. 2).

– Probiotic bacteria, their cell walls or PFM induce signals in the intestine that improve the behavior of the immune system and the host's health.

– The IECs would be the main target of the probiotics, and together with the innate immune cells associated with the intestine would modulate the mucosal and systemic immunity.

– Probiotic bacteria appeared as an effective tool for the maintenance of the intestinal homeostasis and the stimulation of the mucosal immune system, both at the gut and distant sites.

## References

- 1 Hooper LV, Littman DR, Macpherson AJ: Interactions between the microbiota and the immune system. *Science* 2012;336:1268–1273.
- 2 Fuller R: Probiotics in man and animals. *J Appl Bacteriol* 1989;66:365–378.
- 3 Hill C, Guarner F, Reid G, et al: Expert consensus document. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014;11:506–514.
- 4 George Kerry R, Patra JK, Gouda S, et al: Benefaction of probiotics for human health: a review. *J Food Drug Anal* 2018;26:927–939.
- 5 Park MS, Kwon B, Ku S, et al: The efficacy of bifidobacterium longum BORI and lactobacillus acidophilus AD031 probiotic treatment in infants with rotavirus infection. *Nutrients* 2017;9:pii:E887.
- 6 Acurcio LB, Sandes SHC, Bastos RW, et al: Milk fermented by *Lactobacillus* species from Brazilian artisanal cheese protect germ-free mice against *Salmonella Typhimurium* infection. *Benef Microbes* 2017;8:579–588.
- 7 Mallina R, Craik J, Briffa N, et al: Probiotic containing *Lactobacillus casei*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* (ACTIMEL) for the prevention of *Clostridium difficile* associated diarrhoea in the elderly with proximal femur fractures. *J Infect Public Health* 2018;11:85–88.
- 8 Hungin AP, Mulligan C, Pot B; European Society for Primary Care Gastroenterology, et al: Systematic review: probiotics in the management of lower gastrointestinal symptoms in clinical practice – an evidence-based international guide. *Aliment Pharmacol Ther* 2013;38:864–886.
- 9 Fujimura T, Kinoshita J, Makino I, et al: Gastric cancer – state of the art in Japan. *Rozhl Chir* 2012;91:346–352.
- 10 Aragón F, Carino S, Perdígón G, et al: Inhibition of growth and metastasis of breast cancer in mice by milk fermented with *Lactobacillus casei* CRL 431. *J Immunother* 2015;38:185–196.
- 11 So SS, Wan ML, El-Nezami H: Probiotics-mediated suppression of cancer. *Curr Opin Oncol* 2017;29:62–72.
- 12 Kumar M, Verma V, Nagpal R, et al: Anticarcinogenic effect of probiotic fermented milk and chlorophyllin on aflatoxin-B<sub>1</sub>-induced liver carcinogenesis in rats. *Br J Nutr* 2012;107:1006–1016.
- 13 Fábrega MJ, Rodríguez-Nogales A, Garrido-Mesa J, et al: Intestinal anti-inflammatory effects of outer membrane vesicles from *Escherichia coli* nissle 1917 in DSS-experimental colitis in mice. *Front Microbiol* 2017;8:1274.
- 14 Velez EM, Maldonado Galdeano C, Carmuega E, et al: Probiotic fermented milk consumption modulates the allergic process induced by ovalbumin in mice. *Br J Nutr* 2015;114:566–576.
- 15 Nelson HS: Allergen immunotherapy now and in the future. *Allergy Asthma Proc* 2016;37:268–272.
- 16 He J, Zhang F, Han Y: Effect of probiotics on lipid profiles and blood pressure in patients with type 2 diabetes: a meta-analysis of RCTs. *Medicine (Baltimore)* 2017;96:e9166.
- 17 Abdelhamid AG, Esaam A, Hazaa MM: Cell free preparations of probiotics exerted antibacterial and antibiofilm activities against multidrug resistant *E. coli*. *Saudi Pharm J* 2018;26:603–607.
- 18 Majeed M, Nagabhushanam K, Arumugam S, et al: *Bacillus coagulans* MTCC 5856 for the management of major depression with irritable bowel syndrome: a randomised, double-blind, placebo controlled, multi-centre, pilot clinical study. *Food Nutr Res* 2018;4:62.
- 19 Wan ML, Forsythe SJ, El-Nezami H: Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. *Crit Rev Food Sci Nutr* 2018:1–14.
- 20 Wilson KH, Perini F: Role of competition for nutrients in suppression of *Clostridium difficile* by the colonic microflora. *Infect Immun* 1988;56:2610–2614.
- 21 Galdeano CM, Perdígón G: Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. *J Appl Microbiol* 2004;97:673–681.
- 22 Artis D, Grenics RK: The intestinal epithelium: sensors to effectors in nematode infection. *Mucosal Immunol* 2008;1:252–264.
- 23 Sansonetti PJ: War and peace at mucosal surfaces. *Nat Rev Immunol* 2004;4:953–964.
- 24 De Moreno de LeBlanc A, Dogi CA, Galdeano CM, et al: Effect of the administration of a fermented milk containing *Lactobacillus casei* DN-114001 on intestinal microbiota and gut associated immune cells of nursing mice and after weaning until immune maturity. *BMC Immunol* 2008;9:27.
- 25 Mack DR, Ahrne S, Hyde L, et al: Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells in vitro. *Gut* 2003;52:827–833.
- 26 Mattar AF, Teitelbaum DH, Drongowski RA, et al: Probiotics up-regulate MUC-2 mucin gene expression in a Caco-2 cell-culture model. *Pediatr Surg Int* 2002;18:586–590.
- 27 Otte JM, Podolsky DK: Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G613–G626.
- 28 Kim Y, Kim SH, Whang KY, et al: Inhibition of *Escherichia coli* O157:H7 attachment by interactions between lactic acid bacteria and intestinal epithelial cells. *J Microbiol Biotechnol* 2008;18:1278–1285.
- 29 Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, et al: Probiotic mechanisms of action. *Ann Nutr Metab* 2012;61:160–174.
- 30 Russell JB, Diez-Gonzalez F: The effects of fermentation acids on bacterial growth. *Adv Microb Physiol* 1998;39:205–234.
- 31 Nielsen DS, Cho GS, Hanak A, et al: The effect of bacteriocin-producing *Lactobacillus plantarum* strains on the intracellular pH of sessile and planktonic *Listeria monocytogenes* single cells. *Int J Food Microbiol* 2010;141(suppl 1):S53–S59.
- 32 Sankaran-Walters S, Hart R, Dills C: Guardians of the gut: enteric defensins. *Front Microbiol* 2017;8:647.

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- 33 Furrie E, Macfarlane S, Kennedy A, et al: Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005;54:242–249.
- 34 Resta-Lenert S, Barrett KE: Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut* 2003;52:988–997.
- 35 Dai C, Zhao DH, Jiang M: VSL#3 probiotics regulate the intestinal epithelial barrier in vivo and in vitro via the p38 and ERK signaling pathways. *Int J Mol Med* 2012;29:202–208.
- 36 Cazorla SI, Maldonado-Galdeano C, Weill R, et al: Oral administration of probiotics increases paneth cells and intestinal antimicrobial activity. *Front Microbiol* 2018;9:736.
- 37 Habil N, Abate W, Beal J, et al: Heat-killed probiotic bacteria differentially regulate colonic epithelial cell production of human  $\beta$ -defensin-2: dependence on inflammatory cytokines. *Benef Microbes* 2014;5:483–495.
- 38 Miyazaki Y, Kamiya S, Hanawa T, et al: Effect of probiotic bacterial strains of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* on enteroaggregative *Escherichia coli*. *J Infect Chemother* 2010;16:10–18.
- 39 Ness IF, Diep DB, Ike Y: Enterococcal Bacteriocins and Antimicrobial Proteins that Contribute to Niche Control; in Gilmore MS, Clewell DB, Ike Y, Shankar N (eds). Boston, Massachusetts Eye and Ear Infirmary, 2014.
- 40 Khan H, Flint S, Yu PL: Enterocins in food preservation. *Int J Food Microbiol* 2010;141:1–10.
- 41 Chaikhram P, Apichartsrangkoon A, Jirattananangri W, et al: Influence of encapsulated probiotics combined with pressurized longan juice on colon microflora and their metabolic Enterococcal bacteriocins and Antimicrobial proteins that contribute to niche control activities on the exposure to simulated dynamic gastrointestinal tract. *Food Res Int* 2012;49:133–142.
- 42 Maldonado Galdeano C, Novotny Nuñez I, Perdígón G: Malnutrition: role of the diet on the microbiota and in the functioning of the gut immune system. *SM J Public Health Epidemiol* 2016;2:1023–1029.
- 43 Li J, Sung CY, Lee N, et al: Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. *Proc Natl Acad Sci U S A* 2016;113:E1306–E1315.
- 44 Galdeano CM, Perdígón G: The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity. *Clin Vaccine Immunol* 2006;13:219–226.
- 45 Corr SC, Li Y, Riedel CU, et al: Bacteriocin production as a mechanism for the anti-infective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci U S A* 2007;104:7617–7621.
- 46 Fujimura S, Watanabe A, Kimura K, et al: Probiotic mechanism of *Lactobacillus gasseri* OLL2716 strain against *Helicobacter pylori*. *J Clin Microbiol* 2012;50:1134–1136.
- 47 Kim JY, Young JA, Gunther NW, et al: Inhibition of *Salmonella* by bacteriocin-producing lactic acid bacteria derived from U.S. kimchi and broiler chicken. *J Food Saf* 2015;35:1–12.
- 48 De Moreno De Leblanc A, Maldonado Galdeano C, Dogi CA, et al: Adjuvant effect of a probiotic fermented milk in the protection against *Salmonella enteritidis* serovar typhimurium infection: mechanisms involved. *Int J Immunopathol Pharmacol* 2010;23:1235–1244.
- 49 Acurcio LB, Bastos RW, Sandes SHdC, et al: Protective effects of milk fermented by *Lactobacillus plantarum* B7 from Brazilian artisanal cheese on a *Salmonella enterica* serovar Typhimurium infection in BALB/c mice. *J Funct Foods* 2017;33:436–445.
- 50 Mukherjee S, Joardar N, Sengupta S, et al: Gut microbes as future therapeutics in treating inflammatory and infectious diseases: lessons from recent findings. *J Nutr Biochem* 2018;16:111–128.
- 51 Hao Q, Lu Z, Dong BR, et al: Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev* 2015; CD006895.
- 52 Barbieri N, Herrera M, Salva S, et al: *Lactobacillus rhamnosus* CRL1505 nasal administration improves recovery of T-cell mediated immunity against pneumococcal infection in malnourished mice. *Benef Microbes* 2017;30:393–405.
- 53 Leccese Terraf MC, Juarez Tomás MS, Rault L, et al: In vitro effect of vaginal lactobacilli on the growth and adhesion abilities of uropathogenic *Escherichia coli*. *Arch Microbiol* 2017;199:767–774.
- 54 Verhoeven V, Renard N, Makar A, et al: Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study. *Eur J Cancer Prev* 2013;22:46–51.
- 55 Lemme-Dumit JM, Polti MA, Perdígón G, et al: Probiotic bacteria cell walls stimulate the activity of the intestinal epithelial cells and macrophage functionality. *Benef Microbes* 2018;9:153–164.
- 56 McFarland LV: Probiotics and diarrhea. *Ann Nutr Metab* 2010;57(suppl):10–11.
- 57 Xiong N, Hu S: Regulation of intestinal IgA responses. *Cell Mol Life Sci* 2015;72:2645–2655.
- 58 Phalipon A, Cardona A, Kraehenbuhl JP, et al: Secretory component: a new role in secretory IgA-mediated immune exclusion in vivo. *Immunity* 2002;17:107–115.
- 59 Mantis NJ, Cheung MC, Chintalacharuvu KR, et al: Selective adherence of IgA to murine Peyer's patch M cells: evidence for a novel IgA receptor. *J Immunol* 2002;169:1844–1851.
- 60 Rey J, Garin N, Spertini F, et al: Targeting of secretory IgA to Peyer's patch dendritic and T cells after transport by intestinal M cells. *J Immunol* 2004;172:3026–3033.
- 61 Favre L, Spertini F, Corthésy B: Secretory IgA possesses intrinsic modulatory properties stimulating mucosal and systemic immune responses. *J Immunol* 2005;175:2793–2800.
- 62 Kadaoui KA, Corthésy B: Secretory IgA mediates bacterial translocation to dendritic cells in mouse Peyer's patches with restriction to mucosal compartment. *J Immunol* 2007;179:7751–7757.
- 63 Mantis NJ, Rol N, Corthésy B: Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol* 2011;4:603–611.
- 64 Fernández MF, Boris S, Barbes C: Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J Appl Microbiol* 2003;94:449–455.
- 65 De Moreno de LeBlanc A, Maldonado Galdeano C, Chaves S, et al: Oral administration of *L. casei* CRL 431 increases immunity in bronchus and mammary glands. *Biolife Ed. ISSN 1721–727X. Eur J Inflamm* 2005;3:23–28.
- 66 Hooper LV, Macpherson AJ: Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol* 2010;10:159–169.
- 67 Foligné B, Parayre S, Cheddani R, et al: Immunomodulation properties of multi-species fermented milks. *Food Microbiol* 2016;53(Pt A):60–69.
- 68 Takeda S, Kawahara S, Hidaka M, et al: Effects of oral administration of probiotics from mongolian dairy products on the Th1 immune response in mice. *Biosci Biotechnol Biochem* 2013;77:1372–1378.
- 69 Sheikh A, Giti H, Heibor MR, et al: *Lactobacillus delbrueckii* subsp. *Bulgaricus* modulates the secretion of Th1/Th2 and Treg cell-related Cytokines by PBMCs from patients with atopic dermatitis. *Drug Res (Stuttg)* 2017;67:724–729.
- 70 Perdígón G, Maldonado Galdeano C, Valdez JC, Medici M: Interaction of lactic acid bacteria with the gut immune system. *Eur J Clin Nutr* 2002;56(suppl 4):S21–S26.
- 71 Dogi CA, Galdeano CM, Perdígón G: Gut immune stimulation by non pathogenic Gram(+) and Gram(-) bacteria. Comparison with a probiotic strain. *Cytokine* 2008;41:223–231.
- 72 Kelchtermans H, Billiau A, Matthys P: How interferon-gamma keeps autoimmune diseases in check. *Trends Immunol* 2008;29:479–486.
- 73 Jiang K, Cao S, Cui JZ, Matsubara JA: Immuno-modulatory effect of IFN-gamma in AMD and its role as a possible target for therapy. *J Clin Exp Ophthalmol* 2013;suppl 2:71–76.
- 74 Petrof EO, Kojima K, Ropeleski MJ, Musch MW, Tao Y, De Simone C, Chang EB: Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. *Gastroenterology* 2004;127:1474–1487.
- 75 Kelly D, Campbell JI, King TP, et al: Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat Immunol* 2004;5:104–112.

- 76 Sichetti M, De Marco S, Pagiotti R, et al: Anti-inflammatory effect of multistrain probiotic formulation (*L. rhamnosus*, *B. lactis*, and *B. longum*). *Nutrition* 2018;53:95–102.
- 77 Maldonado Galdeano C, Novotny Núñez I, de Moreno de LeBlanc A, et al: Impact of a probiotic fermented milk in the gut ecosystem and in the systemic immunity using a non-severe protein-energy-malnutrition model in mice. *BMC Gastroenterol* 2011;11:64.
- 78 Núñez IN, Galdeano CM, Carmuega E, et al: Effect of a probiotic fermented milk on the thymus in BALB/c mice under non-severe protein-energy malnutrition. *Br J Nutr* 2013; 110:500–508.
- 79 Sanders ME: Probiotics: definition, sources, selection, and uses. *Clin Infect Dis* 2008; 46(suppl 2):S58–S61; discussion S144–S151.
- 80 de Moreno de LeBlanc A, Chaves S, Carmuega E, et al: Effect of long-term continuous consumption of fermented milk containing probiotic bacteria on mucosal immunity and the activity of peritoneal macrophages. *Immunobiology* 2008;213:97–108.
- 81 Fu L, Song J, Wang C, et al: *Bifidobacterium infantis* potentially alleviates shrimp tropomyosin-induced allergy by tolerogenic dendritic cell-dependent induction of regulatory T cells and alterations in gut microbiota. *Front Immunol* 2017;10:1536.
- 82 Jerzynska J, Stelmach W, Balcerak J, et al: Effect of *Lactobacillus rhamnosus* GG and vitamin D supplementation on the immunologic effectiveness of grass-specific sublingual immunotherapy in children with allergy. *Allergy Asthma Proc* 2016;37:324–334.
- 83 Turner JH, Adams AS, Zajac A: Probiotics in prevention and treatment of allergic rhinitis. *Am J Rhinol Allergy* 2015;29:e224.
- 84 Torres S, Fabersani E, Marquez A, et al: Adipose tissue inflammation and metabolic syndrome. The proactive role of probiotics. *Eur J Nutr* 2018, Epub ahead of print.
- 85 Ettinger G, MacDonald K, Reid G et al: The influence of the human microbiome and probiotics on cardiovascular health. *Gut microbes* 2014;5:719–728.
- 86 Swami O, Shah NJ: Role of probiotics in diabetes: a review of their rationale and efficacy. *EMJ Diabet* 2017;5:104–110.
- 87 de Moreno de Leblanc A, Perdigón G: The application of probiotic fermented milks in cancer and intestinal inflammation. *Proc Nutr Soc* 2010;69:421–428.
- 88 Russo F, Linsalata M, Orlando A: Probiotics against neoplastic transformation of gastric mucosa: effects on cell proliferation and polyamine metabolism. *World J Gastroenterol* 2014;20:13258–13272.