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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^+ 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

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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 phagocyted 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 adaptative 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 mucosas like bronchi and urogenital mucosas [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 pathogens 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^+ 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 $CD4^+$ T cells, and αβ T cell receptor-expressing intraepithelial $CD8\alpha\beta^+$ T cells [66]. In healthy mice and humans, the presence of commensal microorganisms in the intestine is tolerated without an acute neutrophils infiltrate. $CD4^+$ 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. $CD4^+$ 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-γ and TNF-α 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-κB and interfere with proteasome function, or promote nuclear export of NF-κB subunit RelA, through a PPAR-γ-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 $CD4^+$ and $CD8^+$ 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 10^6 CFU/mL or gram and that a total of 10^8–10^9 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. However, the mechanisms mediated for the alleviation of allergy have not been described. In a respiratory allergy experimental model, Velez et al. [14] demonstrated that probiotics induce a clear Th1 balance favoring the production of IgG instead of IgE immunoglobulin and increasing the levels of IL-10 and IFN-γ cytokines. Besides, by a co-localization study, the authors postulate that the Th1 cells have been shown to be responsible for the IFN-γ release.

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].

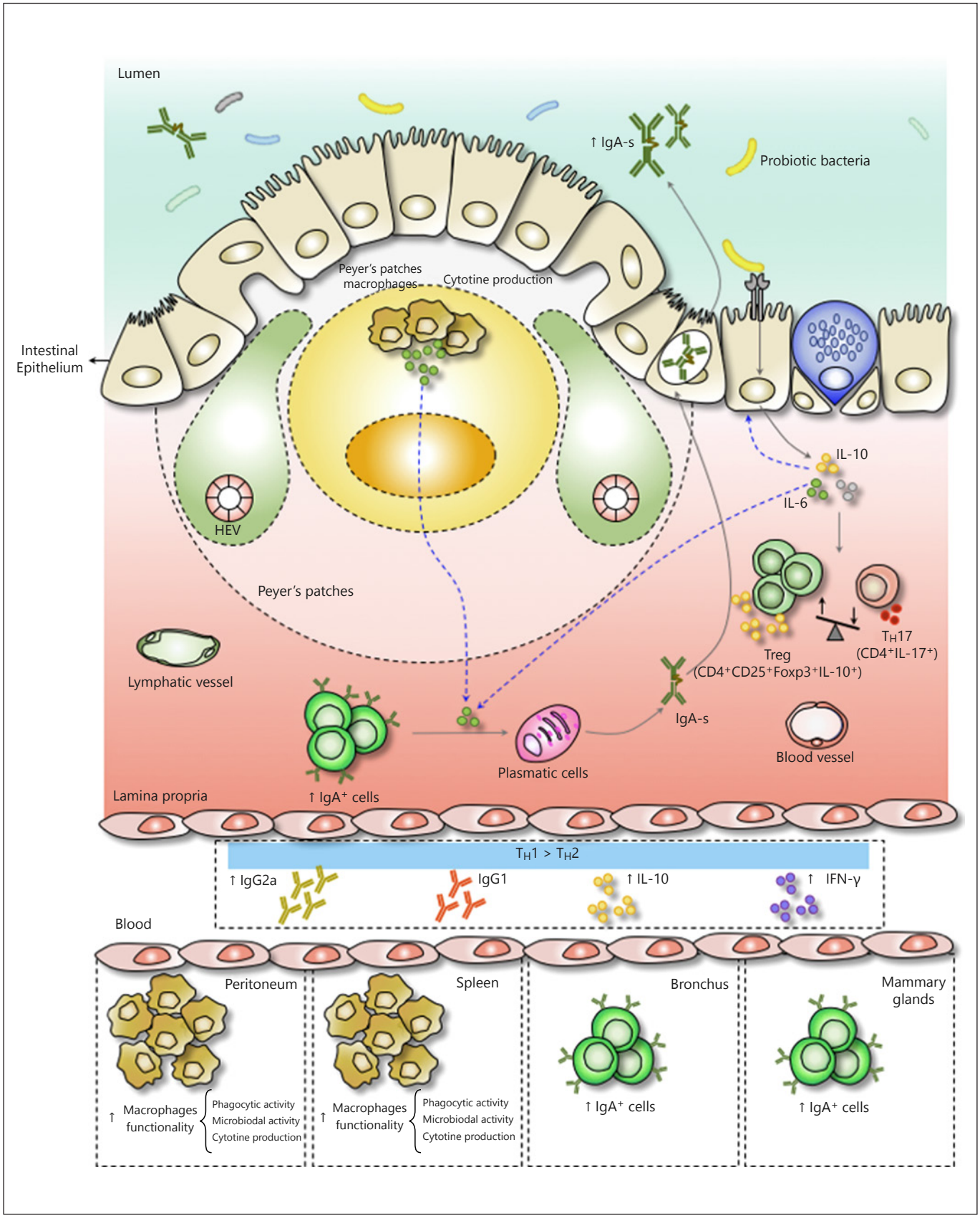
Conclusion

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

– 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 chang-



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es in the gut immune cells, increasing the number of macrophages and DCs of the lamina propria, and enhancing 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$^+$ 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 adaptative immune response.

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

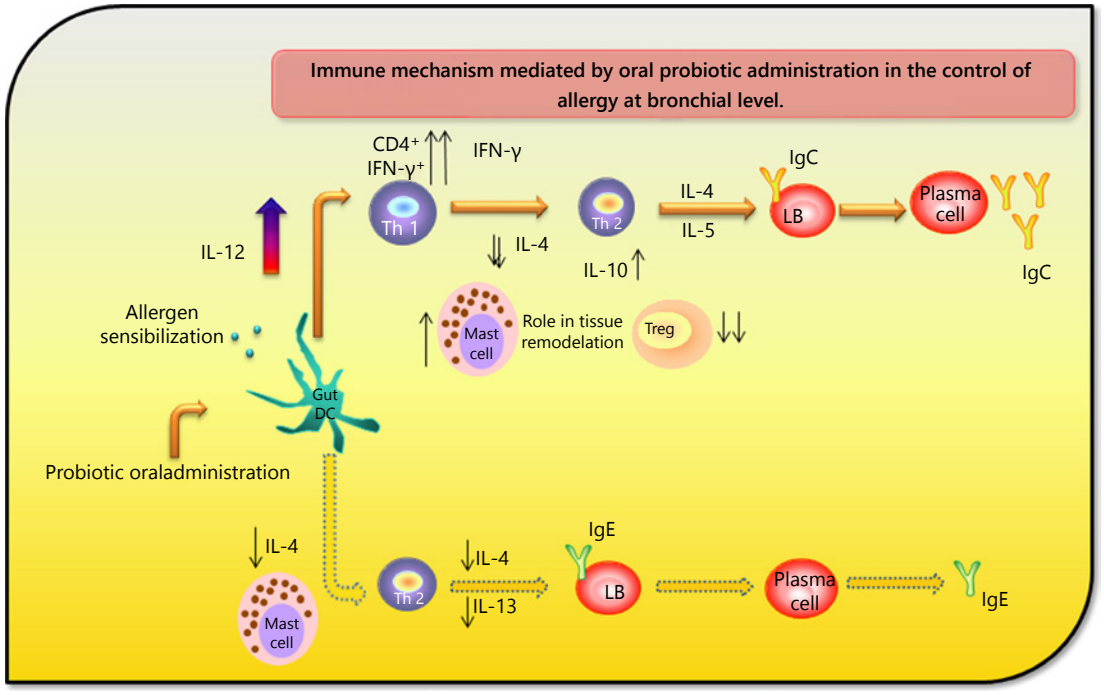

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Fig. 2. Immune mechanisms mediated by oral probiotic administration to control allergy at bronchial level. The probiotic oral administration induces activation of DCs at the gut level, with an IL-12 release that balances the adaptative response to a Th1 profile at the bronchus. The increase in the expression of CD4 and IFN-γ 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.

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 microenvironment 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$^+$) that maintain the immune homeostasis in the gut mucosa (unpublished data). PPs macrophages 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-γ that play an important role in the immunomodulation. PP, peyer patch; Treg, T regulatory.

– 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.

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Disclosure Statement

The authors have no financial conflicts of interest.

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