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Immunomodulatory Potentials of Probiotics: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Author DCN designed the study, searched the literature and wrote the draft versions of the manuscript. Author MCU supervised the literature search and edited the lay-out. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

In recent years, research has focused on natural mechanisms for the management, treatment, and curing of human infections and diseases. One of such natural methods is the application of probiotics, which are live microorganisms which when administered in adequate amounts confer a health benefit on the host. The beneficial effects associated with probiotics were originally thought to be a result of improvements in the intestinal microbial balance, however, there are shred evidence that probiotics can also provide benefits by modulating the immune functions. The ability of these probiotics, majorly the *Lactobacillus* and *Bifidobacterium* species to boost the immune system is proposed to be a result of their interactions with the cells of the immune system. They have been reported to stimulate various parts of the immune system, through several mechanisms enhancing their functions. It has also been established that the effects of probiotic bacteria may also result from soluble factors from these microbes that alter epithelial permeability or mediate activation, maturation or survival of dendritic cells, B and T-cells. Probiotic bacteria, their cell wall components, and other stimulating molecules have been shown to have significant effects on the functionality of the immune systems through the activation of multiple immune mechanisms. This study is aimed at describing the immunological mechanisms of probiotics and their beneficial effects on the host immune system.

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1. INTRODUCTION

The word probiotic is derived from a Greek word which means "pro-life" [1]. In 1965, Lilly and Stillwell first used the term 'probiotic' to describe substances secreted by one organism which stimulates the growth of another [2]. In 2002, the Food and Agricultural Organization of the United Nations/World Health Organization proposed that probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host [3]. Presently, research is focused on natural mechanisms for managing, treating and curing human infections and diseases as a result of the several side effects associated with the use of chemotherapeutic agents and synthetic drugs. Probiotics known as a group of beneficial microbes are currently emerging as one of such natural mechanisms.

Most commonly used probiotic microorganisms belong to the genera *Lactobacillus* and *Bifidobacteria*, however, some strains of *Escherichia coli*, *Bacillus* species, and the yeast *Saccharomyces* are also used [4-6]. Recently, *Clostridium butyricum* was also approved for probiotic use in the European Union [7]. Some of the reported probiotic microorganisms include; *Lactobacillus delbruecki* subsp.*bulgaricus, L. rhamnosus* GG, *L. plantarum, Bifidobacterium bifidum* and *B. infantis* [8,9]. *B. adolescentis* BBMN23 and *B. longum*BBMN68 [10]. Others include *Streptococcus thermophilus, Escherichia coli, Saccharomyces bourladii* [6,11], *Saccharomyces cereviciae* [12], some species of *Lactococcus* and *Enterococcus* [13].

Reported benefits of probiotics include: maintaining the balance of the intestinal flora, increasing lactose tolerance and synthesis of B complex vitamins, absorption of calcium thus maintenance of intestinal homeostasis [1]. Others are anti-mutagenic/anticancer activity, cholesterol-lowering effect, secretion of anti-pathogen substances and enhancement of the immune system/responses [14-16].

The integrity of any eukaryotic organism depends not only on the proper expression of its genes but also on its freedom from /and defense against invading microorganisms [17]. The human body is equipped with defense mechanisms which serve to protect against harmful agents and invading pathogens. It is because of the effectiveness of these defensive mechanisms that both man and animals have survived for so many years. A good example of this is the phagocytosis of bacteria by macrophages and other phagocytic cells of the immune system. However, the host does not depend solely on its immune system to protect it from these agents of diseases.

One of the most important characteristics of probiotics is the regulation of host immune response by manipulating the host immune response towards the infectious microbes and can be useful in the treatment of infectious diseases [18]. Innovative approaches had been attempted as an alternative to vaccines as immune boosters, and these include the use of live biotherapeutic agents (probiotics) such as yeasts and bacteria. Probiotics have been used successfully to improve the host immune response in different health conditions [19]. It is, therefore, the aim of this review to explore the effect of probiotics on the immune system and describe the immunological mechanisms in modulating the host immune system.

2. BRIEF HISTORY OF PROBIOTICS

The concept of probiotics evolved in the late 1800s and early 1900s [20]. Different microorganisms have been used for their supposed ability to prevent and cure diseases, which made Lilly and Stillwell derive the word probiotics in 1965 [21]. The original observation of the beneficial role played by probiotic bacteria was first reported at the beginning of the 20th century by Russian Scientist and Nobel laureate Elie Metchnikoff. He suggested that it would be possible to modify the gut flora and replace harmful microbes with useful ones and thus proposed that the consumption of yogurt containing *Lactobacillus* would result in a decrease in toxin-producing bacteria in the gut and an increase in the life span of the host [8,20]. Henry Tissier, a French pediatrician in the year 1900 observed that children with diarrhea had in their stool a low number of bacteria characterized by a particular Y shaped morphology. These were named *Bacillus bifiduscommunis* and were later assigned to the genus *Bifidobacterium* (bifid*).* The "bifid" bacteria were on the contrary abundant in healthy children [22]. In 1906, he suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora [8,20,22]. Thus the works of Metchnikoff and Tissier were the first to

make scientific suggestions about the probiotic uses of bacteria. The first reported clinical trials were done in the 1930s on the effect of probiotics on constipation [3].

The associated effect of probiotics on the immune system came into play in 1984, when Baalmear et al. [23] observed that animals with complete gut flora have increased phagocytic activity compared with germ-free animals. Similarly, Perdigon et al. [24] discovered that a particular probiotic species *Lactobacillus casei* was active in stimulating phagocytic activity when administered to mice. In 1987, Brandtzaeg et al. suggested that the effect of lactic acid bacteria could be a result of the absorption of the cells or their products by macrophages and transportation to deeper lying lymphatic follicles where they interact with immunocompetent cells [25]. In an *in vitro* experiment carried out in 1989, Fuller found that *Lactobacillus* was successful in modulating inflammatory diseases, enhanced barrier functions and stimulated immunity [26]. Nussler and Thomson in 1992 reported that lactic acid bacteria and their products can interact with immune cells, leading to the production of cytokines that have a manifold effect on immune and non-immune cells [27]. To date, several types of research relating probiotics to the immune system have been carried out with several mechanisms being proposed to elucidate such immuno-stimulating relationships.

3. SAFETY ASSESSMENT OF PRO-BIOTICS FOR HUMAN USE

Strains of lactic acid bacteria (LAB) have a long history of safe use. Different species of *Lactobacillus* and *Enterococcus*, has been consumed frequently since the consumption of fermented milk as food [8]. Probiotic species such as *Lactobacillus acidophilus* have been safely used for several years [8]. Nevertheless, there is a need for the safety aspects to be always considered and possible adverse effects should be continuously evaluated [28]. Members of the genera *Lactococcus*, *Bifidobacteria*, and *Lactobacilli* are most commonly given the "generally regarded as safe" (GRAS) status, while members of the genera *Streptococcus, Enterococcus* and some other genera of LAB are considered opportunistic pathogens [8,9,29]. The safety of probiotics has been considered in reviews and clinical reports which have recorded low cases of human bacteremia [29]. In France, it was estimated that the risk of *Lactobacillus* infection is about one case per 10 million people over a century of probiotic consumption [8,30]. Surveillance studies by Adams and Marteau in 1995 supported the safety of commercial LAB [31]. In 1998, Salminen et al. reported that no harmful effects were observed in controlled clinical studies with Lactobacilli and Bifidobacteria [32]. Further evidence of poor opportunistic pathogenicity of probiotics were provided in clinical studies where certain probiotics was safely administered to immunocompromised patients, premature infants, elderly and patients with Crohn's disease and there were no recorded side-effects [31]. In a recent randomized human study involving the elderly, Lefevre et al. reported that the probiotic product was safe and well-tolerated [16]. Different probiotic formulations have been administered to a considerable large number of individuals suffering from different conditions, under controlled conditions and have been proven to be without associated risks [9]. Considering their widespread use, documented correlations between adverse events and probiotic consumption are very few [8,31].

3.1 Properties of Probiotic Organisms

To be able to exhibit health benefits, probiotics should be able to survive the harsh conditions of the stomach and GI tract of humans after consumption. Some of the properties of an ideal probiotic microorganism include [9,33,34]:

i. Ability to survive the passage through the digestive system.
ii. Ability to adhere to the epithelial cells of the mucosa.
iii. Non-pathogenicity and non-toxicity.
iv. Tolerance to food additives and stable in the food matrix.
v. Excluding or reducing pathogenic adherence.
vi. Multiply and produce acids, hydrogen peroxide and bacteriocins antagonistic to pathogen's growth.
vii. Maintenance of viability in large numbers.

4. PROBIOTICS AND THE INNATE IMMUNE SYSTEM

Consumption of probiotics initiates a host response due to the interaction with intestinal enterocytes, as intestinal cells are known to produce various immunomodulatory molecules when stimulated by bacteria [35]. Oral introduction of lactobacilli can enhance non-specific host resistance to microbial pathogens

and thereby facilitate the exclusion of pathogens in the gut. Lactobacilli modulate the immune response of the host by its interaction with the immune cells and the intestinal epithelium [36]. Several strains of live lactic acid bacteria have been shown to induce *in vitro* the release of the pro-inflammatory cytokines, tumor necrosis factor α, and interleukin 6, reflecting stimulation of nonspecific immunity, [37]. Studies have shown that the *Lactobacillus casei* DN114001 strain induces mucosal immune stimulation, reinforces the non-specific barrier and modulates the innate immune response in the gut, thereby maintaining the intestinal homeostasis [38].

4.1 Effect of Probiotics on Phagocytic Cells

It has been discovered that animals with complete gut flora have increased phagocytic activity compared with germ-free animals [11,39]. Presumably, the normal flora prevents invading organisms from adhering to host cells by covering binding sites, thereby easily exposing them to phagocytic cells (neutrophils, monocytes, macrophages) [2]. Oral introduction of *Lactobacillus casei* and *Lactobacillus bulgaricus* activates the production of macrophages and administration of *L. casei* and *Lactobacillus acidophilus* activates phagocytosis in mice [40]. Similarly, enhanced phagocytosis had been long reported in humans by *L. acidophilus* Lal [41]. Phagocytosis is responsible for early activation of the inflammatory response before antibody production [17]. Phagocytic activity results in the further recruitment of immunocompetent cells and the generation of an inflammatory response [5]. The phagocytic activity of RAW264.7 macrophages murine model is enhanced after inoculation with *Bifidobacterium adolescentis* BBMN23 or *B. longum* BBMN68 [5]. Immune stimulation by these probiotics on the macrophages could be attributed to the enhanced macrophage activity on the components of the immune system. It has been observed that there is an increase in the phagocytic activity of peritoneal macrophage after days of feeding with probiotic cultures [2,42]. Ordinarily, at the earlier stage of infection, the macrophages are recruited to the infected site to engulf the microbes, and when macrophages are activated after bacterial recognition by toll-like receptors (TLRs), they consequently produce high level of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-8, IL-6 and chemokines which recruits more macrophages and other immune cells e.g. neutrophils and basophils [43]. Studies show that probiotics mostly belong to Gram-positive bacteria, and contain lipoteichoic acids and thick peptidoglycan cell wall components (Fig. 1). These cellular components can activate macrophages to secrete cytokines or important mediators which could as well trigger other immune components leading to the stimulation of the immune system [43,44]. There is also a report that oral delivery of *L. casei* probiotic strains to mice could activate mononuclear phagocytes for increased phagocytic activity and lysozyme production [45]. They express non-specific esterase, lysosomal hydrolases, and ectoenzymes, thereby contributing to non-specific uptake of invading materials [43,45,46]. Human studies have confirmed these effects in circulating phagocytes of adult subjects including the elderly (Table 1.) Continuous engulfment by the phagocytes strengthens the immune cells and keeps them at alert [5].

4.2 Probiotic Effects on Inflammatory Response

It has been shown that the administration of *Lactobacillus plantarum*at the dose of 10^{10} CFU/day significantly increased neutrophils, macrophages, and fibroblasts [48], (Table 1). In the inflammatory response, the neutrophils are the first cells to be lured, followed by the macrophages for the engulfment of the infecting pathogen. The fibroblast halts and prevents further spread of the infection [48]. This has also been observed and reported that *in vitro*, *Lactobacillus* has been used to modulate inflammatory diseases, enhance barriers functions and stimulate immunity [26]. Some probiotic bacteria have been reported to induce a pattern of dendritic cell (DC) maturation, characterized by the release of small amounts of tumor necrotic factor α and IL-12, with increased levels of IL-10, and inhibit the generation of pro-inflammatory TH_1 cells [49]. The most patent anti-inflammatory effects by a probiotic is produced by bifidobacteria, which upregulated IL-10 production by dendritic cells in a dose-dependent manner [45].

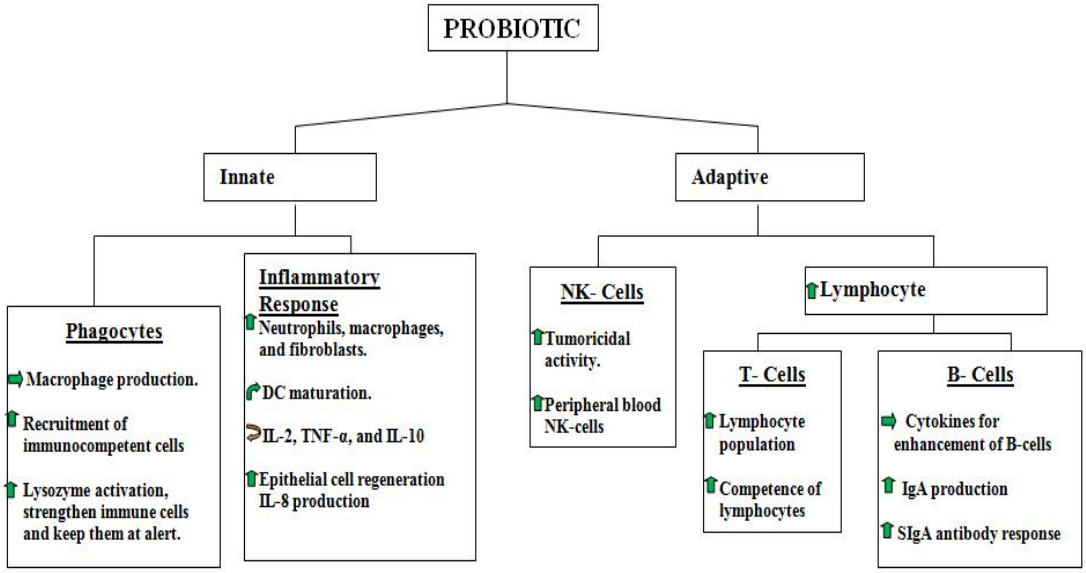

Fig. 1. Mechanisms of immunomodulatory actions of probiotic bacteria
Key⇧; Increase/ Enhance, ➡; Activate/ Stimulate, ↱; Induce, ⮌ ; Release

Table 1. Clinical evidences of immune stimulation by probiotics [47,48]

Probiotics	Immunological Functions	Subjects
Lactobacillus acidophilus La1, *L. rahmnosus*HN001, *B. bifidum* Bb12, *B. lactis*HN019	Phagocytic activity of blood mononuclear and polymorphonuclear cells	Healthy adults and elderly volunteers
*Lactobacillus casei*Shirota, *B. lactis*HN019	The tumoricidal activity of blood mononuclear cells	Healthy adults and elderly volunteers; patients with colorectal cancer
Lactobacillus brevis Labre, *B. lactis*HN019	Production of interferons by peripheral blood mononuclear cells	Healthy adults and elderly volunteers
Lactobacillus plantarum	Increased neutrophils, macrophages, and fibroblast	Adult volunteers
Lactobacillus rhamnosus GG	Anti-rotavirus antibody responses	Children with rotavirus
*L. rahmnosus*GG	Antibody responses following vaccination	Adult volunteers
Bacillus subtilis CU1	Increased the levels of secretory IgA in stools and saliva, high serum IFN-gamma	Elderly during common infectious disease

According to a report by Nikolov in 2012, the inflammation-suppressing properties of probiotics may be able to:

(i) Counteract some of the inflammation-aggravating bacteria. This will decrease the inflammatory response;
(ii) Improve the barrier effect of the mucosa, which will inhibit the translocation of inflammation-inducing luminal contents into the body;
(iii) Directly interact with pro-inflammatory processes [50].

4.3 Stimulation of Cytokines by Pro-biotics

Cytokines are chemical messengers used by both innate and adaptive immunity. Research has shown that live probiotic strains induce the production of protective cytokines that enhance epithelial cell regeneration and inhibit epithelial

cell apoptosis [51]. Cytokine-induced apoptosis was prevented in intestinal epithelial cells in the presence of *L. rhamnosus* GG [48]. Apoptosis which is programmed cell death was prevented by *L. rhamnosus* GG, but the mechanism is not yet fully known. The inhibition of apoptosis enhances the survival of intestinal cells and promotes proliferation during recovery from epithelial injury [52].

Probiotics induce pro-inflammatory cytokine release *in vitro*, such as IL-6 and tumor necrosis factors (TNFs) [53]. This is in agreement with reports by Huang et al. [54] that *Bacillus* strains could stimulate systemic and intestinal IFN- γ production in mice. Zhu et al. [5], who reported that *L. casei* had been shown to induce the cytokine IL-6 production, also demonstrated that *B. adolescentis* BBMN23 is a strong stimulator for TNF production. IL-6 and TNF are cytokines usually secreted by macrophages when activated [43]. Stimulation of these pro-inflammatory and other cytokines by probiotic may be as a result of the immunological responses their presence caused in the body. In the mucosa, probiotic bacteria induce the secretion of cytokines from Intestinal Epithelial Cells (IEC) in a strain-specific manner. The strain specificity could be due to soluble factors produced by probiotic that might modulate cytokine production by peripheral blood mononuclear cells (PBMC) [2]. The IECs serve as the initial point of contact between the host and intestinal microbes and they communicate expansively with commensal bacteria and probiotics. This interaction influences the inflammatory signaling pathway in IECs. The IL-6 and IL-8 released by the epithelial cells are pro-inflammatory, so an intense epithelial stimulation can favor an inflammatory immune response [55]. This cytokine produced by IEC, macrophages and T cells, can induce the terminal development of B cells in plasmatic cells, which express IgA. The cytokines released by Th2 cells are involved in the induction of the IgA immune response [56]. Findings based on the use of cell lines as experimental models reported that the quality and dose of probiotic preparations could impact the IL-8 production by enterocytes [57]. IL-8 appears to be a major cytokine produced by enterocytes following an encounter with probiotics. The IL-8 cytokine primarily functions as a neutrophil chemo-attractant [35]. The macrophages and other immune cells stimulate the production of these cytokines when they come in contact with the probiotic microorganisms, thereby enhancing the immune system for the elimination of any available pathogen in the case of infection or invasion by pathogens.

5. PROBIOTICS AND THE ADAPTIVE IMMUNE CELLS

The majority of the studies concerning probiotic effects on lymphocytes function has utilized animal models. According to Aattouri et al. [58], oral ingestion of lactic acid bacteria such as lactobacilli and bifidobacteria strains by rats and mice increases lymphocyte proliferation and interferon production.

5.1 Stimulation of B-lymphocytes by Probiotics

Earlier studies using rat models by Naidu et al., [59] reveals that lactic acid bacteria administered orally increased the number of antibody-secreting cells, including those in the intestinal mucosa with enhanced B-cells proliferation and antibody production (IgA and IgG). This could be a result of the cell wall components of these bacteria leading to the stimulation of antibodies and cytokines for the proliferation of B-cells (Fig. 1). It has been reported that interactions between host cells and bacteria or their structural components may lead to modulation of T- or B-cell-mediated immune responses, either locally or systemically [60]. When these B-cells are proliferated in response to probiotic, they differentiate into plasma cells producing more antibodies [17]. Early animal studies showed that probiotics were able to enhance systemic antibody responses to parenterally delivered foreign antigen in mice [47,57]. In a study on rats co-colonized with *L. plantarum* and *Escherichia coli*, Herias et al. [61], reported a higher circulating concentration of total IgA and *E. coli* specific IgA and IgM compared with rats which were colonized with *E. coli* alone. If in this study rats were colonized with *E. coli* and it stimulated the production of IgA and IgM. Then when it was co-colonized with *L. plantarum*, and an increase in circulating IgA was recorded, then it could probably be that *L. plantarum* also trigger the production of IgA. Also indicating that synergistic use of probiotics may yield an increased immune effect. Probiotics have also been reported to boost overall SIgA antibody responses [16]. This results in a significant enhancement of systemic antibody response and thereby triggering intestinal immunity and subsequent elimination of the evading pathogens. This is because most SIgA recognizes and opsonizes bacteria in the lumen, thus preventing their access to the lamina propria (LP) [60,62]. SIgA is important in the

maintenance of gut microbiota homeostasis and the protection of the gastrointestinal and respiratory tracts against pathogens, as it is the main immunoglobulin class in human external secretions [16]. The mechanisms whereby probiotics modulate immune responses leading to tolerance or SIgA activation appear to be highly dependent on the strains [49]. In human studies, *L. rhamnosus* GG and *B. breve* YIT4046 were shown to stimulate anti-rotavirus antibodies in response to rotavirus diarrhea in children (Table 1).

5.2 Stimulation of T-lymphocytes by Probiotics

Research has shown that T-helper lymphocyte ($CD4^+$) numbers are increased in the gut-associated lymphoid tissue (GALT) following oral delivery of *L. casei* [11,13]. Providing evidence that probiotic stimulation can increase the size of the lymphocyte population. In a report, orally administered lactic acid bacteria in rats revealed increased numbers of T- lymphocytes, CD4+ cells and also enhanced lymphocyte proliferation [59]. The biological property of probiotic bacteria involved in lymphocyte proliferation is their capacity to affect immune cell redistribution by improving the competence of lymphatic endothelial cells to trap T lymphocytes [63].

5.3 Stimulation of Natural Killer Cells by Probiotics

Oral delivery of *L. rhamnosus* HN001 or *L. casei* Shirota to mice has been shown to increase *ex vivo* natural killer cell tumoricidal activity [45,47]. This proposes that the consumption of these probiotics enhances the destruction of tumor cells by stimulating natural killer (NK) cells, thereby reducing tumor risks. In human studies, *B. lactis*HN019 has been demonstrated to up-regulate peripheral blood NK cells mediated cytotoxicity against tumor cells (Table 1).

6. MECHANISMS OF IMMUNE MODULATION BY PROBIOTICS

Studies have demonstrated that specific chemical compounds isolated from bacteria can induce specific immune responses (Figs. 1, 2 & Table 2) and thus provide the scientific basis for a molecular description of the immunological effects observed after the administration of probiotics. Excluding the extracellular bacteria products, a major role in immunomodulatory activity should be mediated by the structural components of the cell, particularly the cell envelope. This is the outermost structures that the immune cells come into contact with first and includes the cell wall or S-layer proteins, capsules and pellicle [64].

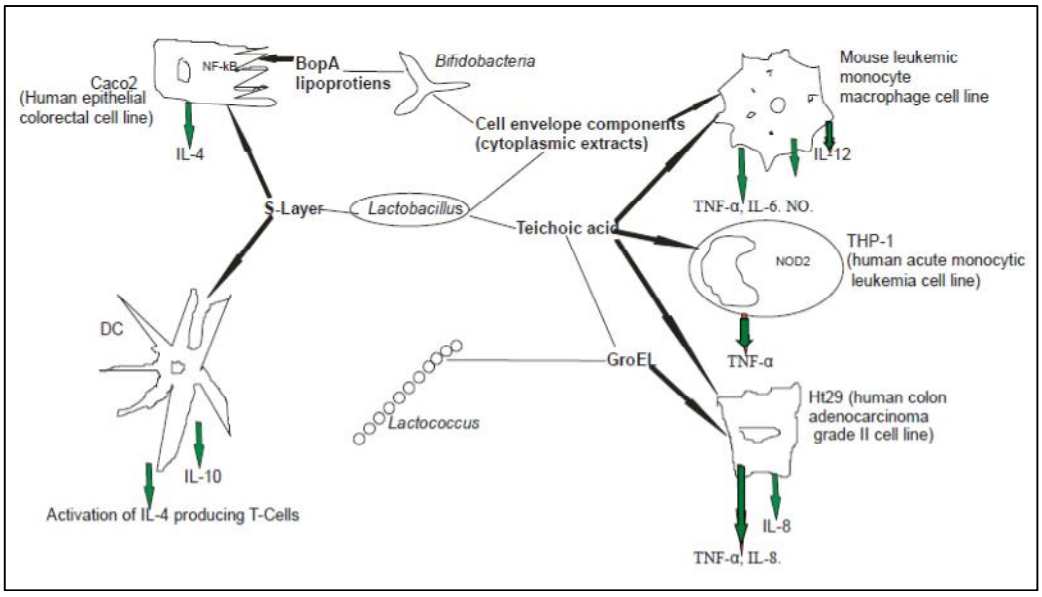

Fig. 2. Molecules or parts of probiotic bacterialcells demonstrated to modulate host immune and epithelial cells

Findings indicated that both cell wall and cytoplasm can be recognized and stimulate the immune system [33]. Because of the limitation that bacteria cannot pass through the epithelial cells, it can be uptaken by microfold cells (m-cells), and only the antigenic particles or products of degradation of the bacteria can make contact with the immune cells by pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMP) [65]. Therefore, PAMP can explain the different production of cytokines induced by bacteria, demonstrating the different components of the cell wall causing the distinct immune-boosting effects. In a study, RAW264.7 macrophages were exposed to heat-killed probiotic *Bifidobacterium spp., L. acidophilus, L. bulgaricus, L. casie, L. gasseri, S. thermophilus* including the cell envelope components and cytoplasmic extracts of these bacteria and they stimulated macrophages to produce TNF-α, IL-6 and nitric oxide (NO) [45], suggesting that bioactive compounds are potentially located everywhere in the bacteria cell.

In the intestinal fluid, probiotics influence the production of antimicrobial peptides (AMPs) by Paneth cells, the production of mucus by goblets cells as well as the level of secretory IgA that is under the dependence of a proliferation-inducing ligand (APRIL) produced by IEC. Probiotics affect the expression level of TLRs on DC as wells as immune cells in Peyer's patches, leading to a production of a wide range of cytokine regulating the evolvement of the immune response.

7. IMMUNOMODULATORY MOLECULES OF PROBIOTIC BACTERIA

7.1 Bacterial Cell Wall Components

Cell wall components of *L. casei*are anti-inflammatory [48]. Peptidoglycan (PGN) and lipopolysaccharide (LPS) are well known potent activators of immune responses (Fig. 2). Peptidoglycan (PGN) is the main constituent of Gram-positive bacterial cell wall, accounting for up to 90% of their weight, whereas it constitutes only 15-20% of the cell wall of Gram-negative bacteria [69]. Specialized conserved pattern recognition receptors (PRR) on the host cell membranes, such as Toll-like receptors (TLRs) and the nucleotide-binding domain (NOD) proteins are the primary sensors of the innate immune system and recognize microbe-associated molecular patterns, including PGN and LPS [37,50,69]. In particular, TLR4 is a specialized receptor for LPS, whereas both NOD1 and NOD2 recognize muramyl peptides released by PGN [37]. The different immune stimulation by cell wall components is shown in Fig. 2 and Table 2. In Gram-positive bacteria cell walls, molecules are protruding from the external surface of the PGN layer known as teichoic acids (TAs). TAs are phosphodiester polymers of glycol or ribitol and can be covalently linked to either peptidoglycan (wall teichoic acid, WTAs) or the cytoplasmic membrane (lipoteichoic acid, LTAs). LTAs purified from *L. casei* YIT 9029 and *L. fermentum*YIT 0159 were demonstrated to induce elevated levels of TNF-α in mouse RAW264.7 macrophages mediated by TLR2 (Fig. 2) [45].

7.2 Other Immunomodulatory Molecules of Probiotic Bacteria

It has been established that the effects of probiotic bacteria may also result from soluble factors that alter epithelial permeability or mediate activation, maturation or survival of dendritic cells [70].

7.2.1 Surface layer (S-layer)

Probiotics can interact with the host immune system through their surface layer, a monomolecular crystalline envelope produced by the self-assembly of protein or glycoprotein sub-units on the outer cell surface [45]. S-layers are commonly found in prokaryotes and makeup to 10-15% of the total protein content of a cell [71]. S-layer protein A (SLpA) released from *L. acidophilus* NCFM cells has been demonstrated to be recognized and bound by dendritic cell-specific intercellular adhesion molecule-3-grabbing non-intergrin (DC-SIGN), a C-type lectin receptor presents on both macrophages and dendritic cells [66]. It was observed that *L. acidophilus* NCFM expressing SLpA was captured by DC-SIGN on DC, and thus activated the IL-4 producing T-cells. These data were confirmed by an experiment performed with purified SLpA protein, which ligated to DC-SIGN and induced IL-10 expression by DCs in the presence of lipopolysaccharide [66]. Nuclear Factor kappa-light chain enhancing activated B-cells (NF-kB), is triggered in the presence of S-layer by human epithelial colorectal adenocarcinoma cell line (Fig. 2). NF-kB is a protein complex that is found in almost all animal cells, which controls the transcription of DNA. It is also involved in cellular responses to stimuli

Table 2. Immunomodulatory molecules of probiotic microorganisms

Probiotics	Molecules	Effects
Lactobacillus casei *Lactobacillus plantarum*	Peptidoglycan	General immune stimulation [33,48]
Lactobacillus casei *Lactobacillus fermentum*	Lipoteichoic acid	Increased levels of TNF-α in mouse macrophage cell [45].
Bifidobacteriumlongum	DNA	Increased cytokine IL-10 [45].
Lactobacillusacidophilus	Surface layer	Activation of IL-4 producing T-cells [66].
*B. bifidum*MIMBb75	BOpA protein	Induce production of IL-8 by colorectal cell line [67,68].
*Escherichia coli*Nissle 1917	Flagellin	Induce production of IL-8 by colorectal cell line [68].
S. thermophiles *L. plantarum*	Cell wall-associated polysaccharide (CAPs)	General immune stimulation [33,48].

such as bacterial and viral antigen with the production of cytokines. NF-KB is the key signaling channel in the inflammatory signaling pathway [2,45].

7.2.2 BOpA protein and flagellin

BOpA is a cell surface-associated lipoprotein of *B. bifidum* MIMBb75 that mediates adhesion to the human Caco-2 intestinal epithelial cells. Upon purification from *B. bifidum*MIMBb75 strain, BOpA has been demonstrated to induce the production of IL-8 by Caco-2 cells in a dose-dependent manner [67]. Flagellin, a structural protein released from *E. coli*Nissle 1917 strain, has been reported to increase the production of the pro-inflammatory cytokine IL-8 in Caco-2 cells [68].

7.2.3 Genomic DNA

It has been shown that prokaryotic DNA contains an unmethylatedCpG motif that can activate immune responses *in vitro* and *in vivo* [45]. DNA mixture isolated from the probiotic mixture VSL#3 containing 8 lyophilized lactic acid bacteria strains generated non-inflammatory responses from epithelial and immune cells [37,72]. In an earlier study, Lammers et al. [73] reported that bacterial DNA extracted from bifidobacterial cultures of the commercial product, VSL#3 influenced cytokine production by peripheral blood mononuclear cells (PBMCs), increasing IL-10. The anti-inflammatory effect of genomic DNA from VSL#3 bacteria was also confirmed in an *in vivo* murine study, which demonstrated that TLR9 signaling was essential in mediating this anti-inflammatory effect [74]. It has been suggested that the immunological effect observed with bifidobacterial genomic DNA is favored by the high guanine-cytosine (GC) content of the *Bifidobacterium* genes (58-61%), which explains the availability of different CpG motifs in the genomes of these bacteria [73].

7.2.4 Cytoplasmic extracts

Cytoplasmic extracts from different probiotic bacteria have demonstrated to stimulate several cytokines involved in immune functions, including Nitric Oxide (NO) (Fig. 2) [45].

It is very important to note that the potential bioactivity of specific bacterial components can be masked by other cell structures, and the effect of a single molecule can be influenced by the presence of additional bioactive substances. In support of this concept, a study by Kaji et al. [75] identified TAs as a key factor for triggering the synergism of inducing IL-10 production. They demonstrated that TAs alone weakly induced IL-10 production, but when macrophages sensed WTAs or LTAs in the presence of *L. casei*shirota strain, these stimuli cooperatively induced potent production of IL-10.

8. THE ROLE OF MICROBIOTA AND PROBIOTICS IN REGULATING IMMUNE RESPONSES IN CANCER PATIENTS

The susceptibility and development of cancer is a result of a complex interaction between gene regulation and the environment [76,77]. Gut microbiota is critical for intestinal immune maturation, protecting the host against pathogens and damaging inflammatory reactions [78], and probiotics (and prebiotics) present more common ways to establish and maintain healthy microbiomes. Lactic acid bacteria (LAB) species constitute members of healthy human gut microbiota. Recent studies have reported that

certain LAB strains are capable of inhibiting tumor progression [79,80]. Generally, the anti-tumor mechanisms of LAB appear to be by the modulation of the immune response and the induction of cellular apoptosis. In two independent studies by Konishi et al. [79] and Baldwin et al. [81], they reported that two strains of *L. casei* decreased tumor cell proliferation and enhance apoptosis in allograft models of colorectal cancer. In a more recent study, the oral administration of the probiotic strain *L. casei* BL23 reduced the onset of chemically induced tumors by the stimulation of IL-12 (Fig. 2) or NK-cell cytotoxicity (Fig. 1) mechanisms [82]. In the same study, they demonstrated the protective effects of *L. casei* BL23 in different mouse models of cancer, including colorectal-associated cancer (CAC) and the TC-1 allograft model. Similarly, the Intratumoral inoculation of 3 mg of heat-killed *Propionibacterium acnes* in subcutaneous melanoma promoted local and systemic Th1 and Tc1 responses associated with *in situ* granuloma formation and tumor regression [83]. *P. acnes* is recognized by TLR2 on monocytes, macrophages, and DCs, leading to the activation of IL-12 promotor [84]. According to Delia et al. [85] and Touchefeu et al. [86], preparations containing *B. bifidum*, *L. acidophilus*, *L. casei*, and the VSL#3 formulation containing *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* spp. have been proven to reduce radiation-induced gut toxicity, such as diarrhea.

Reports are suggesting that genetically modified probiotic microorganisms may have better effects. A *L. acidophilus* strain harboring a deletion in the phosphoglycerol transferase gene and unable to synthetize LTA prevented the progression of colonic polyps in Apc^{Dflox} mice [87]. Elafin-overexpressing *L. casei* and *L. lactis* reduced colitis in mice and *ex vivo* in inflamed epithelial cells from human colitis [88]. *L. gasseri* genetically modified to overexpress superoxide dismutase was reported to decrease colitis in IL-10-deficient hosts [89]. These findings and reports further raise the possibility that probiotics, if properly considered could become an adjuvant therapy for cancer treatment.

9. SPECIAL OBSERVATION

In a very recent study carried out in 2019, it was reported that probiotics can have different effects on the immune system in males, compared with female piglets [90]. It is important to understand that immunity differs considerably with sex. Accurate development of the immune system is crucial in guaranteeing it responds properly to both harmful and harmless stimulation throughout life, and this development, even during the early days of life, is dependent on sex [91]. In the study, the immune cells, antibodies, and other immune-related molecules were different in males and females in response to probiotic supplementation. Female pigs produced more of the immunoglobulins IgA and IgM in their lymph tissue, while in male pigs, the process occurred in the large intestine [90]. This proposes that, during infancy, females may have greater potential for local immune regulation than their male counterparts. This finding implies that specific probiotics may be more beneficial for girls, whilst others could produce improved health outcomes for boys. Given the primary differences in immune development between males and females, taking sex into account could provide a means to progress the effectiveness of probiotics for pharmaceutics and other therapies that act on the immune system.

10. CONCLUSION AND FUTURE PERSPECTIVES

Accumulating researches have shown that probiotics have effects on the immune system. These effects can be seen in their ability to trigger or stimulate the immune cells and suppress certain immune processes. Meantime, research is still ongoing to completely understand the mechanisms involved in immune modulation by probiotics. A probiotic-induced immune stimulation is a complex interplay of the host-microbe interactions. The immune responses (innate and adaptive) can be modulated by probiotic bacteria in a strain- and dose-dependent manner. However, there is limited knowledge for *in vivo* use, safety and effect in immunocompromised individuals and newborns. Thus, improved knowledge of probiotics and its effect on the host immune system by various mechanisms will promote proper strain selection for a specific prophylactic or therapeutic use, ultimately leading to more personalized therapy. There is also a need for more controlled trials with sufficient numbers of volunteers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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