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# Research advances in the immunomodulatory mechanisms of probiotics on non-specific and specific immunity

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ABSTRACT

Probiotics can regulate the body's immune system through both non-specific and specific immunity, thereby regulating host health. In terms of non-specific immune regulation, probiotics can activate the intrinsic immune system, regulate the mucosal barrier function, and play an immune role by influencing the activity of intrinsic immune cells such as macrophages, dendritic cells and natural killer cells, as well as their differentiation and maturation; in terms of specific immune regulation, probiotics play a role in regulating the immunoglobulin level and the maturation of B cells. Probiotics can also regulate T-cell differentiation according to the condition of the body, thus regulating specific immunity. Many studies have focused on the role of probiotics in metabolism and nutrition, and the mechanisms involved in the immunomodulatory role of probiotics have only been partially described. This review summarises the role of common probiotics such as *Lactobacillus plantarum* and *Lactobacillus rhamnosus* in immunomodulation as well as their mechanisms, describing the currently known mechanisms of immunomodulation by probiotics in improving the host immune system. A deeper understanding of probiotics and their specific mechanisms of action will facilitate the use of probiotics for immunomodulation in clinical medicine, functional foods, and other areas. This will also contribute to the development and research of engineered probiotics, next-generation probiotics, and other new functional probiotics with immunomodulatory effects.



## 1. Introduction

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[1]. Driven by technological advancements and innovation, the probiotics industry has seen an expanding application in both the food industry and medical field. Currently, several bacteria and fungi have been utilized to maintain human health, and certain cell types have been evaluated for their role as beneficial probiotic agents, such as *Lactobacillus* and *Bifidobacterium*. Consequently, *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces boulardii* are among the most common bacterial and fungal species involved in probiotics[2]. Probiotics are known for enhancing the body's immunity, inhibiting the growth of pathogenic microflora, and influencing the metabolism and absorption of substances. In the past few years, the role of probiotics in modulating body immunity has attracted increasing attention[3]. Their immunomodulatory effects have been confirmed in various diseases, such as infectious diarrhea, allergies, inflammatory bowel disease, and irritable bowel syndrome[4].

Probiotics are becoming increasingly valued for their safety, reliability and excellent performance in clinical applications[5]. The global probiotic market is reportedly growing at a rate of 15%–20% per year, and the relationship between probiotics and human health and disease prevention has become a key direction in academic research[5]. They are regarded as one of the most valuable options for the restoration

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of microbial diversity and the treatment of infections caused by antibiotic-resistant bacteria or invasive viruses[6]. Probiotics are specific, so their functions are complex and varied, and different probiotics have different functions, which determines the diversity of probiotics in the process of immunomodulation. Consequently, probiotics possess immunomodulatory and numerous other therapeutic mechanisms, which are effective in the treatment of a diverse range of diseases. Probiotics are also regarded as a viable alternative to antibiotics, offering a potential alternative treatment option[7]. Antibiotics have been extensively employed to prevent and treat gastrointestinal infections in livestock. However, their utilization has resulted in the development of resistance, which can also exert long-lasting effects on the human body and disrupt gut flora[8]. Despite the recognized beneficial effects of probiotics on human health, barriers to their utilization remain, such as the fact that taking probiotics to eliminate treatment-related side effects common in adult cancer patients can lead to the development of other diseases[9]. Consequently, it is still necessary to clarify the exact mechanism of probiotic action, select appropriate strains for targeted therapy, and determine the effective dosages. At the same time, elucidating the regulatory mechanism of probiotics can facilitate the development of personalized probiotics for specific populations and enable probiotics to assume a more efficacious regulatory role. Therefore, it is important to investigate the immunomodulatory mechanisms of probiotics on the organism.

A growing body of research has identified probiotics as having the potential to maintain host health and modulate the immune system. Probiotics have been demonstrated to regulate non-specific and specific immunity by modulating the activity of dendritic cells, macrophages, granulocytes, epithelial cells, and the production of the corresponding cytokines and chemokines, as well as the corresponding immunoglobulin-producing cells and the subsequent secretion of immunoglobulins[10]. Probiotics can therefore modulate the host's immune system and exert beneficial effects, thereby preventing and treating associated diseases. Nevertheless, the mechanism of interaction between ingested probiotics and immune cells is not well described. Besides, there are differences in the therapeutic efficacy of probiotics due to individual differences, medication use, and causes of disease. This article provides a comprehensive overview of the relationship between probiotics and immune cells, as well as their regulatory effects on the immune system, based on the latest research findings, and identifies suitable conditions for different bacterial strains. Additionally, some mixed probiotics and multi-species probiotics provide greater benefits in comparison to single strains of probiotics. Consequently, the article searches for more probiotic combinations that can work synergistically by revealing the different immunomodulatory mechanisms of probiotics.

Probiotics modulate immunity by regulating non-specific and specific immunity, as shown in Fig. 1. For non-specific immunity, probiotics primarily function by safeguarding the mucosal barrier's integrity, fortifying it, and enhancing mucosal adherence. They also play a crucial role in inhibiting the attachment of pathogens, effectively removing pathogens from the gut through competitive exclusion, and modulating the activity of innate immune cells, such as macrophages[11]. For specific immunity, they exert their regulatory role by influencing the proliferation and differentiation of B cells and the polarity of T cells[11]. This paper explains the immunomodulatory effects of probiotics in the host from the perspectives of mediated non-specific and specific immunity.

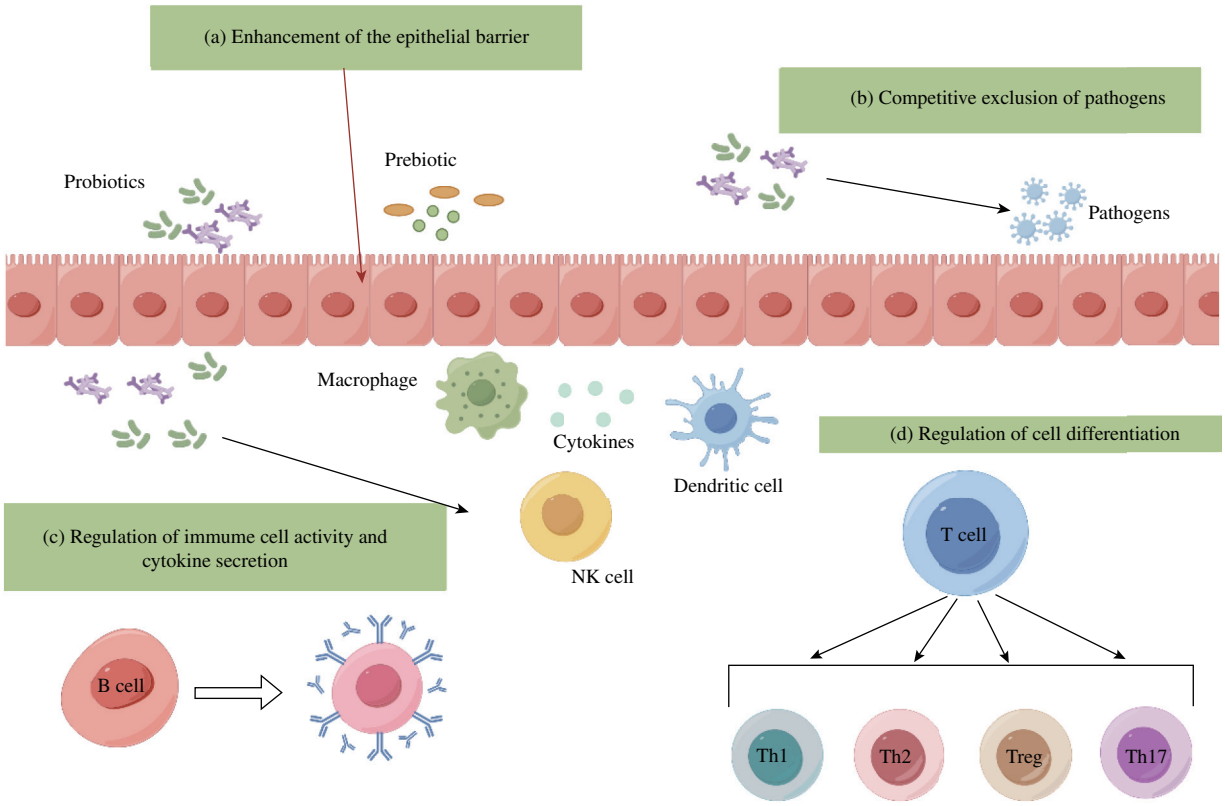


**Fig. 1** Regulatory effects of probiotics on the immune system[11].

## 2. Probiotics and non-specific immunity

Non-specific immunity, also known as innate or intrinsic immunity, is the body's initial defense system against infections, involving various components such as mucosal barriers, biological flora, skin, and intrinsic immune cells and their cytokines. Probiotics boost the body's immune response by strengthening mucosal barrier functions, attaching to and impeding the growth of harmful bacteria, influencing the behavior of immune cells, and stimulating the generation of immune factors.

### *2.1 Mucosal barrier function*

Mucous membranes in the organism are located primarily on the inner surface of the gastrointestinal tract, the respiratory tract, the genitourinary tract, and exocrine glands, and are the first line of defense against pathogens. The gastrointestinal tract, being the body's most vulnerable site to pathogenic microorganisms, makes the intestinal mucosal barrier's defensive function crucial for the body. Probiotics support barrier functionality by strengthening mucosal components, tight junctions (TJ), and innate defenses, maintaining mucosal layer stability, and thus preventing and protecting against diseases, as shown in Table 1. This paper will discuss the role of probiotics in the body regarding mucosal barrier regulation, focusing on 3 aspects: mucus secretion, reinforcement of the mucosal barrier, and modulation of mucosal immunity.

#### *2.1.1 Culture and treatment of HepaRG cell*

Probiotics produce metabolites beneficial to the host, regulate mucin gene expression, and promote goblet cell proliferation. By enhancing goblet cell function, probiotics promote mucin secretion, affecting the nature of the mucosal layer and, consequently, modulating the immune system. The production of intestinal mucus primarily relies on goblet cells, with mucins secreted by them being crucial components of the mucosal layer[19]. The content of mucin polymers is a vital indicator for assessing the protective function of the mucosal barrier.

Ample evidence suggests that probiotics generate host-beneficial metabolites, enhancing the intestinal barrier to maintain host health. Butyrate produced by probiotics appears to be the most effective short-chain fatty acid in regulating mucin production. It significantly upregulates the K-ras signaling protein, leading to the activation of most kinases in the mitogen-activated protein kinase (MAPK) signaling pathway downstream, including MAPK-interacting kinase (MNK)1/2, ribosomal s6 kinase 2 (RSK2), p38-regulated/activated protein kinase, and mitogen- and stress-activated protein kinase. The upregulation of ras also activates p70S6K kinase expression through the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) survival pathway. The upregulated MNK1/2, p70S6K, and RSK2 kinases stimulate mucin synthesis by modifying ribosomal proteins or the translation initiation mechanism. Downstream kinases not only promote protein synthesis and cell growth but also indirectly stimulate goblet cell proliferation and survival by post-translational modifications of key transcription factors such as Myc, CREB, c-Fos, and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB)[20]. The differing properties and functions of various probiotic strains result in the production of distinct short-chain fatty acids (SCFAs). SCFAs also regulate the body's mucin levels according to the body's specific conditions. It was found that *Enterococcus faecium* was able to increase butyrate content and utilization in obese mice, thereby enhancing the intestinal epithelial barrier by increasing the level of acidic mucins[18]. In contrast, *Lactiplantibacillus plantarum* APsulloc331261 increased butyrate levels in allergic airway inflammation mice, and butyrate inhibited mucus secretion by suppressing gene expression of the mucin5AC[21]. *Bifidobacterium dentium* also synthesizes acetate, which is highly expressed in goblet cells through the acetate receptor G protein-coupled receptor (GPR)43. Acetate directly regulates the GPR43 receptor to stimulate mucin 2 production. Additionally, a lower intestinal pH, reduced by acetate, can trigger MUC2 aggregation in goblet cells. Both low pH and GPR43 activation stimulate intestinal secretory cells to release serotonin. This activates serotonin 4 receptors on goblet cells, stimulating the release of mucin 2 and trefoil factor 3 (TFF3), with TFF3 mediating epithelial repair[22].

In addition to promoting mucin production through signaling pathways, the metabolic products of probiotics also enhance the secretion of other active factors in goblet cells to maintain mucin integrity. Probiotics influence gene transcription in goblet cells by affecting signaling pathways and upregulating the expression of active factors (TFF3), Fc gamma binding protein (FCGBP), and anterior gradient 2 (AGR2). FCGBP, a crucial component of mucin granules, forms disulfide bonds with TFF3 after covalent or non-covalent interactions, contributing to the formation of a stable mucus layer. The gastrointestinal mucus layer's form and location are determined by covalently bound FCGBP; its absence leaves the mucus layer indistinguishable[23]. AGR2, a disulfide isomerase located in the endoplasmic reticulum, is responsible for the post-transcriptional regulation of mucin 2. The mucosal barrier of human colonic epithelial cells and gastrointestinal health is improved by *Streptococcus thermophilus* UASt-09's upregulation of goblet cell-related factors (TFF3, ELMβ and AGR2)[24]. On the other hand, *Lactobacillus plantarum* UALp-05 and *Lactobacillus acidophilus* DDS-1-treated cells resulted in significant down-regulation of AGR2 and TFF3 levels, respectively[24]. It appears that the capacity to specifically activate the antimicrobial peptides TFF3 and RELMβ is dependent on the specific strain, and not all probiotics can promote the production of goblet cell-related factors. Some only enhance mucin 2 production without maintaining or improving the integrity of the produced mucin 2 through active factor production, thus potentially failing to strengthen the mucus barrier function.

Probiotics also protect the integrity of the mucosal barrier by enhancing the function of goblet cells. They increase the body's nutrient content, and mitochondria decompose these nutrients to release energy. The protein part of mucin is synthesized in the endoplasmic reticulum and processed by the Golgi apparatus before being carried to the mucosal surface. Consequently, probiotics increase the number of mitochondria, Golgi apparatus, and endoplasmic reticulum in goblet cells, and the cells contain an abundance of mucin granules, thereby altering the structure of goblet cells to enhance their function. Some viruses often target goblet cells, reducing mucin secretion. *Lactobacillus casei* can enhance goblet cell function, thus reducing viral infections[19]. After oral administration of a mixture of *Bacillus licheniformis* and *Bacillus subtilis*, mucin 4-resistant piglets can

**Table 1**
Immunomodulatory effects of probiotics on the mucosal barrier.

| Probiotic strain | Experimental model | Intervention methods | Mode of action | References |
|---|---|---|---|---|
| *L. acidophilus*, *B. Subtilis* and *Saccharomyces cerevisiae* | *E. coli* K99-infected calves | Calves was fed probiotics every day from the next day ($7.0 \times 10^9$ CFU/g; 2 g/calf) after orally challenged with *E. coli* K99 | Improves intestinal immunity, increases mRNA expression of *ZO-1* and *occludin* in the mucosa, and promotes the recovery of intestinal mucosal function. | [12] |
| *L. plantarum* RG14 | 12 newly weaned lambs | The group received 0.9% (*V/m*) postbiotics in the diet | Decreases expression of pro-inflammatory cytokines IL-1β and TNF and anti-inflammatory cytokine *IL-10* mRNA in the intestinal mucosa, increases production of antimicrobial peptides and up-regulates the mucosal barrier through up-regulation of TJ proteins such as TJP-1, CLDN-1 and CLDN-4. | [13] |
| *Bacillus coagulans* | 1-day-old chickens | An average of 1 g bacterial freeze-dried powder which contained $10^8$ CFU viable count was used for each chicken. | Increases the number of goblet cells, reduces levels of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α and increases levels of the SIgA, thereby enhancing innate immunity and maintaining the intestinal mucosal barrier. | [14] |
| *L. delbrueckii* CIDCA 133 | A mouse model of 5-fluorouracil drug-induced mucositis | Mice received doses of 300 μL containing $10^9$ CFU/mL of CIDCA 133 or 300 μL of CIDCA 133 supernatants for 13 days by intragastric gavage. | Promotes expression of mucin 2 and TJ proteins (Ocln, Cldn1, Cldn2, Cldn5, Hp, and Muc2) and reduces neutrophils infiltrating the small intestinal mucosa. | [15] |
| *L. plantarum* HNU082 | Dextran sulfate sodium-induced ulcerative colitis in mice | Mice were given with 200 μL Lp082 ($1 \times 10^9$ CFU/mL) from day1 to day 7. | Increases the content of goblet cells and mRNA expression of mucin 2 to optimize the chemical barrier.<br>Reduces mRNA expression of *claudin-1* and *claudin-2*, increases mRNA expression of *ZO-1* and *ZO-2* and protein content of *ZO-1*, thereby improving the mechanical barrier. | [16] |
| *Lacticaseibacillus rhamnosus* P118 | Chicks infected with *Salmonella* Typhimurium | At the beginning of three days of age, chicks received 0.2 mL phosphate-buffered saline containing $1 \times 10^8$ CFU of the *L. rhamnosus* P118. | Reduces apoptosis thereby significantly increasing the immunity of the intestinal mucosa.<br>Increases levels of TJ proteins (ZO-1 and claudin-1) thereby enhancing the function of intestinal mucosal epithelial cells. | [17] |
| *E. faecium* | Male C57BL/6J mice, fed with standard diet or high-fat diet | *E. faecium* was administered *via* oral gavage, at the dose of $10^8$ CFU/day | Significantly enhances the intestinal epithelial barrier, normalizing the expression of TJ claudin-1 as well as the acidic mucin content altered in obese mice. | [18] |

regulate gut microbiota and enhance goblet cell function to improve intestinal inflammation[25]. Thus some probiotics may maintain the mucosal barrier by enhancing the function of goblet cells. Moreover, the goblet cell-associated antigen passages formed by goblet cells can deliver soluble antigens from the intestinal lumen to dendritic cells, which in turn are presented to T cells and induce adaptive immune responses[26]. In summary, goblet cells have multiple roles in innate and adaptive immunity and are the hub between the two.

### *2.1.2 Reinforcement of the mucosal barrier*

Numerous investigations have discovered that probiotics have a protective effect on the TJ proteins of the mucosal barrier. The role of TJ proteins is to regulate the passage of large water-soluble molecules in intestinal epithelial cells and is the material foundation for the selective permeability of epithelial cells, providing protection against pathogen invasion[27]. Probiotics protect the intestinal mucosa *via* an epidermal growth factor receptor (EGFR)-dependent mechanism[28]. An important intestinal regulator, epidermal growth factor (EGF) is required for intestine development and barrier maintenance. Claudins levels are markedly upregulated by glutamine (Gln) in the EGFR-dependent pathway[29]. EGF serves as a key regulator in the intestine, crucial for both the development and the maintenance of the intestinal barrier. In this context, Gln, through an EGFR-dependent mechanism, significantly enhances the levels of TJ proteins, vital for barrier integrity. The impact of probiotics and EGF on epithelial cells, however, is mediated through distinct signaling pathways. Probiotics effectively mitigate osmotic stress-induced disruption of TJ proteins and barrier dysfunction, primarily through PKC-dependent mechanisms. Furthermore, they offer protection to cellular barriers against stress by upregulating TJ proteins *via* EGFR signaling pathways. Additionally, probiotics have shown the capability in both preventing and reducing the disruption of TJ proteins in intestinal epithelial cells, leveraging EGFR-dependent mechanisms. Research has found that secreted protein P40 can be isolated from *Lactobacillus rhamnosus.* P40 can trans-activate EGFR in intestinal epithelial cells in newborn mice, thereby upregulating the expression of *Claudin 3* mRNA and avoiding damage to TJproteins by pro-inflammatory factors and hydrogen peroxide[30]. After consuming *Clostridium butyricum Prazmowski* supernatant, colitis mice stimulated EGFR

activation, thereby reducing the loss of TJ proteins by hydrogen peroxide[28]. However, consumption of piglets supplemented with *E. faecium* feed did not influence EGFR expression[31].

When the mucosal barrier is compromised, the expression of TJ proteins is suppressed. Probiotics enhance TJ expression, thereby protecting the mucosal barrier. The protective role of probiotics may arise from the coordination of various signaling pathways such as Toll-like receptors (TLR), MyD88, NF-κB, MAPK, PKC, and p38. When intestinal barrier damage occurs, characterized by reduced TJ protein expression, the expression of the pregnane X receptor (PXR) is inhibited. PXR, as a critical transcription factor and exogenous sensor, helps regulate intestinal barrier function. Probiotics mixture (*Bifidobacterium infants*, *L. acidophilus*, *Enterococcus*, and *Bacillus cereus*) enhance PXR expression and elevate the transcription levels of their target genes, such as *Cyp3a11* and *Mdr1a*. PXR overexpression can improve TJ protein expression by inhibiting the phosphorylation of c-Jun N-terminal kinase, thus enhancing intestinal epithelial barrier function[32]. However, probiotics have strain specificity, and not all probiotics can enhance PXR expression. The experiments of Niu et al.[33] found that by screening 320 strains, only 4 strains with the ability to promote the expression of PXR and TJ proteins were obtained. Lipopolysaccharide-induced mucosal barrier injury suppresses TJ protein expression. Probiotics stabilize TJ protein expression by increasing TLR2 expression and competing with lipopolysaccharide (LPS) for TLR4 binding sites. They can also enhance TJ protein expression by inhibiting tumor necrosis factor (TNF-α) cytokine production induced by LPS, thus protecting cells from the downregulation of TJ protein expression induced by lipopolysaccharides[34]. *Weissella cibaria* inhibits NF-κB translocation to the nucleus and deactivates the MLCK-pMLC pathway during lipopolysaccharide exposure. Therefore, it can regulate inflammation and TJ protein expression through the NF-κB-mediated MLCK-pMLC pathway, protecting intestinal epithelial barrier function[35].

Another crucial cause of mucosal barrier damage is the inhibition of TJ protein expression by pathogenic bacterial infection, disrupting the epithelial cell barrier and increasing intestinal mucosal permeability. Probiotics enhance TJ protein expression by occupying sites, competing for nutrients, and inhibiting the invasion and growth of pathogens through various metabolic products and bacteriocins they secrete, thus enhancing the assembly process of tight intercellular connections and reducing cell permeability to protect the intestinal mucosal barrier. Bhat et al.[36] found that exposing Caco-2 cells to *Escherichia coli* reduced the mRNA expression of TJ protein genes, increasing barrier permeability. *Lactobacillus reuteri* upregulated the expression of occludin, TJ protein-1, and claudins, reconstructing the intestinal barrier damaged by *E. coli*. Chicks infected with Salmonella can reduce the proportion of *Salmonella* and balance the diversity of the disrupted intestinal flora after consuming *L. rhamnosus* P118[17].

Probiotics activate multiple signaling pathways to alleviate the abnormal apoptosis of mucosal epithelial cells. Epithelial cells constantly undergo renewal through proliferation, differentiation, and apoptosis. This helps maintain the homeostasis and regeneration of the epithelium while stabilizing the mucosal barrier. However, cytokines and pathogens in the body can induce abnormal apoptosis in intestinal epithelial cells. Studies have found that certain probiotics exhibit a common downstream signaling cascade dependent on TLR2 and TLR10. Probiotic signaling *via* TLR2 contributes to improved intestinal immune tolerance, reduces necrosis, and enhances barrier integrity. Probiotic signaling *via* TLR10 blocks the typical NF-κB cascade response and promotes the PI3/Akt signaling pathway, which reduces cell necrosis and thus aiding in maintaining barrier integrity[37]. In mice treated with *Pediococcus pentosaceus* CECT8330, levels of nuclear NF-κB P65 in colon tissue were reduced, and levels of apoptosis-related proteins Bax and cleaved Caspase-3 significantly decreased, while anti-apoptotic protein Bcl-2 levels increased. Therefore, *P. pentosaceus* CECT8330 inhibits intestinal cell apoptosis and colitis by suppressing the NF-κB pathway[38]. In *Aggregatibacter actinomycetemcomitans*-infected gingival epithelial cells, *L. rhamnosus* Lr32 up-regulated the transcription of the anti-apoptotic gene *BCL-2* and down-regulated the transcription of pro-apoptotic *BAX*[39]. Research has found that apoptotic epithelial cells can regulate regulatory T cells (Tregs). Gut commensals stimulate IFN-β production by dendritic cells, which promotes Treg cell proliferation, but phosphatidylserine on apoptotic epithelial cells inhibits IFN-β production by DCs through inhibitory signaling mediated by the surface glycoprotein CD300a, which inhibits Treg cell proliferation[40]. Therefore, probiotics can regulate not only innate immunity but also adaptive immunity by inhibiting abnormal apoptosis of epithelial cells.

Probiotic metabolic products are also crucial in preventing and alleviating abnormal cell apoptosis. Exopolysaccharides from probiotics exhibit antioxidant and anti-apoptotic activities in epithelial cells. These exopolysaccharides have notable antioxidant activities, such as scavenging 1,1-diphenyl-2-picrylhydrazyl radicals, hydroxyl radicals, and chelating ferrous ions. Through the Keap1/Nrf2 and Bax/Bcl-2 signaling pathways, they effectively mitigate $H_2O_2$-induced oxidative damage in cells, thereby enhancing cell survival rates[41]. Probiotic S-layer proteins regulate the tumor necrosis factor receptor (TNFR) to inhibit cytokine-induced apoptosis. TNFR functions through 2 specific receptors, TNFR1 and TNFR2. Probiotics control signal transduction by inhibiting TNFR1-mediated pathways associated with tissue damage and cell apoptosis. In a TNF-α-induced Caco-2 cell model treated with *L. acidophilus* S-layer proteins, both TNFR1 and TNFR2 were expressed in the control group, leading to cell apoptosis. In the experimental group, only TNFR2 was active, thus inhibiting apoptosis[42]. These pathways facilitate the regeneration of epithelial cells and the regulation of the mucosal immune system. Enteropathogenic *E. coli* induces apoptosis and inhibits autophagy in an intestinal porcine epithelial cell line. Treatment with *Bifidobacterium lactis*-derived EPS attenuated apoptosis and restored autophagy[43]. However, exopolysaccharide can likewise induce apoptosis and inhibit the proliferation of cancer cells. Exopolysaccharide produced by *L. fermentum* YL-11 up-regulates the Bax/Bcl-2 ratio, improves the expression of cleaved PARP protein, and ultimately induces apoptosis in HT-29 cells[44].

### *2.1.3 UHPLC-HRMS analysis*

Probiotics exhibit immunoregulatory properties on the mucosal immune system, enhancing the activity of immune-related cells in the mucosal lamina propria, repairing epithelial cell inflammation, and strengthening mucosal immune functions. The mucosal immune system is an independent immune system with unique structural and functional characteristics, playing a key role in combating infections[45]. Probiotics can act as carriers in the mucosal immune-

delivery system, transferring antigens to stimulate local mucosal immune responses and elevating levels of certain cytokines such as interleukin (IL)-4, interferon gamma (IFN-γ), and IL-17, thus modulating the differentiation of helper T (Th1), T helper 2 (Th2), and T helper 17 (Th17) cells, as depicted in Fig. 2[46]. Adequate delivery of antigens by probiotics not only promotes the differentiation of thcell precursor (Th0) cells into Th1 cells, which activate B cells to secrete secretory immunoglobulin A (SIgA), but also induces T cells to produce cytokines that suppress tumor and viral growth[47]. Low doses of antigens modulate cytokine activity, causing Th0 cells to differentiate into Th2 cells and activating B lymphocytes to secrete immunoglobulin E (IgE). Probiotic delivery of antigens enhances the host's innate immunity, triggering both local mucosal and systemic immune responses for effective immune enhancement. *L. casei*, extensively employed as an antigen delivery vector, is known for its good biosafety[48]. Probiotics promote the development of immune organs in the body, enhancing intestinal immunity and resistance to diseases such as infections and inflammations through various pathways, including promoting macrophages to secrete anti-inflammatory cytokines like IL-10 and transforming growth factor-β (TGF-β), fostering the differentiation and maturation of T and B lymphocytes, regulating the balance of Th1/Th2 and Th17/Tregs, and increasing the contents of IgG, IgM, and IIgA in the intestinal fluid.

Probiotics also modulate mucosal immunity by increasing the synthesis of the antibody molecule SIgA. The level of specific SIgA is considered a standard for evaluating mucosal immunity, with SIgA playing roles in inhibiting pathogen adhesion, anti-infection, immune clearance, anti-allergy, promoting natural antimicrobial factors, which maintain intestinal homeostasis, and shape gut microbiota composition[49]. Probiotics attach to and establish themselves in the intestines, boosting T and B cell responses to antigenic stimuli. This action activates targeted immune responses in the intestinal mucosal-associated lymphoid tissue, elevates the production of SIgA, and strengthens the immune function of the gastrointestinal mucosa. In a study treating mesalazine-induced colitis with probiotics, probiotics improved intestinal barrier function by upregulating the protein and mRNA expression levels of SIgA, thus increasing its content[50]. Studies have found that children consuming milk powder with added *L. paracasei* SD1 probiotics have higher levels of SIgA in their bodies compared to those consuming milk powder without added probiotics[51]. However, some studies have found that infants' consumption of formula containing *Bifidobacterium animalis* did not increase SIgA levels, which tended to increase after consumption of prebiotic formulas[52]. The interaction between SIgA and commensal microorganisms is bidirectional, and probiotics may regulate the distribution of SIgA to keep SIgA at an appropriate level and modulate specific immunity by providing low levels of immune stimulation[53].

### 2.2 *Influence on innate immune cells*

The innate immune system has complex immune mechanisms to defend against foreign infections, thereby maintaining the health of the body. Innate immune cells such as macrophages, dendritic cells, and natural killer (NK) cells are part of the innate immune system. Innate immune cells non-specifically recognize and act on pathogens to prevent the spread of foreign pathogens. Macrophages, dendritic cells, NK cells, and the cytokines they secrete, although capable of destroying pathogens, can disrupt body homeostasis and negatively impact the health of the organism if not properly regulated. Probiotics can activate the body's innate immune system and regulate the activity of innate immune cells, thus preventing viral infections, maintaining the balance of intestinal flora, regulating homeostatic balance, and improving the body's immunity. This article will discuss the role of

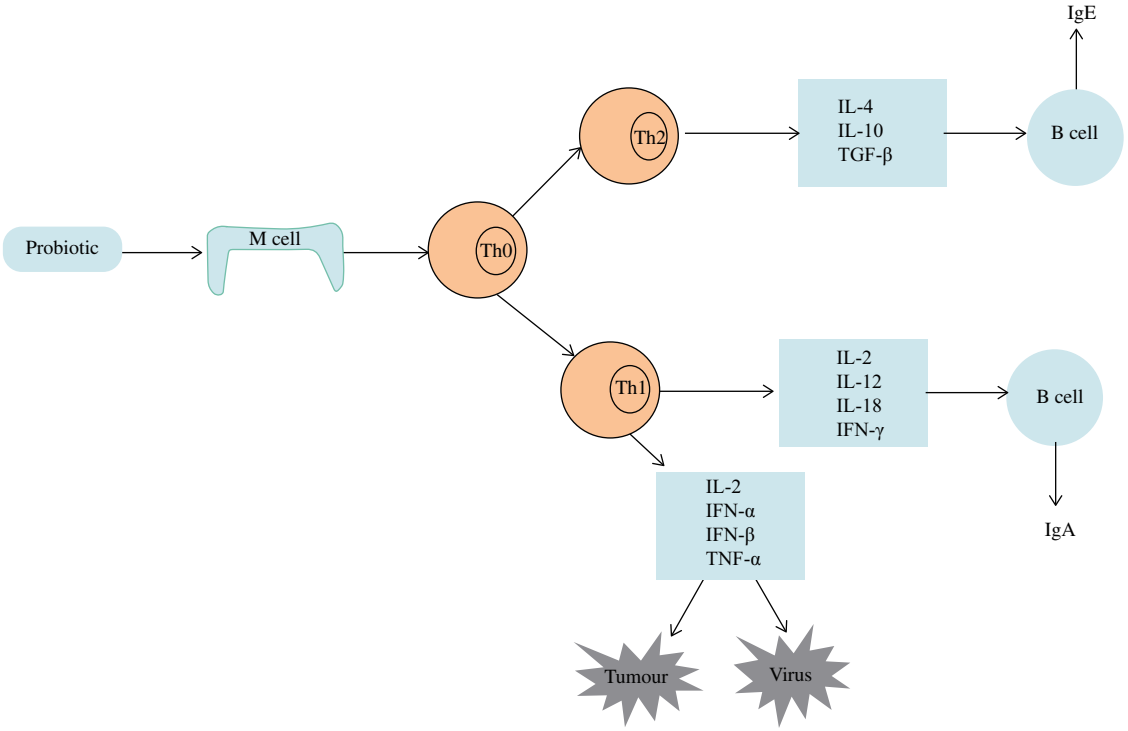


**Fig. 2** Regulatory effects of probiotics on mucosal immunity[46].

probiotics on innate immune cells in terms of their modulation of macrophage, dendritic cell, and NK cell activity and corresponding cytokine secretion.

### 2.2.1 Regulatory role for macrophages

Macrophages, originating from blood monocytes, are widespread innate immune cells and play a crucial role as antigen-presenting cells in the immune system. In recent years, there has a growing amount of research that probiotics can impact the differentiation and activity of macrophages in the body to exert immunological effects, as shown in Table 2.

Probiotics regulate macrophage polarization through various signaling pathways, such as janus kinase-signal transducer and activator of transcription (JAK-STAT), NF-κB, and TLRs[62]. Classical activated macrophages (M1) macrophages are capable of eliminating intracellular pathogens and mitigating pro-inflammatory responses, leading to Th1-type immune responses. In contrast, alternatively activated macrophages (M2) macrophages possess immunoregulatory and potent tissue repair capabilities[63]. Probiotics influence the expression of the signaling regulatory molecule MyD88 mRNA in the TLR2 signaling pathway of macrophages, activating macrophages *via* TLR2-MyD88. TLR-MyD88-induced cell signaling primarily involves downstream pathways MAPKs and NF-κB. Activation of MAPK and NF-κB signaling pathways is essential for M1 macrophage polarization; inhibition of these pathways can prevent M1 macrophage polarization and promote polarization towards M2 macrophages[64]. Probiotics enhance the phosphorylation of MAPK transcription factors in macrophages, which is involved in modulating the expression of inflammatory cytokines. Activation of p38 MAPK induces the production of M1-related inflammatory cytokines, thus inducing M1 macrophage polarization. *L. reuteri* activates the TLR2/MyD88/MAPK signaling pathway, inducing M1 macrophage polarization and the expression of M1-related cytokines[65]. Regarding the NF-κB signaling pathway, activated NF-κB in the form of p65/p50 promotes macrophage polarization towards M1. Probiotics

**Table 2**
Immunological actions exerted by probiotics on macrophages.

| Probiotic strain | Experimental model | Intervention methods | Mode of action | References |
|---|---|---|---|---|
| Heat-inactivated *L. plantarum* CKDB008 | RAW264.7 macrophage cells and LPS-induced RAW264.7 macrophage cells | RAW264.7 macrophage cells were incubated with different concentrations of heat-killed probiotics. | Enhances the phagocytic function of macrophages, induces the production of nitric oxide (NO) and pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β, promotes the clearance of foreign pathogens, and protects and strengthens immune function. | [54] |
| *Bifidobacterium* G9-1 | Maternally separated rats | MS rats were orally administered 50 μL of PBS containing $1 \times 10^8$ CFU BBG9-1 once daily | Significantly improves the amount of macrophages, promotes the conversion to M1-type macrophages, and also increases the expression of IL-6 and IFN-γ, preventing high permeability of the colonic mucosa and aiding in the treatment of irritable bowel syndrome. | [55] |
| *B. subtilis* and *Enterococcus faecalis* | Septicemic C57BL/6J mice induced by ligature puncture of the cecum | Mice were orally given with 200 μL/day probiotic capsule 1 week prior to perform CLP surgery. | Inhibits the activation of macrophages and their conversion from M type to M1 type, reduces levels of the pro-inflammatory cytokines IL-6 and TNF-α, thereby alleviating sepsis and reducing intestinal damage and inflammation. | [56] |
| *Lactobacillus johnsonii* | Collagenase-induced experimental OA mice | *L. johnsonii* was administered by intraperitoneal injection every day and LJ-MVs were administered tail intravenously every 2 days for a period of 42 days. | Hinders the migration of macrophages, prevents their polarization to M1 type, and reduces the inflammatory mediators they produce, while increasing the macrophage M2/M1 proportion, achieving relief from osteoarthritis inflammation and cartilage loss. | [57] |
| *Lactobacillus bulgaricus* DTW1 and *S. thermophilus* DWT4 | M2 macrophage | RAW 264.7 cells were treated for 1, 6 and 12 h separately with LDB-ST at a dose of 10 MOI. | Induces macrophage polarization from M2 type to M1 type and exerts anti-tumor effects by facilitating pro-inflammatory tumor-associated macrophages, playing a role in tumor suppression. | [58] |
| Heat-killed *Lactobacillus brevis* KCTC 12777BP | RAW264.7 murine macrophage cells | RAW264.7 macrophage cells were treated with LBB at 10, 20, and 40 μg/mL, and the media was collected after 6 h. | Exerts an immunostimulatory effect, directly increasing the phagocytic activity of macrophages against bacterial particles, and induces macrophages to produce TNF-α, IL-6, and NO, achieving the effect of defending against pathogens by enhancing host immunity. | [59] |
| *Bacillus amyloliquefaciens* SC06 | *Salmonella enterica* Typhimurium-infected C57BL/6 mice | The BaSC06 powder ($10^5$ CFU/g diet) was mixed uniformly with other feed components. | Induces polarization of M1 and M2 macrophages, can directly promote M1 polarization, thus enhancing phagocytosis and bactericidal activity against *Salmonella* Typhimurium. Probiotics also significantly alleviate inflammation caused by *Salmonella* Typhimurium by promoting M2 macrophage polarization. | [60] |
| *L. acidophilus* LA1 | Mouse colitis was induced using dextran sulfate sodium. | LA1 re-resuspended in sterile PBS to optimal concentration. | Promotes polarization towards M2-type macrophages, upregulates the expression of IL-10 and inhibits the production of pro-inflammatory cytokines, reduces symptoms of colitis, and exerts anti-colitis effects. | [61] |

regulate the NF-κB signaling pathway by controlling signal transducer and activator of transcription (STAT)3, influencing the transcription of the nuclear transcription factor peroxisome proliferator-activated receptor γ (PPARγ). PPARγ has a negative regulatory effect on immune and inflammatory responses, binding to NF-κB and causing its nuclear export, thereby inhibiting the NF-κB signaling pathway[66]. Therefore, probiotics regulate macrophage M1/M2 polarization trends by modulating PPARγ transcription. *L. casei* activates PPARγ, effectively reducing M1 polarization and pro-inflammatory cytokines and inhibiting NF-κB-mediated inflammation[67]. *L. plantarum* RS-09 induces M1 macrophage polarization through activation of the TLR2/NF-κB signaling pathway against S Typhimurium infection in mice[68]. *L. acidophilus* and its metabolite ursodeoxycholic acid may promote Treg polarization and inhibit M1 macrophage polarization by activating the Rap Gap/PI3K-AKT/NF-κB signaling pathway[69]. So *L. acidophilus* and its metabolite ursodeoxycholic acid can modulate both non-specific and specific immunity to alleviate ulcerative colitis. Thus probiotics can modulate macrophage polarization according to the organism, leading to immunomodulation.

Probiotics also regulate macrophage polarization by modulating cytokine secretion within the body *via* signaling pathways. Typically, IFN-γ and LPS induce polarization towards M1 macrophages, while polarization towards M2 macrophages is usually induced using IL-4 and IL-13. In the JAK-STAT signaling pathway of probiotics, phosphorylation of signal transducer proteins STAT3 and suppressor of cytokine signaling 3 (SOCS3) jointly induces upregulation of IL-10 receptor-mediated signaling, reducing the expression of pro-inflammatory cytokines. Consequently, IL-4 and IL-13 anti-inflammatory cytokines promote the activation of STAT6, upregulating the expression of SOCS1, inhibiting the action of STAT1, and leading to macrophage polarization towards M2. Conversely, IFN-γ pro-inflammatory factors can also activate STAT1 to promote the expression of SOCS3, leading to downregulation of STAT3, thereby inducing macrophage polarization towards M1[70]. *L. plantarum*-derived extracellular vesicles can induce the secretion of IL-10 as well as IL-1β and GM-CSF in human skin organ cultures, thereby contributing to the polarization of macrophages towards the M2 state[71]. *L. reuteri* and its metabolite-aminobutyric acid inhibit LPS-stimulated increase in IL-1β secretion, thereby suppressing macrophage polarization toward the M1 phenotype by inhibiting activation of NOD-like receptor thermal protein domain associated protein 3 inflammasome[72]. Probiotics have a broad and complex immunomodulatory role; they have a dual regulatory effect on macrophage polarisation, both promoting and inhibiting it.

Probiotics simultaneously enhance the body's immune capability by increasing the phagocytic capacity of macrophages. Upon pathogen invasion, macrophages can detect and phagocytose pathogens and mobilize nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase and mitochondria to release a large amount of reactive oxygen species (ROS) into the phagosome to kill and clear pathogens[73]. Probiotics induce an increase in NADPH oxidase subunit expression and enhance ROS production, thereby improving the bactericidal ability of macrophages by promoting the release of substances with phagocytic capability. Higher concentrations of *L. reuteri* induce an increase in the expression of NADPH oxidase subunits (p47 phox and gp91 phox) within 6 h, enhancing ROS production capability and potentially reducing harmful effects associated with excess nitrogen species by inhibiting NO production[74]. Probiotics induce macrophages to release the cytokines TNF-α and IL-6 through signaling pathways; both have a cytotoxic effect on cancer cells, causing tumor necrosis. Treatment of macrophages with *L. fermentum* NA-3 induces the production of IL-6, TNF-α, and ROS, thereby enhancing the phagocytic ability of macrophages and limiting pathogen survival[75]. Enhanced phagocytosis likewise modulates specific immunity, and enhanced phagocytosis will better present antigens to T and B cells and promote memory cell production.

### *2.2.2 Regulation of dendritic cells by probiotics*

Probiotics influence both *in vitro* and *in vivo* immunomodulation by producing tolerogenic dendritic cells (tolDCs). Dendritic cells (DCs) are the most effective professional antigen-presenting cells in the human body, serving as a crucial link between innate and adaptive immunity and playing a vital role in promoting immune defense and maintaining immune tolerance[76]. Probiotics can interact with pattern recognition receptors (PRRs) in ulcerative colitis (UC), which bind to pathogen-associated molecular patterns (PAMPs). The binding of PRRs activates various intracellular signaling pathways, inducing the production of tolDCs, or through the secretion of soluble compounds, inducing tolDC production[77]. TolDCs mainly exert immune tolerance by inhibiting effector T cell responses and inducing regulatory T cell activation. They are extensively employed in the cure of organ transplantation and autoimmune illnesses. *L. paracasei* can induce DCs to generate tolDCs, which can inhibit Th1-driven inflammation and the proliferative activity of $CD4^+$ T cells, thereby alleviating atherosclerosis[78]. In an experimental autoimmune encephalomyelitis model, Lactibiane Iki can induce DC to generate tolDC, but Vivomixx can only reduce the number of DCs expressing co-stimulatory molecules and cannot induce tolDC generation[79]. The specificity of strains determines that not all probiotics are capable of inducing tolDC production. TolDC has the function of regulating the balance of $CD4^+$ T cells, so probiotics can achieve the role of regulating specific immunity while inducing tolDC.

Probiotics activate various signaling pathways to induce DC maturation and modulate cytokine production. Probiotics enhance the expression of core transcription factors in the NF-κB, MAPK, and interferon regulatory factor (IRF3) signaling pathways, including Rela, AP1, and IRF3, thus activating NF-κB, MAPK, and IRF3 signal transduction. NF-κB, a critical transcription factor in DC maturation, and the MAPK signaling pathway also play roles in inducing DC maturation, regulating the expression of key surface molecules[80]. Probiotics can induce DC maturation by activating TLR2/4-mediated NF-κB and MAPK signals, which upregulates the expression of DC maturation markers such as CD80, CD86, and major histocompatibility complex class II (MHC-II) molecules[81]. In an experiment identifying the regulatory effect of *B. subtilis* on grass carp dendritic cells, expressions of CD83, CD80/86, and MHC-II molecules were detected in grass carp[82]. The study assessed the expression of these molecular markers in dendritic cells stimulated by *B. subtilis* contrasted with the control group, and a marked increase in CD83 and CD80/86 expressions was observed, thus regulating the maturation of DCs. It has been suggested that probiotics can modulate the immune response by controlling

DC maturation and thus regulating the release of pro-inflammatory cytokines, but specific properties may provide therapeutic options for the treatment of certain diseases. After treatment of the Caco-2 by *Lactobacillus delbrueckii* and *L. rhamnosus*, *L. delbrueckii* stimulated only the expression of CD83, whereas *L. rhamnosus* was more effective in inducing DC maturation[83]. Consequently, it is not the case that all probiotics are able to regulate DC maturation. The NF-κB and MAPK signaling pathways are also vital for cytokine production. Probiotics, by activating the NF-κB signaling pathway, induce the generation of IL-6, IL-8, and TNF-α and promote IL-10 production mediated by extracellular signal regulated kinase (ERK) and TGF-β production mediated by P38 MAPK, ERK, and c-Jun N-terminal kinase. *L. paracasei* CNCM I-4034 activates TLR signaling pathways, significantly increasing the release of pro-inflammatory cytokines and stimulating the innate immune system[84].

### 2.2.3 Regulation of NK cells

NK cells, innate lymphoid cells, mediate immune surveillance and clearance of virus-infected and tumor-transformed cells. Probiotics can modulate NK cells, thus exerting an immunoregulatory effect, as shown in Table 3.

Probiotics, by activating immune cells to produce cytokines, promote the proliferation of NK cells. In the absence of other activation signals, probiotics induce the production of pro-inflammatory and anti-inflammatory cytokines, chemokines, and growth factors. These cytokines stimulate signaling pathways that promote NK cell proliferation. IL-15 is crucial for the survival and proliferation of NK cells. The IL-15 receptor (IL-15R) comprises 3 subunits: the α, β, and γ c chains. IL-15Rα has a high affinity for IL-15, forming an IL-15Rα-IL-15 complex. This complex can then be trans-presented to NK cells expressing the β-γ c chains. The signaling of the β-γ c chains activates various pathways, including JAK1/3-dependent Stat5 phosphorylation, which triggers transcriptional responses and cell survival pathways, and another pathway involving PI3K-Akt-mTOR signaling *via* p70-S6 kinase activation of ribosomal protein S6, leading to cell proliferation and thereby promoting NK cell proliferation[91]. In NK cells, IL-2 activates the JAK-STAT, PI3K-AKT, and Ras-RAF/MAPK signaling pathways, which stimulates the phosphorylation of Stat5a/b, Tor, AKT (T308), and Hsp60 proteins in these pathways. This upregulates telomerase expression, enhancing the expansion time of NK cells and thus increasing their proliferation capability[92]. Immunodeficient mice orally administered 3 strains of probiotics (*L. plantarum* CJLP243, CJW55-10, and CJLP475) showed that all 3 strains promoted the secretion of IFN-γ, IL-1β, IL-6, IL-12, and TNF-α and increased NK cell proliferation[93].

Probiotics can also enhance NK cell activity by activating immune cells to produce cytokines. IL-15 transmits signals through the JAK-STAT pathway, particularly by activating JAK1/3, STAT5, and STAT3 through phosphorylation. Additionally, IL-15 has been shown to utilize the downstream PI3K and MAPK pathways in conjunction

**Table 3**
Immunomodulatory effects of probiotics on NK cells.

| Probiotic strain | Experimental model | Intervention methods | Mode of action | References |
|---|---|---|---|---|
| *W. cibaria* JW15 | 100 nondiabetic subjects | Over an 8-week test period, the probiotic group consumed 4 capsules that contained 2.5 × $10^9$ CFU of *W. cibaria* JW15 per capsule twice daily (2 capsules in the morning, and 2 capsules in the evening). | *W. cibaria* JW15 improves immune ability by enhancing NK cell activity. | [85] |
| *L. rhamnosus* HDB1258 | Mice with LPS-induced systemic inflammation | HDB1258 (LL, 1 × $10^8$ CFU/mouse·day; LH, 1 × $10^9$ CFU/mouse·day)was orally given once a day for 14 days from next day after the final treatment with LPS. | Activates the host's innate immunity to strengthen the immune response, increases NK cell cytotoxicity, and modulates the expression of IL-10 and TNF-α in the gut microbiota and immune cells to suppress inflammation, thus maintaining a balanced immune response. | [86] |
| Probiotic AJ2 | Cancer patients | Cancer patients were given oral supplementation of 125 billion CFU/capsule, and in total, 3 capsules/day for 4 weeks. | It increases NK cell secretion of functional IFN-γ, improving NK cell function in Malignant tumour patients and enhancing their ability to differentiate tumors, potentially inhibiting tumor cell growth. | [87] |
| *Lactobacillus* | 20 patients with IBS | Take capsules composed of *Lactobacillus* spp., larch arabinogalactan, and colostrum for 21 consecutive days (twice a day, 2 capsules each time). | Increases the amount of activated NK cells and lowers the number of IL-6 and IFN-γ, affecting the local and systemic immune response of patients with conditions like irritable bowel syndrome and maintaining intestinal immune homeostasis. | [88] |
| *L. rhamnosus* LM1019 | Mmunosuppressed mice induced by cyclophosphamide | Mmunosuppressed mice were fed 2 doses of LM1019 ($10^8$ or $10^9$ CFU/head) from day 3 to day 7. | Induces an increase in pro-inflammatory cytokine levels, including IFN-γ and IL-12, promoting NK cell activation. Possesses potential immunostimulatory properties, enhancing immune function. | [89] |
| *L. paracasei* | 200 nondiabetic subjects over 60 years in age with white blood cell levels between 4 × $10^3$/μL and 10 × $10^3$/μL. | The test group consumed 1 bottle (120 mL) of dairy yogurt containing *L. paracasei* (*L. casei* 431) at 12.0 × $10^8$ CFU/day | Significantly increases NK cell activity, IL-12, and IgG1 levels. Enhances NK cell function and IFN-γ concentration to improve immune function, particularly in populations with impaired immune function. | [90] |

with the IL-15R. Through these mechanisms, IL-15 can regulate the gene expression of anti-apoptotic and pro-inflammatory mediators, thereby enhancing the activation and survival of NK cells. interferon-α (IFN-α) further upregulates the expression of the immune signaling proteins CD100 and IFN-γ in NK cells and increases the expression of CD100 and its receptors, plexin-B1/B2. NK cells recognize pathogen-infected cells through the CD100-plexin-B1/B2 interaction, enhancing NK cytotoxicity[94]. IFN-α also increases the expression of IL-15R and IL-15-mediated signal transduction, indirectly augmenting cell activity by enhancing IL-15's signaling and functional effects. NK cells were originally named for mediating spontaneous cytotoxicity in non-specific immunity, but recent studies have demonstrated that they can also modulate adaptive immune responses by producing cytokines. NK cells can improve DC maturation, effector function, and cross-presentation, thereby promoting T cell responses. Furthermore, NK cells can secrete cytokines during T cell initiation and promote the differentiation of naive $CD4^+$ T to Th1 cells[95].

IL-12 and IL-2 also enhance NK cell activity by activating signaling pathways. The receptor dimer formed by IL-12 binding to its receptor activates when adjacent to JAKs. Activated JAKs phosphorylate tyrosine residues on the cytokine receptor itself, and the activated receptor complex undergoes phosphorylation with SH2 domains, forming homodimers and heterodimers and entering the nucleus to initiate gene transcription and expression, thereby promoting the production of cytotoxic substances and enhancing NK cell cytotoxicity[96]. IL-2 enhances NK cell responsiveness to IL-12 by increasing the IL-12 receptor and IL-12 signaling pathway-related factors, indirectly activating NK cells[97]. Furthermore, IL-2 induces the proliferation of NK and T cells through the JAK1, JAK3, STAT1, STAT3, and STAT5 signaling pathways, thereby enhancing NK cell cytotoxicity. In mice infected with influenza, consumption of *Bifidobacterium longum* MM-2 significantly increased the gene expression of NK cell activators (such as IFN-γ, IL-2, IL-12, and IL-18) in the lungs, as well as significantly enhancing NK cell activity in the lungs and spleen. Even in uninfected mice, MM-2 significantly increased IFN-γ production and spleen NK cell activity[98]. After consuming yogurt containing *L. paracasei*, *B. lactis*, and *L. plantarum*, serum levels of IL-12 and IFN-γ were elevated in nondiabetic patients, which activated NK cell-mediated cytotoxicity[90]. Nevertheless, supplementation with *L. casei* Shirota in individuals with low NK cell activity does not result in an increase in NK cell activity and modulates other immune functions[99]. Consequently, the regulatory effects of distinct probiotics on NK cell activity are also disparate.

## 3. Probiotics and specific immunity

The characteristics of adaptive immunity include specificity, immune memory, and self- and non-self recognition[100]. The adaptive immune system primarily consists of 2 mechanisms: humoral immunity and cellular immunity, mediated by antibodies and cells, respectively. The essential elements of adaptive immunity are T and B cells. B cells are closely related to humoral immune responses, whereas T cells are essential for cell-mediated immune responses. T cells and B cells have been shown to interact with probiotics, potentially altering their functions and responses. The following sections discuss the impact of probiotics on specific immunity, focusing on B cell immunoglobulin secretion and maturation and T cell differentiation.

### *3.1 Humoral immunity*

Probiotics regulate humoral immunity by modulating the levels of immunoglobulins in the body. Numerous studies have found that probiotics have a regulatory effect on humoral immunity, as illustrated in Fig. 3[101]. IgG, IgM, and IgA are the most common serum immunoglobulins, and their levels are often measured to assess humoral immunity. Probiotics activate transcription factors through TLR2 and TLR4 signaling, triggering IL-6 production, which is crucial for B-cell immunoglobulin production. IL-6 is a cytokine with immunoglobulin-switching properties, promoting the final differentiation of Ig-secreting B cells and thereby increasing the secretion of IgM, IgG, and IgA. Thus, probiotics enhance immunoglobulin secretion through the TLR signaling pathway. Four different *Lactobacillus* strains found in Korean fermented foods were found to increase the ratio of IgA-secreting B cells *via* the TLR/IL-6 signaling pathway, promoting IgA secretion[102]. Sakai et al.[103] discovered that *Lactobacillus gasseri* SBT2055 significantly increased the amount of IgA in co-cultures of B cells and bone marrow-derived dendritic cells by activating the TLR2 signal. *L. gasseri* SBT2055 stimulated DCs to produce the cytokines TGF-β, B-cell activating factor (BAFF), IL-6, and IL-10. TGF-β and BAFF induce IgA class-switch recombination, while IL-6 and IL-10 induce the differentiation of IgA-producing plasma cells in B cells. Allergic diseases are characterized by elevated serum IgE levels. Probiotics can induce the production of IFN-γ, IL-10, and IL-12 while reducing the production of IL-4 by whey protein, thereby suppressing serum IgE. IL-4 is an essential cytokine for increasing IgE synthesis in the body. IL-4 stimulates lymphocytes to induce the switch of precursor B cells to IgE, while IL-12 can inhibit IL-induced IgE synthesis through an IFN-γ-dependent mechanism. IFN-γ is a known inhibitor of IgE synthesis. High concentrations of IL-4 can completely inhibit the production of IFN-γ and induce IgE synthesis in mixed lymphocytes, while IL-12 can induce a substantial quantity of IFN-γ production, even in the presence of high concentrations of IL-4[104].

Probiotics enhance B cell development by upregulating transcription factor forkhead box O1 (FOXO1) and key target gene expression. FOXO1 plays a critical role in the early precursor development and terminal differentiation of B cells. The deficiency of FOXO1 causes impaired B cell development due to decreased expression of key target genes like *EBF1*, IL-7 receptor, recombination activating gene (*RAG*)*1*, *RAG2*, adenosine deaminase, and *L*-selectin. *L. rhamnosus* upregulates *FOXO1* and *RAG2* gene expression, downregulates the cell cycle and promotes early B cell development. Its p40 protein activates the EGF/AKT/NF-κB signaling pathway, inducing intestinal epithelial cells to secrete functional a proliferation-inducing ligand, which promotes B-cell IgA production[105]. Probiotics also function by increasing B cell numbers, enhancing the number of memory B cells in response to certain vaccines. Enani et al.[106] found that, contrasted with the control group, mice treated with *B. longum* CCUG 52486 for 4 weeks before influenza vaccination had increased IgG memory B cells and total IgG B cells numbers.

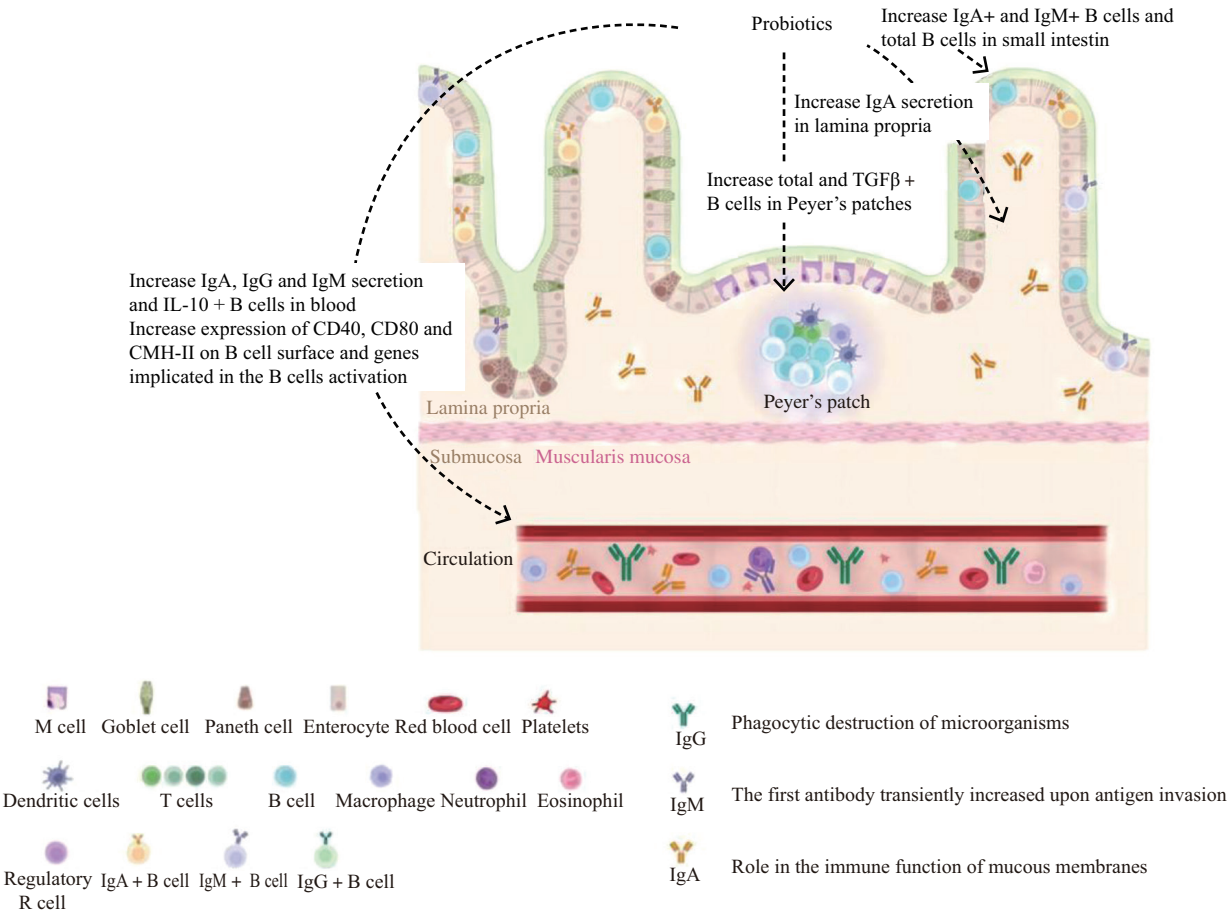


**Fig. 3** Regulation of humoral immunity by probiotics[101].

### 3.2 *Cellular immunity*

#### 3.2.1 *Regulation of treg numbers*

The beneficial effects of probiotics on certain diseases, such as allergies or colitis, are attributed to their ability to increase the number of Tregs. Tregs play a role in maintaining immune homeostasis, for instance, by regulating self-tolerance, tumor immunity, allergies, and transplant rejection reactions. Irregularities in Treg numbers can lead to the loss of tolerance and autoimmune diseases[107]. One of the pathways by which probiotics maintain immune homeostasis is by producing SCFAs like acetate, propionate, and butyrate. Probiotic-produced SCFAs induce T cell differentiation into Treg cells by inhibiting histone deacetylases and enhancing acetylation at the forkhead box P3 (FoxP3) site and protein. SCFAs also upregulate the expression of inducible cyclooxygenase-2 (*COX-2*) mRNA, increasing the production of prostaglandin (PG)E2 in Kupffer cells. PGE2 not only participates in Treg expansion but also mediates their suppressive activity. Thus, probiotics regulate PGE2's regulatory factor COX-2, indirectly modulating Tregs and various inflammatory and autoimmune diseases. Butyrate activates the GPR109A signal, giving colonic antigen-presenting cells anti-inflammatory properties, thereby inducing Treg and IL-10-producing T cell differentiation[108]. Mice supplemented with *L. rhamnosus* show increased levels of butyrate in the intestine and serum, raising the amount of Tregs in the intestine and bone marrow[109]. In an experiment treating rat colitis with *B. longum*, *B. longum* also increased the proportion of Tregs in rat spleens, thereby raising the levels of IL-10 in the body and the IL-10/IL-12 ratio while reducing the content of IL-12, IL-17, and IL-23 in the serum, thus alle*via*ting colitis symptoms[110].

#### 3.2.2 *Regulating the Th1/Th2 balanc*

Probiotics exert immunoregulatory effects by affecting T-cell differentiation. Th1 cells activate macrophages and neutrophils, promoting inflammatory responses by secreting IL-12, IFN-γ, and TNF-α. Th2 cells secrete IL-4, IL-5, and IL-13, activating mast cells and eosinophils involved in allergic responses. Probiotics influence the Th1/Th2 balance, participating in the modulation of the immune system[111]. Probiotics promote cytokine secretion, which acts on intracellular signaling pathways, thereby inducing T cell differentiation. IL-12 drives Th1 cell differentiation by activating STAT4. IL-12 binds to the IL-12R on T cells, inducing tyrosine phosphorylation of Jak2 and tyrosine kinase 2 (Tyk2), thereby phosphorylating IL-12R and providing the transcription factor STAT4 a docking site. Phosphorylated STAT4 dimerizes, activates the IL-12/STAT4 pathway, inducing Th1 differentiation, and the STAT4 homodimers translocate to the nucleus, promoting gene transcription of Th1 cytokine IFN-γ[112]. IL-4 binding to its receptor leads to phosphorylation and dimerization of STAT6, activating the transcription of the main Th2 regulatory factor, GATA3. GATA3, the primary transcription factor for Th2 cell differentiation, binds to

different regions of the *IL-4* gene site, influencing IL-4 expression through epigenetic modifications of Th2 cytokine gene sites. Elevated IL-4 production enhances GATA3 expression, establishing a positive feedback loop and further inducing Th2 cell polarization[113]. *L. paracasei* BBM171 regulates the balance between Th1 and Th2 cells by releasing IL-12, which reduces pro-inflammatory cytokine IL-17 levels, and increases anti-inflammatory cytokine IL-10 levels to improve allergic reactions[114]. In CP-induced immune-impaired mice, *L. casei* NCU011054 can regulate T-bet/GATA-3 and IFN-γ/IL-4 *via* the TLRs/NF-κB signaling pathway, thus regulating the Th1/Th2 balance and maintaining the body's immune function[115]. The application of MV of *E. coli* Nissle 1917 to $CD4^+$ T cells and DCs resulted in the maturation of DCs, which in turn stimulated the secretion of INF-γ and IL-12[116]. These cytokines were found to be effective in the differentiation of T cells into Th1 cells. Consequently, certain probiotics are capable of regulating the Th1/Th2 balance by influencing non-specific immunity. However, it was also found that after *L. acidophilus* and *Bacillus clausii* were applied to Th1- and Th2-biased mice, *L. acidophilus* was able to effectively regulate the Th1/Th2 balance, while *B. clausii* could only ameliorate the Th2 dysregulation, but not the Th1-biased mice[117]. Consequently, probiotics are specific to the target host, and not all probiotics are capable of regulating the Th1/Th2 balance.

### 3.2.3 *Regulating the Th17/Treg balance*

Probiotics can also influence the Th17/Treg balance in the host's immune system by producing relevant cytokines, as shown in Fig. 4[118]. IL-6 activates STAT3's tyrosine/serine phosphorylation through the cytokine receptor pathway, and STAT3's phosphorylation promotes the synthesis of retinoic acid-related orphan receptor γt (RORγt), the main transcription factor for IL-17 cytokine expression and Th17 lymphocyte differentiation, driving T cells toward Th17 subgroup differentiation[119]. The combined action of IL-2 and TGF-β promotes tyrosine phosphorylation of STAT5, which in turn promotes the synthesis of transcription factor Foxp3. Increased expression of Foxp3 drives T cells toward Treg subgroup differentiation[120]. When probiotics induce Th0 differentiation into Tregs, the clinical effect is negative regulation of the host immune system. On the other hand, when probiotics induce Th0 differentiation into Th17, the clinical impact of probiotics is positive regulation of the host immune system[111]. Probiotics exert biological effects by regulating cytokine secretion (IL-2, IL-6, and TGF-β), which effectively promotes Treg differentiation and inhibiting Th17 differentiation in DSS-colitis mice, and regulates the Treg/Th17 ratio, thus benefiting the development and maturation of the immune system[121]. The administration of *L. rhamnosus* 2016SWU.05.0601 to OVA-sensitised mice resulted in the upregulation of T-bet and Foxp3 and downregulation of GATA3 and RORγT[122]. This led to the transfer of Th2 and Th17 phenotypes to Th1 and Treg, respectively. In a mouse model of PM2.5-induced lung inflammation, *L. rhamnosus* was observed to restore the expression of ROR-γt and Foxp3 to normal levels, thereby regulating the Th17/Treg balance[123]. In a mouse model of allergy, *L. acidophilus* has been observed to upregulate the expression levels of Foxp3 and TGF-β, thereby increasing the number of Treg[124]. The consumption of *L. casei* BL23 in untreated mice has been observed to increase the expression of cytokines IL-6 and IL-17 and to shift Treg to Th17, thereby conferring protection against colorectal cancer[125]. Although strains are specific to a particular strain, the conditions under which they act also affect the immunomodulatory effects of probiotics.

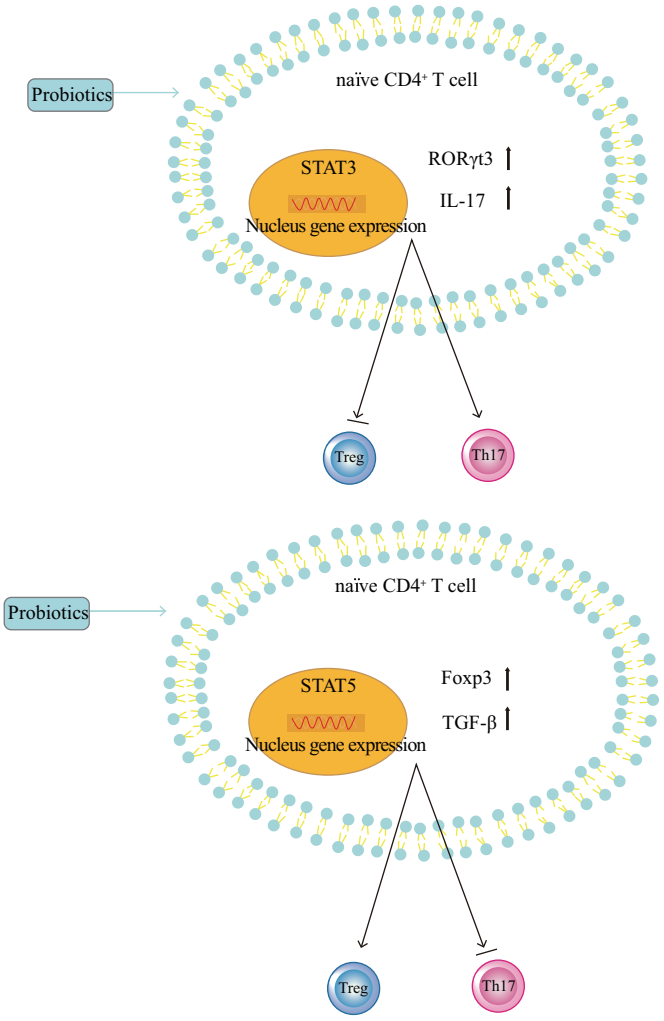


**Fig. 4** Probiotic regulation of the Th17/Treg balance[118].

## 4. Conclusion and future perspectives

Probiotics play an immunoregulatory role by modulating both nonspecific and specific immunity. Their functions include promoting the production of cytokines by epithelial cells, which increases mucin secretion, enhancing phagocytic and NK cell activity, activating T cells, and stimulates the production of IgA. Increasingly, probiotics are being used as biotherapeutic agents and dietary supplements for human consumption. They are available in various forms, such as capsules, sachets, tablets, granules, chewable tablets, liquids, or freeze-dried suspensions. They can colonize the gastrointestinal tract, improve the microecological balance of the body, and induce immune responses. All these characteristics may positively impact the host's immunity. The therapeutic scope of probiotics has expanded considerably in recent years, and their immunomodulatory effects have been demonstrated to be efficacious in addressing a range of health issues, not just limited to maintaining gut health. Probiotics have been demonstrated to be particularly effective in addressing intestinal issues, but they also have great potential for addressing

a range of other conditions, including respiratory series diseases, allergic reactions, skin disorders, and more[126]. Probiotics are widely utilized in the pharmaceutical and food industries, with dairy products remaining the predominant form of probiotic consumption[127]. The addition of lactic acid bacteria to dairy products not only extends the shelf life of fermented products but also acts as an antimicrobial agent against numerous pathogens within the human body, thereby improving overall health[128]. Furthermore, research into non-dairy probiotic foods has been ongoing. Some probiotic fermented foods have been demonstrated to not only increase the levels of vitamins and minerals (potassium, calcium and sodium), but also to improve anti-hypertensive, antioxidant and antimicrobial properties[128].

Extensive preclinical research on the application of probiotics has been conducted, and studies aimed at characterizing and isolating new potential probiotic candidates are ongoing. Clinical trials are also continuously underway. Uncovering various probiotic resources and identifying specific probiotics for different diseases is key to their safe and effective use. The development and utilization of probiotic resources are currently hot topics in the health industry, but there are still shortcomings. Despite the numerous benefits of conventional probiotics for the host, they are still subject to limitations such as strain-specific dysfunction and variable efficacy under different host conditions[129]. These limitations emphasize the need to develop new strategies for engineering probiotics. Nevertheless, there are still many challenges in engineering probiotics, such as the safety and genetic stability of probiotics in the host. To improve the performance and efficiency of engineered probiotics for better immunomodulation by incorporating probiotic studies on the mechanism and function of organismal immunomodulation. Furthermore, a novel trend is the utilization of next-generation probiotics. Next-generation probiotics are defined as microorganisms with probiotic properties that are not included in the commonly used probiotic spectrum[130]. Moreover, the suitability and safety of these probiotics for host populations may be more stringent than that of conventional probiotics, and the mechanisms by which they are associated with disease and health conditions have not been fully elucidated. The study and summary of the immunomodulatory mechanisms of traditional probiotics provide a foundation for future in-depth research into the mechanisms of action of the next generation of probiotics.

While their application holds broad prospects, the mechanisms of action and signaling pathways of probiotics are not yet fully elucidated. Therefore, future investigations should concentrate on mechanistic studies to further understand their bioactivity both *in vitro* and *in vivo*. To confirm the efficacy of probiotics in treating specific diseases, new clinical trials should be undertaken. These trials should focus on determining the appropriate dosages, the duration of treatment, and identifying potential side effects. Additionally, the trials aim to discover new probiotic strains that are clinically effective and reliable.

## Conflict of interest

The authors declare that they have no conflict of interest.

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