

Immune Modulation by Probiotics

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Abstract Probiotics are defined as live microorganisms, which when administered in adequate amounts confer health benefits on the host. Lactic acid bacteria and *Bifidobacterium* strains are the most common groups of bacteria with claimed probiotic properties. These bacterial strains have been conventionally incorporated into food and beverage products as dietary adjuncts, aimed at promoting gastrointestinal health. Meanwhile, a growing number of studies have also revealed that probiotic strains could exert beneficial health effects beyond the gut, mainly attributed to their peculiar immunomodulatory properties. Probiotic strains are capable of modulating the innate and adaptive immune response through both immunostimulation and immunoregulation and can thereby exert prophylactic and therapeutic effects on the host. Indeed, experimental evidences have demonstrated that administration of live probiotics and/or probiotic-derived products can be potentially applied in the prevention and/or treatment of a wide range of non-gastrointestinal diseases, such as metabolic disorders, allergic and inflammatory skin disorders, respiratory diseases, osteoporosis, male hypogonadism, and rheumatoid arthritis. However, more clinical trials on the efficacy of different probiotic strains in the prevention and treatment of these health conditions are needed to generate more definitive results. The exact mechanisms by which specific probiotic strains can stimulate and/or regulate immune functions remain to be elucidated. Nonetheless, recent advances in biotechnology have provided rapid ways to explore possible immunomodulatory mechanisms of probiotics. Altogether, this chapter provides a succinct summary of the updated evidence on the immunomodulatory effects of probiotics and discusses their possible mechanisms of action. This chapter also presents the future directions to promote a better understanding of the underlying immunomodulatory actions of probiotics.

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1 Introduction

In 1953, Werner Kollath introduced the term “probiotika” for “active substances that are essential for a healthy development of life” (Guarner et al. 2005). This term evolved to “probiotic,” which is derived from the Greek word “biotikos,” meaning “for life.” The term probiotic was first used by Lilly and Stillwell (1965) to describe “substances produced by one microorganism that stimulate the growth of another.” However, Parker (1974) applied the word probiotic to describe animal feed supplements that improved animal health and eventually introduced a new definition as “organisms and substances that contribute to intestinal microbial balance.” Since then, probiotics have received an increasing interest for the promotion of human and animal gastrointestinal health. Thereafter, Fuller (1989) revised Park’s definition by focusing on microorganisms and redefined probiotic as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.” The definition of probiotics has been re-modified throughout the years until 2001, when the joint Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended the adoption of the definition of probiotics as “live microorganisms, which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO 2001). This definition has been continuously retained and reinforced by an expert panel from the International Scientific Association for Probiotics and Prebiotics recently, as it is still relevant and capable of accommodating current and anticipated applications (Hill et al. 2014).

In order for a microorganism to be classified as a probiotic, it must fulfill the following criteria: (1) must be able to survive in the intestinal transit, (2) must be able to adhere to mucosal surfaces and colonize the intestine, (3) must be resistant to technological processes, (4) must be safe for consumption, (5) must possess documented beneficial health effects, and (6) must be taxonomically identified at the genus, species, and strain level (Borchers et al. 2009). The most common microorganisms with claimed probiotic properties are bacteria, *Bifidobacterium*, and lactic acid bacteria (LAB) strains, including the genera of *Lactobacillus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, and *Pediococcus*. Additionally, *Saccharomyces*, a yeast genus, has also been identified as a microorganism associated with probiotic properties. Meanwhile, it is extremely important to note that the health-promoting effects of probiotics are strain-specific.

Lactobacillus and *Bifidobacterium* strains are among the primary candidates of probiotic bacteria inhabiting the gastrointestinal tract and are widely used as dietary adjuncts owing to their long history of safe use. There is unequivocal evidence that administration of specific strains of probiotic bacteria can help in the maintenance of a balanced intestinal microbiota and therefore potentially promote overall host health (Butel 2014). Dairy products have been considered as a desirable delivery vehicle for delivering probiotic bacteria to the human gastrointestinal tract (Xiao et al. 2014). Hence, probiotic strains are most often incorporated in dairy-based food and beverages globally, including fermented milk, yogurt, ice cream, frozen yogurt, cheeses, pasteurized milk, and kefir. Nevertheless, non-dairy probiotic products are also increasingly being developed due to the increase in vegetarianism

as a dietary habit, lactose intolerance, and the cholesterol content of dairy products (Céspedes et al. 2013).

Among the many purported health benefits attributed to the administration of probiotics, the ability of probiotics to modulate the gut immune system has attracted more attention from the probiotic community. The intestinal microbiome plays an important role in the maintenance of the host immune homeostasis. It has been reported that probiotics may induce the production of intestinal defensin and immunoglobulin that inhibit the growth of pathogens, thereby maintaining a balanced intestinal microbiome (Thomas and Versalovic 2010). Probiotics have also been demonstrated to stimulate the production of cytokines, which can promote the activation of epithelial cells and both innate and adaptive immune cells, thus leading to the maintenance of a healthier intestinal microbiome and immune homeostasis (Hemarajata and Versalovic 2013). On the other hand, Furness et al. (1999) have mentioned that 70–80 % of the host's immune cells are located in the gut. Therefore, in addition to the local gut immunomodulatory effects, probiotics may also extend its immunomodulatory activity to the systemic levels. Increasing interest has been focused on proving the previously proposed hypothesis that probiotics may stimulate and/or regulate the immune system beyond the gut, such as on the gut-liver axis and the gut-brain-skin axis (Arck et al. 2010; Smith et al. 2014). Indeed, compelling evidence has demonstrated that live probiotics and/or probiotic-derived bioactives extend its beneficial roles by modulating the immune system beyond the gut (Bowe and Logan 2011; Saulnier et al. 2013). This chapter documents up-to-date in vitro and in vivo evidences of immunomodulatory effects of probiotics in health and non-gastrointestinal diseases and discusses their possible molecular and cellular mechanisms of action.

2 Probiotics and Immune Modulation

Innate and adaptive arms of the immune system work together to maintain the host immune homeostasis and to protect against external insults and undesirable inflammation (Yang and Polk 2011). However, imbalance of innate and adaptive immune responses can result in cell and tissue damage that further contribute to diseases. Experimental studies have proposed that probiotics can modulate humoral components and hematopoietic cells (natural killer cells, mast cells, neutrophils, macrophages, T cells, B cells, and dendritic cells) in both the innate (aspecific) and adaptive (specific) immune systems (Fig. 1), thereby promoting host defense.

Dong et al. (2012) have evaluated the immunomodulatory potential of live *Lactobacillus* and *Bifidobacterium* strains in human peripheral blood mononuclear cells in vitro. The authors found that *L. casei* Shirota, *L. rhamnosus* GG, *L. plantarum* NCIMB 8826, *L. reuteri* NCIMB 11951, *B. longum* SP 07/3, and *B. bifidum* MF 20/5 not only induced the expressions of CD69⁺ on T lymphocytes, T helper (Th) cells, cytotoxic T (Tc) cells, and natural killer cells but also CD25⁺ on T lymphocytes and NK cells, in a strain-dependent manner. These strains also

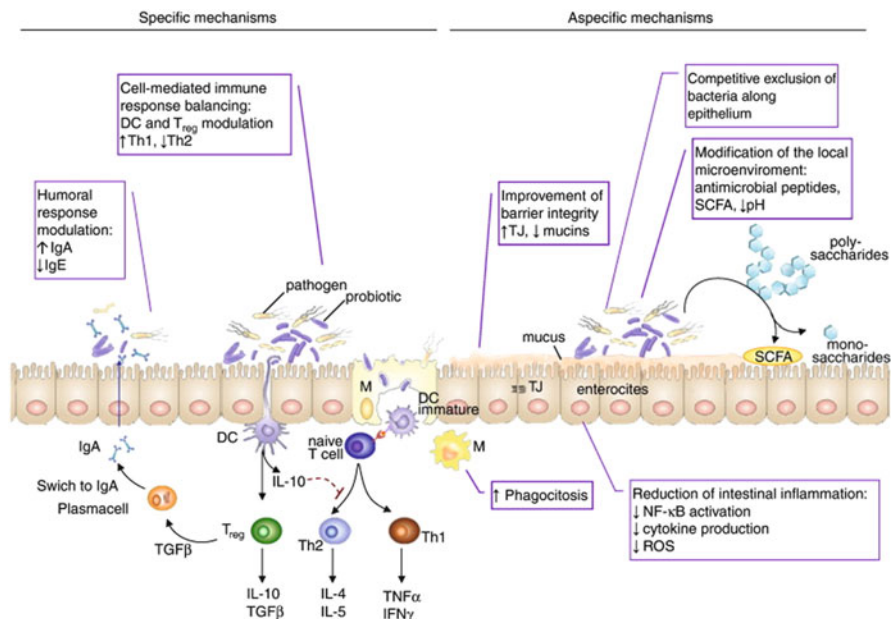


Fig. 1 Immunomodulatory actions of probiotics. Specific mechanisms: involvement of probiotics in cell-mediated and humoral immune responses. Aspecific mechanisms: enhancement of epithelial barrier function, competitive exclusion of bacteria along epithelium, modification of local microenvironment, and reduction of intestinal inflammation. Reprinted from Iacano et al. (2011) with permission from Elsevier (License number: 3537870510625)

increased the production of cytokines (IL-1 β , IL-6, IL-10, TNF- α), granulocyte-macrophage colony-stimulating factor, and macrophage inflammatory protein-1 α (MIP-1 α). It has also been reported that 16-month-old male Swiss mice orally administered with milk fermented with *L. fermentum* MTCC 5898 (3×10^9 CFU/day) for a period of 2 months showed significantly higher neutrophil phagocytosis and lower humoral antibodies (IgG₁/IgG_{2a} ratio and IgE levels) compared to the control, thereby demonstrating the potential of probiotics to improve immune functions in aging subjects (Sharma et al. 2014).

Macrophages serve as one of the immune sentinel cells with both protective and pathogenic roles in host defense (Galli et al. 2011). They express pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domains (NODs) that recognize pathogen-associated molecular patterns (PAMPs) expressed by pathogens, which subsequently lead to the activation of a range of immune responses, including phagocytosis and cytokine production. It has been reported that peptidoglycans of *L. johnsonii* JCM 2012 and *L. plantarum* ATCC 14917 inhibited IL-12 production by macrophages that was induced by *L. casei* through PRRs such as TLR2 and NOD2, as well as suppressed IL-12p40 mRNA (Shida et al. 2009). The overproduction of IL-12, which subsequently results in the overstimulation of Th₁ cells, is considered as one of the main factors that contribute to autoimmune and inflammatory diseases. In addition, IL-12p40 is a subunit of IL-23

that regulates the proliferation and maintenance of Th₁₇ cells, which plays a major role in the development of inflammatory diseases. Hence, findings in this study suggest that the ability of peptidoglycans to downregulate the production of IL-12 and IL-23 may be useful for the treatment of inflammatory diseases. On the other hand, cell wall extracts of *B. adolescentis* BBMN23, *B. longum* BBMN68, and *L. salivarius* Ren were shown to increase phagocytic activity and production of nitric oxide, IL-6, and TNF- α , thereby enhancing the overall activities of macrophage RAW264.7 cells (Zhu et al. 2011). Oral administration of dahi containing *L. acidophilus* LaVK2 ($2\text{--}20 \times 10^7$ CFU) and *B. bifidum* BbVK3 ($2\text{--}20 \times 10^7$ CFU/g) for 4 months was also found to improve production of reactive oxygen intermediates (ROI), phagocytic activity, and adherence indices of peritoneal macrophages in aged mice, thus potentially improving immune functions in aging individuals (Kaushal and Kansal 2014).

Probiotics also modulate the function of mast cells, which specialize in stimulating or suppressing homeostatic or pathophysiological responses. Mast cells are well known for their role in allergic inflammation due to the ability of allergen-specific IgE to activate high-affinity IgE receptor (Fc ϵ R1) on the cell surface, which leads to the release of mediators and cytokines (Forsythe et al. 2012). Using global microarray analysis (Affymetrix GeneChip[®] Human Genome U133 Plus 2.0 Array), Oksaharju et al. (2011) have found that *L. rhamnosus* LGG and Lc705 downregulated genes encoding mast cell activation and mediator release [high-affinity IgE receptor subunits α (*Fc ϵ R1a*) and γ (*Fc ϵ R1g*) and histamine H4 receptor (*Hrh4*)] and immune responses (IL-8, TNF- α , CCL2, and IL-10) and thus could be applied in the cases of allergy mediated by mast cell activation. Oral administration of *L. rhamnosus* JB-1 (1×10^9 CFU/daily) for 9 days in healthy male Sprague-Dawley rats have also revealed that this probiotic strain systemically inhibited mast cell membrane potassium current and degranulation (enhanced mast cell stabilization) by inhibiting β -hexosaminidase (mast cell mediator release) in response to the IgE-mediated activation, thus reducing passive cutaneous anaphylaxis and peritoneal mast cell responses to the KCa3.1 opener, a potent therapeutic target in a wide range of autoimmune diseases (Forsythe et al. 2012).

Dendritic cells are critical antigen-presenting cells for naïve T cells and generation of the T cell response. In vitro studies have demonstrated that *L. rhamnosus* Lcr35 can modulate the immunological functions of human dendritic cells. High dose of *L. rhamnosus* Lcr35 (MOI, 100) has been shown to upregulate nearly 1700 genes mainly involved in the immune response, with threefold changes (Evrard et al. 2011). Flow cytometry and ELISA analyses have also further revealed that *L. rhamnosus* Lcr35 upregulated the maturation of dendritic cell membrane phenotypes (CD86, CD83, HLA-DR, and TLR4) and increased the pro-Th₁/Th₁₇ cytokine levels in a dose-dependent manner. Despite pro-Th₁/Th₁₇ cytokines mediating inflammation and autoimmune diseases, in vivo studies have proposed that upregulation of Th₁/Th₁₇ responses could alleviate certain inflammation or allergic diseases (Lin et al. 2009). IgE-dependent allergies are diseases characterized by imbalance of Th₁/Th₂ responses. Yu et al. (2010) have demonstrated that *L. rhamnosus* Lcr35 is capable of regulating the impaired Th₁/Th₂ cytokine profile

by modulating the Th₁/Th₂ responses toward the Th₁ (/Th₁₇) balance in dendritic cells. These findings suggest that *L. rhamnosus* Lcr35-treated dendritic cells have a higher potential of antigen presentation and co-stimulation and therefore may induce immune responses as required during inflammation and/or allergies. Till date, this is the first report of probiotics found to have a pro-Th₁/Th₁₇ effect on dendritic cell maturation that merits further investigation. On the other hand, You et al. (2014) have conducted a study to evaluate the effect of *B. longum* bv. *infantis* CCUG 52486, *B. longum* SP 07/3, *L. rhamnosus* GG, and *L. casei* Shirota on dendritic cell function in an allogeneic mixed leukocyte reaction model, using peripheral blood obtained from healthy young (20–30 years) and old (55–75 years) subjects. The authors found that these strains increased the expression of CD40, CD80, and CCR7 in both young and old dendritic cells and only enhanced cytokine production (TNF- α and TGF- β) in old dendritic cells. Additionally, probiotic treatment was also found to stimulate the activation of young T cells activation by old dendritic cells, suggesting that probiotics may be able to modulate dendritic cell function in aging individuals.

Since probiotics are mainly used to maintain healthy intestines, a large proportion of research attention has focused on exploring the mechanisms of intestinal immunomodulation by probiotics. Probiotics have been found to exhibit their immunomodulatory effects in a strain-dependent and cell subset-specific manner. Suda et al. (2014) have evaluated the potential of *L. jensenii* TL2937 in modulating the immune response in cocultures of porcine intestinal epithelial cells and antigen-presenting cells from porcine Peyer's patches. The authors found that *L. jensenii* TL2937 not only stimulated the expression of IL-10, MHC class II, Bcl-3, and CD80/CD86 in CD172a⁺CD11R1⁻ and CD172a⁺CD11R1^{high-} antigen-presenting cells but also suppressed TLR4 activation and subsequent inflammatory response by upregulating negative regulators (MKP1, Bcl3, and A20) of TLR4 in porcine intestinal epithelial cells. Smelt et al. (2013) have also demonstrated that specific probiotic strains exhibited specific immune responses in specific sites of the intestine in vivo. Although *L. plantarum* WCFS1, *L. lactis* MG1363, and *L. salivarius* UC118 increased the numbers of CD11c⁺MHC class II⁺ dendritic cells in the mice Peyer's patches, *L. salivarius* UCC118 was the only strain which exhibited immunoregulatory effects by decreasing the number of effector T cells and increasing the number of regulatory T cells (Treg cells; CD4⁺CD25⁺Foxp3⁺ T cells) in the small intestine lamina propria. IgA is an important intestinal humoral component, which is secreted from B cells in gut-associated lymphoid tissues (Mora et al. 2006). Sakai et al. (2014) have revealed that oral administration of diet containing *L. gasseri* SBT 2055 (1.0×10^9 CFU/g/daily) for 5 weeks activated the TLR2 signaling pathway and increased the rate of IgA⁺ cells and IgA production in both the lamina propria and Peyer's patch of the small intestine in healthy mice, as well as stimulated the expression of TGF- β in bone marrow-derived dendritic cells. These findings suggest that *L. gasseri* SBT 2055 could enhance host immune responses and protect against intestinal inflammation. In addition to animal studies, a number of clinical trials have also been conducted to elucidate the immunomodulatory actions and to evaluate the efficacy of probiotics in healthy populations (Table 1).

Table 1 Clinical evidences on the immunomodulatory activity of probiotic strains in healthy populations

Treatment	Probiotic strain	Subject	Dose and duration (intervention)	Immune response elicited	References
Enhancement of humoral responses in preschool children	<i>L. plantarum</i> IS-10506	12 healthy Indonesian children (aged 12–24 months)	Diet containing <i>L. plantarum</i> IS-10506 at a dose of 2.3×10^{10} CFU/g; daily for 90 days (randomized, double-blind, placebo-controlled, pre-post trial)	– Fecal sIgA excretion ↑	Surono et al. (2014)
Enhancement of immunomodulatory factors in breast milk	<i>B. lactis</i> HN019 <i>L. rhamnosus</i> HN 001	69 pregnant women; <i>L. rhamnosus</i> HN001 ($n = 34$) and <i>B. lactis</i> HN019 ($n = 35$)	Capsule powder containing <i>B. lactis</i> HN019 at a dose of 9×10^9 CFU/capsule or <i>L. rhamnosus</i> HN001 at a dose of 6×10^9 CFU/capsule; daily for 2–5 weeks before delivery and 6 months in lactation period	– Cord blood IFN- γ levels ↑ – Detectable blood IFN- γ levels† – TGF- β 1 levels† in early breast milk (week 1) – IgA ↑ in breast milk	Prescott et al. (2008)
Maintenance of intestinal immune homeostasis in adult	<i>L. rhamnosus</i> CNCMI-4036	20 healthy volunteers enrolled in three cities in Spain (average age of 28 years)	Capsule containing <i>L. rhamnosus</i> CNCMI-4036 at a dose of 9×10^9 CFU; daily for 30 days (multicenter, randomized, double-blind, placebo-controlled trial)	– IL-4 ↑ – IL-10 ↑ – IL-12 ↓ – IL-10/IL-12 ↑ – TNF- α /IL-10 ↓	Plaza-diaz et al. (2013)
Modulation of anti-inflammatory cytokine profile in elderly	<i>L. casei</i> Shirota	Sixteen healthy volunteers, 10 females and 6 males (aged 55–74 years)	Probiotic drink containing <i>L. casei</i> Shirota at a dose of 1.3×10^{10} CFU; daily for 4 weeks (randomized placebo-controlled, single-blind cross-over trial)	– NK cell activity ↑ – CD25 T cells ↓ – IL-10/IL-12 ↑	Dong et al. (2013)
Modulation of pro- and anti-inflammatory	<i>L. rhamnosus</i> GG ATCC 53103	Fifteen elderly volunteers (aged 66–80 years)	Capsule containing <i>L. rhamnosus</i> GG ATCC 53103 at a dose of 1×10^{10} CFU/	– IL-8 ↓, and returning to baseline 1 month after discontinuation	Hibberd et al. (2014)

(continued)

Table 1 (continued)

Treatment	Probiotic strain	Subject	Dose and duration (intervention)	Immune response elicited	References
Treatment profile in elderly			capsule; twice daily for 28 days and followed through day 56 (Phase 1 open-label clinical trial)	– No significant effects on IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13, TNF- α , and IL-6	
Modulation of NK cell activity in healthy men	<i>L. casei</i> Shirota	35 healthy male Caucasian nonsmokers (aged 18–60 years)	Probiotic drink containing <i>L. casei</i> Shirota at a dose of 1.95×10^{10} CFU; daily for 4 weeks	– No significant effects on NK cell numbers, cytokine profile, and peripheral blood mononuclear cells	Seifert et al. (2011)

3 Non-gastrointestinal Diseases Modulated by Probiotics

The potential immunomodulatory roles of probiotics in non-gastrointestinal diseases are still emerging and might offer promising novel applications in the near future. Results from preliminary studies have proposed that probiotics may modulate immune responses in diseases such as metabolic syndrome, respiratory diseases, allergic and inflammatory skin disorders, osteoporosis, rheumatoid arthritis, and male hypogonadism, which will be further discussed in later sections (3.1–3.6). Although immunomodulatory effects of probiotics represent a new avenue for discovering the potential use of probiotics in non-gastrointestinal health, a number of clinical trials have already been performed. However, more clinical studies are needed in order to establish the immunomodulatory role of probiotics in the treatment of non-gastrointestinal diseases.

3.1 Metabolic Syndrome

Metabolic syndrome is a constellation of multiplex factors, such as abdominal obesity, hyperlipidemia, hypertension, and nonalcoholic fatty liver disease that raises the risk of type 2 diabetes mellitus and cardiovascular diseases (Okubo et al. 2014; Fig. 2). It has been estimated that 25 % of the global population is suffering from metabolic syndrome (Shivakumar et al. 2014). Accumulating

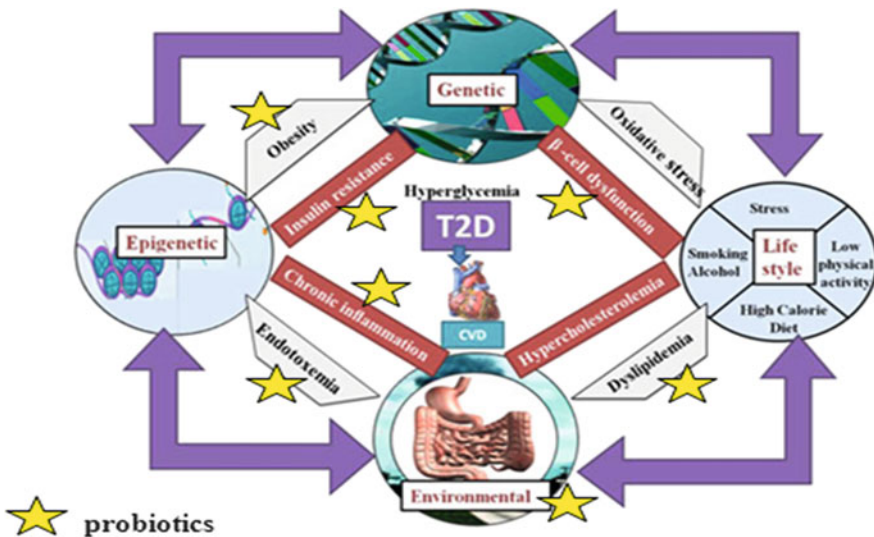


Fig. 2 Probable immunological actions of probiotics to reduce the risk factors that contribute to type 2 diabetes mellitus and cardiovascular diseases. Reprinted from Panwar et al. (2013) with permission from John Wiley and Sons (License number: 3522340228488)

evidence has shown that altered gut microbiota mediated low-grade inflammation which contributed to the onset of metabolic syndrome and related diseases, via mechanisms responsible for intestinal barrier dysfunction (Cani et al. 2012; Zhang et al. 2014). Thus, the administration of probiotics appears to be a promising approach to treat metabolic syndrome owing to their ability to modulate gut microbiota and to restore impaired intestinal barrier function. Indeed, to date, probiotic approaches that target the gut microbiota have shown the most positive results in the prevention and treatment of abdominal obesity, hypertension, hyperlipidemia, and nonalcoholic fatty liver disease via immune-mediated mechanisms. Preliminary findings have shown that probiotics are capable of stimulating and regulating local and systemic immune responses in a diet-induced metabolic syndrome murine model. However, their potential warrants further investigation and clinical trials.

3.1.1 Abdominal Obesity

An increased high-fat diet has been linked to the development of abdominal obesity and increased risk of type 2 diabetes mellitus (Kaur 2014). Recently, Núñez et al. (2014) have conducted a study evaluating the effect of *L. casei* CRL 431 on intestinal and humoral responses using a high-fat-induced mouse model. The mice were orally administered a balanced diet or high-fat diet supplemented with milk, fermented milk containing *L. casei* CRL 431 ($8 \pm 2 \times 10^8$ CFU/mL/daily), water suspension containing *L. casei* CRL 431 ($2 \pm 1 \times 10^8$ CFU/mL/daily), or water for 60 days. The authors found that while both the administration of fermented milk or water suspension containing *L. casei* CRL 431 enhanced the phagocytic activity of macrophages, only fermented milk containing *L. casei* CRL 431 increased the number of macrophages and IgA⁺ cells in the small intestine. These results indicate that fermented milk containing *L. casei* CRL 431 can attenuate high-fat-diet-induced obesity by modulating mucosal immunity altered by obesity.

TNF- α is known as a cytokine that can stimulate the apoptosis of adipocytes and inhibit the synthesis of fatty acid synthase (Cawthorn and Sethi 2008). It has been reported that oral administration of *L. rhamnosus* PL 60 (1×10^7 or 1×10^9 CFU/mouse; daily) for 8 weeks significantly downregulated both TNF- α and fatty acid synthase mRNA expression levels in epididymal white adipose tissues, leading to an antiobesity effect in high-fat-diet-induced mice (Lee et al. 2006). Meanwhile, Park et al. (2013a, b) have also demonstrated that the mRNA expression of TNF- α , IL-1 β , IL-6, and MCP1 (pro-inflammatory genes) in adipose tissues was downregulated in high-fat-diet-induced mice upon receiving *L. curvatus* HY 7601 (5×10^9 CFU/day) or *L. plantarum* KY 032 (5×10^9 CFU/day) treatment for 8 weeks. Recently, it has also been reported that oral administration of *L. gasseri* SBT 2055 (5×10^8 CFU/g; daily) for 24 weeks downregulated mRNA expression of CCL2 and CCR2 (pro-inflammatory genes) in epididymal adipose tissues of high-fat-induced mice (Miyoshi et al. 2014). These findings collectively suggest

that probiotic strains may also attenuate inflammation of adipose tissues in high-fat-induced obesity by suppressing pro-inflammatory immune responses (TNF- α , IL-1 β , IL-6, MCP1, CCL2, and CCR2). On the other hand, Yoo et al. (2013) have conducted an animal study involving 50 C57BL/6J mice that were fed with high-fat high-cholesterol diet (HFCD) supplemented with *L. plantarum* KY 1032 (10^{10} CFU/day), HFCD supplemented with *L. curvatus* HY 7601 (10^{10} CFU/day), or HFCD supplemented with *L. plantarum* KY 1032 and *L. curvatus* HY 7601 (10^{10} CFU/day) for 9 weeks. The authors found that only mice orally administered with *L. plantarum* KY 1032 were capable of suppressing plasma TNF- α and IL-1 β (pro-inflammatory cytokine), indicating that probiotics may reduce the incidence of systemic low-grade inflammation in high-fat-diet-induced metabolic syndrome.

A reduction in the number of adipose tissue macrophage and macrophage infiltration has been shown to attenuate adipose inflammation and other obesity complications (Cani et al. 2008). Recently, Wang et al. (2015) have conducted an animal study involving 40 male high-fat diet-induced C57BL/6J mice fed with *L. paracasei* CNLM I-4270, *L. rhamnosus* I-3690, or *B. animalis* subsp. *lactis* I-2494 (10^8 CFU/day) for 12 weeks. The authors found that all three probiotic strains significantly reduced the number of adipose tissue macrophages and infiltration of pro-inflammatory macrophages (CD11c⁺ and MMP-12⁺) and may therefore attenuate inflammation in adipose tissues.

In addition to abdominal obesity, several studies have also determined the immune modulating potential of probiotics in the prevention of type 2 diabetes mellitus using an obesity-induced diabetes murine model. Inflammatory cytokines, such as TNF- α , IL-6, and IL-8, can cause insulin resistance and inflammation and consequently onset type 2 diabetes mellitus (King 2008). Hence, suppression of these inflammatory cytokines is a promising therapeutic approach to combat type 2 diabetes mellitus. Interestingly, several studies have demonstrated that probiotics exhibit antidiabetic effects by suppressing Th₁ immune responses. Amar et al. (2011) have found that high-fat-diet-induced diabetic mice orally treated with *B. lactis* 420 (10^9 CFU/day) downregulated inflammatory cytokine (TNF- α , IL-1 β , IL-6, and PAI-1) mRNA in mesenteric adipose tissues, the muscle, and the liver, thereby resulting in improved insulin sensitivity and overall inflammatory status. Another recent study also showed that oral administration of *L. casei* (4×10^9 CFU/day) inhibited the development of type 2 diabetes mellitus by suppressing plasma Th₁-associated pro-inflammatory cytokines (IFN- γ and TNF- α) and downregulating Th₁ responses related to *Tbet* gene mRNA levels in high-fat high-sucrose diet-induced pre-insulin-resistant rats (Zhang et al. 2014). Recently, a randomized, double-blind, controlled clinical trial was conducted on 44 patients (22 for placebo group and 22 for intervention group), BMI ≥ 25 , over a period of 8 weeks to evaluate the effect of probiotic yogurt (3.7×10^6 CFU/g of *L. acidophilus* A5 and *B. lactis* BB12) consumption (300 g/day) on inflammatory biomarkers in patients with type 2 diabetes mellitus. The consumption of probiotic yogurt in the intervention group was found to reduce plasma TNF- α , thus ameliorating the onset of type 2 diabetes (Mohamadshahi et al. 2014).

On the other hand, probiotics can also improve type 2 diabetes by stimulating Th₂ immune responses. Chen et al. (2014) have reported that oral administration of *L. rhamnosus* CCFM 0528 (10⁹ CFU/day) for 12 weeks upregulated IL-4 and IL-10 (Th₂ immune responses) expression and downregulated IL-6, IL-8, and TNF- α (Th₁ immune responses) expression in the spleen, leading to improved glucose tolerance in high-fat-diet, streptozotocin-induced type 2 diabetic mice. Dendritic cells from nonobese diabetic mice have been shown to produce elevated levels of IL-12 that activate IFN- γ -producing T cells which can promote the development of diabetes (Trembleau et al. 1995). Meanwhile, nonobese diabetic dendritic cells stimulated with *L. casei* enhanced IL-10 over IL-12 in vitro, and their transfer reduced the incidence of diabetes in vivo, thus proposing another potential immunological role of probiotics for the treatment of type 2 diabetes mellitus (Manirarora et al. 2011).

3.1.2 Hypertension

Hypertension is one of the central metabolic syndromes that is increasing worldwide at an alarming rate. It has been estimated that the number of adult with hypertension will reach a total of 1.56 billion in the year 2025 (Kearney et al. 2005). Although the exact mechanisms underlying the immunomodulatory potentials of probiotics for hypertension still remain poorly understood, findings in animal studies have proposed that probiotics may attenuate pathogenesis in hypertension by targeting immune responses in the kidney and aorta.

Angiotensin type 1 receptors (AT₁R) specifically activate immune and non-hematopoietic cells to promote pathogenesis in angiotensin II-dependent hypertension mice (Ryan 2013). A mutational analysis has revealed that CD3⁺ T cells and renal macrophages were increased in bone marrow-specific AT₁R knock-out mice associated with exaggerated hypertensive responses (Crowley et al. 2010). Mycophenolate mofetil is a drug that is used to improve hypertension (Herrera et al. 2006). It has been demonstrated that high-protein-diet-induced hypertension in Dahl salt-sensitive rats treated with mycophenolate mofetil reduced renal cortical T cell infiltration, thereby attenuating hypertension (De Miguel et al. 2011). Additionally, Barhoumi et al. (2011) have demonstrated that angiotensin II-induced hypertension is associated with the reduction of Treg cells in the renal cortex, and adoptive transfer to increase Treg cells suppressed TNF- α expression. On the other hand, inhibition of IL-6 has also been shown to downregulate IL-6 expression, T cell, and macrophage cell infiltration in cold-induced hypertension (Crosswhite and Sun 2010). Nonetheless, Chan et al. (2012) have also further revealed that deoxycorticosterone acetate-salt-induced hypertension was accompanied with an increase in the number of macrophage and expression levels of chemokine (CCR2, CC12, CCL7, and CC8) in the aorta. Altogether, these results propose that probiotics may attenuate hypertension by modulating immune cells (T cells, macrophages, Treg cells) and pro-inflammatory cytokines (TNF- α and IL-6) in the kidney, as well as chemokines (CCR2, CCL12, CCL7, and CC8) in the aorta.

3.1.3 Hyperlipidemia

Hyperlipidemia is the term used to describe the increased concentration of any or all lipids in the plasma (Stocks et al. 2005). Elevated very-low-density lipoproteins in the plasma can contribute to the risk of atherosclerosis. Apolipoprotein E-deficient mice (ApoE^{-/-}) are characterized by elevated very-low-density lipoproteins and are widely used as murine models of atherosclerosis, steatosis, and hyperlipidemia (King et al. 2010). Interestingly, Mencarelli et al. (2012) have used this model to evaluate the potential of a probiotic cocktail (VSL#3) consisting of eight probiotic strains (*L. acidophilus* MB 443, *L. delbrueckii* subsp. *bulgaricus* MB 453, *L. casei* MB 451, *L. plantarum* MB 452, *B. longum* Y10, *B. infantis* Y1, *B. breve* Y8, and *Streptococcus salivarius* subsp. *thermophilus* MB 455) in protecting against low-grade intestinal inflammation in the development of atherosclerosis. The ApoE^{-/-} mice were orally administered with VSL#3 (20×10^9 CFU/kg/day) or 0.2 % dextran sulfate sodium (DSS) filtered drinking water containing VSL#3 (20×10^9 CFU/kg/day) six days a week for 12 weeks. The authors found that VSL#3 downregulated the expression of TNF- α and RANTES (inflammatory mediators) in both the colonic mucosa and mesenteric adipose tissues of ApoE^{-/-} mice challenged with DSS. VSL#3 treatment was also shown to stimulate the expression of nuclear receptors (PPAR- γ and VDR) of positive regulators in the intestine and to increase IL-10 (anti-inflammatory cytokine) production from CD5⁺T cells in the spleen. Another set of results in this study also highlighted that the administration of VSL#3 downregulated the expression of inflammatory mediators (ICAM-1, VCAM, and RANTES) in the aorta, as well as decreased the percentage of CD35⁺ cells in circulating macrophages of ApoE^{-/-} mice challenged with DSS, thereby preventing the generation of new plaques in the thoracoabdominal aortas and inhibiting the extension of atherosclerotic plaques (Fig. 3). Altogether, these results demonstrate that VSL#3 can potentially protect subjects with low-grade inflammation of hyperlipidemia against atherosclerosis by decreasing local and systemic inflammatory immune responses (aorta, intestine, adipose tissue, and plasma) while stimulating the expression of nuclear receptors of positive regulators and IL-10-producing T lymphocytes.

3.1.4 Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease is the common cause of chronic liver disease worldwide that increases with the prevalence of obesity, dyslipidemia, and insulin resistance (Kneeman 2012). Numerous studies have highlighted a causative relationship between liver (local) and systemic inflammation and nonalcoholic fatty liver disease. The invariant natural killer T (iNKT) cells are hepatic lymphocytes that play a role in the pathogenesis of nonalcoholic fatty liver disease (steatosis and nonalcoholic steatohepatitis). Although the exact mechanisms of NKT cells in liver diseases remain unclear, iNKT cells are capable of balancing the production of

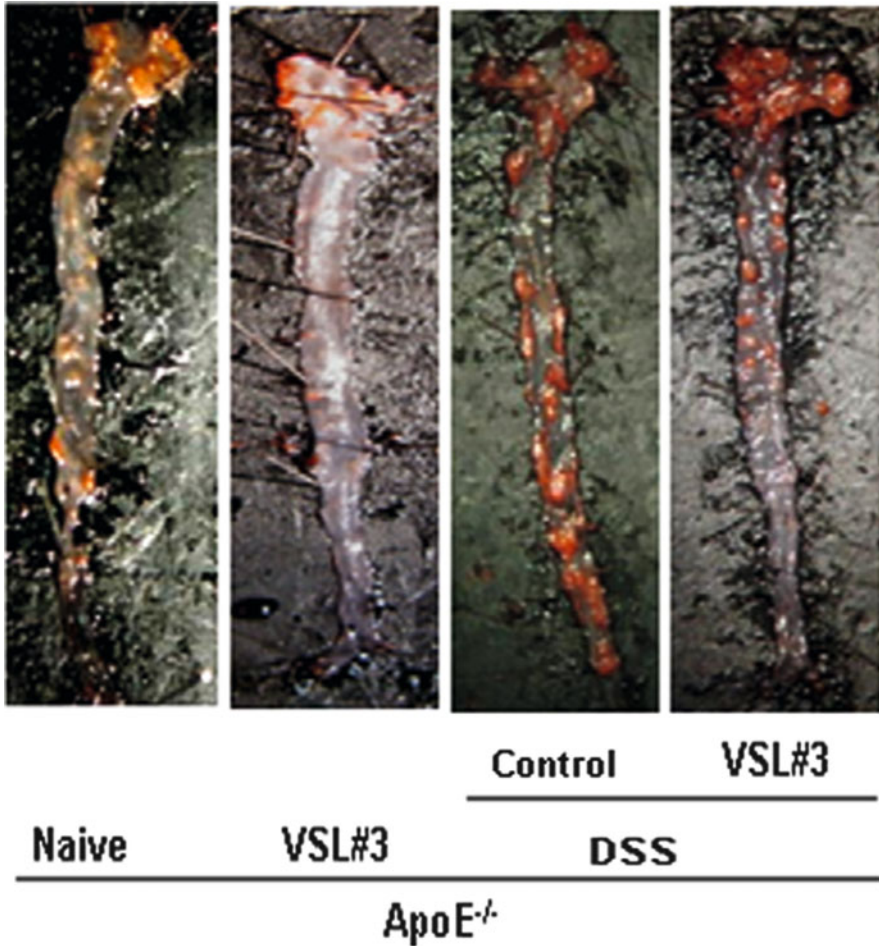


Fig. 3 Effect of VSL# 3 administration on aortic plaques in ApoE^{-/-} mice. The lipids in the vessel wall were stained with Sudan IV. ApoE^{-/-} mice administered with VSL#3 for 12 weeks decreased the number of new plaques in thoracoabdominal aorta and inhibited the extension of atherosclerotic plaques. Reprinted from Mencarelli et al. (2012); free access from PLOS

pro-inflammatory and anti-inflammatory cytokines (Seki et al. 2000). On the other hand, it has been reported that chronic liver inflammation is mediated by the I κ B- β /NF- κ B signaling pathway, where increased NF- κ B activity was associated with upregulated expression of inflammatory cytokines (TNF- α , IL-1 β , and IL-6) in the liver of high-fat-diet-induced steatosis mouse model (Cai et al. 2005). Therefore, *i*NKT cells and I κ B- β /NF- κ B signaling pathway could be promising targets in treating nonalcoholic fatty liver damage and inflammation.

Ma et al. (2008) have evaluated the potential of VSL#3 (a mixture of lyophilized *Bifidobacterium*, *Lactobacillus*, and *Streptococcus thermophilus*) to improve

high-fat-diet-induced hepatic steatosis mice. The authors found that oral administration of VSL#3 (1.5×10^9 CFU/day) daily for 4 weeks significantly improved high-fat-diet-induced steatosis by stimulating IL-4 that contributes to an improvement in hepatic NKT cell depletion. Elevated TNF- α expression was found to activate I κ B activity (represented by ratio of phosphor-I κ B- α /total I κ B- α) in high-fat-induced hepatic steatosis mice. Nevertheless, VSL#3 treatment and adoptive transfer of NKT cells suppressed TNF- α expression associated with reduced I κ B activity and downstream signal for I κ B- α (NF- κ B p65 activity). Similarly, Liang et al. (2013) have also found that high-fat-diet mice orally administered with lipid extracts of VSL#3 for 4 weeks stimulated hepatic *i*NKT cells, suggesting that probiotic strains in VSL#3 may contain glycolipid antigens that directly confer effector functions to hepatic *i*NKT cells. Matrix metalloproteinases (MMPs) play an important role in the pathogenesis of liver diseases (Kurzepa et al. 2014). According to Esposito et al. (2009), VSL#3 treatment also suppressed the hepatic gelatinase activity of MMP-2 and MMP-9, thereby inhibiting liver inflammatory damage in rats receiving high-fat diet.

Several clinical studies have also been performed to evaluate the potential of probiotics for the treatment of nonalcoholic fatty liver disease, ranging from children to adult populations. Malaguarnera et al. (2012) have conducted a double-blind and randomized study to determine the effects of oral administration of *B. longum* with fructo-oligosaccharides in 66 patients (aged of 30–65 years) with abnormal hepatic steatosis. The authors found that patients who received *B. longum* with fructo-oligosaccharides and lifestyle modification (diet and exercise) had significantly decreased serum TNF- α and nonalcoholic steatohepatitis activity index compared to patients who received lifestyle modification alone. Furthermore, Eslamparast et al. (2014) have evaluated the effects of synbiotic capsules (Protexin) containing 2×10^8 CFU of seven probiotic strains (*L. casei*, *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. longum*, and *L. bulgaricus*) in a 28-week randomized, double-blind, placebo-controlled trial involving 52 patients with nonalcoholic fatty liver diseases. Synbiotic intervention significantly decreased TNF- α in plasma and NF- κ B p65 in peripheral blood mononuclear cells. However, Vajro et al. (2011) reported that consumption of *L. rhamnosus* GG (12×10^9 CFU/day) for 8 weeks showed no significant effect on TNF- α production in twenty obese children (aged of 10.7 ± 2.1 years) with persisting hypertransaminasemia and ultrasonographic bright liver. Altogether, these findings warrant further investigation.

3.2 Respiratory Diseases

Respiratory diseases are a major leading cause of mortality and morbidity throughout the world, while respiratory infections have been reported to account for over four million deaths per year in low- or middle-income countries (World Health Organization 2008; Zar and Ferkol 2014). Thus, effective strategies are needed to

Table 2 Clinical trials on the immunological potential of probiotics for the treatment of respiratory diseases

Treatment	Probiotic strain	Subject	Dose and duration (intervention)	Immune response elicited	References
Prevention of asthma	<i>L. rhamnosus</i> GG ATCC 53103	131 children (aged 6–24 months old) with at least two wheezing episodes and family history of atopic disease	<i>L. rhamnosus</i> LGG (10^{10} CFU/day; 6 months) (prospective double-blind, randomized study)	– No significant effect on IgE levels	Wickens et al. (2008)
Breathing illness	<i>L. fermentum</i> VRI-003	20 healthy elite male distance runners	Freeze-dried powder in gelatin capsules containing <i>L. fermentum</i> -VRI-003 (1.2×10^{10} CFU; twice a day; 72 days)	– No significant effect on IgA, IgA1, IL-4, and IL-12 levels – Plasma IFN- γ ↑	Cox et al. (2010)
Prevention of influenza	<i>L. fermentum</i> CECT 5716	50 volunteers (aged 22–56 years)	Capsule containing <i>L. fermentum</i> CECT5716 (1×10^{10} CFU/day; 28 days), 2 weeks before and after vaccination, respectively	– No significant effect on IFN- γ , IL-10 – Specific anti-influenza IgA ↑	Olivares et al. (2007)

properly tackle the burden of respiratory diseases. In brief, there are a growing number of in vitro and animal studies suggesting that probiotics may modulate pulmonary immune responses, thereby attenuating respiratory diseases, in particular asthma and influenza viral infections. However, clinical trials addressing the immunomodulatory potentials of probiotics in subjects with asthma and influenza are still very limited (Table 2).

3.2.1 Asthma

Asthma is a chronic inflammatory disease of the airways, with an estimated 300 million people of all ages and all ethnic backgrounds worldwide suffering from asthma (Masoli et al. 2004). Several studies have highlighted that Th₂ lymphocytes, Th₁₇ cells, as well as their cytokines (IL-4, IL-5, IL-12, IL-17A,

IL-17F, and IL-23) are key inflammatory mediators that play critical roles in orchestrating the asthma pathogenesis (Li and Hua 2014). Thus, targeting these components may be a promising immunotherapeutic approach for the treatment of asthma. Interestingly, Jan et al. (2011) have demonstrated that oral administration of *L. gasseri* A5 (4×10^6 CFU/daily) starting from 2 weeks before sensitization for 4 weeks not only significantly decreased IL-17A production but also reduced TNF- α production and activation-regulated chemokines in bronchoalveolar lavage fluids of *Dermatophagoides pteronyssinus*-sensitized and *Dermatophagoides pteronyssinus*-challenged mice. Furthermore, the number of IL-17-producing alveolar macrophages was also decreased in *L. gasseri* A5-treated mice compared to untreated mice, suggesting that *L. gasseri* A5 treatment is capable of downregulating Th₁₇ pro-inflammatory responses and improving asthma.

In addition to immunosuppressive effects on Th₁₇ pro-inflammatory responses, probiotics (*L. rhamnosus* Lcr35, *L. rhamnosus* GG, *B. lactis* BB12, and *L. reuteri* strains) may attenuate asthmatic responses via a Treg cell-mediated mechanism (Jang et al. 2012; Karimi et al. 2009; Feleszko et al. 2007). The daily oral administration of *L. rhamnosus* Lcr35 at a dose of 1×10^9 CFU/mL for 7 days before the first sensitization was found to increase the percentage of Treg cells in the mouse spleen and also decrease Th₂ (IL-4, IL-13, and IL-5) and Th₁ (IFN- γ) cytokines in the mouse serum (Jang et al. 2012). Administration of anti-CD25 monoclonal antibody was also shown to inhibit the protective effects of *L. rhamnosus* Lcr35, confirming that Treg cells are essential targets in mediating the immune response of *L. rhamnosus* Lcr35 in asthma. Additionally, oral administration of *L. rhamnosus* Lcr35 (1×10^9 CFU/mL/daily; 1 week) prior to sensitization was also found to significantly decrease ovalbumin-specific IgE production in ovalbumin-induced murine model of asthma, to suppress Th₂ responses (CCL11, Th2-related chemokines) and to increase Treg cells following the adoptive transfer of *L. rhamnosus* Lcr35-treated dendritic cells (Yu et al. 2010; Kim et al. 2013). These studies collectively suggest that *L. rhamnosus* Lcr35 can stimulate dendritic cell activation and suppress the Th₂ responses by upregulating Treg cells in the murine model of asthma. However, the efficacy of *L. rhamnosus* Lcr35 in subjects with asthma still remains unclear. Similar to *L. rhamnosus* Lcr35, *L. reuteri* strains have also been shown to increase the number of Treg cells in the spleen and to suppress Th₂ responses (TNF, MCP-1/CCL2, IL-5) in an ovalbumin-induced murine model (Karimi et al. 2009).

3.2.2 Influenza

Influenza is a respiratory viral infectious disease caused by RNA viruses, belonging to the family of *Orthomyxoviridae* (influenza viruses). In vivo studies have proposed that probiotics may attenuate influenza virus infections by stimulating NK cells, which are major cellular components of the innate immunity that can recognize and control a broad spectrum of pathogens, including viruses. Intranasal administration of heat-killed *L. pentosus* S-PT84 (200 μ g/day) for 3 days

significantly increased IFN- γ and IL-12 (Th1 antiviral cytokines) production in mediastinal lymph node cells and bronchoalveolar lavage fluids, subsequently stimulating NK cell activity in the lung of mice infected with influenza virus PR8 (mouse-adapted H1N1 strain) (Izumo et al. 2010). A similar conclusion has also been drawn in another study by Harata et al. (2010), where daily intranasal administration of *L. rhamnosus* GG (200 $\mu\text{g/day}$) for three consecutive days upregulated mRNA expression of IL-1 β , monocyte chemotactic protein (MCP), and TNF- α following activation of lung NK cells in mice infected with influenza virus A (H1N1). On the other hand, Park et al. (2013a, b) have also demonstrated that intranasal (10^9 CFU/mouse) or oral (10^9 CFU/daily for 10 days) administration of *L. plantarum* DK119 2055 can protect mice from influenza A/PR8 virus infection by increasing CD11c⁺ dendritic and macrophage cells and stimulating IFN- γ and IL-12 production. Altogether, these studies suggest that probiotics most probably exhibit their antiviral effects against influenza virus A by stimulating macrophage- and dendritic cell-mediated immune responses and NK cell activation.

3.3 Allergic and Inflammatory Skin Disorders

Contemporary studies have focused on the possible deployment of probiotics for alleviating allergic and inflammatory skin disorders due to their ability to balance intestinal microbiota, which ameliorates the immune system at both the local and systemic levels. Preliminary data suggest that probiotics could produce dermal bioactives such as bacteriocins and may thereby enhance the skin immune defense (Tan et al. 2014). A bacteriocin, CBT-SL5 produced from *E. faecalis* at a concentration of 100 ng/mL, was found to suppress *P. acnes*-induced NF- κ B translocation, mRNA expression, and protein production of IL-8 in vitro, highlighting its potential as a topical agent for acne vulgaris (Jin et al. 2008). On the other hand, Pinto et al. (2011) have also demonstrated that plantaricin A, synthesized by *L. plantarum*, significantly induced proliferation and migration of human keratinocytes and increased expression of TGF- β 1, VEGF-A, and IL-8, indicating a function in accelerating wound healing (Pinto et al. 2011). At a low concentration (10 $\mu\text{g/mL}$), plantaricin A was also found to increase antioxidant defenses of human keratinocytes and mRNA expression levels of filaggrin, involucrin, β -defensin 2, and TNF- α , which can promote antioxidant defenses, barrier functions, and antimicrobial activity of the skin.

The possible immunomodulatory actions of probiotics have also been investigated using animal models. Hacini-Rachinel et al. (2009) have revealed that oral administration of *L. casei* (DN-114 001) reduced the number of CD8⁺ effector T cells and increased the recruitment of CD4⁺ effector T cells and Treg cells in the skin of mice with antigen-specific-induced skin inflammation. *L. casei* (DN-114 001) treatment also enhanced the IL-10 production from Treg cells in skin-draining lymph nodes of hapten-sensitized mice, thus suggesting its potential to treat allergic skin diseases in human. It has also been further reported that oral administration of

L. rhamnosus Lcr35 increased Treg cells, leading to the suppression of IL-4 production, and thymic stromal lymphopoietin (TSLP), which can promote Th₁ cytokines that initiate the inflammation cascade in ovalbumin-induced atopic dermatitis SK-1 hairless mice via a mechanism that may be associated with Treg cells (Kim et al. 2012). Moreover, *L. rhamnosus* 1.3724 treatment was also found to prevent the development of atopic dermatitis in NC/NgaTnd mice by stimulating IFN- γ in skin, while *L. sakei* probio 65 treatment reduced plasma IgE level and IL-4 production in the spleen of sensitized mice (Tanaka et al. 2009; Park et al. 2008). On the other hand, several studies have also reported that probiotics can attenuate skin inflammation via the gut-skin axis. Inoue et al. (2007) have revealed that *L. johnsonii* NCC533 treatment can prevent the development of atopic dermatitis by stimulating intestinal IgA production, whereas Sawada et al. (2007) have suggested that oral administration of heat-treated *L. rhamnosus* LGG upregulated IL-10 mRNA expression levels in both Peyer's patches and mesenteric lymph nodes in maternal and infant NC/Nga mice (a model of human atopic dermatitis) and may thus delay the onset and prevent the development of atopic dermatitis in human.

In addition to animal studies, Guéniche et al. (2010) have performed an ex vivo study to evaluate the immunomodulatory potential of *L. paracasei* CNCM I-2116 on substance P-induced skin inflammation. The authors found that *L. paracasei* CNCM I-2116 inhibited mast cell degranulation and TNF- α production induced by substance P ex vivo, thereby accelerating skin barrier recovery. Moreover, clinical trials have been performed in patients with other skin disorders, particularly on atopic dermatitis (Table 3). However, several clinical trials showed that probiotic strains were incapable of modulating immune responses in subjects with atopic dermatitis. Guéniche et al. (2009) have also conducted a randomized, double-blind, placebo-controlled trial to determine the immunomodulatory effects of probiotics in 57 volunteers upon exposure to ultraviolet (2×1.5 minimal erythema dose). The authors reported that volunteers who ingested *L. johnsonii* NCC 533 daily for 8 weeks had significantly increased production of regulating cytokines and growth factors such as TGF- β , which lead to the preservation of cutaneous immune homeostasis.

3.4 Osteoporosis

Estrogen deficiency is the primary risk factor of developing osteoporosis, a degenerative skeletal disease in postmenopausal women. In osteoporosis, osteoclastic bone resorption exceeds osteoblastic bone formation, thus leading to bone loss. Interestingly, probiotic treatment may attenuate osteoporosis in a postmenopausal woman by suppressing inflammatory cytokines (TNF- α and IL-1 β) and T cell subsets (Treg and CD4⁺ T lymphocytes) involved in osteoclastogenesis. Recently, Ohlsson et al. (2014) have demonstrated that oral administration of either *L. paracasei* DSM 13434 or a cocktail of three strains (*L. paracasei* DSM 13434, *L. plantarum* DSM 15312, and DSM 15313) for 6 weeks, starting 2 weeks before

Table 3 Clinical trials on the immunological potential of probiotics for the treatment of allergic and inflammatory skin disorders

Treatment	Probiotic strain	Subject	Dose and duration (intervention)	Immune response elicited	References
Prevention of eczema and atopic dermatitis in infants	<i>L. rhamnosus</i> HN001 <i>B. animalis</i> subsp. <i>lactis</i> HN019	512 pregnant women (mother or infant's father had a history of treated asthma, eczema, or hay fever)	<i>L. rhamnosus</i> HN001 (6×10^9 CFU/day) <i>B. animalis</i> subsp. <i>lactis</i> HN019 (9×10^9 CFU/day) Mother: 35-week gestation until 6 months if breastfeeding Infant: birth until 2 years (double-blind, randomized placebo-controlled trial of infants)	– IgE ↓	Wickens et al. (2008)
Atopic dermatitis in infants	<i>L. rhamnosus</i> GG ATCC 53103	131 children with at least two wheezing episodes and a first-degree family history of atopic disease (aged 6–24 months old)	<i>L. rhamnosus</i> LGG (10^{10} CFU/day for 6 months) (prospective double-blind, randomized study)	– No significant effect on TGF-β and IgE levels	Rose et al. (2010)
Children with mild-to-moderate atopic dermatitis	<i>L. casei</i> <i>L. rhamnosus</i> <i>L. plantarum</i> <i>B. lactis</i>	100 children with mild-to-moderate atopic dermatitis (aged 2–9 years)	Juice containing each strain (1×10^9 CFU; twice a day; 6 weeks) (randomized, double-blind, placebo-controlled, parallel trial)	– No significant effects on IL-4; IL-10 and TNF-α in serum	Yang et al. (2014)
Atopic dermatitis in infants	<i>B. breve</i> M-16V	90 full-term infants with atopic dermatitis (aged 0–7 months)	Galacto- and fructo-oligosaccharide mixture containing <i>B. breve</i> M-16V (1.3×10^9 CFU/day; 12 weeks) (double-blind, placebo-controlled multicenter trial)	– No significant effect on IgE-associated atopic dermatitis	van der Aa et al. (2010)
Incidence of eczema in children	<i>B. bifidum</i> W23 <i>B. lactis</i> W52 <i>L. lactis</i> W58	102 pregnant mothers during the last 6 weeks of pregnancy and postnatally for 12 months to their offspring	Freeze-dried powder containing three probiotic strains (3×10^9 CFU/day; 3 months) (double-blind, randomized, placebo-controlled trial)	– No significant effect on IgE level – No significant effect on IL-10 production – IL-5 ↓ – IL-13 ↓	Niers et al. (2009)
Chronic infected leg ulcers	<i>L. plantarum</i> ATCC 10241	34 patients (14 patients with type 2 diabetes) (aged 40–70 years)	Gauze pad containing <i>L. plantarum</i> ATCC 10241 (1×10^5 CFU/day for 10 days)	– IL-8 ↓	Peral et al. (2010)

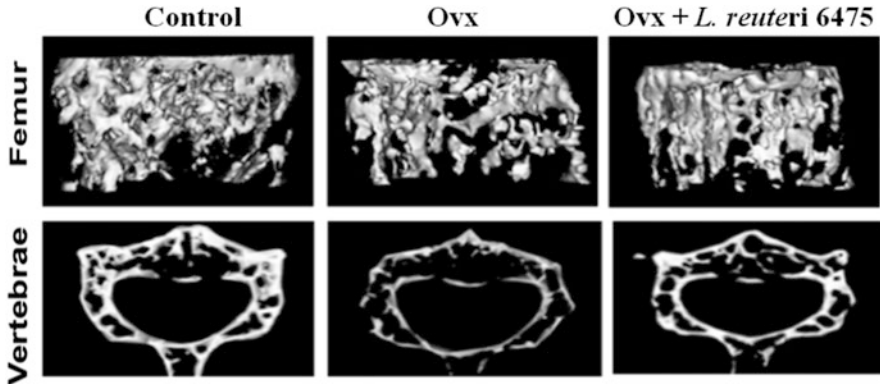


Fig. 4 Micro-computed tomography isosurface images of femur and vertebral trabecular bone of mice. Ovariectomized (ovx) mice treated with *L. reuteri* 6475 for 4 weeks display femur and vertebral trabecular bone volumes that are similar to ovary-intact control mice. Reprinted from Britton et al. (2014) with permission from John Wiley and Sons (License number: 3534800317389)

ovariectomy, downregulated gene expression of TNF- α and IL-1 β , maintained the number of Treg cells, and increased TGF- β 1 production in ovariectomized mice. TGF- β 1 has been shown to stimulate Treg cells, thereby inhibiting osteoclast differentiation and functions. Meanwhile, Britton et al. (2014) have also reported that ovariectomized mice fed with *L. reuteri* 6475 (1×10^9 CFU/mL; three times per week) for 4 weeks reduced the number of ovariectomized-induced CD4⁺ T lymphocytes, which prevented femur and vertebral trabecular bone loss (Fig. 4).

3.5 Rheumatoid Arthritis

Rheumatoid arthritis is a chronic autoimmune inflammatory disease in which the Th₁/Th₂ balance shifts toward Th₁ (pro-inflammatory responses), leading to joints deformities with destruction of cartilage and bone (Geusens et al. 2006). Owing to the immunosuppressive effect of probiotics, their potential use in reducing the pro-inflammatory responses were investigated in rats with type II collagen-induced rheumatoid arthritis. So et al. (2008a) have demonstrated that oral administration of *L. casei* (5×10^9 CFU/dose; 3 times/week) at day 7 after induction and continued for 11 weeks suppressed CD4⁺ T cells from releasing type II collagen reactive pro-inflammatory components, including IFN- γ , TNF- α , IL-1 β , IL-2, IL-6, IL-12, IL-17, and Cox-2. Meanwhile, *L. casei* treatment also upregulated IL-10 expression and subsequently reduced the translocation of NF-KB, which is a key transcription factor that stimulates the production of certain pro-inflammatory cytokines (Driessler et al. 2004). Hence, *L. casei* may suppress pro-inflammatory cytokines by stimulating IL-10 production from CD4⁺ T cells. In another study,

coadministration of *L. casei* (2×10^{10} CFU, 500 mg/kg) and collagen type II (250 mg/kg; glucosamine, 250 mg/kg) three times a week for 12 weeks has also been shown to suppress pro-inflammatory cytokines (IL-12, IL-17, IFN- γ , TNF- α , IL-1 β , IL-2, and IL-6) and stimulate anti-inflammatory cytokines (TGF- β and IL-10) in mice with type II collagen-induced rheumatoid arthritis (So et al. 2008b). Meanwhile, this treatment also increased the numbers of Treg cells, upregulated Foxp3 expression, and suppressed collagen type II-reactive Th₁-type IgG isotypes, IgG2a, and IgG2b. Although the ability of probiotics to suppress Th₁-type cellular and humoral immune responses seems promising for the treatment of rheumatoid arthritis, more studies are needed to justify their potential.

3.6 Male Hypogonadism

High-energy and high-fat-diet-induced obesity has been shown to cause an increased risk for male hypogonadism (Cebler et al. 2010). Recently, Poutahidis et al. (2013) demonstrated that the pro-inflammatory cytokine IL-17 alone is capable of implicating a chronic inflammatory pathway in male hypogonadism, while probiotics can attenuate the incidence of male hypogonadism. Oral administration of *L. reuteri* ATCC PTA 6475 inhibited diet-induced obesity by restoring the Treg/Th₁₇ balance and suppressing the systemic pro-inflammatory cytokine IL-17, thus demonstrating its potential in preventing male hypogonadism (Poutahidis et al. 2013). Poutahidis et al. (2014) then evaluated the anti-inflammatory effect of *L. reuteri* ATCC PTA 6475 in sustaining reproductive fitness in aging mice. Interestingly, the authors found that oral administration of *L. reuteri* ATCC PTA 6475 (3.5×10^5 CFU/mouse/day) at 2 months of age significantly increased the seminiferous tubule cross-sectional area, conspicuous Leydig cell area, and testicular weight of aging mice (aged of 5 months old). The beneficial effect of *L. reuteri* in the testicular health of 12-month-old mice was also recapitulated by blocking IL-17 signaling, indicating that systemic pro-inflammatory cytokine IL-17 could be a potential immunological target of probiotics in male hypogonadism. Recently, Al-Asmakh et al. (2014) have also reported that the gut microbiota plays a role in testicular health by modulating the permeability of the blood-testis barrier. Considering that probiotics can promote healthy gut microbiota to maintain immune homeostasis, probiotics may also be able to modulate the permeability of the blood-testis barrier and contribute to testicular health.

4 Future Trends

Despite the extensive use of probiotics as immune modulators in the treatment of gastrointestinal disorders, recent trends in probiotic research have clearly indicated a tendency toward discovering the potential applications of probiotics in

non-gastrointestinal diseases. There are a growing number of studies suggesting that probiotics may stimulate and/or regulate gastrointestinal immune responses at both the local and systemic levels, thereby exerting beneficial effects beyond the gut (Vandenplas et al. 2014). Until today, the precise mechanisms of local and/or systemic immunomodulation by probiotics remain largely unknown. The turning point came in year 2009, when Ventura et al. (2009) suggested that probiogenomics could pave the way for the identification of genes and/or cell constituents in probiotics responsible for immune responses. Since then, omics high-throughput techniques, such as transcriptomics, proteomics, and metabolomics are continuously being introduced in the field of probiotics. Specifically, probiogenomics and these high-throughput analyses can be combined and used to bridge the mechanistic gap between genotype and phenotype, which could provide valuable insights into the way probiotics modulate the gastrointestinal and non-gastrointestinal immune responses in the human host (Sánchez et al. 2013). Meanwhile, we are approaching a new frontier, where computational (in silico) tools can be applied to screen and predict the immunological targets of probiotics through the discipline of immunoinformatics (Flower 2007). By using this approach, the immunogenicity of immunogenic substances of probiotics can be predicted based on the sum of predicted binding energies. On the other hand, Wendelsdorf et al. (2012) have also developed an in silico gut, known as ENteric Immunity Simulator (ENISI) for the studies of the inflammatory and regulatory immune responses in the gastrointestinal tract. With ENISI, we can now better understand immunological mechanisms of gastrointestinal pathogens in the gut and facilitate subsequent screening of probiotics that can be potentially deployed in the treatment of gastroenteric infections. Altogether, these prerequisites could not only facilitate and hasten the discovery of plausible and new immunity mechanisms of probiotic strains but also lead to a boost in the development of immunobiotic products in the near future. Nonetheless, a large number of clinical trials are still needed to determine the immunological efficacy and safety of beneficial probiotic strains for specific health claims.

5 Conclusions

In addition to regulating the gastrointestinal mucosal immunity, probiotics have the ability to modulate the activity of immune cells in both the innate and adaptive immune systems. Hence, probiotics represent a potential breakthrough therapeutic approach for other organs and systemic autoimmune disorders. It appears that immunostimulatory and/or immunoregulatory effects of probiotics on the human host are strain-specific and operate through a specific mechanism. Certainly, recent advances in experimental and computational tools, as well as animal models, can now be applied to unravel the mechanisms underpinning the immunomodulation effect of probiotic strains and to accelerate future development of probiotic-based products.

Conflict of Interest No.

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