Probiotics and probiotic-based vaccines: A novel approach for improving vaccine efficacy

Nesa Kazemifard1†, Abolfazl Dehkohneh2,3† and Shaghayegh Baradaran Ghavami1*

1Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran, 2Department 4 – Materials and the Environment, Bundesanstalt für Materialforschung und –prüfung (BAM), Berlin, Germany, 3Department of Biology Chemistry Pharmacy, Freie Universität Berlin, Berlin, Germany

Introduction

Vaccination is defined as the stimulation and development of the adaptive immune system by administering specific antigens. Vaccines’ efficacy, in inducing immunity, varies in different societies due to economic, social, and biological conditions. One of the influential biological factors is gut microbiota. Cross-talks between gut bacteria and the host immune system are initiated at birth during microbial colonization and directly control the immune responses and protection against pathogen colonization. Imbalances in the gut microbiota composition, termed dysbiosis, can trigger several immune disorders through the activity of the adaptive immune system and impair the adequate response to the vaccination. The bacteria used in probiotics are often members of the gut microbiota, which have health benefits for the host. Probiotics are generally consumed as a component of fermented foods, affect both innate and acquired immune systems, and decrease infections. This review aimed to discuss the gut microbiota’s role in regulating immune responses to vaccination and how probiotics can help induce immune responses against pathogens. Finally, probiotic-based oral vaccines and their efficacy have been discussed.

KEYWORDS
probiotics, vaccine, vaccine efficacy, probiotic-based vaccines, gut microbiota, adaptive immunity
the immune system; hence, its composition might affect how individuals respond to vaccinations (6, 7).

Gut microbiota develops alongside host development and is affected by genetics and environmental factors, and is an integral part of the human body (8, 9). The microbiota interacts with the host in many ways. Cross-talks between gut bacteria and the host immune system are initiated at birth during microbial colonization (10). This interaction promotes the intestinal epithelial barrier, immune homeostasis, protects from pathogen colonization (11), and inhibits deleterious inflammatory reactions that would harm both the host and its gut microbiota (12). Gut lymph nodes, lamina propria, and epithelial cells (mucosal immune system) form a protective barrier for the integrity of the intestinal tract (13). Therefore the gut microbiota composition can affect the normal mucosal immune system (14).

During gut microbiota development, especially in early life, various factors can affect and alter its composition. For instance, the human gut changes considerably during the first 2 years of life as children grow from breast milk-dominated diets to solid foods and are exposed to vast numbers of bacterial species (15). Therefore undernourished children have been reported to have less mature gut microbiota compared to healthy children (16). Diet serves as a significant factor in gut microbiota composition in adults too. Various studies reported that a higher-fat diet in healthy adults appeared to be associated with unfavorable changes in gut microbiota, fecal metabolomics profiles, and plasma pro-inflammatory factors, which might result in long-term adverse consequences for health (17–19). In addition, metabolic diseases such as diabetes can alter the gut microbiome and disrupt gut bacterial equilibrium (20). Other factors, including physical activity, mental health, and obesity may also affect gut microbiota composition (21–23).

Imbalances in the gut microbiota composition, termed dysbiosis, can trigger several immune disorders through the activity of the adaptive immune system (24). For example, recent studies on this subject reported that germ-free (GF) mice had a reduced number of Th1 and Th17 cells. Th17 cells, which are grouped as CD4+ effector T cells that secrete IL-17, play an important role in host defense against extracellular pathogens and the development of autoimmune diseases (25–27). Moreover, in dysbiotic gut microbiota, the number of inducible Foxp3 Helio-Tregs (iTregs) is reduced significantly in colonic lamina propria (28). Other studies indicate that excessive use of antibiotics disrupting gut microbiota hemostasis in young children might delay or impair the proper development of IgG response and immune memory that profoundly impacts adulthood (29). This review highlighted studies about the relationship between gut microbiota and related immune responses after vaccination and the impact of gut microbiota dysbiosis on VE.

**Gut microbiota and vaccine efficacy**

Cross-talk between the gut microbiome and the immune system by producing various metabolites and antimicrobial peptides directly regulates innate and adaptive immunity (30) and its failure to regulate inflammatory responses could increase the risk of developing inflammatory conditions in the gastrointestinal tract (31). Therefore the gut microbiota impacts the efficacy of various immune system-related interventions, including prevention of human immunodeficiency virus (HIV) infection (32, 33), cancer immunotherapy (34–36), and dysregulation in gut microbial composition associated with autoantibodies production and autoimmune diseases (37–40). Several studies were designed to evaluate the relationship between gut microbiota and immune responses to assess vaccine efficiency. A study by Pulendran et al. showed that antibiotic consumption resulted in a 10,000-fold reduction in gut bacterial composition and reduced specific neutralization and binding antibody responses against the influenza vaccine, and a significant association between bacterial species and metabolic phenotypes in the gut was displayed in this study (41). Furthermore, infants who received oral polio vaccine (OPV), intramuscular tetanus-hepatitis B, and intradermal Bacillus Calmette–Guérin (BCG) vaccines had detectable levels of *Bifidobacterium longum* (B. longum) and displayed higher specific T cell responses, serum IgG and fecal polio-specific IgA levels. In contrast, a higher relative abundance of *Enterobacteriales* and *Pseudomonadales* was associated with lower specific T cell responses and serum IgG levels (6, 42). Another study on infants receiving BCG, OPV, tetanus toxoid (TT), and hepatitis B virus confirmed the previous results that *Bifidobacterium* abundance in early infancy might increase the protective effects of vaccines by enhancing immunologic memory (7). The concurrent presence of non-polio enterovirus (NPEV) and oral polio vaccination can affect VE and reduce OPV seroconversion (43).

One of the critical factors in VE is the expression of Toll-like receptor 5 (TLR5) within 3 days after vaccination, which correlates to the amount of hemagglutination inhibition (HAI) titers 4 weeks after influenza vaccination (44, 45). TLR5 is a cell receptor for the recognition of flagellin and stimulates inflammatory signaling and immune responses (46). In addition, trivalent inactivated influenza vaccination of *Trls−/−* mice resulted in reduced antibody titers. TLR5-mediated sensing of the microbiota also impacted antibody responses to the inactivated polio vaccine (47). NOD2 (Nucleotide-binding oligomerization domain 2), an intracellular pathogen recognition sensor, is associated with the immune system and VE stimulation (48, 49). Recognition of symbiotic bacteria by NOD2 in CD11c-expressing phagocytes helps the mucosal adjuvant activity of cholera toxin (CT), as confirmed by a study on mice (50).
One of the most influential factors that lead to dysregulation of gut microbiota dysbiosis is antibiotic exposure (51). In 1 study, it is demonstrated that antibiotics-induced dysbiosis in infant mice (but not adults) leads to impaired antibody responses and promotes ex vivo cytokine recall responses (52). Antibiotic-treated mice models also showed impaired oral immunization in response to cholera toxin (53) and dysregulation in the generation of anti-viral macrophages, virus-specific CD4 and CD8 T cells, and antibody responses following respiratory influenza virus infection (54, 55). Gut dysbiosis induced by antibiotics significantly decreased the activation of CD4+ T cells and CD8+ T cells and declined the level of memory of CD4+ T cells and CD8+ T cells in secondary lymphoid organs of the vaccinated animals (56). In a study on human adults with impaired microbiome induced by antibiotics, reduced antibody response to TIV in subjects with low pre-existing immunity to influenza virus was observed (41). However, adults receiving Rotavirus (RV), Pneumo23, and TT vaccines with antibiotics consumption showed increased fecal shedding of RV and changes in gut bacteria beta diversity which is associated with RV vaccine immunogenicity boosting (57). Although antibiotics consumption could not improve the immunogenicity of OPV in human infants, the reduction of enteropathy and pathogenic intestinal bacteria biomarkers were reported (58).

The composition of gut microbiota and its diversity are associated with the response of the immune system to vaccines. In this case, a study on specific pathogen-free layer chickens (SPF) showed that shifts in gut microbiota composition might result in changes in cell- and antibody-mediated immune responses to vaccination against influenza viruses (59, 60). Other experiments on adults receiving an HIV vaccine showed the immunogenicity of the vaccine was correlated with microbiota clusters (61). On the contrary, another study on human adults reported no differences in overall gut microbiota community diversity between humoral responders and non-responders to the oral Salmonella Typhi vaccine (62). Co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) in pig models revealed that high growth outcomes were associated with several gut microbiome characteristics, such as increased bacterial diversity, increased relative abundance of Bacteroides pectinophilus, decreased Mycoplasmaaceae species diversity, higher Firmicutes:Bacteroidetes ratios, increased relative abundance of the phylum Spirochaetes, reduced relative abundance of the family Lachnospiraceae, and increased Lachnospiraceae species (63). Diet is also influential on the gut microbiome and vaccine efficacy. A study showed that a gluten-free diet was associated with a reduced anti-tetanus IgG response, and it increased the relative abundance of the anti-inflammatory Bifidobacterium in the mice model (64).

Humans harbor several latent viruses, including cytomegalovirus (CMV) implicated in the modulation of host immunity (65). However, there is an insufficient understanding of the influence of lifelong persistent latent viral infections on the immune system (66). In a rhesus macaques model, subclinical CMV infection increased butyrate-producing bacteria and lower antibody responses to influenza vaccination (67).

Oral RV vaccines have the potential role in reducing the morbidity and mortality of RV infection that causes diarrhea-related death in children worldwide, but RV vaccines showed significantly lower efficacy in low-income countries (68, 69). A comparison between infants in India and Malawi and infants born in the UK showed that ORV immune response was significantly impaired among infants in the former. This result is linked with their gut microbiome composition, in which microbiota diversity was significantly higher among Malawan infants, while Indian infants had high Bifidobacterium abundance (70). Despite low RVV immunogenicity which was also reported in rural Zimbabwean infants, it was not associated with the composition or function of the early-life gut microbiome (71). Human gut microbiota transplanted pig models vaccinated with attenuated RV showed significantly enhanced IFN-γ producing T cell responses and reduced regulatory T cells and cytokine production (72). Moreover, poor diet decreased total Ig and HRV-specific IgG and IgA antibody titers in serum or ileum and it increased fecal virus shedding titers in human infant microbiome transplanted pig models (57, 73, 74). In a study on rural Ghana’s infants, RVV response was associated with an increased relative abundance of Streptococcus gallocclyticus, decreased relative abundance of phylum Bacteroidetes and higher Enterobacteria/Bacteroides ratio (75). Another study reported that RVV response correlates with a higher relative abundance of bacteria belonging to Clostridium cluster XI and Proteobacteria (76). Bacteroides thetaiotaomicron is also associated with anti-rotavirus IgA titer (77). However, a study on Nicaraguan Infants reported a limited impact of gut microbial taxa on response to oral RVV (78).

Recent studies indicated that dysbiosis might be relevant in systemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. Khan et al. indicated an association between dysbiosis and severe inflammatory response in coronavirus disease 2019 (COVID-19) patients. Decreased Firmicutes/Bacteroidetes ratio, induced by the depletion of Faecalibacterium prausnitzii (F. prausnitzii), Bacteroides plebeius (B. plebeius), and Prevotella, which utilize fiber, and a relative increase in Bacteroidetes species is associated with raised serum IL-21 levels and better prognosis (79). A study on a cohort of 100 patients revealed that the composition of the gut microbiome in patients with COVID-19 correlates with disease severity, plasma concentrations of several inflammatory cytokines, and tissue-damaged associated chemokines. Patients with COVID-19 are recommended to consume beneficial microorganisms with immunomodulatory potentials, such as F. prausnitzii, Eubacterium rectale, and several Bifidobacterium species, and
### TABLE 1  Probiotics’ effect on immune responses and vaccine efficacy.

| Probiotic strain | Participants | Vaccine | Effects of probiotics on vaccine response | Reference |
|------------------|--------------|---------|-----------------------------------------|-----------|
| **B. longum BB536** | Human infants | DTP (diphtheria, pertussis, and tetanus) | An increase in the ratio of IFN-γ/IL-4 secretion cells in the BB536 supplementation group | (92) |
| **L. paracasei 431** | Human adults | Inactivated trivalent influenza vaccine | No difference in A/H1N1, A/H3N2, and B strain-specific IgG/No difference in A/H1N1, A/H3N2, and B strain-specific IgA levels in saliva / No difference in seroconversion rates 3 w after vaccination | (118) |
| **L. rhamnosus GG** | Human pregnant women | Combined diphtheria-tetanus-acellular pertussis-Haemophilus influenza type b vaccine | Lower pneumococcal-specific IgG levels/Lower seroconversion rates for pneumococcal serotypes /Lower tetanus toxoid-specific IgG levels/No difference in Hib-specific IgG levels/Higher tolerogenic T regulatory (Treg) responses | (117) |
| **L. paracasei MoLac-1 (heat-killed)** | Human adults | Inactivated trivalent influenza vaccine | No differences in natural killer cell activity, neutrophil bactericidal or phagocytosis activity/No difference in IgA, IgG, and IgM levels/Higher H3N2 specific IgG levels/No difference in seroconversion rates | (119) |
| **B. lactis BB-12 / L. paracasei 431** | Human adults | Inactivated trivalent influenza vaccine | An increase in influenza-specific IgG levels/Higher seroconversion rates for IgG/Higher influenza-specific IgA levels in saliva /No differences in NK-cells activity, number of CD4+ T-lymphocytes and phagocytosis/No differences in INF-γ, IL-2, and IL-10 levels | (95) |
| **LGG and inulin** | Human adults | Nasal attenuated trivalent influenza vaccine | Increased seroprotection rate to the H3N2 strain, but not to the H1N1 or B strain | (106) |
| **B. longum BL999 /L. rhamnosus LPR** | Human infants | Hepatitis B Virus (HBV), DTP | An improvement in HepB surface antibody responses in subjects receiving monovalent and a DTPa-HepB combination vaccine at 6 months but not those who received 3 monovalent doses | (93) |
| **B. bifidum /B. infantis/ B. longum/ L. acidophilus** | Human infants | Measles-Mumps-Rubella-Variella vaccine | Higher overall seroconversion rates/No difference in specific seroconversion rates for rubella, mumps, measles, varicella/No difference in the rate of treatment-related adverse effects between the two groups | (96) |
| **L. acidophilus CRL431/ L. rhamnosus GG** | Human adults | Oral polio vaccine | An increase in poliovirus neutralizing antibody levels/ Increase in poliovirus-specific IgA and IgM levels /No change in poliovirus-specific IgG levels | (108) |
| **L. casei GG** | Human infants | Oral rotavirus vaccine | Higher number of rotavirus-specific IgM secreting cells/ Higher IgA seroconversion rates /Higher IgM seroconversion rates | (107) |

(Continued)
TABLE 1 (Continued)

| Probiotic strain | Participants | Vaccine | Effects of probiotics on vaccine response | Reference |
|------------------|--------------|---------|------------------------------------------|-----------|
| *Escherichia coli* Nissle 1917 | Ciprofloxacin (Cipro)-treated Gn piglets colonized with a defined commensal microbiota (DM) | Virulent human rotavirus (HRV) | An increase in the numbers of total immunoglobulin-secreting cells, HRV-specific antibody-secreting cells, activated antibody-forming cells, memory antibody-forming B cells, and naive antibody-forming B cells/ A Decreased in levels of pro-inflammatory but increased levels of immuno-regulatory cytokines and increased frequencies of Toll-like receptor-expressing cells | (109) |
| *Lactobacillus GG* | Human gut microbiome transplanted neonatal Gn pig | Attenuated HRV vaccine | Significantly enhancement in HRV-specific IFN-γ producing T cell responses to the AttHRV vaccine. Neither doses of LGG significantly improved the protection rate, HRV-specific IgA and IgG antibody titers in serum, or IgA antibody titers in intestinal contents | (72) |
| *L. plantarum* | 24-Month-old children | - | An increase in fecal sIgA titer /A Significant positive correlation between TGF-β1, TNF-α, and fecal sIgA | (100) |
| *B. longum* + glucose-oligosaccharide | Human adults | Influenza seasonal vaccine | Significantly higher number of senescent (CD28–CD57+) helper T cells/Significantly higher plasma levels of anti-CMV IgG and a greater tendency for CMV seropositivity/Higher numbers of CD28–CD57+ helper T cells | (94) |
| *L. plantarum* GUANKE (LPG) | Mice | SARS-CoV-2 vaccine | Enhancement of SARS-CoV-2 neutralization antibodies production/A boost in specific neutralization antibodies >8-fold in bronchoalveolar lavage and >2-fold in sera when LPG was given immediately after SARS-CoV-2 vaccine inoculation /Persistence in T-cell responses | (103) |
| *Lactococcus lactis* strain plasma (LC-Plasma) | Human adults | Dengue fever (DF) | Significant reduction in the cumulative incidence days of DF-like symptoms/Significantly reduced severity score in the LC-Plasma group | (111) |
| *Lactobacillus plantarum* Probi-88 | *In vitro* and *in silico* study | SARS-CoV-2 infection | A significant inhibition in the replication of SARS-CoV-2 and the production of reactive oxygen species (ROS) levels/A significant reduction in inflammatory markers such as IFN-α, IFN-β, and IL-6 | (104) |
| *Lactobacillus* probiotic | Chickens | Herpesvirus of turkeys vaccine | An increase in the expression of major histocompatibility complex (MHC) II on macrophages and B cells in spleen/A decrease in the number of CD4+CD25+ T regulatory | (99) |
| Probiotic strain | Participants | Vaccine  | Effects of probiotics on vaccine response                                                                 | Reference |
|-----------------|--------------|----------|----------------------------------------------------------------------------------------------------------|-----------|
| *Escherichia coli*  
 *Nissle (EcN) 1917* | Malnourished piglet model transplanted with human infant fecal microbiota (HIFM) | HRV vaccine | Increased frequencies of activated plasmacytoid dendritic cells (pDC) and activated conventional dendritic cells (cDC)/increase frequencies of systemic activated and memory antibody-forming B cells and IgA+ B cells in the systemic tissues/Increase in the mean numbers of systemic and intestinal HRV-specific IgA antibody-secreting cells (ASCs), as well as HRV-specific IgA antibody titers in serum and small intestinal contents | (110)   |
| *Bacillus velezensis* | Pigeons | Pigeon circovirus (PiCV) | Significant reduction in the PiCV viral load in the feces and spleen of pigeons/Up-regulation in Interferon-gamma (IFN-γ), myxovirus resistance 1 (Mx1), signal transducers and activators of transcription 1 (STAT1), toll-like receptor 2 (TLR2) and 4 (TLR4) gene expression | (115)   |
| *Lactococcus lactis*  
 NZ1330 | BALB/c Mouse Model | Allergy to Amaranthus retroflexus pollens | Significantly reduction in the serum IgE level/Best performance in terms of improving allergies to Th1 and Treg responses | (112)   |
| *L. acidophilus; L. plantarum; Pediococcus pentosaceus; Saccharomyces cerevisiae; B. subtilis; R. licheniformis long-chain inulin (lcITF) and L. acidophilus W37 (LaW37)* | Broiler chickens | Salmonella Enteritidis (SE) vaccine | Diminished the negative effect of live vaccine growth performance/reduced mortality rate, fecal shedding, and re-isolation of SE from liver, spleen, heart, and cecum | (102)   |
| *L. rhamnosus GG* (LGG) | Piglets | Salmonella Typhimurium strains (STM) | Enhanced vaccination efficacy by 2-fold /Higher relative abundance of Prevotellaceae and lower relative abundance of Lactobacillaceae in feces/Increased the relative abundance of fecal lactobacilli was correlated with higher fecal consistency | (105)   |
| *Clostridium butyricum and Saccharomyces boulardii* | Piglets | - | Increased the plasma concentrations of IL-23, IL-17, and IL-22, as well as the plasma levels of anti-M.hyo and anti-PCV2 antibodies/Decreases in inflammation levels and oxidative stress injury, and improvement of intestinal barrier function | (116)   |
| *L. rhamnosus GG* (LGG) | Patients with type 1 diabetes | Betapropiolactone- whole inactivated virus | Reduction in the inflammatory responses (i.e., IFN-γ, IL17A, IL-17F, IL-6, and TNF-α)/Significantly | (120)   |
The effects of probiotics on vaccine efficacy

Probiotics are live commensal microorganisms that have positive benefits for the host that are generally consumed as a component of fermented foods. They have an impact on both innate and adaptive immune systems and decrease infections (87, 88). A meta-analysis comprising 1,979 adults showed that probiotics and prebiotics effectively promote immunogenicity by influencing seroconversion and seroprotection rates in adults vaccinated with influenza vaccines (89).

Bifidobacteria (BIF) is one of the probiotics and beneficial bacteria for human and animal health, having roles in the prevention of infection, modulation of lipid metabolism, and reduction of allergic symptoms by stimulating the host's mucosal immune system and systemic immune response (90, 91). Consumption of B. longum BB536 in newborns showed an increase in the number of interferon-γ (IFN-γ), a representative cytokine for T helper 1 response, secretion cells, and the ratio of IFN-γ/IL-4 secretion cells (92). In addition, a combination of B. longum BL999 and Lactobacillus rhamnosus (L. rhamnosus) [LPR (CGMCC1.3724)] consumption after Hepatitis B vaccination resulted in improved antibody responses (93). The results of a study on adults who received seasonal influenza vaccines was the same. Probiotic consumption (B. longum bv. infantis CCUG 52,486, combined with a prebiotic gluco-oligosaccharide) could improve total antibody titers and seroprotection (94). Bifidobacterium lactis BB-12 and Lactobacillus paracasei (L. paracasei) 431 improved specific Antibody titers and seroconversion rates after influenza vaccination but there was no difference in INF-γ, IL-2, and IL-10 levels (95). In a randomized placebo-controlled, double-blinded prospective trial, the effect of probiotics [Bifidobacterium bifidum, B. infantis, B. longum, and Lactobacillus acidophilus (L. acidophilus)] on vaccination efficacy could not be proven statistically (96).

Strains of Lactobacillus are a subdominant component of the commensal human intestinal microbiota and are identified as a potential driving force in the development of the human immune system (97). They exert early immunostimulatory effects that may be directly linked to the initial inflammation responses in human macrophages (98). Chickens who
FIGURE 1
How to build a probiotic-based vaccine: 1. Extract the antigen gene from the pathogen, 2. Amplify the gene by polymerase chain reaction (PCR), 3. Build a recombinant expression plasmid by ligating antigen gene into a proper plasmid, 4. Transfect recombinant plasmid into a probiotic.
(Continued)
received *Lactobacillus* spp as probiotics showed an increased major histocompatibility complex (MHC) II expression on macrophages and B cells. The number of CD4 + CD25 + T regulatory cells was also reduced in the spleen (99). In a study, the probiotic function of *Lactobacillus plantarum* (*L. plantarum*) was assessed and the results showed that fecal secretory immunoglobulin A (sIgA) titer significantly increased in the probiotic group infants (100). Another study on chicken models showed that a mixture of probiotic *Lactobacillus acidophilus*; *Lactobacillus plantarum*; *Pediciococcus pentosaceus*; *Saccharomyces cerevisiae*; *Bacillus subtilis*, and *Bacillus licheniformis* in broiler chickens resulted in the diminished adverse effect of live vaccine, reduced mortality rate, fecal shedding, and re-isolation of *Salmonella Enteritidis* (SE) from liver, spleen, heart, and cecum against SE vaccine (102). On this subject, oral administration of *L. plantarum* GUANKE (LPG) on mice models acted as a booster for COVID-19 vaccination and boosted >8-fold specific neutralization antibodies in bronchoalveolar lavage (BAL) and >2-fold in serum (103). An in-vitro and in-silico study showed that *L. plantarum* could reduce inflammatory markers such as IFN-α, IFN-β, and IL-6 and block virus replication by interaction with SARS-CoV-2 helicase (104). *L. acidophilus* W37 (LaW37) with long-chain inulin (lcITF) was also used as a probiotic in a study on piglets and increased two-folded vaccine efficacy against *Salmonella Typhimurium* strains (STM) (105).

A pilot study on adults who received the influenza vaccine reported that *L. rhamnosus GG* (LGG) could be an influential adjuvant to improve influenza vaccine immunogenicity (106). LGG also improves T cell responses but not antibody production on human gut microbiota (HGM) transplanted gnotobiotic (Gn) pig model vaccinated with AttHRV (72). However, specific RV antibody production was stimulated in infants who received LGG (107). Another study confirms that the combination of *L. acidophilus* CRL431 and LGG enhanced IgA and IgM (but not IgG) production after OP vaccine (108).

Other types of probiotics have been studied on this subject as well. For example, *Escherichia coli Nissle* (EcN) 1917 was used to colonize antibiotic-treated and human infants fecal microbiota transplanted Gn piglets and immune response was evaluated to human Rotavirus (HRV). As a result, the humoral and cellular immune responses were enhanced, and EcN biofilm increased the frequencies of systemic memory and IgA + B cells (109, 110). Likewise, the *Lactococcus lactis* strain decreased severity and symptoms in volunteers with Dengue fever (DF) compared to the placebo group, promoted IFN-γ and TGF-β cytokines secretion, and reduced serum IgE and IL-4 cytokine levels in mice models (111, 112). *Bacillus toyonensis* (*B. toyonensis*) BCT-7112 was also enabled to improve the humoral immune response of ewes against the clostridium perfringens epsilon toxin (rETX) vaccine and boost higher neutralizing antibody titers (113). *B. toyonensis* and *Saccharomyces boulardii* also successfully boosted antibody production and expression of IFN-γ, IL2, and Bcl6 genes in *Clostridium chauvoei* vaccinated sheep (114). Likewise, *Bacillus velezensis* significantly reduced the pigeon circovirus (PiCV) viral load in the feces and spleen of pigeons and promoted TLR 2&4 expression (115). Fecal microbiome transplantation with *Clostridium butyricum* and *Saccharomyces boulardii* treatment in piglets not only improved plasma concentrations of IL-23, IL-17, IL-22 and specific antibodies against *Mycoplasma hyopneumoniae* (M. hyo) and Porcine Circovirus Type 2 (PCV2), but also decreased the inflammation levels and oxidative stress injury, and improved intestinal barrier function (116).

Although several studies reported a positive effect of *Lactobacillus* on VE, some studies yielded different results. For example, maternal LGG supplementation showed decreased specific antibody responses in tetanus, Haemophilus influenza type b (Hib), and pneumococcal conjugate (PCV7) vaccinated infants (117). Also, probiotic consumption containing *Lactobacillus strains* (*L. paracasei* and *Lactobacillus casei* (*L. casei*) 431 showed no effects on the immune response to the influenza vaccine but shortened the duration of respiratory symptoms (118). Another study on *L. paracasei* and MoLac-1 (heat-killed) supplemented diet reported the same results, and these probiotics could not boost immune responses after vaccination (119). A recent study also assessed LGG consumption impact on influenza vaccine efficacy in type 1 diabetic (T1D) children and reported no significant improvement in humoral response in the probiotic group (120).

In conclusion, although some studies show that probiotics are inefficient in boosting the immune system and increasing vaccine efficacy, most studies demonstrated the positive effects of probiotics on promoting vaccine immunity and protecting the gut barrier simultaneously (Table 1).

### Probiotic-based vaccines

One efficient way to increase VE, produce a better immune response to an antigen, and reduce attenuated vaccine risk is to utilize recombinant antigens in gut microbiota vectors. Based on this idea, several probiotic-based vaccines were developed...
IgA antibodies production against TGEV spike glycoprotein induction of local mucosal immune responses and IgG and expressing TGEV spike glycoprotein. Results on mice revealed volunteers with this probiotic-based vaccine (chickens (proteins derived from the H9N2 virus successfully induces of animals that received spike-protein subunit VP8 in the mouse model. The serum Frontiers in (Kazemifard et al. /three./three/eight/nine/fmed./two/zero/two/two/nine/four/zero/four/five/four.

**Lactobacillus casei** strains are known for their immune stimulatory effect and have been used as probiotics for many years. A genetically engineered **L. casei** oral vaccine expressing dendritic cell (DC)-targeting peptide for Porcine epidemic diarrhea (PED) resulted in significantly elevated levels of anti-PEDV specific IgG and IgA antibodies in mice and piglets (133, 134). Yoon et al. expressed poly-glutamic acid synthetase A (pgsA) protein from HPV-16 L2 in **L. casei**, and interestingly, L2-specific antibodies had cross-neutralizing activity against diverse HPV types in the mouse model (135). Recombinant **L. casei** was also used for immunizing piglets against TGEV. As a result, solid cellular response, switching from Th1 to Th2-based immune responses, and IL-17 expression in systemic and mucosal immunity was reported (136). In another study, α, β1, and β2 toxoids of *Clostridium perfringens* expressed in **L. casei** ATCC 393 vector and elevated the levels of antigen-specific mucosa sIgA and sera IgG antibodies with exotoxin-neutralizing activity were seen in rabbit models (137). A different study used this probiotic expressing the VP2 protein of infectious pancreatic necrosis virus (IPNV) and reported induction of local mucosal and systemic immune responses in rainbow trout juveniles (138).

Other strains of *lactobacillus* are used in this technique as well. Oral recombinant **Lactobacillus** vaccine containing VP7 antigen of porcine rotavirus (PRV) showed stimulation in the differentiation of dendritic cells (DCs) in Peyer's patches (PPs) significantly, increased serum levels of IL-4 and IFN-γ and production of B220+ B cells in mesenteric lymph nodes (MLNs). Also, it increased the titer levels of the VP7-specific antibodies in mice models (139). Recombinant **L. Plantarum** expressing H9N2 avian influenza virus used for specific pathogen-free (SPF) 3-week-old chickens and could elicit humoral and cellular immunity (140). Shi et al. showed excessive serum titers of hemagglutination-inhibition (HI) antibodies in mice, and robust T cell immune responses in both mouse and chicken H9N2 vaccinated models by Recombinant **L. plantarum** (141). **L. Plantarum** NC8, expressing oral rabies vaccine G protein fused with a DC-targeting peptide (DCgp), resulted in more functional maturation of DCs and a strong Th1-biased immune response in mice (142). A recent study utilized **L. Plantarum** for developing SARS-CoV-2 food-grade oral vaccine. The results indicated that the spike gene could be efficiently expressed on the surface of recombinant **L. Plantarum** and displayed high antigenicity (143). As a novel approach for vaccination against SARS-CoV-2, **L. plantarum** strain expressing the SARS-CoV-2 spike protein was used, and high yields for S protein were obtained in an engineered probiotic group in vitro (143). In murine models, **Lactobacillus pentosus** expressing D antigenic site of spike glycoprotein transmissible gastroenteritis
coronavirus (TGEV) could induce IgG and slgA against this virus (144). Recombinant Lactobacillus rhamnosus that contains Koi herpesvirus (KHV) ORF81 protein in vaccinated fish was also successfully generated antigen-specific IgM with KHV-neutralizing activity (145). Another study used Lactobacillus acidophilus vector with the membrane-proximal external region from HIV-1 (MPER) and secreted interleukin-1β (IL-1β) or expressed the surface flagellin subunit C (FltC) as adjuvants, and reported as an improved vaccine efficacy and immune response against HIV-1 in mice (146). These studies demonstrated that probiotics have a potential role in acting as a shuttle for recombinant oral vaccines and successfully promoting the immune system against pathogens, and improving intestinal condition simultaneously.

Future perspective

There is no doubt that gut microbiota significantly impacts human metabolism and the immune system. Even further, some scientists consider gut microbiota as an endocrine organ in the human body. Probiotics are part of gut microbiota that have health benefits and promote immune responses. Based on the impact of gut mucosal immunity in the humoral immune response to vaccination, using probiotics as an immune booster next to oral vaccines can lead to better immunity, and probiotic-based recombinant vaccines promise a better generation of recombinant vaccines. Although a few human studies were performed on this subject, probiotics and probiotic-based recombinant vaccines’ efficacy on immunity against pathogens is promising. Such a new oral vaccine against SARS-CoV-2 infection was developed by Symvivo Corporation (a Vancouver-based Biotech Company) using Bifidobacteria longum, for expressing spike protein (named bacTRL-Spike), and it is under investigation in phase 1 clinical trials (NCT04334980). However, more studies need to be performed to detect the effectiveness of probiotics and engineered probiotic vaccines in clinical trials and investigate their role in human immunological pathways to ensure their safety and durable immunity.

Author contributions

NK: literature search, writing, and drawing of figures. AD and SB: literature search. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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