

## Probiotics and Vaccination in Children

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### Abstract

Immunisation is one of the most beneficial and cost-effective disease prevention measures. However several immunisations are associated with suboptimal seroconversion rates and so the protective effect is not optimal. In the last two decades the concept about the use of probiotic bacteria as novel mucosal adjuvants has engendered a lot of interest due to our increased immunological understanding and the availability of various techniques to enhance existing vaccine specific-immune responses. Mostly in developing countries, many people still die every year from vaccine-preventable diseases such as pneumonia and diarrhea. To date, emphasis has been placed on identifying novel vaccine antigens and adjuvants that induce stronger protective immune responses, as well as developing mucosally-administered vaccines. We would have enormous benefits in allowing safe administration of vaccines in remote areas and we may overcome the necessity for multiple doses. The precise mechanism of action of probiotics is not fully understood, but several animal and human studies have proven immunomodulatory effects involving both the humoral and cellular components of the host's immune system. This review discusses whether dietary supplementation with oral probiotics enhances the immune response of infants after routine vaccinations and also evaluates clinical effects of probiotics in adults. Further well designed, randomized, placebo-controlled studies are needed to understand fully the immunomodulatory properties of probiotics, whether the effects exerted are strain and age-dependent, and their clinical relevance in enhancing protection following vaccination.

**Keywords:** Infants; Immunization; Vaccine; Response; Mucosally-administered vaccines; Seroconversion rates; Bacteria

### Introduction

The development of vaccines, one of the most important medical interventions for improving the human health, dates back to the 19th century after the discovery by Koch and Pasteur that several infectious and potentially lethal diseases were caused by microorganisms. The studies on microbiological characteristics of pathogens and mechanisms of immune response established the principles for the development of the first type of vaccines through the isolation, inactivation and the following inoculations of infectious agents. Over the centuries, the spread of different diseases and the improvement in scientific research promote the development of new strategies of vaccination [1].

Current vaccines can be divided in two groups: live attenuated and inactivated. The first group includes vaccines against pathogens as smallpox, yellow fever, rubella, mumps and measles. They consist of weakened versions of the pathogen and mimic the protective immunity induced in people who survive live infection.

Several types of vaccines belong to the second group composed of toxoid vaccines against diphtheria and tetanus, carbohydrate vaccines against pneumococcus, conjugate vaccines against *Haemophilus influenzae* type B and meningococcus and subunit vaccines against recombinant hepatitis B virus. In order to enhance and modulate the

quality of a specific immune response, this kind of vaccine often contains different molecules used as adjuvants, which includes aluminium salts, surface-active substances, polyanions, bacterial extracts and, recently, also probiotics [2,3].

### Immunological Mechanisms of Vaccination

The effectiveness of vaccines is based on immunological memory that can be defined as a heightened immune response directed against a previously encountered microorganism and characterised by an increased number of antigen-specific cells and their capacity to respond to a secondary stimulation, through both antibody production and T cell responses [4].

The active immunization that results after vaccination is the consequence of the exposure of the host to an antigen followed by the stimulation of humoral and cell-mediated components of immune response enhancing the ability of the host to react to a second exposure to the same antigen.

Vaccines induce a specific immune response in the host through the activation of both innate and acquired immune cells. Antigen vaccines are able to recognize and activate PRRs (Pattern Recognition Receptors), including TLRs (Toll-like Receptors), on the surface of APCs (Antigen Presenting Cells), like Dendritic cells (DCs) and macrophages. This activation induces the development of a T cell-specific response, but also of a direct B cells antibody response [5].

The interaction between DCs and T cells through PRRs and TLRs induces the clonal expansion of T cells, usually regulated by a DCs-dependent cytokine network, including interleukin (IL)-12 and IL18, that results in interferon (IFN)- $\gamma$  production by T cells and particularly CD8<sup>+</sup>T cell expansion. Not only receptor activation, but also DCs subsets and local micro-environmental, influence the differentiation of CD4<sup>+</sup> T naïve cells and the consequent immune response [6].

In fact, after vaccine-specific activation, CD4<sup>+</sup> naïve T cells differentiate into many different T helper cell subpopulations such as Th1, Th2, Th9, Th17, Th21, TFH, and Treg cells, or in short-lived effector and memory cells. Moreover innate cells can induce activated T cells to migrate into mucosal tissues inducing a mucosal immunity [7].

Both activated T cells and innate immune cells can drive B cells to proliferate as plasma cells and undergo immunoglobulin class switching. Innate immune cells also regulate the strength and persistence of antibody responses through a mechanism involving TLR signaling and MyD88 and TRIF pathways in DCs. Most of the activated plasma cells have a short life, while only a small portion of them survive as memory cells for many years. These long-lived plasma cells are able to secrete antibodies in an antigen-independent way, maintaining constant antibody titres in serum and other body fluids. After a second encounter with the same antigen, memory B cells rapidly proliferate and differentiate resulting in an enhanced secretion of higher antibody levels with increased antigen affinity [8].

The development of memory B cells and persistent plasma cells is also regulated by the germinal center, with the interaction of DCs and PRRs and the recruitment of several signaling molecules as CD40, IL21, PD-1 and BAFF (B cell-activation factor). In particular, TF<sub>H</sub> cells have an important role in the regulation of memory B cells and persistent plasma cells development, enabling CD4<sup>+</sup> T cells to home in the follicles where they promote the differentiation of germinal center B cells, through the up-regulation of IL21 production and CXCL13 receptor (CXCR5) expression [9].

## Vaccination and Mucosal Immunity

Mucosal immune system represents the first line of defence against external pathogens playing a key role as barrier that protect the host from environmental injuries. The Mucosa-Associated Lymphoid Tissue (MALT), characterised by a network of tissues, immune cells and effector molecules, is the principal site of interaction between the host and the commensal bacteria of intestinal microflora [10].

It is anatomically organized in lymphoid micro-compartments such as the Peyer patches, the mesenteric lymph nodes, tonsils and adenoids which represent the most important mucosal inductive sites where immune responses are initiated, acting independently from the systemic immune apparatus [11].

The MALT is composed of cells from the innate and acquired immune system, including APCs (macrophages and DCs), neutrophils, NK cells, mast cells, as well as T and B cells that contribute in different ways to host defence against pathogens and initiating adaptive mucosal immune response.

TLR activation of mucosal APCs promotes both the initiation of a pro-inflammatory response against external pathogens, or the suppression of systemic immunity against non-pathogen antigens like

food proteins and microflora bacterial antigens, inducing oral tolerance [12].

Adaptive mucosal immune response is mainly mediated by secretory IgA (sIgA) antibodies, whose protease resistance makes this immunoglobulin subclass particularly suitable for functioning in mucosa secretion. Mucosal sIgA production is mediated by T helper cells and regulated by the synergic action of transforming growth factor (TGF)- $\beta$ , IL10 and IL4 which promote B cell switching to IgA production [13].

Moreover, mucosal cytotoxic T lymphocytes (CTL) have a crucial role in immune responses against enteric or respiratory viruses and intracellular parasites [14].

Most infections affect or start at a mucosal level of gastrointestinal and respiratory tracts and for this reason is now widely used in a mucosal route of vaccination. In fact, the topical mucosal application of a vaccine appears to be important for protection against non-invasive pathogens usually resistant to serum antibodies or passive passage across an epithelium.

Mucosal vaccines are particularly effective because they are able to mimic some characteristics of mucosal pathogens, such as the ability to adhere mucosal surfaces and M cells, to survive in lumen environments, to invade organized mucosal lymphoid tissue, to stimulate innate response and evoke adaptive immune response appropriate for the target pathogen [15].

The development of those vaccines requires efficient antigen delivery and adjuvant systems, in order to protect the vaccine from enzymatic digestion and elimination and encourage interaction with mucosal inductive sites or M cells.

## Maturation of Immune system, Gut Microbiota and Probiotics

Development of the immune system, since the first day of life, is strongly influenced both by the exposure to external antigens and the interaction between immune system and bacterial antigens of gut microbiota.

Soon after birth the correct colonization of gastrointestinal tract and microbial exposure give the most important environmental stimulation for the postnatal maturation of the immune system. Microbial activation of regulatory pathways through TLRs induces the growth and proliferation of APCs and T regulatory cells, essential for the regulation of T cell responses and in particular Th1/Th2 balance. The immune system is able to recognize microbial antigens of commensal bacterial and establishes a state of tolerance towards them [16].

Commensal bacteria are able to establish a symbiotic relationship with the host. In particular they not only facilitate absorption of nutrients, facilitating the hydrolysis of some otherwise indigestible carbohydrates, but also protect against intestinal colonization of pathogens, do not express virulence factors, and suppress proinflammatory processes as NF- $\kappa$ B pathway [17]. Moreover gut microbiota supports immune responses against viral infections through the reduction of PRR ligand release and the following up-regulation of pro-inflammatory cytokine production, such as IL-1 $\beta$  [18].

Among commensal bacteria, probiotics are defined as *“Live microorganisms which when administered in adequate amounts*

confer a health benefit on the host”[19]. Probiotics are able to colonize the gastrointestinal tract, interacting with intestinal epithelial cells (IEC) and macrophages and strengthening the mucosal barrier against pathogens [20].

Probiotics are able to indirectly modulate immune response influencing the composition of gut microbiota. They also have direct immunomodulatory activities, in particular they increase NK activity, they can induce cytokine production and the expression of co-stimulatory molecules by APCs and the maturation of DCs [21]. Their properties are not only species-specific, but also strain specific and the administration of probiotic preparation composed by different bacterial strains synergistically potentiate their action.

Due to their immunodulatory properties, probiotics are often used as a support in the therapy for allergic diseases, but also for the prevention of intestinal dysbiosis after antibiotic therapy and for the immune reconstitution of patients in critical illness.

## Probiotics and Vaccines

Probiotics have been shown to be immunomodulatory and can affect antibody responses following vaccination. Immunisation is one of the most beneficial and cost-effective disease prevention measures. However several immunisations are associated with suboptimal seroconversion rates and so the protective effect is not optimal. Oral probiotics given to infants during the period of immunization may improve the seroconversion rates [22].

To date, emphasis has been placed on identifying novel vaccine antigens and adjuvants that induce stronger protective immune responses, as well as developing mucosally-administered vaccines [23].

In this regard within the last two decades the concept about the use of probiotic bacteria as novel mucosal adjuvant has engendered a lot of interest due to our increased immunological understanding and the availability of various techniques to enhance existing vaccine specific-immune responses.

Probiotic bacteria have been suggested to confer a range of health benefits both in children [24-29] and adults [30]. Among the possible mechanisms explaining these effects is direct or indirect modulation of the intestinal immune system. Specific probiotic strains have indeed been shown to enhance local immunity through innate cell surface pattern recognition receptors or via direct lymphoid cell activation [31,32].

The reason for this lies in the discovery of the major immune-modulating role played by gut microbiota in the gastrointestinal tract, lactobacilli and bifidobacteria, in particular [33,34].

Although experimental data have shown that the endogenous microbiota plays a significant role in shaping development of the immune system [35-37]. The precise mechanism of action of probiotics is not fully understood, but several animal and human studies have proven immunomodulatory effects involving both the humoral and the cellular components of the host's immune system [22,38]. This review discusses whether dietary supplementation with oral probiotics enhances the immune response of infants after routine vaccinations and also evaluates clinical effects of probiotics in adults.

## Probiotics as Vaccine Adjuvants

The efficacy of vaccines is the result of a combination of factors that include the effectiveness of the specific vaccine; the type of adjuvant

included in the vaccine and the achievement of vaccine delivery which is in turn is influenced by cost and feasibility of route of administration.

The term adjuvant comes from the latin “adjuvare” meaning “to help”. Adjuvants are critical components of vaccines as they help the immune system respond to the vaccine by several proposed mechanisms such as immunomodulation via cytokine regulation as well as depot formation, which allows for sustained release at a site of injection to maintain a continual source of immune stimulation [39].

Potential vaccine adjuvants are a broad range of compounds such as mineral salts, saponins, liposomes, and particulate compounds [40-42]. The most commonly used **vaccine adjuvant** in humans since 1926 is alum [43]. The mechanisms of action of current human approved adjuvants such as alum are controversial and have several limitations [44]. To date, adjuvants capable of augmenting mucosal immune responses have had variable and limited success. The most studied of these, the mutant cholera and *E. coli*-derived toxin molecules, have been shown to promote elevated IgA responses to vaccine antigens but are associated with toxicity despite recent attempts to address this [45-49]. One of the advantages of using bacterial compounds as adjuvants is their ability to directly interact with and signal to the innate and adaptive immune systems via specific Toll-like receptors (TLRs). In particular, TLR ligands have shown promise as effective mucosal vaccine adjuvants. The **TLR4** agonist, monophosphoryl lipid A (MPL), derived from *Salmonella* minnesota-isolated LPS, combined with alum in the AS04 formulation, is currently the only vaccine adjuvant with demonstrated ability to enhance mucosal immune responses to vaccine antigen that is licensed in the U.S. for use in the human papillomavirus vaccine [50,51].

## Evidence from Clinical Trials of Probiotic Effect on Vaccine Immunity

Randomized, placebo-controlled clinical trials (RCTs) investigating the effectiveness of concomitant probiotics administration on the response to vaccination in infants, adults and some studies involving experimental animals have been evaluated.

Lactic acid bacteria (LAB) microbial communities normally present in the intestine of most animals play an important role in humans and other animals as immunomodulators. Probiotic microorganisms include the LAB *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* (LGG). Specifically, lactobacilli are reported to enhance the effectiveness of several candidate mucosal vaccines for malaria, **HIV**, and infantile diarrhea but these have predominately been examined in preclinical studies involving experimental animals [52-54].

There is some evidence that suggests LGG has an immunostimulating effect on oral rotavirus vaccination. One study examined the influence of *Lactobacillus casei* strain GG (currently known as *Lactobacillus rhamnosus* (GG or LGG) on the oral rotavirus vaccine. In the study, 2-5-month-old infants were given LGG or a placebo immediately before receiving the oral rotavirus vaccine (D x RRV) and for the subsequent 5 days. LGG significantly increased the number of rotavirus-specific immunoglobulin M (IgM) antibody secreting cells 8 days after vaccination, and a trend for higher rotavirus-specific IgA antibody titres was also observed in the probiotic group compared with the placebo group (P=0.05) [55].

In a small RCT, adults consumed either *L. rhamnosus* GG (LGG) or *L. paracasei* CRL431 orally for five weeks and immunized with a live attenuated oral poliovirus vaccine (containing serotypes 1,2 and 3). Probiotics increased poliovirus neutralizing antibody titers to poliovirus serotypes 1 and 2 (for LGG) and to serotype 3 (CRL431) [56].

In another study, LGG increased protective hemagglutinin inhibition titers in more adults than placebo following immunization with a live attenuated nasal influenza vaccine (LAIV) [57]. So *Lactobacillus* GG is potential as an important adjuvant to improve influenza vaccine immunogenicity.

In two studies involving experimental animals it has been reported two RCT that investigated the impact of colonization by probiotics. In the first study, it was investigated the effects of *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis* Bb12 (Bb12) on B lymphocyte responses to an attenuated human rotavirus (HRV) Wa strain vaccine in a neonatal gnotobiotic pig model. The findings suggest that soluble mediators such as CD14 (sCD14), cytokines, growth factors, and lactoferrin affect initial probiotic colonization, and together, they modulate neonatal antibody responses to oral attenuated human rotavirus vaccine in complex ways [58]. The other one examined the effects of co-colonization with *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis* Bb12 (Bb12) on 3-dose vaccination with attenuated HRV and challenge with virulent human rotavirus (VirHRV) were assessed in 4 groups of gnotobiotic (Gn) pigs: Pro+Vac (probiotic-colonized/vaccinated), Vac (vaccinated), Pro (probiotic-colonized, non-vaccinated) and Control (non-colonized, non-vaccinated). The results show that in the neonatal Gn pig disease model, selected probiotics contribute to immunomaturation, regulate immune homeostasis and modulate vaccine and virulent HRV effects, thereby moderating HRV diarrhea [59]. In contrast, adults treated with LGG or *L. lactis* for seven days and immunized with an oral *Salmonella typhi* Ty21a vaccine exhibited no significant changes in total or *S. typhi*-specific IgG, IgM, or IgA antibody-secreting cells (ASCs) although LGG did stimulate *S. typhi*-specific IgA ASCs in a greater number of subjects than *L. lactis* or placebo [60]. Moreover, neutrophil CR3 expression was up-regulated by *L. lactis*, suggesting that this probiotic enhances innate rather than adaptive immunity. These results indicate that probiotics may influence differently the immune response to oral *S. typhi* vaccine and that the immunomodulatory effect of probiotics is strain dependent. Similarly, adults treated with one of seven different probiotic strains (*B. lactis* Bi-07 and Bi-04, *L. acidophilus* La-14 and NCFM, *L. plantarum* Lp-115, *L. paracasei* Lpc-37, and *L. salivarius* Ls-33) had no difference in antigen-specific IgA or IgM levels following oral *Vibrio cholerae* vaccination, while a trend towards higher cholera-specific IgG levels was observed [61]. Some strains of probiotics demonstrated a faster immune response measured with serum immunoglobulin indicators, especially IgG, although overall vaccination was not influenced [61].

Another study evidenced no significant effect on vaccine responses by probiotics. It has evaluated with *Bifidobacterium breve* strain Yakult (BBG-01), given for 4 weeks, regarding the response to oral cholera vaccine in 2–5-year-old Bangladeshi children [62]. There were a significantly lower proportion of responders in the probiotic group for some viral-specific IgA antibodies compared with the placebo group.

Taylor et al. determined whether probiotic dietary supplementation in the first 6 months of life could modify allergen- and vaccine-specific

immune responses. The probiotic *Lactobacillus acidophilus* LAVRI-A1 (Probiomics) was fed to allergy-prone infants for the first 6 months of life and the response to tetanus vaccine was assessed at 2, 4 and 6 months [63]. The probiotic decreased the IL-10 response to tetanus toxoid antigen at 6 months compared with the placebo group and reduced IL-5 and transforming growth factor- $\beta$  (TGF- $\beta$ ) release by peripheral blood mononuclear cells (PBMCs) following stimulation with staphylococcal enterotoxin B (SEB). However, antibody responses to the vaccine were not reported.

In the study by West et al. [64], it was aimed at determining the impact of *Lactobacillus* F19 (LF19) during weaning on infections and IgG antibody responses to routine vaccines. 4-month-old infants were provided with a cereal containing *Lactobacillus paracasei* ssp. Paracasei strain F19 (LF19), or the same cereal without probiotic, daily for 9 months. The infants were immunized with DTaP (diphtheria, tetanus toxoid and acellular pertussis), polio and Hib vaccines at 3, 5.5 and 12 months. There was no significant effect of the probiotic on antibody titres to Hib, diphtheria and tetanus antigens measured before and after the second and third doses of vaccines. However, adjustment for breastfeeding duration suggested that the probiotic enhanced anti-diphtheria antibody titres in infants' breastfed for less than 6 months. A similar effect was observed for tetanus antigen, but there was no effect of LF19 on Hib vaccination.

In the study by Youngster et al. 8–10 month-old infants were provided with a probiotic formulation comprising *Lactobacillus acidophilus* ATCC4356, *Bifidobacterium bifidum* DSMZ20082, *Bifidobacterium longum* ATCC157078 and *Bifidobacterium infantis* ATCC15697 (Altman Probiotic Kid Powder) for 5 months in total, beginning 2 months prior to vaccination against mumps, measles, rubella and varicella (MMRV) [22]. While there was no significant difference in protective antibody titers to each individual vaccine component, when all antibody results were combined, there was a trend towards a greater percentage of infants reaching protective IgG antibody titers 3 months post-vaccination in the probiotic group [22].

Most vaccines are currently administered via the parenteral route either intramuscularly or subcutaneously. Therefore, probiotics also need to be able to enhance parenteral vaccine responses if they are to be of clinical benefit. Indeed, supplementation with a *Bifidobacterium longum* BL999 and *Lactobacillus rhamnosus* LPR mix to infants during the first six months of life doubled the serum anti-HBsAgIgG concentrations compared to placebo following a standard three-dose hepatitis B vaccination schedule, although this difference was not statistically significant [66]. In this study all infants received a monovalent HepB vaccine at birth and 1 month of age, and at 6 months they received either the monovalent HepB vaccine or a hexavalent diphtheria-tetanus-acellular pertussis (DTaP) combination vaccine containing a HepB component. There was a trend for the probiotic mix to increase HepB virus surface antibody (HBsAb) responses in those infants receiving HepB +DTaP, but, such as reported above, this was not statistically significant, and there was no effect of probiotics in infants receiving the monovalent HepB.

In the study by Olivares et al. adults were given *L. fermentum* CECT5716 and an inactivated trivalent influenza vaccine. The vaccination induced an increase in T-helper type 1 cytokine concentrations and in T-helper and T-cytotoxic proportions in both groups. In the case of the probiotic group, a significant increase in antigen specific immunoglobulin A was detected [66].

In a larger randomized clinical trial (RCT), treatment of adults with *B. lactis* BB-12 but not *L. paracasei* 431 significantly elevated influenza-specific IgG, IgG1, and IgG3 levels while both probiotics induced similar influenza-specific salivary IgA responses to placebo [67].

A similar effect was observed in another study. In this study to determine whether the size of the intestinal bifidobacterial population can influence the immune response to poliovirus vaccination, from birth to 4 months, infants were given a fermented formula containing *Streptococcus thermophilus* and *B. breve* or a standard formula (placebo) [68]. The results indicate that poliovirus-specific IgA levels in the feces were increased following pentavalent vaccine [diphtheria, tetanus, polio, *Haemophilus influenzae* type b (Hib), and pertussis vaccines] compared to placebo treatment, although the authors did not examine the adjuvant effect for the other administered vaccines.

The timing of probiotic administration is an important parameter to consider when evaluating their adjuvant effects. In particular, maternal (prenatal) treatment is suggested to be more effective as it provides added advantages to the infant via breast-feeding at a critical time when the neonatal immune system is rapidly developing.

In a randomized placebo-controlled double-blind allergy-prevention trial it was reported that a mixture of four probiotics combined with the pre-biotic galactooligosaccharide (GOS) on antibody responses to diphtheria, tetanus and *Haemophilus influenzae* type b (Hib) vaccines in 6-month-old infants [69]. Mothers of unborn children at increased risk for atopy received the probiotics during their

last month of pregnancy, and the same mixture was given in combination with GOS syrup to their newborns for 6 months. A protective Hib-specific IgG antibody response (>1 mg/ml) occurred more frequently in the probiotic group (16 of 29 infants) compared with the placebo group (6 of 25 infants), but there were no significant differences in vaccine-specific antibody titres between groups.

Another study suggests that maternal LGG supplementation may not be beneficial in terms of improving vaccine-specific immunity in infants. The effects of the probiotic, *Lactobacillus rhamnosus* GG (LGG) on immune responses to tetanus, *Haemophilus influenzae* type b (Hib) and pneumococcal conjugate (PCV7) vaccines in infants were investigated. This study was conducted as part of a larger clinical trial assessing the impact of maternal LGG supplementation in preventing the development of atopic eczema in infants at high-risk for developing allergic disease. Maternal LGG supplementation was associated with reduced antibody responses against tetanus, Hib, and pneumococcal serotypes contained in PCV7 but not total IgG levels. Maternal LGG supplementation was also associated with a trend to increased number of tetanus toxoid-specific T regulatory in the peripheral blood compared to placebo-treated infants. As probiotic immune effects can be species/strain specific, these findings do not exclude the potential use of other probiotic bacteria to modulate infant immune responses to vaccines [70].

In Table 1 the summary of the studies on probiotics adjuvants effects is shown.

Mucosally-administred vaccines		
Authors	Probiotics and vaccines	Biological effects
Mercenier et al. 2000 (preclinical studies involving experimental animals) [53]	<i>lactic acid bacteria</i> (LAB): <i>Lacto coccuslactis</i> , <i>Streptococcus gordonii</i> and <i>Lactobacillus spp</i> mucosal vaccines for malaria	It has been shown that systemic and mucosal antigen-specific immune responses can be elicited in mice through the nasal route using the three LAB systems under study.
Aldovini and Young et al. 1991 (preclinical studies involving experimental animals) [54]	<i>L. lactis</i> vaccine with the V2-V4 loop of the HIV virus	Induced humoral and cell-mediated immune response is sufficient to provide immunity against an HIV Envexpressing vaccinia virus challenge in mice
Isolauri et al. 1995 (in infants)	<i>L. casei</i> GG oral rotavirus vaccine	Increased levels of rotavirus-specific serum IgA.
de Vrese et al. 2005 (in adults) [55]	<i>L. rhamnosus</i> GG (LGG) or <i>L. paracasei</i> CRL431 live attenuated oral <b>poliovirus</b> vaccine (containing serotypes 1, 2, and 3)	Higher serum <b>neutralizing antibody</b> titers to poliovirus serotypes 1 and 2 (for LGG) and to serotype 3 (CRL431).
Davidson et al. 2011 (in adults) [56]	<i>L. rhamnosus</i> GG live attenuated nasal <b>influenza vaccine</b>	Increased protective <b>hemagglutinin</b> inhibition titers.
Fang et al. 2000 (in adult ) [60]	<i>L. rhamnosus</i> GG or <i>L. lactis</i> oral <i>Salmonella typhi</i> Ty21a	LGG stimulated <i>S. typhi</i> -specific IgA; <i>L. lactis</i> increased CR3 receptor expression on neutriphilis.
Chattha et al. 2013 ( studies involving experimental animals) [58]	<i>Lactobacillus rhamnosus</i> GG (LGG) and <i>Bifidobacterium lactis</i> Bb12 (Bb12) attenuated (Att) human rotavirus (HRV) Wa strain vaccine	Higher mean serum IgA HRV antibody titers and intestinal IgA antibody secreting cell numbers in Att-HRV vaccinated pigs; In vaccinated pigs without col/milk, probiotic colonization did not affect IgA HRV antibody titers.
Paineau et al.2008 (in adults) [61]	seven different probiotic strains ( <i>B. lactis</i> Bi-07 and Bi-04, <i>L. acidophilus</i> La-14 and NCFM, <i>L. plantarum</i> Lp-115, <i>L. paracasei</i> Lpc-37, and <i>L. salivarius</i> Ls-33)	No effect on antigen-specific IgA or IgM; A trend towards higher cholera-specific IgG levels was observed.

	oral <i>Vibrio cholera</i> vaccination	
Matsuda et al. 2011 (in infants) [62]	<i>Bifidobacterium breve</i> Ykult (BBG-01) oral inactivated cholera vaccine	No significant difference.
Taylor et al. 2006 (in infants) [63]	<i>Lactobacillus acidophilus</i> LAVRI-A1 allergen vaccine specific	Reduced production of IL-5 and TGF-beta; no significant effects of probiotics on either Type 1(Th1) or Type 2 (h2) T helper cell responses to allergens or other stimuli.
West et al. 2008 (in infants) [64]	<i>Lactobacillus</i> F19 (LF19) DTaP (diphtheria and tetanus toxoid and acellular pertussis), polio and Hib-conjugate vaccines	No difference in days with infectious symptoms; Days with antibiotic prescriptions were fewer and enhanced anti- diphtheria toxin (D) in the LF19 group;
Vlasova et al. 2013 (experimental animals) [59]	<i>Lactobacillus rhamnosus</i> GG (LGG) and <i>Bifidobacterium lactis</i> Bb12 (Bb12) attenuated HRV and challenge with virulent human rotavirus (VirHRV)	Selected probiotics contribute to immunomaturation, regulate immune homeostasis and modulate vaccine and virulent HRV effects, thereby moderating HRV diarrhea.
<b>Parenterally-administred vaccines</b>		
Soh et al. 2010 (in infants) [65]	<i>Bifidobacterium longum</i> BL999 and <i>Lactobacillus rhamnosus</i> LPR mix standard three-dose hepatitis B vaccination schedule	Doubled the serum anti-HBsAgIgG concentrations (this difference was not statistically significant).
Olivares et al.2007 (in adults) [66]	<i>L. fermentum</i> CECT5716 inactivated trivalent influenza vaccine	Higher <b>TNF-<math>\alpha</math></b> , total IgG and IgM, as well as influenza-specific IgA .
Rizzardini et al. 2011 (in adults) [67]	<i>B. lactis</i> BB-12 and <i>L. paracasei</i> 431 inactivated trivalent influenza vaccine	Elevated influenza-specific IgG, IgG1, and IgG3 levels (B. lactisBB-12); influenza-specific salivary IgA responses (both probiotics).
Mullie et al. 2004 (in infants) [68]	<i>Streptococcus thermophiles</i> and <i>B. breve</i> Pentacoq <sup>®</sup> vaccination diphtheria, tetanus, polio, <i>Haemophilus influenza</i> type b (Hib), and pertussis vaccines	Increased poliovirus-specific IgA levels in the feces.
Youngster et al. 2011 (in infants) [22]	<i>Lactobacillus acidophilus</i> ATCC4356, <i>Bifidobacterium bifidum</i> DSMZ20082, <i>Bifidobacterium longum</i> ATCC157078 and <i>Bifidobacterium infantis</i> ATCC15697 mumps, measles, rubella and varicella vaccine (MMRV)	No significant difference in protective antibody titres to each individual vaccine component.
<b>Prenatal treatment</b>		
Kukkonen et al. 2006 [69]	Probiotic combination (LGG, <i>L. rhamnosus</i> LC705, <i>B. breve</i> Bbi99, and <i>Propionibacterium freudenreichii</i> ) to mothers in the last four weeks until delivery and to their infants (together with a prebiotic, galacto-oligosaccharides) for the first six months <i>Haemophilus influenza</i> type b (Hib) vaccines	Higher serum levels of Hib-specific IgG in infants.
Licciardi et al. 2013 [70]	<i>L. rhamnosus</i> GG(LGG) tetanus, <i>Haemophilus influenzae</i> type b (Hib) and pneumococcal conjugate (PCV7) vaccines	Reduced antibody responses against tetanus, Hib and pneumococcal serotypes contained in PCV, but not total IgG levels.

**Table 1:** Summary of probiotics adjuvants effects.

## Conclusion

The majority of studies investigating the impact of probiotics on responses to vaccination have been conducted in healthy adults and there are limited studies in infants and the effects are not clear. There is strong evidence that probiotics reduce the incidence and duration of diarrhoeal infection among infants and adults [71].

Two studies monitored the incidence and duration of cold and flu-like symptoms following influenza vaccination has indeed identified a lower incidence of infections among that receiving probiotic treatment [56,66]. Influenza vaccination provides a particularly useful tool because it is used in routine clinical practice in elderly people, in whom seroprotection and seroconversion rates are low and correlate with poor protection.

There are trends towards better responses to vaccination in some of the studies, but effects are clearly limited. Although some studies are comparable in terms of duration of the intervention, age and characteristics of the infants, the probiotics administered are different in every case. Further research is required to compare the effects of different probiotics within a standardized study design.

Further well designed, randomized, placebo-controlled studies are needed to understand fully the immunomodulatory properties of probiotics, whether the effects exerted are strain and age-dependent, and their clinical relevance in enhancing protection following vaccination.

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