Vaccines & Vaccination

del Giudice et al., J Vaccines Vaccin 2014, 5:3
http://dx.doi.org/10.4172/2157-7560.1000226

Review Article open Access

Probiotics and Vaccination in Children

Michele Miraglia del Giudice[1], Salvatore Leonardi[2*], Francesca Galdo[1], Annalisa Allegorico[1], Martina Filippelli[2], Teresa Arrigo3, Carmelo Salpietro[3], Mario La Rosa[2], Chiara Valsecchi[4], Sara Carlotta Tagliacarne[4] , Anna Maria Castellazzi[4] and Gian Luigi Marseglia[4]

[1]*Department of Pediatrics F. Fede, Second University of Naples, Naples, Italy*

[2]*Department of Medical and Pediatric Science, University of Catania, Catania, Italy*

[3]*Department of Pediatric Sciences, University of Messina, 98100 Messina, Italy*

[4]*Department of Pediatrics, Foundation IRCCS Policlinico San Matteo, University of Pavia, Italy*

[*]**Corresponding author:** Salvatore Leonardi, Department of Medical and Pediatric Science, University of Catania, Catania, Italy, Tel: 095738302; E-mail: leonardi@unict.it

Received date: 19 March 2014; **Accepted date:** 24 April 2014; **Published date:** 26 April 2014

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)-γ production by T cells and particularly $CD8^+$T cell expansion. Not only receptor activation, but also DCs subsets and local micro-environmental, influence the differentiation of $CD4^+$ T naïve cells and the consequent immune response [6].

In fact, after vaccine-specific activation, CD4+ 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_H cells have an important role in the regulation of memory B cells and persistent plasma cells development, enabling $CD4^+$ 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)-, 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-kB 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-1β [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 adjuvantshas 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 discusseswhether 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 caseistrain 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 1,2and 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 Bl-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 cholera**e 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* LAVR1-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 6months compared with the placebo group and reduced IL-5 and transforming growth factor-β (TGF-β) 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. Paracaseistrain 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 1month 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 influenza* 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]	*lactic acid bacteria* (LAB): Lacto coccuslactis, *Streptococcus gordonii* and *Lactobacillus spp* 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]	*L. lactis* 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)	*L. casei* GG oral rotavirus vaccine	Incresed levels of rotavirus-specific serum IgA.
de Vrese et al. 2005 (in adults) [55]	*L. rhamnosus* GG (LGG) or *L. paracasei* CRL431 live attenuated oral **poliovirus** vaccine (containing serotypes 1, 2, and 3)	Higher serum **neutralizing antibody**titers to poliovirus serotypes 1 and 2 (for LGG) and to serotype 3 (CRL431).
Davidson et al. 2011 (in adults) [56]	*L. rhamnosus* GG live attenuated nasal **influenza vaccine**	Increased protective **hemagglutinin** inhibition titers.
Fang et al. 2000 (in adult) [60]	*L. rhamnosus* GG *or L. lactis* oral *Salmonella typhi* Ty21a	LGG stimulated S. typhi-specific IgA; L. lactis incresed CR3 receptor expression on neutriphilis.
Chattha et al. 2013 (studies involving experimental animals) [58]	*Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis* 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 (*B. lactis* Bi-07 and Bl-04, *L. acidophilus* La-14 and NCFM, *L. plantarum* Lp-115, *L. paracasei* Lpc-37, and *L. salivarius* Ls-33)	No effect on antigen-specific IgA or IgM; A trend towards higher cholera-specific IgG levels was observed.

	oral *Vibrio cholera* vaccination	
Matsuda et al. 2011 (in infants) [62]	*Bifidobacteriumbreve Ykult* (BBG-01) oral inactivated cholera vaccine	No significant difference.
Taylor et al. 2006 (in infants) [63]	*Lactobacillus acidophilus* 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]	*Lactobacillus* 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]	*Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis* 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.
Parenterally-administred vaccines		
Soh et al. 2010 (in infants) [65]	*Bifidobacterium longum* BL999 and *Lactobacillus rhamnosus* 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]	*L. fermentum* CECT5716 inactivated trivalent influenza vaccine	Higher **TNF**-α, total IgG and IgM, as well as influenza-specific IgA .
Rizzardini et al. 2011 (in adults) [67]	*B. lactis* BB-12 and *L. paracasei* 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]	*Streptococcus thermophiles and B. breve* Pentacoq® vaccination diphtheria, tetanus, polio, *Haemophilus influenza* type b (Hib), and pertussis vaccines	Increased poliovirus-specific IgA levels in the feces.
Youngster et al. 2011 (in infants) [22]	*Lactobacillus acidophilus* ATCC4356, *Bifido bacterium bifidum* DSMZ20082, *Bifidobacterium longum* ATCC157078 and *Bifidobacterium infantis* ATCC15697 mumps, measles, rubella and varicella vaccine (MMRV)	No significant difference in protective antibody titres to each individual vaccine component.
Prenatal treatment		
Kukkonen et al. 2006 [69]	Probiotic combination (*LGG, L. rhamnosus LC705, B. breveBbi99, and Propionibacterium freudenreichii*) to mothers in the last four weeks until delivery and to their infants (together with a prebiotic, galacto-oligosaccharides) for the first six months *Haemophilus influenza* type b (Hib) vaccines	Higher serum levels of Hib-specific IgG in infants.
Licciardi et al. 2013 [70]	*L. rhamnosus* GG(LGG) tetanus, *Haemophilus influenzae* 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.

References

1. Janeway CA, Travers P, Walport M, Shlomchik M (2007) Immunobiology. (7th edn), Garland Science.
2. Pérez O, Romeu B, Cabrera O, González E, Batista-Duharte A, et al. (2013) Adjuvants are Key Factors for the Development of Future Vaccines: Lessons from the Finlay Adjuvant Platform. Front Immunol 4: 407.
3. Coffman RL, Sher A, Seder RA (2010) Vaccine adjuvants: putting innate immunity to work. Immunity 33: 492-503.
4. Kurtulus S, Tripathi P, Hildeman DA (2013) Protecting and rescuing the effectors: roles of differentiation and survival in the control of memory T cell development. Front Immunol 3: 404.
5. Pulendran B, Ahmed R (2006) Translating innate immunity into immunological memory: implications for vaccine development. Cell 124: 849-863.
6. Kolumam GA, Thomas S, Thompson LJ, Sprent J, Murali-Krishna K (2005) Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. J Exp Med 202: 637-650.
7. Vitaliti G, Leonardi S, Miraglia Del Giudice M, Salpietro A, et al. (2012) Mucosal immunity and sublingual immunotherapy in respiratory disorders. Journal of biological regulators and homeostatic agents. 26: S85-93.
8. Manz RA, Hauser AE, Hiepe F, Radbruch A (2005) Maintenance of serum antibody levels. Annu Rev Immunol 23: 367-386.
9. Deenick EK, Ma CS, Brink R, Tangye SG (2011) Regulation of T follicular helper cell formation and function by antigen presenting cells. CurrOpinImmunol 23: 111-118.
10. Gill N, Wlodarska M, Finlay BB (2010) The future of mucosal immunology: studying an integrated system-wide organ. Nat Immunol 11: 558-560.
11. Mowat AM, Donachie AM, Parker LA, Robson NC, Beacock-Sharp H, et al. (2003) The role of dendritic cells in regulating mucosal immunity and tolerance. Novartis Found Symp 252: 291-302.
12. Iwasaki A, Medzhitov R (2010) Regulation of adaptive immunity by the innate immune system. Science 327: 291-295.
13. Asano T, Kaneko H, Terada T, Kasahara Y, Fukao T, et al. (2004) Molecular analysis of B-cell differentiation in selective or partial IgA deficiency. ClinExpImmunol 136: 284-290.
14. Staats HF, Bradney CP, Gwinn WM, Jackson SS, Sempowski GD, et al. (2001) Cytokine requirements for induction of systemic and mucosal CTL after nasal immunization. J Immunol 167: 5386-5394.
15. Mayer L, Shao L (2004) Therapeutic potential of oral tolerance. Nat Rev Immunol 4: 407-419.
16. Hooper LV, Gordon JI (2001) Commensal host-bacterial relationships in the gut. Science 292: 1115-1118.
17. Caballero-Franco C, Keller K, De Simone C, Chadee K (2007) The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am J PhysiolGastrointest Liver Physiol 292: G315-322.
18. Pang IK, Iwasaki A (2012) Control of antiviral immunity by pattern recognition and the microbiome. Immunol Rev 245: 209-226.
19. http://www.who.int/foodsafety/publications/fs_management/probiotics/en/
20. Thomas CM, Versalovic J (2010) Probiotics-host communication: Modulation of signaling pathways in the intestine. Gut Microbes 1: 148-163.
21. Niers LE, Hoekstra MO, Timmerman HM, van Uden NO, de Graaf PM, et al. (2007) Selection of probiotic bacteria for prevention of allergic diseases: immunomodulation of neonatal dendritic cells. ClinExpImmunol 149: 344-352.
22. Youngster I, Kozer E, Lazarovitch Z, Broide E, Goldman M (2011) Probiotics and the immunological response to infant vaccinations: a prospective, placebo controlled pilot study. Arch Dis Child 96: 345-349.
23. Licciardi PV, Tang ML (2011) Vaccine adjuvant properties of probiotic bacteria. Discov Med 12: 525-533.
24. Van Niel CW, Feudtner C, Garrison MM, Christakis DA (2002) Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. Pediatrics 109: 678-684.
25. Saavedra JM (2007) Use of probiotics in pediatrics: rationale, mechanisms of action, and practical aspects. NutrClinPract 22: 351-365.
26. del Giudice MM, Leonardi S, Maiello N, Brunese FP (2010) Food allergy and probiotics in childhood. J Clin Gastroenterol 44 Suppl 1: S22-25.
27. Miraglia Del Giudice M, Maiello N, Decimo F, Fusco N, D' Agostino B, et al. (2012) Airways allergic inflammation and L. reuterii treatment in asthmatic children. J BiolRegulHomeost Agents 26: S35-40.
28. Lionetti E, Francavilla R, Castellazzi AM, Arrigo T, Labò E, et al. (2012) Probiotics and Helicobacter pylori infection in children. J BiolRegulHomeost Agents 26: S69-76.
29. Castellazzi AM, Valsecchi C, Caimmi S, Licari A, Marseglia A, et al. (2013) Probiotics and food allergy. Ital J Pediatr 39: 47.
30. Santosa S, Farnworth E, Jones PJ (2006) Probiotics and their potential health claims. Nutr Rev 64: 265-274.
31. Isolauri E, Sütas Y, Kankaanpää P, Arvilommi H, Salminen S (2001) Probiotics: effects on immunity. Am J ClinNutr 73: 444S-450S.
32. Cross ML (2002) Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. FEMS Immunol Med Microbiol 34: 245-253.
33. del Giudice MM, Brunese FP (2008) Probiotics, prebiotics, and allergy in children: what's new in the last year? J Clin Gastroenterol 42 Suppl 3 Pt 2: S205-208.
34. del Giudice MM, Rocco A, Capristo C (2006) Probiotics in the atopic march: highlights and new insights. Dig Liver Dis 38 Suppl 2: S288-290.
35. Maeda Y, Noda S, Tanaka K, Sawamura S, Aiba Y, et al. (2001) The failure of oral tolerance induction is functionally coupled to the absence of T cells in Peyer's patches under germfree conditions. Immunobiology 204: 442-457.
36. Yamanaka T, Helgeland L, Farstad IN, Fukushima H, Midtvedt T, et al. (2003) Microbial colonization drives lymphocyte accumulation and differentiation in the follicle-associated epithelium of Peyer's patches. J Immunol 170: 816-822.
37. Macpherson AJ, Harris NL (2004) Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 4: 478-485.
38. del Giudice MM, Leonardi S, Ciprandi G, Galdo F, Gubitosi A, et al. (2012) Probiotics in childhood: allergic illness and respiratory infections. J Clin Gastroenterol 46 Suppl: S69-72.
39. Schijns VE (2000) Immunological concepts of vaccine adjuvant activity. CurrOpinImmunol 12: 456-463.
40. Cox JC, Coulter AR (1997) Adjuvants--a classification and review of their modes of action. Vaccine 15: 248-256.
41. Singh M, O'Hagan D (1999) Advances in vaccine adjuvants. Nat Biotechnol 17: 1075-1081.
42. Stertman L, Strindelius L, Sjöholm I (2004) Starch microparticles as an adjuvant in immunisation: effect of route of administration on the immune response in mice. Vaccine 22: 2863-2872.

43. Mbow ML, De Gregorio E, Ulmer JB (2011) Alum's adjuvant action: grease is the word. Nat Med 17: 415-416.
44. Kool M, Fierens K, Lambrecht BN (2012) Alum adjuvant: some of the tricks of the oldest adjuvant. J Med Microbiol 61: 927-934.
45. Holmgren J, Czerkinsky C, Eriksson K, Mharandi A (2003) Mucosal immunisation and adjuvants: a brief overview of recent advances and challenges. Vaccine 21 Suppl 2: S89-95.
46. Lawson LB, Norton EB, Clements JD (2011) Defending the mucosa: adjuvant and carrier formulations for mucosal immunity. CurrOpinImmunol 23: 414-420.
47. Skene CD, Sutton P (2006) Saponin-adjuvanted particulate vaccines for clinical use. Methods 40: 53-59.
48. Sugai T, Mori M, Nakazawa M, Ichino M, Naruto T, et al. (2005) A CpG-containing oligodeoxynucleotide as an efficient adjuvant counterbalancing the Th1/Th2 immune response in diphtheria-tetanus-pertussis vaccine. Vaccine 23: 5450-5456.
49. van Ginkel FW, Jackson RJ, Yuki Y, McGhee JR (2000) Cutting edge: the mucosal adjuvant cholera toxin redirects vaccine proteins into olfactory tissues. J Immunol 165: 4778-4782.
50. Evrard B, Coudeyras S, Dosgilbert A, Charbonnel N, Alamé J, et al. (2011) Dose-dependent immunomodulation of human dendritic cells by the probiotic Lactobacillus rhamnosus Lcr35. PLoS One 6: e18735.
51. Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, et al. (2012) Bifidobacteriuminfantis 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. Gut 61: 354-366.
52. Amdekar S, Dwivedi D, Roy P, Kushwah S, Singh V (2010) Probiotics: multifarious oral vaccine against infectious traumas. FEMS Immunol Med Microbiol 58: 299-306.
53. Mercenier A, Müller-Alouf H, Grangette C (2000) Lactic acid bacteria as live vaccines. Curr Issues MolBiol 2: 17-25.
54. Aldovini A, Young RA (1991) Humoral and cell-mediated immune responses to live recombinant BCG-HIV vaccines. Nature 351: 479-482.
55. Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T (1995) Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by Lactobacillus casei GG. Vaccine 13: 310-312.
56. de Vrese M, Rautenberg P, Laue C, Koopmans M, Herremans T, et al. (2005) Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination. Eur J Nutr 44: 406-413.
57. Davidson LE, Fiorino AM, Snydman DR, Hibberd PL (2011) Lactobacillus GG as an immune adjuvant for live-attenuated influenza vaccine in healthy adults: a randomized double-blind placebo-controlled trial. Eur J ClinNutr 65: 501-507.
58. Chattha KS, Vlasova AN, Kandasamy S, Esseili MA, Siegismund C, et al. (2013) Probiotics and colostrum/milk differentially affect neonatal humoral immune responses to oral rotavirus vaccine. Vaccine 31: 1916-1923.
59. Vlasova AN, Chattha KS, Kandasamy S, Liu Z, Esseili M, et al. (2013) Lactobacilli and bifidobacteria promote immune homeostasis by modulating innate immune responses to human rotavirus in neonatal gnotobiotic pigs. PLoS One 8: e76962.
60. Fang H, Elina T, Heikki A, Seppo S (2000) Modulation of humoral immune response through probiotic intake. FEMS Immunol Med Microbiol 29: 47-52.
61. Paineau D, Carcano D, Leyer G, Darquy S, Alyanakian MA, et al. (2008) Effects of seven potential probiotic strains on specific immune responses in healthy adults: a double-blind, randomized, controlled trial. FEMS Immunol Med Microbiol 53: 107-113.
62. Matsuda F, Chowdhury MI, Saha A, Asahara T, Nomoto K, et al. (2011) Evaluation of a probiotics, Bifidobacteriumbreve BBG-01, for enhancement of immunogenicity of an oral inactivated cholera vaccine and safety: a randomized, double-blind, placebo-controlled trial in Bangladeshi children under 5 years of age. Vaccine 29: 1855-1858.
63. Taylor AL, Hale J, Wiltschut J, Lehmann H, Dunstan JA, et al. (2006) Effects of probiotic supplementation for the first 6 months of life on allergen- and vaccine-specific immune responses. ClinExp Allergy 36: 1227-1235.
64. West CE, Gothefors L, Granström M, Käyhty H, Hammarström ML, et al. (2008) Effects of feeding probiotics during weaning on infections and antibody responses to diphtheria, tetanus and Hib vaccines. Pediatr Allergy Immunol 19: 53-60.
65. Soh SE, Ong DQ, Gerez I, Zhang X, Chollate P, et al. (2010) Effect of probiotic supplementation in the first 6 months of life on specific antibody responses to infant Hepatitis B vaccination. Vaccine 28: 2577-2579.
66. Olivares M, Díaz-Ropero MP, Sierra S, Lara-Villoslada F, Fonollá J, et al. (2007) Oral intake of Lactobacillus fermentum CECT5716 enhances the effects of influenza vaccination. Nutrition 23: 254-260.
67. Rizzardini G, Eskesen D, Calder PC, Capetti A, Jespersen L, et al. (2011) Evaluation of the immune benefits of two probiotic strains Bifidobacteriumanimalis ssp. lactis, BB-12® and Lactobacillus paracasei ssp. paracasei, L. casei 431® in an influenza vaccination model: a randomised, double-blind, placebo-controlled study. Br J Nutr 107: 876-884.
68. Mullie C, Yazourh A, Thibault H, Odou MF, Singer E, et al. (2004) Increased poliovirus-specific intestinal antibody response coincides with promotion of Bifidobacteriumlongum-infantis and Bifidobacteriumbreve in infants: a randomized, double-blind, placebo-controlled trial. Pediatr Res 56: 791-795.
69. Kukkonen K, Nieminen T, Poussa T, Savilahti E, Kuitunen M (2006) Effect of probiotics on vaccine antibody responses in infancy--a randomized placebo-controlled double-blind trial. Pediatr Allergy Immunol 17: 416-421.
70. Licciardi PV, Ismail IH, Balloch A, Mui M, Hoe E, et al. (2013) Maternal Supplementation with LGG Reduces Vaccine-Specific Immune Responses in Infants at High-Risk of Developing Allergic Disease. Front Immunol 4: 381.
71. Lomax AR, Calder PC (2009) Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. Curr Pharm Des 15: 1428-1518.