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Modulation of vaccine response by concomitant probiotic administration

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Evidence suggests that probiotic bacteria modulate both innate and adaptive immunity in the host, and in some situations can result in reduced severity of common illnesses, such as acute rotavirus infection and respiratory infections. Responses to vaccination are increasingly being used to provide high quality information on the immunomodulatory effects of dietary components in humans. The present review focuses on the effect of probiotic administration upon vaccination response. The majority of studies investigating the impact of probiotics on responses to vaccination have been conducted in healthy adults, and at best they show modest effects of probiotics on serum or salivary IgA titres. Studies in infants and in elderly subjects are very limited, and it is too early to draw any firm conclusions regarding the potential for probiotics to act as adjuvants in vaccination. Although some studies are comparable in terms of duration of the intervention, age and characteristics of the subjects, most differ in terms of the probiotic selected. Further well designed, randomized, placebo-controlled studies are needed to understand fully the immunomodulatory properties of probiotics, whether the effects exerted are strain-dependent and age-dependent and their clinical relevance in enhancing immune protection following vaccination.

Introduction

Evidence suggests that probiotic bacteria reduce the risk, and in some cases duration or severity, of infections [1]. In infants, a number of studies have demonstrated that probiotics reduce the clinical symptoms of diarrhoeal disease, particularly in acute rotavirus infection [2–6]. Most studies have shown that lactobacilli and bifidobacteria reduce the risk of antibiotic-associated diarrhoea, but data relating to duration and severity are inconsistent, perhaps due to some influence of the condition that the patient was being treated for [1]. Consumption of some probiotic strains may reduce incidence and/or severity of respiratory infections in children [7–9], adults [10] and in the elderly [11], although evidence is limited and studies investigating prevention of common respiratory illnesses have produced mixed results [1]. A recent systematic review identified 14randomized controlled trials (RCTs) investigating the effects of probiotics for the prevention of upper or lower respiratory infections [12]. The review concluded that 10 of these RCTs demonstrated no evidence of beneficial effects of probiotics on the incidence of respiratory disease, five out of six providing relevant data demonstrated a reduction in symptom severity, and three out of nine reported a shorter clinical course of infection in the probiotic groups [12].

Response to vaccination is increasingly being used as a surrogate for the response to infection and can therefore provide information on the immunomodulatory effects of dietary components, including probiotics, in humans [13]. Vaccine efficacy can be assessed by levels of vaccine-specific antibodies in the serum following vaccination, which directly correlate with protection and are described as a 'gold-standard' for determining the influence of probiotics on immune responses [14]. This review discusses the theoretical basis for modulation of immune response to vaccination by probiotics, and describes published studies investigating the impact of concomitant probiotic administration on the response to vaccination in infants, adults and elderly individuals.

Theoretical basis for modulation of immune response to vaccination by probiotics

Probiotics may influence immune function by direct and indirect actions. Direct effects include changes to the gut microbiota and alteration of the profile of pathogen-associated molecular patterns (PAMPs) presented to the gut associated lymphoid tissue. Indirect effects could arise from microbial products such as short chain fatty acids [15]. Evidence from animal models indicates that the resident gut microbiota shapes anti-viral defences and modulates the outcome of viral infections, with germ free mice more susceptible to a number of infections, including influenza [16]. Experiments with specific pathogen-free (SPF) mice treated with antibiotics support this. Antibiotic-treated SPF mice given a sub lethal dose of PR8 virus had impaired generation of virus specific antibody, cluster of differentiation 4+ (CD4+) and CD8+ T cell responses and delayed viral clearance [16]. Treatment with antibiotics also reduced migration of respiratory dendritic cells from the lung to the draining lymph node during influenza infection and, as a result, there was a reduction in the priming of naïve antigen specific CD8+ T cells [17].

Animal studies using antibiotics have identified specific classes of bacteria involved in maintaining immunity against viral infection. For example, neomycin almost completely eliminates *Lactobacillus* spp. and results in impairment of influenza-specific CD8+ T cell responses, suggesting that neomycin sensitive bacteria in the gut support the immune response to influenza infection [16].

Gut microbes are also suggested to support immune responses against viral infections through inflammasome-mediated cytokine release. Antibiotic-treated mice have reduced levels of interleukin-1β (IL-1β) secretion in the lung during influenza infection, suggesting that gut-resident bacteria support cytokine production [16]. It has been speculated that gut microbes release low concentrations of pattern recognition receptor (PRR) ligands, which provide signals for inflammasome-mediated cytokine release (for example, in the lung during influenza infection). These in turn regulate the activity of respiratory dendritic cells during activation of adaptive immunity against the virus [16]. Evidence that gut-resident bacteria play a role in shaping immune defences form the basis for the hypothesis that probiotics may modulate responses to infection or vaccination. However, the mechanisms by which probiotics modulate the immune system, particularly in the context of vaccination, are not clear. A recent animal study demonstrated that the probiotic, *Lactobacillus gasseri*, led to diversification of B cell populations in the lamina propria of the murine colon *in vivo*. This organism was proposed as a vaccine vector for oral immunization against mucosal pathogens [18]. Another study demonstrated that *Lactobacillus paracasei* subsp. *paracasei* NTU 101 fed daily to mice for 3 to 9 weeks induced stronger interactions between CD4+ T cells and dendritic cells and enhanced proliferation of CD4+ T cells and B cells [19].

Thus, there is compelling evidence that resident bacteria in the gastro-intestinal tract influence the immune response to viral infections. However specific data relating to vaccination responses is lacking. The following sections review published studies investigating the impact of concomitant probiotic administration on the response to vaccination in humans.

Studies in infants

Oral vaccines

Two studies investigated the effects of probiotics on responses to oral vaccines in infants. One study examined the influence of *Lactobacillus casei* strain GG (currently known as *Lactobacillus rhamnosus* GG or LGG) on the oral rotavirus vaccine [20], and the other examined the effect of the *Bifidobacterium breve* strain in Yakult (BBG-01) on the oral cholera vaccine [21] (see Table 1). In the first 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 [20]. 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 ($P = 0.05$).

In contrast, there was no effect of *Bifidobacterium breve* strain Yakult (BBG-01), given for 4 weeks, on the response to oral cholera vaccine in 2–5-year-old Bangladeshi children [21]. There were significantly lower proportion of responders in the probiotic group for some viral-specific IgA antibodies compared with the placebo group. This was particularly evident in the younger infants.

Parenteral vaccines

Five studies investigated the effects of probiotics on responses to parenteral vaccines in infants (Table 1). Kukkonen *et al.* [22], investigated the effect of a mixture of four probiotics combined with the prebiotic galacto-oligosaccharide (GOS) on antibody responses to diphtheria, tetanus and *Haemophilus influenza* type b (Hib) vaccination in allergy-prone infants. Pregnant mothers 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. Vaccines were administered at 3, 4 and 5 months and antibody titres were measured at 6 months. A protective Hib-specific IgG antibody response (>1 μg ml^{-1}) 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.

Table 1

Studies investigating the effects of probioics on vaccine responses in infants

Probiotic(s)	Vaccine(s)	Study design	Outcomes	Reference
***Lactobacillus casei* strain GG (*Lactobacillus rhamnosus* GG or LGG) 5×10^{10} (cfu) twice daily**	Oral DxRRV rhesus-human reassortant rotavirus vaccine	Infants (2–5 months) given probiotic ($n = 29$) or placebo ($n = 28$) as a powder reconstituted in 5 ml water after vaccination and for next 5 days	• Increase in IgM antibody secreting cells in LGG vs. placebo group ($P = 0.02$) • Trend for higher IgA in LGG group ($P = 0.05$)	Isolauri *et al*. (1995) [20]
***Bifidobacterium breve* (BBG-01) 4×10^9 cfu day^{-1}**	Oral cholera vaccine Dukoral®	Infants (2–5 years) given probiotic ($n = 64$) or placebo ($n = 60$) for 4 weeks, vaccination on days 21 and 35	• Significantly lower proportion of responders in the probiotic group for some viral-specific IgA antibodies compared with the placebo group ($P = 0.016$ for viral CTB specific IgA)	Matsuda *et al*. (2011) [21]
***Lactobacillus rhamnosus* GG (LGG) (ATCC 53103) 5×10^9 cfu, *Lactobacillus rhamnosus* (LC705) 5×10^9 cfu, *Bifidobacterium breve* (Bbi99) 2×10^8 cfu, *Propionibacterium freudenreichii* ssp. *Shermanii* JS 2×10^9 cfu day^{-1} (twice daily for pregnant mothers)**	Parenteral diphtheria, tetanus and whole cell pertussis (DTwP), *Haemophilus influenzae* type b (Hib) vaccines	Pregnant mothers given probiotics or placebo capsules for last month of pregnancy; same mixture as a powder given in combination with prebiotic GOS sugar syrup to newborns for 6 months ($n = 47$ probiotic, $n = 40$ placebo group). DTwP vaccines given at 3, 4 and 5 months, Hib vaccine at 4 months	• Higher frequency of Hib-specific IgG antibody response in the probiotic (16 of 29 infants) vs. placebo (6 of 25 infants, $P = 0.023$) • Trend for higher Hib-specific IgG antibody titres ($P < 0.064$) • Diphtheria- and tetanus-specific IgG the titres comparable between groups	Kukkonen *et al*. (2006) [22]
***Lactobacillus acidophilus* LAVRI-A1 3×10^9 cfu day^{-1}**	Parenteral tetanus vaccine	Newborn infants given probiotic ($n = 58$) or placebo ($n = 60$) for 6 months as a powder; vaccinations at 2, 4 and 6 months	• Lower IL-10 responses to tetanus antigen in probiotic *vs*. placebo group ($P = 0.03$)	Taylor *et al*. (2006) [23]
***Lactobacillus paracasei* ssp. *paracasei* strain F19 1×10^9 cfu day^{-1}**	Parenteral diphtheria, tetanus toxoid and acellular pertussis (DTaP), polio and Hib vaccines	Infants (4 months) given cereals with probiotic ($n = 89$) or placebo ($n = 90$) for 9 months; vaccines administered at 3, 5.5 and 12 months	• No significant differences in vaccine specific antibody titres between groups • When adjusted for breastfeeding duration the probiotic enhanced anti-diphtheria antibody titres in infants breastfed for < 6 months ($P = 0.024$) • A similar trend for tetanus antigen ($P = 0.035$) but no difference for Hib	West *et al*. (2008) [24]
***Bifidobacterium longum* BL999 1×10^7 cfu, *Lactobacillus rhamnosus* LPR 2×10^7 cfu day^{-1}**	Schedule A: Monovalent hepatitis B (HepB) vaccine at dose 1 and 2, DTaP vaccine containing HepB component at dose 3; Schedule B: monovalent HepB vaccine for all doses	Newborn infants given probiotic or placebo for 6 months; vaccinations according to schedule A (probiotic $n = 29$, placebo $n = 28$) or B (probiotic $n = 77$, placebo $n = 68$) at 0, 1 and 6 months	• Trend for probiotic supplementation to increase HepB virus surface antibody (HBsAb) responses in infants on schedule A ($P = 0.069$) • No effect of probiotic on antibody titres in infants on schedule B	Soh *et al*. (2010) [25]
***Lactobacillus acidophilus* strain ATCC4356, *Bifidobacterium bifidum* DSMZ20082, *Bifidobacterium longum* ATCC157078, *Bifidobacterium infantis* ATCC15697 3×10^9 cfu day^{-1} (Altman Probiotic Kid Powder)**	Parenteral mumps, measles, rubella and varicella (MMRV) vaccine	Infants (8–10 months) given the probiotics ($n = 25$) or placebo ($n = 22$) as a powder for 5 months, starting 2 months prior to vaccination	• No difference in vaccine-specific IgG antibody titres between probiotic and placebo groups • When combining all the antibody results, more infants reached protective IgG antibody titres 3 months post-vaccination in probiotic *vs*. placebo group ($P = 0.052$)	Youngster *et al*. (2011) [26]

cfu, colony-forming units.

In a similar study, 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 [23]. 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-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*. [24], 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.

Soh *et al*. [25] examined the response to hepatitis B (HepB) vaccination in allergy-prone infants fed formula supplemented with *Bifidobacterium longum* BL999 and *Lactobacillus rhamnosus* LPR or a control formula without probiotics for the first 6 months of life. 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 this was not statistically significant, and there was no effect of probiotics in infants receiving the monovalent HepB.

Finally, in the study by Youngster *et al*. [26], 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) [26]. While there was no significant difference in protective antibody titres 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 titres 3 months post-vaccination in the probiotic group [26].

Summary of studies in infants

There are a limited number of studies investigating the influence of probiotics on vaccination in infants and the effects are not clear. At best, 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.

Studies in adults

Seven studies have investigated the influence of probiotics on response to vaccination in adults, four of these employing oral vaccines, two employing parenteral vaccines, and one a nasally administered vaccine (Table 2).

Oral vaccines

Fermented milk containing *Lactobacillus acidophilus* La1 and *Bifidobacterium* Bb12 consumed for 3 weeks significantly increased the vaccine-specific serum IgA titres to an attenuated *Salmonella typhi* Ty21a oral vaccine given on days 7, 9 and 11 [27]. In a separate study, LGG taken for 7 days tended to increase vaccine-specific IgA antibodies to the *Salmonella typhi* Ty21a oral vaccine administered on days 1, 3 and 5 [28]. However, there was no effect of *Lactococcus lactis*, and no effect of either probiotic on the numbers of vaccine specific IgA, IgG or IgM antibody secreting cells 7 days post-vaccination [28]. Vaccine-specific IgA titre to an oral poliovirus vaccine was increased by LGG and *Lactobacillus paracasei* ssp. *paracasei* (CRL431) during a 5 week intervention, with the live attenuated poliomyelitis virus vaccine being administered on day 8 [29]. Those receiving the probiotics had a significantly greater increase in neutralizing antibodies compared with a placebo group. There was also a minor effect on poliovirus serotype-1-specific IgG and on serotype-2-and -3-specific IgM antibody titres [29]. Strain specific effects of probiotics on response to an oral cholera vaccine were explored by Paineau *et al*. [30]. Healthy adult volunteers were assigned to one of seven probiotics (members of the genera *Lactobacillus* and *Bifidobacterium*) or placebo for 21 days and received the oral cholera vaccine on days 7 and 14. *Bifidobacterium lactis* Bi-04 and *Lactobacillus acidophilus* La-14 significantly increased vaccine-specific serum IgG antibody levels on day 21, and there was a similar trend for *Bifidobacterium lactis* Bi-07 and *Lactobaacillus plantarum* Lp-115. However, there were no significant effects of probiotics on vaccine-specific serum IgA or IgM antibodies [30].

Parenteral vaccines

Three studies have examined the potential adjuvant properties of probiotics given in conjunction with parenteral or nasally administered influenza vaccines (Table 2). In the first study, *Lactobacillus fermentum* CECT5716, taken for 4 weeks, significantly increased titres of influenza virus-specific plasma IgA (but not IgM or IgG) to the inactivated trivalent influenza vaccine for the vaccine campaign of 2004/2005, administered 2 weeks into the intervention [31]. Additionally, the incidence of influenza-like illnesses for 5 months post-vaccination were lower in the probiotic group compared with the control group [31]. In the second study, both *Bifidobacterium animalis* ssp. *lactis* (BB-12®) and *Lactobacillus paracasei* ssp. *paracasei* (*L. casei* 431®) taken for 6 weeks increased influenza vaccine-specific serum IgG and vaccine-specific salivary sIgA titres after vaccination at 2 weeks in the 2008/2009 campaign [32]. There was no effect of either probiotic on vaccine-specific serum IgA or IgMon plasma cytokine concentrations or on parameters of innate immunity [32]. Finally, LGG taken for 28 days immediately after receiving a nasally administered trivalent live attenuated influenza vaccine from the campaign

Table 2

Studies investigating the effects of probioics on vaccine responses in adults

Probiotic(s)	Vaccine(s)	Study design	Outcomes	Reference
***Streptococcus thermophilus*, *Lactobacillus acidophilus* La1 and *Bifidobacterium* Bb12 5×10^9 cfu day^{-1}**	Oral attenuated *Salmonella typhi* Ty21a vaccine	Healthy adults given probiotic yoghurt ($n = 16$) or no intervention ($n = 14$) for 3 weeks; vaccination on days 7, 9 and 11	• Greater increase in vaccine-specific serum IgA antibody titre in probiotic *vs.* control group ($P = 0.04$)	Link-Amster *et al.* (1994) [27]
***Lactobacillus* GG (LGG) 4×10^{10} cfu day^{-1} or *Lactococcus lactis* (*L.lactis*) 3.4×10^{10} cfu day^{-1}**	Oral attenuated *Salmonella typhi* Ty21a vaccine	Healthy adult volunteers receiving LGG ($n = 10$), *L. lactis* ($n = 10$) or placebo ($n = 9$) for 7 days; vaccination on days 1, 3 and 5	• No significant difference in vaccine-specific IgA, IgG or IgM antibody secreting cells between groups • Trend for higher vaccine-specific IgA antibody in LGG group	Fang *et al* (2000) [28]
***Lactobacillus rhamnosus* GG (LGG)** *Lactobacillus paracasei* ssp. *paracasei* strain CRL431 (CRL431) 1×10^{10} cfu day^{-1}	Oral attenuated poliomyelitis virus types 1, 2 and 3 vaccine	Healthy males given yogurt with LGG ($n = 21$), CRL431 ($n = 21$) or no probiotic ($n = 22$) for 5 weeks; vaccination on day 8	• Significantly greater increase in neutralizing antibodies and enhanced poliovirus-specific IgA titre in probiotic groups *vs.* placebo group ($P < 0.036$) • Probiotics had a minor effect on poliovirus serotype-1-specific IgG and on serotype-2- and –3-specific IgM antibody titres	de Vrese *et al.* (2005) [29]
***Lactobacillus* strains: *L. acidophilus* La-14, *L. acidophilus* NCFM®, *L. plantarum* Lp-115, *L. paracasei* Lpc-37, *L. salivarius* Ls-33, *Bifidobacterium* strains: *B. lactis* Bl-04, *B. lactis* Bi-07 1×10^{10} cfu twice daily**	Oral cholera vaccine Dukoral®	Healthy adults assigned to one of seven probiotics ($n = 9$ for each) or placebo ($n = 20$) for 21 days; vaccination on days 7 and 14	• Significantly higher vaccine-specific serum IgG antibody levels on day 21 in subjects given probiotics *B. lactis* Bl-04 and *L. acidophilus* La-14 *vs.* control group ($P = 0.01$) • Similar trend for *B. lactis* Bi-07 and *L. plantarum* Lp-115 supplementation	Paineau *et al.* (2008) [30]
***Lactobacillus fermentum* (CECT5716) 1×10^{10} cfu day^{-1}**	Parenteral inactivated trivalent influenza vaccine for the campaign of 2004/2005	Healthy adults given probiotic ($n = 25$) or placebo ($n = 25$) for 4 weeks; vaccination on day 14	• Probiotic increased vaccine-specific IgA antibodies post-vaccination ($P < 0.05$) • Incidence of influenza-like illnesses for 5 months post-vaccination lower in the probiotic *vs.* placebo group ($P < 0.05$ for last month)	Olivares *et al.* (2007) [31]
***Bifidobacterium animalis* ssp. *lactis* (BB-12®) or *Lactobacillus paracasei* ssp. *paracasei* (L.casei 431®) 1×10^9 day^{-1}**	Parenteral attenuated trivalent influenza vaccine for the campaign of 2008/2009	Healthy adults given probiotic ($n = 53$ for BB-12®, $n = 56$ for *L.casei* 431®) or placebo ($n = 102$) for 6 weeks; vaccination at week 2	• Significantly greater increase in vaccine-specific IgG antibody titre in probiotic groups *vs.* placebo ($P < 0.001$ for IgG1 and IgG3) • Significantly greater mean-fold increases for vaccine-specific secretory IgA antibody in saliva in BB-12® group ($P = 0.035$) and *L.casei* 431® group ($P = 0.017$) *vs.* placebo group	Rizzardini *et al.* (2011) [32]
***Lactobacillus* GG (LGG) 1×10^{10} cfu and 295 mg prebiotic inulin twice daily**	Nasally administered attenuated trivalent influenza vaccine for the campaign of 2007/2008	Healthy adults given probiotic ($n = 21$) or placebo ($n = 21$) for 28 days after vaccination	• LGG significantly increased seroprotection rate to the H3N2 strain at day 28 ($P = 0.048$), but not to the H1N1 or B strain • No effect on seroconversion rates at day 56	Davidson *et al* (2011) [33]

cfu, colony-forming units.

of 2007/2008 significantly increased seroprotection (haemagglutinin inhibition [HAI] antibody titre = 40) to the H3N2 virus strain, but not to the H1N1 or B strain at day 28 [33]. However, at day 56 the rates of seroconversion (at least a four-fold rise in HAI antibody titre) were not significantly different.

Summary of studies in adults

Overall, some studies in adults demonstrate an increase in vaccine-specific serum IgA concentrations, but this is not entirely consistent. Effects on other Ig subclasses and on seroprotection/seroconversion are unclear.

Studies in elderly subjects

Three studies examined the effect of probiotics on the response to parenteral influenza vaccines in elderly subjects (Table 3). This is a particularly important group for consideration because of the impact of immunosenes-

Table 3

Studies investigating the effects of probioics on vaccine responses in elderly subjects

Probiotic(s) used	Vaccine(s) used	Study design	Outcomes	Reference
***Lactobacills paracasei* (NCC 2461) 1×10^9 cfu and 6 g prebiotic fructo-oligosaccharide as part of a daily nutritional formula**	Parenteral trivalent influenza vaccine and pneumococcal vaccine containing 23 serotypes	Elderly subjects (≥70 years) given either nutritional formula containing a range of nutrients and vitamins plus the probiotic NCC 2461 and prebiotic for 6 months or no supplement; vaccination after 4 months	• No effect on antibody response to vaccines • Significantly lower incidence of infection after 12 months, in particular respiratory illnesses, in treatment group *vs*. controls ($P = 0.034$)	Bunout *et al*. (2004) [35]
***Lactobacillus paracasei* ssp. *paracasei* (Actimel®) 10^{10}cfu/100 g bottle twice daily**	Parenteral inactivated trivalent influenza virus vaccine (2005–2006 campaign vaccine for pilot study and 2006–2007 for confirmatory study)	Pilot study: probiotic ($n = 44$) or placebo ($n = 42$) consumed for 7 weeks Confirmatory study: probiotic ($n = 113$) or placebo ($n = 109$) consumed for 13 weeks. Vaccination after 4 weeks	• Trend for higher virus-specific antibody titres in probiotic *vs*. control group • Significantly greater seroconversion rate for B strain in main study at 3, 6 and 9 weeks post-vaccination in probiotic *vs*. placebo group ($P = 0.02$)	Boge *et al*. (2009) [36]

cfu, colony-forming units.

cence on response to vaccination, and the consequences of respiratory infections in older people [34]. Bunout *et al*. [35], examined the effects of a complete nutritional formula containing a range of nutrients and vitamins plus the probiotic *Lactobacillus paracasei* (NCC 2461) and the prebiotic fructo-oligosaccharide for 6 months on the response to influenza and pneumococcal vaccines (given at 4 months) in free-living Chilean subjects over 70-years-old. At 12 months there was a significantly lower incidence of infection, in particular, respiratory infection, in the treatment group compared with the control group, but there was no effect on antibody responses to either vaccine [35]. Boge *et al*. [36], conducted an intervention trial of a probiotic drink containing *Lactobacillus paracasei* ssp. *paracasei* (Actimel®) on the response to influenza vaccination in healthy elderly volunteers (>70 years). This trial was conducted in two phases: a pilot study in 2005–2006 (probiotic/placebo consumed for 7 weeks), followed by a confirmatory study in 2006–2007 (probiotic/placebo consumed for 13 weeks), with the inactivated influenza virus vaccine being administered during the fourth week of intervention. H1N1 was the only vaccine strain common to both phases of the study, with the H3N2 and B strains being different between vaccination seasons. In both phases of the trial, the probiotic group exhibited higher virus-specific antibody titres post-vaccination compared with the control group, although these differences were only statistically significant within the confirmatory phase [36]. The intensity of the probiotic effect was vaccine subtype-dependent, with the most pronounced enhancement for the influenza virus H3N2 strain in the pilot and the B strain in the confirmatory study. Seroconversion rates within the probiotic group in the confirmatory phase were significantly higher for the B strain at 3, 6 and 9 weeks post-vaccination compared with the placebo group ($P = 0.02$), but there was no effect of the probiotic on seroconversion for the H1N1 or H3N2 strains [36]. It is perhaps pertinent to note that the B strain is known to show major human variability, and the effects on this subtype therefore need to be interpreted with caution. The third study is not included in Table 3 because of an unusual study design, which makes the data very difficult to interpret [37]. In this small study, 27 elderly subjects consumed a test food containing *Bifidobacterium longum* BB536 for 5 weeks, with an influenza vaccination (2004/2005 campaign) being given at 3 weeks. At 5 weeks, the subjects were then randomized to either continue on the probiotic, or to consume a placebo for a further 14 weeks. The randomization was stratified for gender and H3N2 titres, but not for overall protection, so that the proportion of subjects with effective titre was 53.8% in the BB536 group and 28.6% in the placebo group [37]. Although the paper reports significantly lower incidence of influenza and fever in the probiotic group, the subject numbers are extremely small, and these data should probably be disregarded.

Summary of studies in elderly subjects

Studies are very limited, and it is too early to draw any conclusions regarding the potential influence of probiotics on the response to influenza vaccination in elderly subjects. More research is required.

Conclusion

The majority of studies investigating the impact of probiotics on responses to vaccination have been conducted in healthy adults, and at best they show modest effects of probiotics on serum or salivary IgA titres. Studies in infants and in elderly subjects are very limited, and it is too early to

draw any firm conclusions regarding the potential for probiotics to act as adjuvants in vaccination. There is strong evidence that probiotics reduce the incidence and duration of diarrhoeal infection among infants and adults [1]. Further studies of the effect of probiotics on the response to rotavirus and cholera vaccination in infants and cholera or Salmonella vaccination in adults would therefore be of interest.

Evidence suggests that probiotics can reduce the duration, but not the incidence, of common respiratory illnesses [1]. Two studies which monitored the incidence and duration of cold and flu-like symptoms following Influenza vaccination have indeed identified a lower incidence of infections among those receiving probiotic treatment [31, 35]. 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. Unfortunately, very few of the studies published to date have reported rates of seroprotection and seroconversion. This information is critical in evaluating the potential clinical benefits of probiotics as adjuvants for vaccination. 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.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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