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Ageing, immunity and influenza: a role for probiotics?

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Influenza is a major cause of death in the over 65s. Increased susceptibility to infection and reduced response to vaccination are due to immunosenscence in combination with medical history and lifestyle factors. Age-related alterations in the composition of the gut microbiota have a direct impact on the immune system and it is proposed that modulation of the gut microbiota using pre- and probiotics could offer an opportunity to improve immune responses to infections and vaccination in older people. There is growing evidence that probiotics have immunomodulatory properties, which to some extent are strain-dependent, and are strongly influenced by ageing. Randomised controlled trials suggest that probiotics may reduce the incidence and/or severity of respiratory infections, although there is limited data on older people. A small number of studies have examined the potential adjuvant effects of selected probiotics for vaccination against influenza; however, the data is inconsistent, particularly in older people. This review describes the impact of age-related changes in the gut on the immune response to respiratory infections and evaluates whether restoration of gut microbial homoeostasis by probiotics offers an opportunity to modulate the outcome of respiratory infections and vaccination against influenza in older people. Although there is promising evidence for effects of probiotics on human health, there is a lack of consistent data, perhaps partly due to strain-specific differences and an influence of the age of the host. Further research is critical in evaluating the potential use of probiotics in respiratory infections and vaccination in the ageing population.

Ageing: Immunity: Influenza: Probiotics

Respiratory infections are a leading cause of mortality and morbidity in elderly people. Increased susceptibility to infections is influenced by many factors, including immunosenescence, nutritional status, poor hygiene and living conditions, medical history, medication use and stress. Reduced biodiversity, compromised stability and larger inter-individual variation in the gut microbiota are also commonly reported to be associated with ageing[1,2]. This review examines how age-related changes in the gut can impact on the immune system, describes evidence suggesting that the gut microbiota influences the immune response to respiratory infections and evaluates whether restoration of gut microbial homoeostasis by probiotics offers an opportunity to modulate the outcome of respiratory infections and vaccination against influenza in older people.

Influence of ageing on the gut microbiota composition

Reduced biodiversity, compromised stability and larger inter-individual variation in the gut microbiota have become widely recognised as a feature of ageing[1–3]. A reduction in total gene counts in faecal samples from 178 elderly subjects in long-term care compared with those in the community illustrates the reduction in gut microbiota biodiversity, which is associated with frailty, morbidity and poor nutritional status in elderly subjects[1]. Also commonly reported is an age-related increase in facultative anaerobes, including streptococci, staphylococci, enterococci and enterobacteria[4–7]. These are sometimes referred to as 'pathobionts'; bacteria, which are present at low concentrations in the healthy gut microbiota, but thrive in inflamed conditions and actively promote the inflammation by producing inflammatory stimuli[7]. It has been suggested that reduced intestinal motility contributes to a compromise in the homoeostatic equilibrium between the gut microbiota and the host immune system in older people[2]. However, the relationship may be reciprocal, since impairment of the gut-associated lymphoid tissue resulting in reduced production of secretory IgA, α-defensins, antimicrobial peptides and mucus may lead to failure to control the resident microbiota, facilitating dysregulated growth[2].

Abbreviations: DC, dendritic cells; NK, natural killer.

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Age-related alterations in the composition of the gut microbiota can influence health through a number of mechanisms. Reduced biodiversity may result in increased colonisation of toxin-producing *Clostridium difficile*, a major nosocomial complication affecting older people[8,9]. Excessive endotoxin production in the gut can promote inflammation[10] and a decrease in butyrate-producing bacterial groups may impair epithelial integrity and remove the protective, trophic and anti-inflammatory effects of butyrate from the colonic epithelium[11–13].

The ageing immune system

The term 'immunosenescence' describes a loss of diversity in the T cell repertoire and senescence or unresponsiveness of oligoclonal T cells, resulting in poor antigen-specific cellular responses, skewing of immune effector pathways and ultimately, poor resistance to infection and response to vaccination[14]. Degeneration of the thymus, the site of T cell maturation, is a hallmark of ageing; by age 50–55 years, the thymus is reduced to approximately 10% of its original capacity and by age 70 years, thymic output of naïve T cells is virtually absent[14]. However, the number of circulating T cells in older people remains constant because of homoeostatic proliferation of both naïve and memory T cells, much of which is antigen-independent. Thymic involution, combined with increased homoeostatic proliferation and expansion of the memory compartment, dramatically reduce T cell receptor diversity. In addition, repeated antigen exposure (for example, chronic antigenic stimulation by persistent Herpes viruses), proliferation of T cells and telomere shortening lead to replicative senescence and as a result, dysfunctional cells increasingly fill the immunological space. Since a diverse T cell repertoire is essential for an effective immune response to new infections and to immunisation, older individuals face a significant challenge in dealing with both[15,16]. It is well established that ageing is associated with an increased susceptibility to infections[17] and that vaccines are considerably less effective in older people[16,18,19], presenting a major public health challenge.

Prolonged exposure to TNF, and perhaps other inflammatory cytokines, promotes immunosenescence; indeed young individuals with chronic inflammatory disease exhibit premature immunosenescence, telomere shortening and reduced thymic output (reviewed in[14]). The term 'inflamm-ageing' has been coined to describe the now well-recognised progressive increase in chronic inflammation associated with ageing. Triggers include microbial components, tissue damage and metabolic stress, which activate inflammasome pathways and lead to the production of inflammatory cytokines and chemokines, acute phase proteins and other inflammatory mediators (Fig. 1)[20]. A number of factors have been identified to modify inflamm-ageing, including diet and the gut microbiota (Fig. 1)[20]. However, paradoxically, a shift towards a more anti-inflammatory profile is not necessarily beneficial, since up-regulation of IL-10 production suppresses interferon γ production and the expression of co-stimulatory molecules on antigen-presenting dendritic cells (DC)[21]. This results in a decrease in the interferon γ:IL-10 ratio, which is associated with impaired clearance of the influenza virus from infected lung tissue and poor response to vaccination[21].

Respiratory infections and response to influenza vaccination in older people

Older people experience more frequent and severe respiratory infections and are at higher risk of associated complications, such as pneumonia[22]. As a result, there is greater morbidity resulting from respiratory infections in elderly individuals, and influenza is a major cause of death in the over 65s[21,22]. The efficacy of influenza vaccination is also significantly impaired by ageing; it is estimated that influenza vaccination protects only 17–53% of elderly individuals compared with 70–90% of young[19,23]. A direct relationship between poor vaccination and immunosenescence is illustrated by the fact that loss of CD28 (an important co-stimulatory molecule on T cells) is one of the most well-recognised features of immunosenescence and is correlated with a poor response to vaccination[24–26].

Probiotics, ageing and immunity

Probiotics are defined as 'live microbial feed ingredients that, when ingested in sufficient quantities, exert health benefits on the consumer'[27]. Most probiotics consist of lactobacilli or bifidobacteria, but an increase in levels of these bacteria in the gut is not necessarily a health benefit in itself and other parameters need to be considered. The same applies to prebiotics, defined as 'selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health'[28] and synbiotics (a combination of the two); simply altering numbers of particular bacterial groups is not necessarily considered sufficient evidence for a health benefit. However, there is some evidence for beneficial effects of probiotics on bowel function, including infection- and antibiotic-associated diarrhoea[29], on respiratory infection (see later) and on immune function[3,30]. Claims relating to positive effects of probiotics on immune function have been difficult to substantiate because many of the immunological markers employed do not in themselves indicate a beneficial physiological effect[31]. Moreover, there is a degree of redundancy within the immune system, such that a reduction in the functional capacity of one component may be compensated for by another[31]. Nevertheless, there is growing evidence that probiotics have immunomodulatory properties and that these properties of probiotics are strain-dependent[32–41]. For example, there is consistent evidence that a number of probiotics enhance natural killer (NK) cell activity *in vitro*[40–43] and there is some

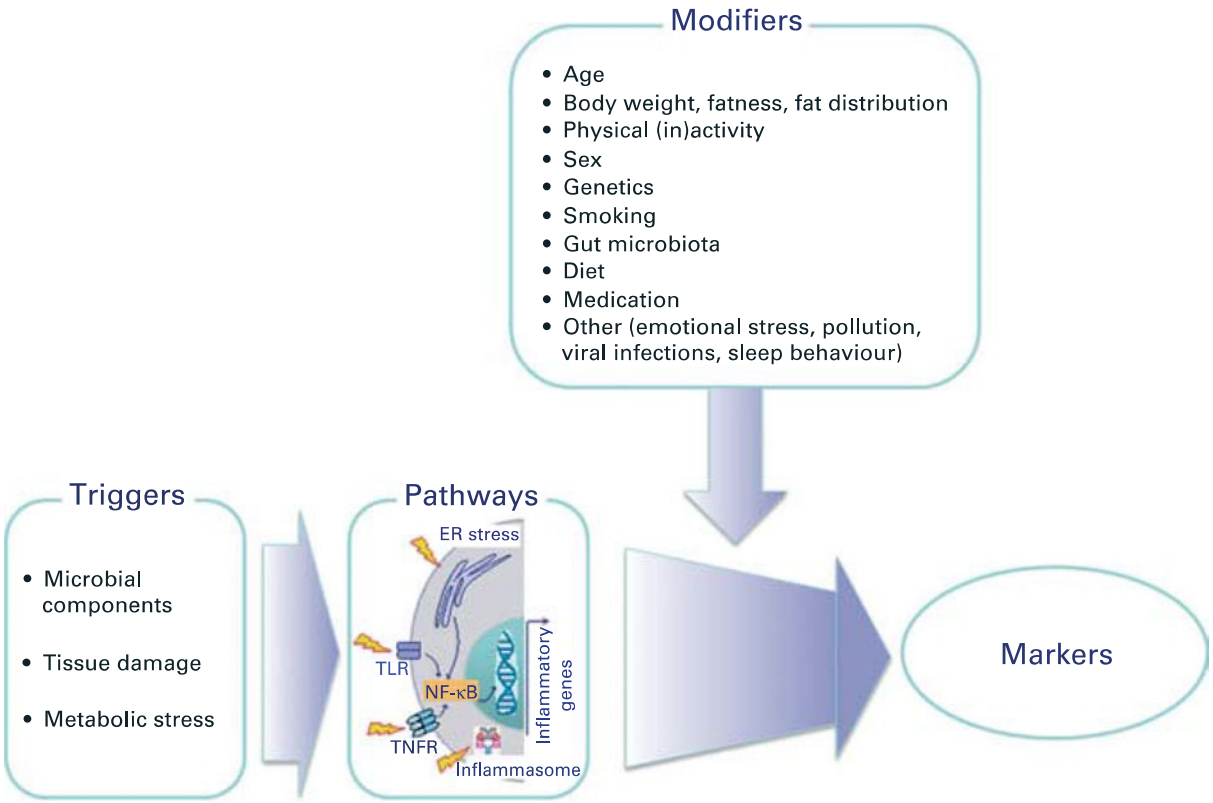

Fig. 1. (colour online) Overview of the triggers and modifiers of inflamm-ageing. ER, endoplasmic reticulum; TLR, Toll-like receptor; TNFR, TNF receptor. Reproduced, with permission, from[20].

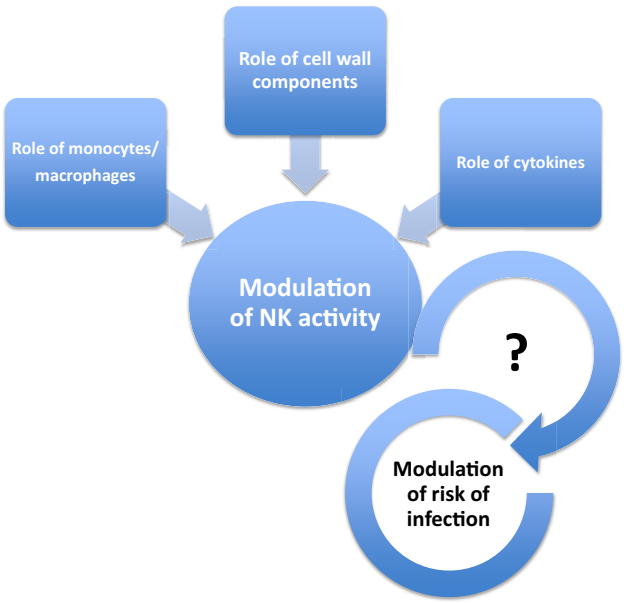

Fig. 2. (colour online) Modulation of natural killer (NK) activity and infection by probiotics. Enhancement of NK activity by probiotics is suggested to require phagocytosis of the bacteria, resulting in induction of cytokines, particularly IL-12. The mechanism involves stimulation by insoluble bacterial cell wall components. Probiotics with cells walls which are particularly resistant to digestion appear to be better inducers of IL-12 and enhancers of NK activity.

supportive evidence that this also occurs *in vivo*[44–46]. Probiotic strains whose cell walls are resistant to digestion appear to be particularly potent enhancers of NK activity; it is suggested that monocytes phagocytose the probiotic bacteria and the insoluble cell wall components induce the production of IL-12, which augments NK cell activity[38,47]. Animal studies suggest that this may ultimately play a role in modulation of the risk of infection (Fig. 2). For example, neonatal and infant mice administered *Lactobacillus casei* Shirota by stomach tube for 3 weeks prior to infection with influenza demonstrated a lower rate of accumulated symptoms, a greater survival rate and lower titres of influenza in nasal washings taken a few days after infection[48]. These correlated with an increase in NK cell activity and production of IL-12[48].

Probiotics have also been demonstrated to modulate cytokine production in a strain-dependent manner. Shida *et al.*[38] propose that probiotics fall into two main categories: those which are 'immunostimulatory', characterised by their ability to induce IL-12 and therefore to augment host defence via enhancement of NK cell activity and T helper 1 pathways, and those which are 'immunoregulatory', characterised by their ability to induce IL-10 and the T regulatory pathway. In general, lactobacilli tend to fall into the immunostimulatory category, whereas bifidobacteria tend to fall in the immunoregulatory category[38,40]. Kechaou *et al.*[49] screened 158 bacterial strains for their ability to modulate the

induction of IL-12 relative to IL-10 in TNFα-activated HT-29 cells or peripheral blood mononuclear cells and then selected one strain which was strongly pro-inflammatory, one which was neutral, and one which was strongly anti-inflammatory to test in a murine model of influenza infection. The outcome was that the most immunostimulatory strain provided the greatest protection against weight loss and symptoms[49].

There is particular interest in the positive influence of probiotics in older people, who are subject to alteration in gut microflora composition, as well as immunosenescence. Although probiotics have been proposed as prime candidates for 'anti-immunosenescence' therapy[50,51], and 'have the potentiality to be involved in the promotion of longevity'[2], there is limited information regarding their influence on those aspects of immunity, which are particularly susceptible to immunosenescence. A study by Moro-Garcia *et al.*[52] demonstrated that *Lactobacillus delbrueckii* subsp. *bulgaricus* 8481 decreased the percentage of $CD8^+CD28^-$ T cells and increased the proportion of naïve T cells between 3 and 6 months compared with baseline in subjects aged >65 years, although the mechanism is not clear. However, there is some direct evidence that the immune response to probiotics is significantly influenced by ageing. You and Yaqoob[41] demonstrated that peripheral blood mononuclear cell from older subjects (age 60–85 years) were more responsive to the immunoregulatory effects (IL-10 induction) of two strains of bifidobacteria than young subjects (age 18–30 years), whereas peripheral blood mononuclear cell from young subjects were more responsive to the immunostimulatory effects (IL-12 induction) of two strains of lactobacilli (Fig. 3). This suggests that the same probiotics might have different effects on health outcomes in young and older subjects. Further studies investigated the effects of the same four probiotics, *Bifidobacterium longum bv. infantis* CCUG 52486, *B. longum* SP 07/3, *Lactobacillus rhamnosus* GG and *L. casei* Shirota, on human DC function in an allogeneic mixed leucocyte reaction model, using DC and T cells from young and older donors in different combinations. The study demonstrated that ageing increased the responsiveness of DC to probiotics, but this was not sufficient to overcome the impact of immunosenescence in the mixed leucocyte reaction, since pretreatment of young or old DC with lipopolysaccharide or probiotics failed to enhance the proliferation of T cells derived from older donors[53].

The choice of probiotic, particularly for older individuals, is a matter of debate. An interesting point of view put forward by Dominguez-Bello *et al.*[54] is that attempting to improve the gut microbiota composition of older people by introducing probiotic strains characteristic of a young microbiota is not necessarily the best strategy, and it may be more appropriate to identify 'successfully aged' donors of probiotic strains, which might survive better in an older host and achieve a more suitable equilibrium with the resident microbiota. *B. longum bv. infantis* CCUG 52486 is an example of a strain which was identified to be present in particularly healthy subjects aged >90 years[55]. It has subsequently been

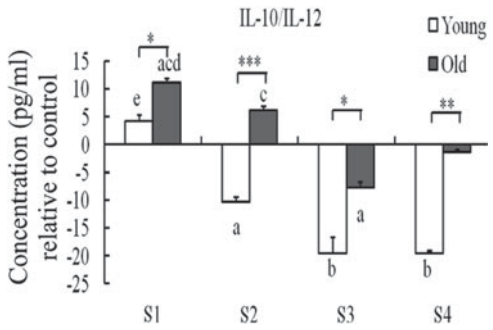

Fig. 3. Effect of probiotics on the IL-10:IL-12 ratio by human peripheral blood mononuclear cells. S1, *Bifidobacterium longum bv. infantis* CCUG52486; S2, *B. longum* SP 07/3; S3, *Lactobacillus rhamnosus* GG; S4, *Lactobacillus casei* Shirota. Data are means with standard errors for *n* 8 samples for each group and are relative to a medium only control. There was a significant effect of age ($P<0{\cdot}001$) and treatment ($P<0{\cdot}001$) on the IL-10:IL-12 ratio (two-way ANOVA). Significant differences are denoted as $^{a}P<0{\cdot}05$, $^{b}P<0{\cdot}01$ relative to the medium control for the same age group; $^{c}P<0{\cdot}05$ relative to *Lactobacillus rhamnosus* GG for the same age group; $^{d}P<0{\cdot}05$ relative to *Lactobacillus casei* Shirota for the same age group; $^{e}P<0{\cdot}05$ relative to all the other strains for the same age group (*post-hoc t* tests with Bonferroni correction). Significant age differences are denoted as $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$ for the same treatment (one-way ANOVA followed by *post-hoc t* tests with Bonferroni correction). Reproduced from[53] with permission.

demonstrated to have particular ecological fitness and anti-pathogenic effects *in vitro*[56] and immunomodulatory effects which are strongly influenced by the age of the host[41,53].

Probiotics and respiratory infections

Emerging evidence suggests that the resident gut microbiota plays an influential role in shaping antiviral defences and modulating the outcome of viral infections[57]. Evidence from animal models indicates that germ-free mice are more susceptible to a number of infections, including influenza[58], and adult mice treated with antibiotics under pathogen-free conditions before challenge with influenza have a critically impaired immune response and delayed clearance of the virus compared with those not treated with antibiotic[58]. The suggested mechanism is via Toll-like receptors and nod-like receptor 3, which activate the nod-like receptor 3 inflammasome in the gut, eliciting indirect stimulation of respiratory immunity[59,60]. There may be additional, as yet uncharacterised, functional links between mucosal surfaces in the gut and respiratory system. Evidence that gut-resident bacteria play a role in shaping immune defences forms the basis for the hypothesis that pre- and probiotics may modulate responses to infection or vaccination. This hypothesis is supported by a number of animal studies, which demonstrate that several probiotic strains prevent dissemination and improve clearance of pathogen in the lung, and increase phagocytic and NK activity in respiratory mononuclear cells[48,49,61–66].

Probiotic bacteria may reduce the incidence and/or severity of respiratory infections in children[67], adults[68]

and in the elderly[69], although evidence is limited and studies investigating prevention of common respiratory illnesses have produced mixed results[59]. A recent systematic review evaluated ten randomised controlled trials with a total of 3451 participants investigating the effects of probiotics for the prevention of upper respiratory tract infections[70]. The review concluded that there was a 42% reduction in the number of participants experiencing at least one episode of acute upper respiratory tract infection, a 47% reduction in the number of participants experiencing three or more episodes of acute upper respiratory tract infection and a 33% reduction in antibiotic usage[70]. However, there was no effect on mean duration of upper respiratory tract infection and there was no data for older people, despite the fact that respiratory infections and associated secondary complications are a significant cause of death in individuals aged over 65 years. Furthermore, since ageing is associated with reduced biodiversity and compromised stability of the gut microbiota[2], older individuals may benefit more from intervention with probiotics.

Probiotics and the immune response to influenza vaccination

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 human subjects[71]. Four out of eight of the studies investigating the impact of probiotics on responses to influenza vaccination were conducted in healthy adults[72–75], while the remaining four were conducted in elderly subjects[76–79]; three of these were conducted in institutionalised individuals[77–79] (Table 1). The largest of the trials conducted in elderly subjects (*n* 362 and 375 on probiotic and placebo, respectively) demonstrated no effect of *L. casei* Shirota on respiratory infections, seroprotection, seroconversion or mean antibody titres[79]. However, a much smaller trial (*n* 14–19 per group)[78] reported a significant increase in influenza-specific IgG and IgA in subjects consuming *L. plantarum* CECT7315 and CECT7316. Boge *et al.*,[77] conducted an intervention trial of a probiotic drink containing *Lactobacillus paracasei* ssp. *paracasei* (Actimel®) on the response to influenza vaccination in healthy elderly volunteers (age >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 to the control group, although these differences were only statistically significant within the confirmatory phase[77]. 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 postvaccination compared to the placebo group ($P=0{\cdot}02$), but there was no effect of the probiotic on seroconversion for the H1N1 or H3N2 strains[77]. 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. Bunout *et al.*,[76] 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 aged over 70 years. 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[76]. A fifth study in elderly subjects is not included in Table 1 because of an unusual study design, which makes the data very difficult to interpret[80]. In this small study, twenty-seven elderly subjects consumed a test food containing *B. longum* BB536 for 5 weeks, with an influenza vaccination (2004/2005 campaign) being given at 3 weeks. At 5 weeks, the subjects were then randomised to either continue on the probiotic, or to consume a placebo for a further 14 weeks. The randomisation was stratified for sex and H3N2 titres, but not for overall protection, so that the proportion of subjects with effective titres was 53·8% in the BB536 group and 28·6% in the placebo group[80]. Although the paper reports significantly lower incidence of influenza and fever in the probiotic group, the subject numbers are extremely small, and this data needs to be interpreted with caution. Thus, 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.

In healthy adults, *Bifidobacterium animalis* ssp. *lactis* (BB-12®) or *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[75]. There was no effect of either probiotic on vaccine-specific serum IgA or IgM on plasma cytokine concentrations, or on parameters of innate immunity, although the authors acknowledge that sampling at 4 weeks after vaccination may have missed changes in immune response to the vaccine[75]. *Lactobacillus* GG taken for 28 d immediately after receiving a nasally administered trivalent live attenuated influenza vaccine from the campaign of 2007/2008 significantly increased seroprotection (haemagglutinin inhibition antibody titre =40) to the H3N2 virus strain, but not to the H1N1 or B strain at day 28[74]. However, at day 56 the rates of seroconversion (at least a fourfold rise in HAI antibody titre) were not significantly different. French & Penny[73] reported significantly higher antibody titres to

Table 1. Studies investigating the effects of probiotics on the response to influenza vaccination

Probiotic(s) used	Vaccine(s) used	Study design	Outcomes	Reference
Lactobacillus casei Shirota ($6{\cdot}5\times10^9$ in 65 ml fermented milk product twice daily)	Parenteral attenuated trivalent influenza vaccine for the campaign of 2007/2008	Healthy men & women ≥65 years from fifty-three nursing homes on probiotic (*n* 362) or placebo (*n* 375) for 176 d during winter 2007–2008. Vaccination at 21 d, sampling at days 1, 50, 176	No effect on respiratory infections 172 subjects seroprotected at baseline; no effect of probiotic on seroprotection, seroconversion or mean Ab titres	[79]
Lactobacillus plantarum CECT7315 and CECT 7316	Parenteral attenuated trivalent influenza vaccine for the Spanish campaign of 2006/2007	Institutionalised elderly subjects aged 65–85 years randomly assigned to 5×10^9 cfu/d (*n* 19) OR 5×10^8 cfu/d (*n* 14) *L. plantarum* CECT 7315/7316 (in a 1:1 ratio) in 20 g powdered skim milk OR placebo (20 g skim milk; *n* 15). Intervention initiated 3 months after vaccination; duration 3 months	Significant increase in influenza-specific IgG only in group receiving high dose. Increase in influenza-specific IgA in both probiotic groups	[78]
Bifidobacterium animalis ssp. *lactis* (BB-12®) or *Lactobacillus paracasei* ssp. *paracasei* (L. casei 431®) 1×10^9/d	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 *v.* placebo ($P<0{\cdot}001$ for IgG1 and IgG3) Significantly greater mean-fold increases for vaccine-specific secretory IgA antibody in saliva in BB-12® group ($P=0{\cdot}035$) and *L. casei* 431® group ($P=0{\cdot}017$) *v.* placebo group	[75]
Lactobacillus GG 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 d after vaccination	*Lactobacillus* GG significantly increased seroprotection rate to the H3N2 strain at day 28 ($P=0{\cdot}048$), but not to the H1N1 or B strain No effect on seroconversion rates at day 56	[74]
Lactobacillus fermentum VR1003	Parenteral inactivated trivalent influenza vaccine 2006 (Australia)	Healthy adults randomised to 1×10^9 cfu/d probiotic capsules (*n* 21) or placebo (*n* 26). 6 weeks intervention, initiated 2 weeks prior to vaccination	Significantly higher median H1N1 titres in probiotic group, but no differences for other subunits Average number of respiratory symptoms reduced (2 *v.* 5 d)	[73]
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)	Elderly subjects in nursing homes. 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 *v.* control group Significantly greater seroconversion rate for B strain in main study at 3, 6 and 9 weeks post-vaccination in probiotic *v.* placebo group ($P=0{\cdot}02$)	[77]
Lactobacillus fermentum (CECT5716) 1×10^{10} cfu/d	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 ($P<0{\cdot}05$) Incidence of influenza-like illnesses for 5 months post-vaccination lower in the probiotic *v.* placebo group ($P<0{\cdot}05$ for last month)	[72]
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 twenty-three 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 *v.* controls ($P=0{\cdot}034$)	[76]

H1N1 following intervention with *Lactobacillus fermentum* VRI 003 for 6 weeks in total (vaccination after 2 weeks), but there were no differences for other subunits. The average number of days of respiratory symptoms was reduced from 5 to 2 d[73]. *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[72]. Additionally, the incidence of influenza-like illnesses for 5 months post-vaccination were lower in the probiotic group compared to the control group[72]. Overall, there is some evidence for adjuvant effects of probiotics in influenza vaccination, but this is currently limited, most of the studies are very small, and there may be strain-specific differences which need to be considered[40,47]. In selecting probiotics with potential adjuvant activity specifically targeted towards older individuals, Dominguez-Bello *et al.*[54] suggest that 'the healthy old rather than the healthy young are the best donors of probiotic species for old individuals'. This argues against the common perception that modulating the microbiota composition of an aged gut to resemble that of a younger individual will be beneficial for intestinal health and immunity. Instead, it is suggested that the aim should be to establish a new equilibrium with a suitably aged microbial community in an older human host[54].

Future perspective

In their recent review, Kau *et al.*[81] propose that 'the time is right and the need is great to understand better the relationships between diet, nutritional status, the immune system and microbial ecology in humans at different stages of life.' They suggest that a combination of metagenomics methods for describing the gut microbiome and the use of gnotobiotics (rearing of animals under germ-free conditions with subsequent exposure to single species) represents a potentially powerful approach to examine relationships between nutritional status and gut microbial communities. It may also provide a mechanistic understanding of the influence of the gut microbiota on the immune system that could be used to inform the design and execution of human studies. One of the challenges in this area is that the effects of probiotics on human health may be strain-specific and, as this review illustrates, dependent on the age of the host. Hypothesis-led research is therefore critical in evaluating the potential use of probiotics in respiratory infections and vaccination in the ageing population.

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Conflicts of interest

None.

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