Review

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Focus: Immunity and the microbiota

Influence of the microbiota on vaccine effectiveness

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Studies of the relationship between the microbiome and the development and function of the immune system are demonstrating novel concepts that could significantly alter the way we treat disease and promote wellness. Several diseases, including inflammatory bowel disease, allergy/asthma, and diabetes, are associated with changes in composition of the microbiome. Recent findings suggest novel complex mechanisms by which the microbiome impacts immune cell development and differentiation. A major implication of these findings is that the composition of microbiome may ultimately affect vaccine efficacy. We explore here the potential role of the microbiome in vaccine responses in the context of our growing understanding of the relationship between the gastrointestinal microbiota, resident immune cell populations, and systemic immunity.

Microbial composition affects the development and function of immune cell populations and vice versa

Microbial colonization of the human body begins at birth, and thereafter humans are colonized by what becomes a resident microbiota throughout life. We have coevolved for millions of years with our microbiota in a symbiotic relationship that is mutually beneficial (reviewed in [1]). Maintenance of this symbiotic relationship is essential for health, whereas its disruption leads to a state termed 'dysbiosis' (microbial imbalance) which predisposes the body to disease [2]. Although it has been known for some time that the intestinal resident microbiota are crucial symbionts (see Glossary) for metabolism and nutrition, mounting evidence suggests a previously unrecognized, mutually beneficial relationship based on crosstalk between the microbiota and the immune system, which impacts upon the behavior of both systems [3]. The significance of this crosstalk is most strikingly illustrated in the gastrointestinal tract of germ-free (GF) mice, which exhibit inappropriate organization and function of mucosal immune tissues (reviewed in [4]). Colonization of GF mice with the microbiota of conventionally raised mice [specific-pathogen free (SPF) conditions] restores many of these deficiencies, validating the requirement of the microbiota for the development and maturation of the gut-associated lymphoid tissue (GALT) [5,6]. Other studies have shown that distinct members of the gut microbiota can have a systemic impact by orchestrating the development of specific immune cell compartments [7–11]. In addition, recent experiments have shown that, in some cases, microbial metabolites can impact upon the cellular networks in the gut, offering mechanistic routes by which the microbiome

Glossary

Enterotype: the classification of humans or of other living organisms with an intestine based on similarities in their gut microbial communities as viewed through multivariate statistical methods.
Enteropathy: the presence of any pathology or inflammation in the intestine.
Mucosal vaccine: immunization through oral, nasal or vaginal routes.
Parenteral vaccine: vaccines administered through a non-mucosal surface (intravenous, intramuscular etc.).
Pathobionts: symbionts that are able to cause disease when the environmental conditions are altered.
Probiotics: viable non-pathogenic microorganisms that confer health benefits by augmenting the activities of commensal microorganisms in the gut. Examples of these activities include biosynthesis of vitamin K, fermentation of indigestible dietary fiber, and competition with pathogenic microorganism for nutrients, among others. Probiotics can modulate the host immune response directly through interaction with intestinal epithelial cells (IEC) or immune cells in the gut, or indirectly through the modulation of the intestinal microbiota. The immunomodulatory effects of probiotics seem to be microbe species- and strain-specific. The most important benefit of probiotics is likely the maintenance of gut homeostasis (sustaining a balanced and beneficial flora). Currently, the most common species of probiotics used are *Lactobacillus* and *Bifidobacterium* species.
Prebiotics: non-digestible dietary supplements (mainly fiber/carbohydrates) that also offer benefit to the host by selectively stimulating the growth of particular species of bacteria (mainly *Bifidobacterium*). Oligofructoses are naturally occurring plant carbohydrates, consisting of fructose polymers and a terminal glucose molecule, and combined with other prebiotics show positive effects. The most extensively used prebiotics are fructans such as inulin, fructo-oligosaccharides, and galacto-oligosaccharides. Because they are indigestible in the small intestine, they are fermented anaerobically in the colon to produce short-chain fatty acids (such as acetate, proprionate, and butyrate) that have been shown to modulate IEC function and favor the growth of other microbiota.
Symbionts: organisms that live in a state of symbiosis – a state in which at least two different organisms reside in a niche and closely associate to the benefit of each organism in the interaction.
Vaccine adjuvants: substances added to the vaccine which can increase the systemic and local immune response to the particular vaccine.
Vaccine efficacy: defined as the percentage reduction of disease incidence in a vaccinated group compared to an unvaccinated group. The vaccine efficacy is measured by double-blinded clinical trial, thereby measuring the 'best-case scenario' under strict and controlled conditions. It is very important to assess the safety of each new vaccine and rigorous assessment is required before regulatory authorities can license a vaccine.
Vaccine effectiveness: a measure of the ability of the vaccine to prevent disease in the 'real world'. Vaccine effectiveness is proportional to the vaccine efficacy, but is influenced primarily by how well the target groups are immunized. Many factors can influence vaccine effectiveness, including the population immunized (age, prior exposure, genetic make-up etc.) and vaccine characteristics (mode of delivery, accessibility, stability etc).

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Table 1. Immunoregulatory effects of microbiota in the GI tract[a].

Microbiota species	Immune regulation	Refs
Segmented filamentous bacteria (SFB)	*Alter T cell subsets in the terminal ileum* SFB induce accumulation of Th17 cells in the terminal ileum, through an unknown mechanism. It is hypothesized that SFB induce the production of SAA in the terminal ileum. This protein could act on lamina propria dendritic cells, which in turn stimulate a Th17 cell inducing environment.	[86]
Bacteroides fragilis (B. fragilis)	*Direct the development of FoxP3$^+$ Tregs in the colon* Polysaccharide A (PSA) from *B. fragilis* mediates conversion of CD4$^+$ T cells into Foxp3$^+$ Tregs. This effect is mediated through TLR2 signaling in T cells and not in dendritic cells. PSA–TLR2$^{-/-}$ interaction results in increased secretion of IL-10 by Tregs, and markedly reduces the expansion of Th17 cells in the gut.	[87–90]
Clostridium cluster IV (*C. leptum* group) and XIVa (*C. coccoides* group)	*Promote the expansion of colonic and systemic Tregs* Clostridia activate IEC to secrete TGFβ and other Treg-inducing molecules such as MMP2, MMP9, MMP13 and IDO, thus increasing the number of Tregs in the colon, but not in the small intestine. Tregs are also increased in the spleen, liver and lungs. Clostridia induction of Tregs seems to be independent of PRR because mice deficient for MyD88, Rip2, or Card9 have normal numbers of Tregs in the colon. Clostridia in the gut also seem to affects systemic sites. Low levels of systemic IgE and IL-4, and high levels of IL-10-producing splenocytes, are found in a model of OVA-induced asthma in animals carrying Clostridia.	[7,11]
Sphingomonas yanoikuyae	*Modulate the phenotype and response of iNKT cells* iNKT cells from GF mice show significant impairment in antigen-stimulated cytokine responses compared to SPF mice. Oral gavage with *S. yanoikuyae* could restore the hyporesponsiveness of iNKT from GF mice. This response is independent of TLR and IL-12 stimulation because mice deficient in these receptors have similar phenotypic changes.	[91]

[a]Abbreviations: Card9, caspase recruitment domain family, member 9, a key inducer of Dectic-1 signaling; IDO, indoleamine 2,3-dioxygenase; IEC, intestinal epithelial cells; iNTK cells, invariant natural killer T cells; MMP, matrix metalloproteinase; Myd88, myeloid differentiation protein 88 a signaling adaptor protein for Toll-like receptors; Rip2, receptor-interacting protein 2 (an adaptor protein for NOD receptors); SAA, serum amyloid A; TGFβ, transforming growth factor.

can guide immune responses. Previously documented microbiota/host immune interactions have been recently received by Kamada *et al.* [12] and are summarized in Table 1.

The immune system also clearly exerts its influence on the microbiota; both innate and adaptive immune components are modified by microbial composition [3]. Microbial sensing through Toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD) innate immunity receptors is crucial to maintain gut microbial homeostasis [13]. Virtually every mouse deficient in these receptors and/or their signaling adaptor proteins (such as MyD88) exhibits altered or dysbiotic microbiota [14] which, in many cases, is sufficient to induce disease [15]. Strikingly, in some cases transfer of this 'dysbiotic microbiota' into wild type hosts is sufficient to transmit disease [16]. Adaptive response effectors, such as secretory immunoglobulin (Ig) A, also have a key impact upon the microbiota. IgA is crucial for compartmentalization of the microbiota to the gut lumen, and IgA-deficient mice have increased microbiota penetration of the intestinal barrier and elevated microbe-specific serum IgG [17,18].

The above examples emphasize the symbiotic nature of the intestinal ecosystem in which the microbiota influence many physiological processes in the host while the host provides a nurturing niche for survival of specific microbial communities. Similar examples of this bidirectional mutualism are emerging in other mucosal tissues [19]. Collectively, this new appreciation of the host and its resident microbial community as a superorganism is changing the way we conceptualize and approach disease and treatment. It will be fundamental in designing prevention strategies and impacts directly upon vaccine development, adjuvants, and vaccine responses. In this review we explore the concept that the microbiota either directly or indirectly influence vaccine responses. We discuss the notion that discrepancies in vaccine effectiveness in different parts of the world could be a consequence of dysbiosis, malnutrition, and overexposure to microorganisms. We summarize recent literature on the impact of probiotics/prebiotics on vaccine efficacy, and finally we highlight two key articles that study the direct influence of the intestinal microbiota on oral vaccination.

Early-life environmental exposures and vaccine responses

The composition of the intestinal microbiota in each adult remains remarkably stable throughout life at the phylum level and in overall function, as seen through metagenomic analyses [20]. However, in humans this community of microbes does not become stable and mature until about 2 years of age (post-weaning), and several studies have shown the microbial composition prior to this time period is highly variable and sensitive to environmental exposures [20,21]. Thus, children under the age of 2 years are much more susceptible to environment-driven alterations in microbiota composition, and this can have lasting consequences on future immune responses.

There are three major environmental variables that affect microbiota early in life: diet (breast fed vs formula fed), delivery method (vaginal vs Caesarean section), and hygiene (clean vs unsanitary environment) [22]. Multiple studies have reported significant reductions of the probiotic microbes *Lactobacillus, Streptococcus*, and *Bifidobacterium* in infants who are not breast-fed [23,24]. Formula-fed infants, by contrast, have an expansion of *Bacteroides* species as well as Enterobactericeae (*Klebsiella* and *E. coli*, for example). Although no clinical trials have studied the impact that these exposures may have on oral or parenteral vaccines, it is likely that these differences in early-life

microbiota composition will be reflected in the immune response of the child to vaccines.

In regions of the world with poor sanitation, where increased fecal-oral bacterial exposure occurs early in life, there is clear evidence of reduced immunogenicity generated via oral vaccines. For example, an early study showed that serum antibody responses to oral cholera vaccines were more pronounced (>twofold) in children from a westernized country with access to proper sanitation, and low infection levels compared to those in a region with poor sanitation and increased enteric disease burden, as assessed in double-blind pediatric trials in Nicaragua and Sweden [25]. Since then, Levine and coworkers have reviewed years of clinical data and concluded that increased fecal-oral bacterial exposures dampened immune responses and efficacy of oral cholera vaccines [26]. Similarly, there is evidence of poor efficacy of rotavirus vaccines in developing countries with poor sanitation compared to industrialized nations [27–29]. Likewise, in areas of India, responses to an oral polio vaccine correlated with the location of the children tested. Children in poorer regions of Northern India elicited lower mucosal immune responses compared to the rest of India, again likely reflecting access to better sanitation in wealthier regions [30]. Thus, irrespective of whether the vaccine is viral or bacterial, its effectiveness in these resource-poor and undernourished settings, where poor sanitation is rampant, is correspondingly poor. Although there may be a nutritional component to this phenomenon (discussed in more detail below), it is also likely that it is driven by poor sanitation. For example, studies in Bangladesh have confirmed that, within populations sharing the same diet, there is wide range of antibody responses to an oral cholera vaccine that cannot be explained solely by nutrient deficiency [31,32]. A subclinical, chronic inflammatory condition in children, termed environmental enteropathy (EE), is now suggested to be the main driver of these effects [26,33]. EE is defined as chronic intestinal inflammation, and dysfunction of the small intestinal barrier, independent of any known infectious etiology [33]. The main features are visible in the small intestine and include villous blunting, increased intestinal permeability, and chronic inflammation, leading to malabsorption and growth stunting even without exposure to diarrheal disease [33]. Immunologically, children with EE have increased infiltration of $CD8^+$ intraepithelial lymphocytes [34]. The small intestinal microbiota of children with EE is shifted and dysbiotic, likely due to an overgrowth of Gram-negative pathobionts including *Klebsiella, E. coli*, and *Bacteroides* [35].

Although the etiology of EE remains unknown, a leading hypothesis is that individuals living in regions with high oral exposures to contaminated water and food are exposed to a larger variety of potentially harmful microorganisms, resulting in an altered intestinal immune system and thus altered responses to antigenic challenge, compared to those without these exposures [36]. In support of this hypothesis, there is a significant difference in T helper (Th)1 cytokine responsiveness to classical TLR agonists in children from poor regions of South Africa as compared to Canadian children [37]. Most strikingly, immune cells in the blood of South African infants aged 2 years are severely hyporesponsive to lipopolysaccharide (LPS) stimulation and secrete low levels of cytokines in response to TLR4 stimulation compared to age-matched children from Canada [38]. Although the mechanisms underlying this phenomenon are unknown, it has been postulated that this is due to differential environmental exposures to TLR ligands in different regions of the world. Because EE presents with small intestinal microbial dysbiosis, barrier defects, and chronic inflammation, oral vaccine antigens may not trigger a protective antibody response to the level of children who present with normal intestinal homeostasis (Figure 1). More studies are necessary to reveal the key issues related to EE and oral vaccine blunting, including the etiology of EE in children, which microbes drive the condition, and a better assessment of the immunological environment in the intestine of children with EE symptoms and their response to vaccines. Thus, animal models are sorely needed to facilitate the development of more efficacious vaccine strategies in populations afflicted with EE.

Taken together, early-life environmental exposures, diet, and sanitation all influence the microbiota composition encountered perinatally. During this time the immune system is plastic and not fully mature. It is not yet clear how this affects downstream vaccine effectiveness. However, in developing countries with poor sanitation, EE may be a major culprit for vaccine failure because it leads to a mucosal immune system that is chronically activated. In this scenario, an oral vaccine may not elicit a response in this population because the immunological function of the small intestinal may be permanently altered due to constant microbial penetration of the epithelium and a dysbiotic microbiota. Aside from sanitation, another major driver of microbial composition in these developing country populations is diet.

Impact of nutritional status on the microbiota and vaccine effectiveness

Malnutrition affects millions of children worldwide and is linked to 1 in 5 deaths in children under the age of 5 years [36,39]. It has been well documented that nutrition can impact upon the function of the mammalian adaptive immune system, and thus the responses to vaccines (reviewed in [40]). Many micronutrients are important for immune function and vaccine effectiveness, including vitamin A and zinc [41,42]. For example, vitamin A deficiency in mice has been shown to modulate trafficking of vaccine-specific $CD8^+$ T cells to the gastrointestinal tract in an ovalbumin (OVA)/simian immunodeficiency virus vaccine model by interfering with retinoic acid-dependent upregulation of mucosal homing integrins in vaccine-specific $CD8^+$ T cells [43]. In addition, lack of vitamin A can alter retinoic acid metabolism, leading to altered trafficking of dendritic cells (DCs) [44] and class II major histocompatibility complex (MHC-II) antigen presentation, each of which could affect DC presentation of vaccine antigens [45]. Furthermore, retinoic acid can serve as a cofactor to promote IgA-producing B cells, by synergizing with interleukin 5 (IL-5) secretion by DCs [46]. Innate lymphoid cells (ILCs) have been implicated as primary

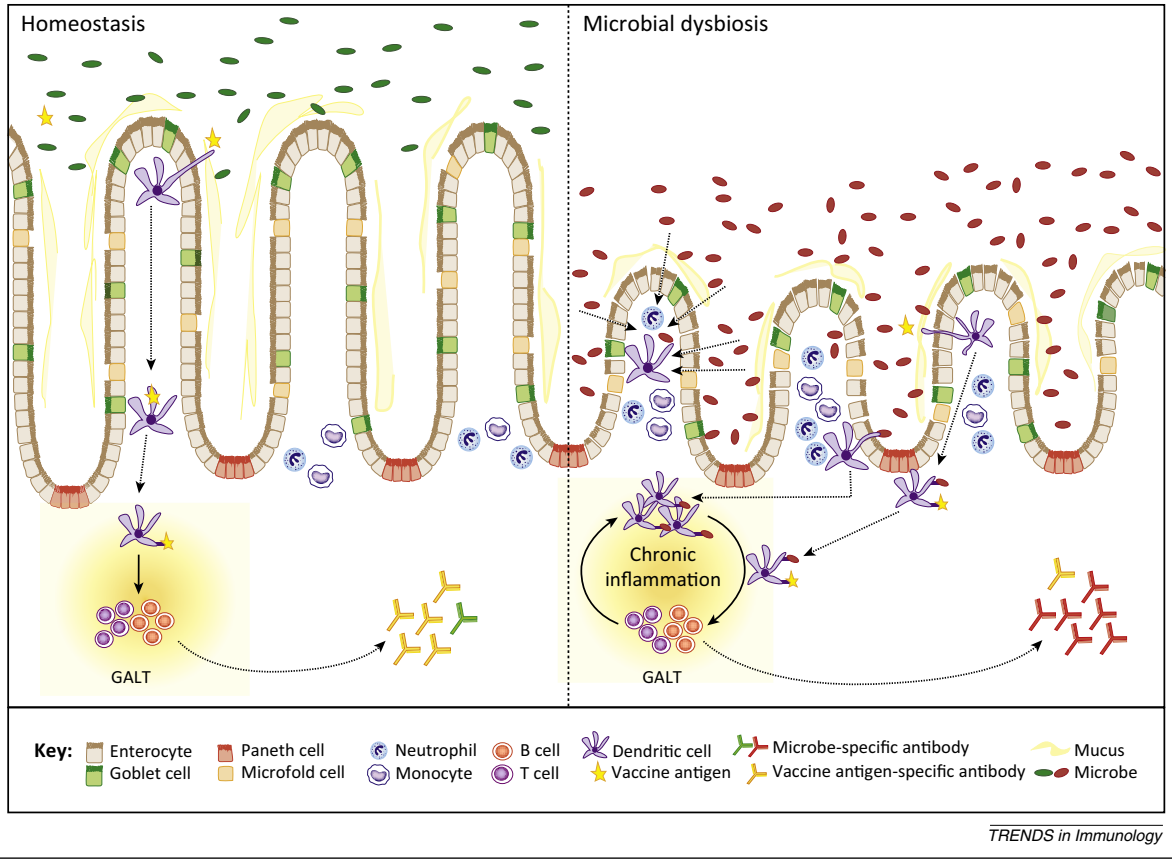

Figure 1. Intestinal homeostasis can affect vaccine effectiveness. In the small intestine of children with disorders such as environmental enteropathy, microbial dysbiosis can lead to tissue damage and thus to gut inflammation. If the dysbiosis persists, this can lead to chronic gut inflammation where there is an increase in intestinal permeability and a decrease in villous length and enterocyte absorption. Immune cells in the gut-associated lymphoid tissue (GALT) respond by creating dendritic cell (DC)-mediated T cell and antibody responses to invading microbiota. This leads to a cycle of inflammation, and the influx of proinflammatory cells, decrease in absorption potential, and shift in the immune populations can have an impact upon the host response to oral antigenic challenge. In this case, microbial translocation leads to an increase in DCs with antigens from the microbiota, and this may dampen the immunogenicity of a vaccine antigen because the immune system is preoccupied with preventing a systemic microbial breach of the intestine. Many oral vaccine antigens are microbial in nature, thus cross-presentation of microbiota antigens in chronic inflammation could affect the downstream antibody responses to the vaccine.

sensors of vitamin A malnutrition because vitamin A deficiency depletes IL-22-producing ILC3 cells, but can upregulate Th2-like cytokines secreted by ILC2 cells, such as IL-13, which can signal the gut immune system to respond to antigens in a Th2-skewed manner [47]. Many immune transcription factors and processes rely on zinc uptake, and in addition zinc helps to form a protective intestinal barrier against microbes [42]. For example, in zinc-deficient mice, serum antibody responses and T cell proliferation were significantly decreased after vaccination with a parental hepatitis B vaccine [48]. In light of the impact of diet on the composition of the intestinal microbiome [49], it is likely that prolonged malnutrition significantly alters the microbiome. How these diet-driven changes in the microbiome impact upon vaccine effectiveness is unknown.

One hypothesis is that expansion of gut pathobionts and inflammatory mediators in a malnourished host could overwhelm the immune system, leading to a gut ecosystem where vaccine antigens do not elicit robust immune responses as effectively (Figure 1). One study measured the response to a hepatitis B parenteral vaccine in celiac disease patients (noted for their expansion of upper intestinal pathobionts) with active or non-active disease, and found that serum IgG antibody titers against the vaccine were significantly blunted only during active disease [50], thereby significantly decreasing the likelihood of a host genetic component.

Several recent reports suggest that malnutrition also has a pervasive impact on the composition of the intestinal microbiota, including an expansion of Proteobacteria and decreased growth rate [51]. It was found [52] that protein deficiency in mice lacking the protein transport enzyme angiotension-coverting enzyme-2 (ACE2) regulates expression of antimicrobial peptides in the small intestine. This in turn affects microbial dysbiosis and predisposes the mice to intestinal inflammation after dextran sulfate sodium (DSS) treatment. ACE2-deficient mice lack expression of the neutral amino acid transporter BoAT1 in the small intestine, and exhibit markedly reduced serum levels of neutral amino acids including the essential amino acid tryptophan [52]. Transfer of microbiota from ACE2-deficient mice to GF mice demonstrated that microbiota composition changes due to malnutrition was sufficient to transfer the inflammatory phenotype to recipient mice [52]. This study provides an example of how the microbiota, nutrition, and the immune system are tightly linked and require homeostasis to function properly. Given the interrelationship between the

microbiota and nutrition, future studies on vaccine responses should also assess microbiota composition because its composition can predispose a host to intestinal damage and immune defects that are negatively correlated to vaccine outcome.

Impact of probiotics and prebiotics on vaccine responses

There is evidence that probiotics exert immunomodulatory effects in both *in vitro* systems and animal models (reviewed in [53]). Thus, an array of nutritional interventions using probiotics have been examined for their effects on vaccine responses in human populations (reviewed in [54–56]). Examples of these studies performed in humans are summarized in the Table 2. The rationale behind the use of probiotics, prebiotics, or a combination of the two for vaccine delivery is that they may improve vaccine efficacy by appropriately stimulating the immune system (adjuvant effect). However, the mechanisms behind the proposed beneficial effects of probiotics in vaccination remain unknown. Although some of these studies report significant increases in vaccine-specific antibodies [57–60], the majority show modest or no effect of probiotics in providing major enhancement of antibody responses to vaccines [61–65]. Moreover, the outcomes of these studies are highly dependent on experimental conditions including strain and dose of probiotics, type of vaccine, and population studied. Overall, owing to the inconsistency of these results, the clinical relevance of the effect of probiotics on vaccine efficacy in human populations remains undefined.

Vaccine-specific serum antibody titers are used as a surrogate measure of vaccine efficacy. Although many studies have shown a trend towards increased serum antibodies after administration of probiotics, these are not significantly higher than those obtained in placebo controls [62,66]. In addition, some probiotic formulations have demonstrated specific effects for one vaccine but not for others. For example, in a study performed in Finland [61], the diet of pregnant mothers was supplemented with a mixture of four probiotics during the last month of pregnancy (summarized in Table 2). After birth, infants received the same mix of probiotics and syrup containing 0.8 g of galacto-oligosaccharides once a day for 6 months. The infants were immunized at ages 3, 4, and 5 months with a triple diphtheria, tetanus, and *Haemophilus influenzae* type b (Hib) vaccine. Serum samples were analyzed at 6 months of age. The probiotic group exhibited a higher frequency of Hib-specific IgG compared to the placebo group. However, no differences in levels of IgG specific for diphtheria or tetanus were observed in probiotic group compared to placebo controls [61]. These differences suggest that probiotic formulations may improve responses to a particular vaccine but not to others. Indeed, it seems that the effects of probiotics are highly specific. For example, in a study performed in USA [57], healthy adults were given *Lactobacillus rhamnosus GG* (LGG) twice daily or placebo for 28 days post-vaccination with trivalent live attenuated influenza vaccine containing an H1N1 resembling that of the 2007–2008 strain, an H3N2-like strain, and a B-like strain. Serum samples analyzed at day 28 for antibody titers against each vaccine component showed a significant increase in H3N2-specific IgG levels in the probiotic group versus the placebo control group. However, no difference in IgG levels specific for the H1N1 or B strains were observed at the same time-point. The above examples illustrate the complexity of the effects of probiotics of vaccine responses.

Recent studies using animal models highlight the complexity of the immune mechanisms that confer protection during vaccination and their modulation through intervention with probiotics. Chattha *et al.* [67] studied the effect of rotavirus vaccines on T cell responses in gnotobiotic piglets colonized with a mixture of LGG and *Bifidobacterium lactis* Bb12 and vaccinated orally with attenuated human rotavirus. Treatment with probiotics resulted in higher serum levels of T helper 1 (Th1) cytokines such as interferon γ (IFN-γ) and IL-12, and reduced levels of Th2 cytokines (IL-4); these cytokine levels correlated with reduced severity of diarrhea and reduced virus shedding upon challenge with virulent rotavirus. Interestingly, increased levels of intestinal T regulatory cells (Tregs) were found in the probiotic group, suggesting that disease protection could be mediated not only by the Th1 response but also by regulatory responses necessary to restore gut homeostasis and reduce inflammation. In summary, there is evidence that probiotics may serve not only as adjuvants for particular types of vaccines but also as facilitators of a return to tissue homeostasis following pathogen challenge.

The dosage of probiotics may also exert an effect. For example, a study was designed to evaluate the effect of the dose of the probiotic *Lactobacillus acidophilus* and responses to oral rotavirus vaccines [68]. Gnotobiotic piglets were administered daily probiotics with a high dose (maximum dose 1×10^9), low dose (maximum dose 1×10^6), or no probiotic. The piglets were then immunized orally with attenuated human rotavirus vaccine at days 5 and 15 of probiotic treatment. A low dose of probiotic promoted higher IFN-γ production by $CD4^+$ and $CD8^+$ T cells in the intestine, in systemic sites, and in the blood, whereas high doses of the same probiotic increased intestinal and systemic Tregs. To examine the vaccine protective effects under the various doses of probiotics, the authors challenged the piglets with a virulent human rotavirus strain. They monitored clinical signs, virus shedding, and diarrhea for 7 days. Neither dose of LGG significantly enhanced protection conferred by the vaccine against rotavirus diarrhea. Thus, different doses of the same probiotic can influence distinct T cells responses induced by rotavirus vaccine. These findings may help to explain some of the controversies regarding supplementation with the same probiotics and the differential outcomes reported using similar formulations with different doses [69]. Thus, to validate probiotics in human trials, a better selection of the strain and dose of probiotics may help to promote better outcomes in vaccine effectiveness.

Only a few studies have evaluated the effect of prebiotics on vaccine responses. Similarly to probiotics, divergent conclusions have been reported, perhaps reflecting differences in methodology, use of different prebiotics, different timing of supplementation, etc. Some formulations of prebiotics have shown positive effects in animal models. Benyacoub *et al.* [70] studied the effect of a fructo-oligosaccharide (FOS):inulin mix on the efficacy of a attenuated murine

Table 2. Summary of recent trials testing the impact of probiotics on the immunogenicity and efficacy of vaccines.

Probiotics	Vaccine	Study design	Results	Refs
Lactobacillus rhamnosus GG (LGG) (ATCC 52103) 1.8×10^{10} cfu ($n = 31$). Maltodextrin was given to the placebo group ($n = 30$)	Parenteral: tetanus, *Haemophilus influenza* type b (Hib) and pneumococcal conjugate vaccine (PCV7)	Pregnant mothers received probiotics or placebo every day from week 36 of gestation until delivery. Blood samples were analyzed at 12 months age of the infants Study performed in Australia	Reduced antibody levels specific for tetanus, Hib and PCV7 were found in the probiotic group. Total IgG was similar in both groups	[65]
LGG (ATCC 52103) 5×10^9 cfu, LGG (LC705) 5×10^9 cfu, *Bifidobacterium breve* (Bbi99) 2×10^8 cfu and *Propionibacterium freudenreichii* ssp. Shermanii JS 2×10^9 cfu ($n = 47$). The placebo group received microcrystalline cellulose and infants received sugar syrup without galacto-oligosaccharides ($n = 40$)	Parenteral: diphtheria, tetanus and whole cell pertussis (DTwP), Hib	Pregnant mothers received a mix of probiotics or placebo capsules twice daily in the last month of pregnancy. After birth, infants received the same mix of probiotics and syrup containing 0.8 g of galacto-oligosaccharides once a day during 6 months. Infant blood samples were analyzed at 6 months of age. Study performed in Finland	No difference for diphtheria and tetanus-specific IgG was found between groups. There was a trend of higher frequency of Hib -specific IgG in the probiotic group	[61]
Bifidobacterium longum (ATCC BAA-999) 1×10^7 cfu *Lactobacillus rhamnosus* LPR 2×10^7 cfu. Probiotics ($n = 29$) or ($n = 28$) placebo for schedule A vaccination. Probiotic ($n = 77$) pr ($n = 68$) placebo for schedule B	Parenteral: schedule A: monovalent hepatitis B (HepB) at doses 1 and 2; dose 3 was a combination of hexavalent DT and acellular pertusis (DTaP) and HepB. Schedule B: only monovalent HepB for 3 doses	Newborns were given daily probiotics or placebo for 6 months. Blood was analyzed for HepB specific IgG at 12 months of age. Study performed in Singapore	Schedule A: there was a trend towards an increase in HepB-specific IgG. Schedule B: no difference in HepB-specific IgG	[66]
Lactobacillus acidophilus (ATCC 4356), *Bifidobacterum bifidum*, (DSMZ 20082), *Bifidobacterium longum* (ATCC 157078) and *Bifidobacterium infantis* (ATCC 15697), dose of 3×10^9 cfu each ($n = 25$). Placebo group received corn flour ($n = 22$)	Parenteral: mumps, measles, rubella, and varicella (MMRV) vaccine. Infants were immunized at ages 3, 4, and 5 months	Infants aged 10 months were given probiotics mix or placebo daily for 5 months. Blood samples were analyzed at 15 months of age. Study performed in Israel	There was no difference in specific IgG responses between the two groups	[64]
Bifidobacterium breve (BBG-01) 4×10^9 cfu ($n = 64$) and cornstarch and hydroxycellulose as placebo controls ($n = 62$)	Oral: cholera vaccine (Dukoral) was administered at days 21 and 35 of the study	Children of 2–5 years of age were administered probiotics or placebo for 4 weeks. Blood and feces samples were obtained on days 14, 28, and 42, and analyzed for IgG and IgA specific for *Vibrio cholera* LPS and cholera toxin B subunit (CTB). Study performed in Bangladesh	Placebo group exhibited higher levels of CTB-specific IgA in serum. The probiotics group showed higher levels of serum LPS-specific IgA and fecal CTB-specific IgA	[62]
Group 1: LGG 4×10^{10} cfu ($n = 21$). Group 2: *Lactobacillus lactis* 3.4×10^{10} cfu ($n = 10$). Group 3: Placebo (ethyl cellulose) ($n = 9$)	Oral: attenuated *Salmonella* Typhi Ty21a (Vivotif) were given to volunteers on days 1, 3, and 5	Adult volunteers received one of the probiotics or placebo for 7 days. Blood samples were obtained before vaccine administration and at day 7 post-vaccination. Study performed in Finland	No differences in Salmonella-specific IgA, IgG, or IgM were detected between the groups	[92]
Group 1: LGG (ATCC 53103), 1×10^{10} cfu ($n = 21$). Group 2: *Lactobacillus paracasei* (CRL431), 1×10^{10} cfu ($n = 21$) and Group 3: placebo ($n = 22$)	Oral: live attenuated poliomyelitis viruses type 1 (LS_{c1}), type 2 (P2712), type 3 ($12_{a1}b$). Vaccinations were administered at day 8	Healthy volunteers aged 20–30 were given a dose of probiotics or placebo once a day for 5 weeks. Blood samples were analyzed for poliovirus serotype-specific IgA, IgG, and IgM at 4 weeks before, immediately before, and 2, 4, and 7 weeks after vaccination. Study performed in Germany	Increased serotype 1 polio-specific IgA were observed in the probiotic groups compared to placebo. No effect was observed for the other polio serotypes	[58]

Table 2 (*Continued*)

Probiotics	Vaccine	Study design	Results	Refs
Group1: *Bifidobacterium lactis* (b1-07). Group2: *Bifidobacterium lactis* (Bl-04). Group 3: *Lactobacillus acidophilus* (La-14). Group 4: *Lactobacillus acidophilus* (NCFM). Group 5*: Lactobacillus plantarum* (Lp-115) Group 6: *Lactobacillus paracasei* (Lpc-37). Group 7: *Lactobacillus salivarius* (Ls33). Dose of 1×10^{10} cfu twice daily, ($n = 9$) for each group. Placebo (maltodextrin), ($n = 20$)	Oral: cholera vaccine (Dukoral) was given at days 7 and 14	Healthy volunteers aged 18–62 were assigned to one of the probiotic groups or to the placebo group for 21 days. Blood and saliva were obtained at day − 21, day 0, day 21, and day 28. Cholera toxin IgA, IgG, and IgM in serum was analyzed at day 0-21 (early response) or days 21–28 (late response. Salivary IgA was analyzed at the same time-points. Study performed in France	Higher vaccine-specific serum IgG was found only in subjects receiving group 2 and 3 probiotic vs placebo, day 7. No differences for serum IgA or IgM were found No difference were found in changes of antibodies in the probiotics groups compared to placebo, during late response	[59]
LGG 1×10^{10} cfu and 295 mg of 295 of inulin twice daily ($n = 21$) Placebo (gelatin and 355 mg inulin) ($n = 21$)	Nasally: trivalent live attenuated influenza vaccine 2007–2008 campaign containing: H1N1-like strain, H3N2-like strain, and B-like strain antigens	Healthy adults were given probiotics or placebo for 28 days post-vaccination. Blood was obtained before immunization and at days 14, 28, and 56, and analyzed for antibody titers for each vaccine component. Study performed in the USA	Significant increase in seroprotection in the LGG group vs placebo for the H3N2 strain. No protection for the H1N1 and B strains at day 28. No effect on seroprotection at day 56 for any strain	[57]
Group 1: *Bifidobacterium animalis* ssp (BB12) in capsule. Group 2: Placebo (capsule), 1×10^{9} cfu daily ($n = 53$). Group 2: placebo capsule ($n = 48$). Group 3: *Lactobacillus paracasei* ssp (431) in acidified milk, 1×10^{9} cfu daily ($n = 56$). Group 4: placebo acidified milk ($n = 54$)	Parenteral: trivalent attenuated influenza vaccine 2007–2008 campaign containing: H1N1-like strain, H3N1-like strain, and B strain. Vaccination at day 15	Healthy adults were given probiotics or placebo for 6 weeks. Blood samples were obtained before and at 6 weeks. Vaccine-specific IgG (IgG1 and IgG3) was analyzed in plasma and saliva. Study performed in Italy	Significant higher vaccine-specific IgG (IgG1 and IgG3) in the probiotics groups 1 and 2 vs placebo. Significant higher salivary IgA in probiotic groups vs placebo. No differences in IgG and IgM	[60]
Group 1: *Lactobacillus plantarum* (CECT 7315/16), 5×10^{9} cfu in powder skim milk ($n = 19$). Group2: *Lactobacillus plantarum* (CECT 7315/16), $5x10^{8}$ cfu in powder skim milk ($n = 14$). Group 3: placebo in powder milk ($n = 15$)	Parenteral: trivalent influenza vaccine, Spanish campaign 2006–2007 containing: H1N1, H3N2, and B strain.	Elderly institutionalized volunteers (65–85 years). Probiotics or placebo were administered 3–4 months after vaccination Blood samples were taken immediately before and 3 months after probiotics consumption. IgA, IgG, and IgM were analyzed in plasma. Study performed in Spain	Significantly higher vaccine-specific IgG was found in the group 1 vs placebo. Higher levels of vaccine-specific IgA were found in groups 2 and 3 vs placebo; no difference was found for IgM	[69]
Lactobacillus paracasei (MoLac-1), heat-killed 1×10^{10} cfu in jelly ($n = 8$). Placebo in jelly ($n = 7$)	Parenteral: trivalent influenza vaccine (H1N1, H3N2, and B strains) was administered 3 weeks after probiotic administration	Elderly volunteers from nursing home received heat-killed probiotics or placebo jelly for 12 weeks. Blood samples were collected at weeks 0, 6, and 12. Study performed in Japan	No significant difference was observed in innate immune response between the probiotics and placebo control groups. There was a trend towards higher vaccine antibody responses in the probiotic vs placebo group	[93]

Salmonella-based vaccine. Supplementing the diet of mice with the prebiotic mixture resulted in higher titers of LPS-specific IgA and flagellin-specific IgG. To evaluate efficacy, prebiotic-supplemented and control mice were infected with virulent strains of *Salmonella*. 73% of mice that received the FOS mix diet survived the infection, whereas only 40% of control mice survived. Similarly, using a model of murine influenza vaccine [71], one study analyzed the immune response after prebiotic administration at different time-points pre- and post-immunization. The prebiotic consisted of short-chain galactose oligosaccharides/long-chain fructo-oligosaccharides

(scGOS/lcFOS) (ratio 9:1) and pectin-derived acidic oligosaccharides (pAOS). Early prebiotic supplementation (14 days before until day 8 post-vaccination) increased vaccine-specific delayed-type hypersensitivity. By contrast, no significant effect was observed when the supplementation started at later time-points. Thus, the timing of prebiotic supplementation appears to be crucial for achieving improved immunomodulatory effects in vaccination interventions.

In contrast to data derived from animal models, the results from human studies using prebiotics and vaccines have not been encouraging. This is exemplified by a multinational study in Western Europe [72] in which the effect of adding a prebiotic mix (scGOS/lcFOS) and (pAOS) on specific vaccine responses to Hib and tetanus immunization was evaluated. The prebiotic mix was added to formula of healthy infants during the first year of life. No differences were found in IgG responses to Hib and tetanus vaccines between infants with or without prebiotic supplementation. Similar results were obtained in an independent study in shantytowns in Peru [73] that evaluated the effects of dietary supplementation with the prebiotic oligofructose on the prevalence of diarrhea and responses to immunization with Hib in infants (6–12 months). Blood samples obtained before immunization with Hib (~5–6 months) and 1 month post-vaccination failed to show an effect of the oligofructose prebiotic on antibody responses to Hib vaccination. Likewise, they failed to show any effect on the prevalence of diarrhea or other infections. Although the authors argued that the lack of a prebiotic effect could reflect the fact that breast milk already contains high concentrations of oligosaccharides, a previously described Western European study [72] failed to show an effect of prebiotics in infants fed with formula. Finally, a Dutch study aimed at evaluating the effect of prebiotics (scGOS/lcFOS) on vaccine responses in preterm infants failed to show an effect of prebiotics on vaccine responses to Diphtheria, tetanus, pertusis, polio, and Hib (DTaP-IPV-Hib) [74]. Although diet (breast or formula feeding) was not taken into account in correlating vaccine-specific antibody responses, both groups (scGOS/lcFOS and placebo) contained similar numbers of breast-fed infants.

Collectively the data suggest that, irrespective of socio-economic status or diet, prebiotics fail to show a positive effect on vaccine effectiveness in human (although no adverse effects were detected either). It is interesting, however, that the same prebiotic supplementation (scGOS/FOS 9:1) was shown to be protective against atopy because it could modulate protective immune response in infants at risk for allergy by reducing IgE levels and lowering the incidence of atopic dermatitis [75]. Similarly, this formulation protects infants with a parental history of atopy from all types of infections including upper respiratory infections and allergic disease [76–78]. Thus, in the absence of an effect of prebiotics on vaccine response, they may still be protective in other immune-mediated diseases, and in particular atopy. It is possible that the microbiota and/or the immune response are selectively altered in infants at high risk for atopy, and this can be modulated through prebiotic interventions.

Taken together, numerous studies have measured antibody titers post-administration of certain probiotics or prebiotics, but these show significant discrepancies that may reflect the type and dose of probiotic/prebiotic administered, the characteristics of the population studied (ethnicity, age of participants, socio-economic status etc.), duration of the interventions, type of vaccine analyzed (parenteral vs mucosal), or other differences in the vaccine schedules utilized in different countries.

Influence of intestinal microbial diversity on vaccine responses

Many factors influence vaccine effectiveness, including nutrition, age, sex, and genetics, (reviewed in [79–81]). However, as noted above, studies correlating intestinal microbiota and its direct influence on vaccine efficacy have been sparse [82] and sometimes discrepant (e.g., supplementation with probiotics/prebiotics) [54]. A few studies have begun to address whether a particular microbial community composition is associated with differential vaccine responses. For example, in a recent study human volunteers were vaccinated orally with a live attenuated *Salmonella* Typhi (Ty21a) vaccine [83]. Vaccination did not disrupt the composition, diversity, or stability of the microbial community. Vaccine-specific responses were assessed by measuring IFN-γ in $CD8^+$ T cells in blood and serum IgG and IgA titers against *S*. Typhi LPS. Only six individuals who received full doses of Ty21a vaccine were included in the analyses, and four unvaccinated served as controls. Four of the six vaccinated individuals exhibited increased $CD8^+$ IFN-γ responses to vaccination. Interestingly, three of these individuals harbored greater community richness and diversity of their microbiota compared with vaccinated individuals showing lower $CD8^+$ IFN-γ ($n = 2$). Analyses of specific bacterial OTUs that could discriminate between high and low IFN-γ-producing individuals revealed more abundant Clostridiales in high IFN-γ-producing individuals. Overall, the authors speculated that vaccine efficacy could potentially be related to the composition of the resident commensal community. This is the first study to make a direct link between microbiota composition and vaccine responses.

In a related study by the same group, Macaques of different geographic origins (with distinct genetic make-up and a different MHC allele repertoire) were immunized with two live attenuated *Shigella disenteriae* (*S. dysenteriae*) vaccine candidates [84]. Protection was evaluated by response to subsequent challenge with wild type *S. dysenteriae*. Three types of immunizations were performed – group 1, vaccinations at days 0 and 28 with candidate 1 ($n = 6$) and challenge at day 56; group 2, vaccinations days 0 and 28 with candidate 2 ($n = 6$) and challenge at day 56; group 3, vaccination at days 0, 2, 4, and 7 ($n = 6$) and challenge at day 28. Controls for each group received saline. Analysis of bacterial communities in feces showed distinct community types in macaques (enterotypes) and, similarly to the previous study, a high level of diversity in the intestinal microbial composition of the macaques correlated with improved protection upon challenge with virulent *S. dysenteriae*. The authors also found that the macaque microbial communities changed

during immunization and challenge with virulent *S. dysenteriae*. Interestingly, macaques that harbored a higher microbial diversity exhibited a more stable microbial community following vaccination and challenge, and this correlated with reduced clinical symptoms of shigellosis. Thus, a more diverse intestinal microbiota may play a protective role in response to enteric pathogens. It is known that enteric pathogens disturb the microbiota to colonize the same niche occupied by commensals [85]. Correspondingly, a more stable microbiome can more effectively resist changes caused by enteropathogens. Studies on the association between the microbiota and vaccine-specific IgG and IgA showed a positive correlation between antibody levels and some microbiota genera. For example, there was a positive correlation with *Oscillospira* and a negative correlation with *Streptococcus* in protective responses. Although many factors such as vaccine regimen (different vaccine strain used) and the different gut bacterial communities of the macaques before the intervention prevent wider comparison, this finding suggests that particular microbiome compositions could influence antibody responses.

The above vaccine studies are the first to use DNA sequence analysis to define the composition of the intestinal microbial communities and correlate these with vaccine-specific responses. The findings suggest that a more diverse intestinal microbiota fosters a more protective immune response to oral vaccines against intestinal pathogens. This highlights the importance of addressing the composition of the intestinal microbiota in human vaccine trials and may help to explain why vaccine trials show considerable discrepancies in different parts of the world [82]. Given technological advances in high-throughput sequencing and microbial community analyses, the feasibility of evaluating

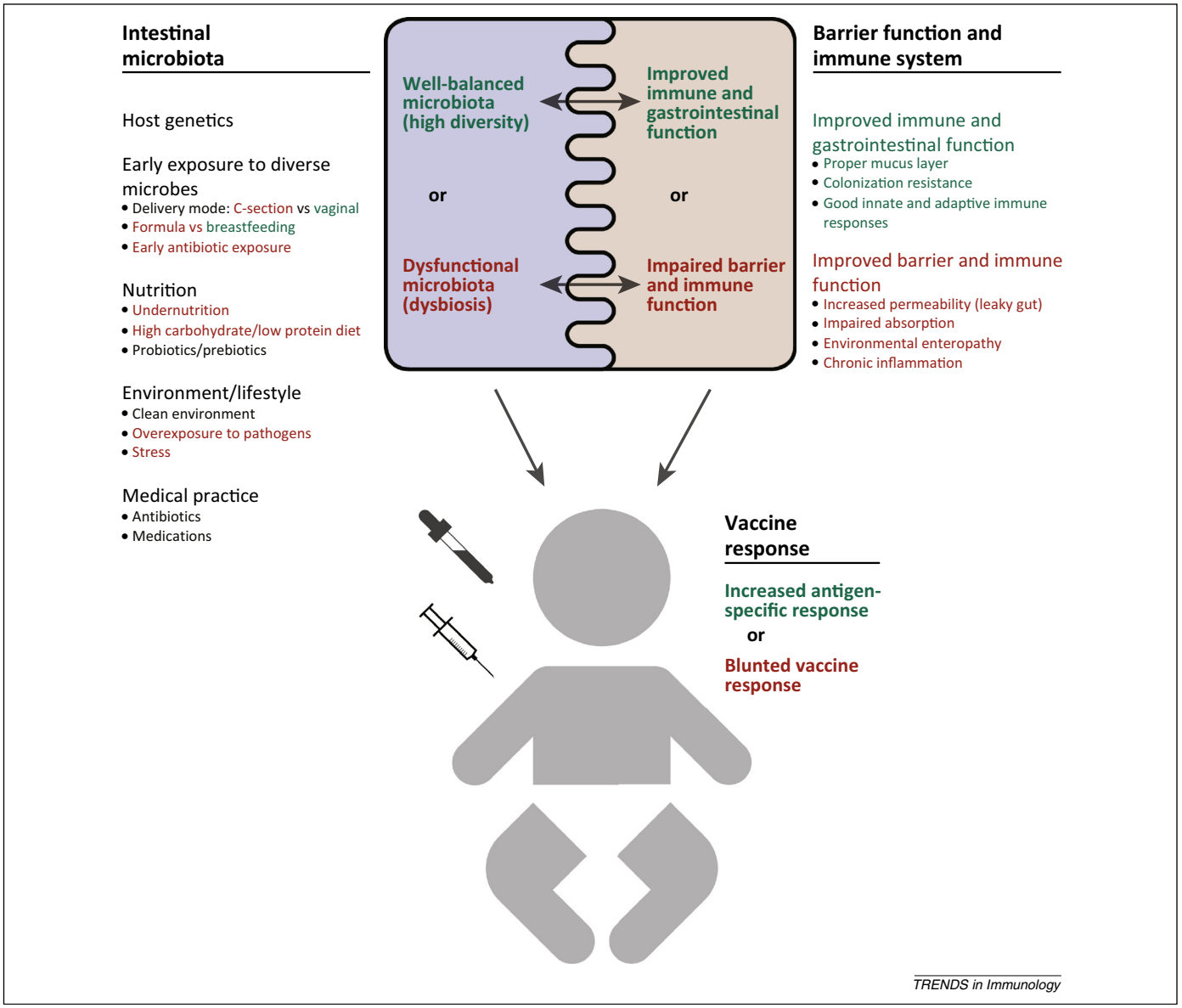

Figure 2. Scheme illustrating how the microbiota may influence vaccine responses. Numerous studies have shown an interconnection between the intestinal microbiota and the immune response. This mutualistic relationship is bidirectional in that the intestinal flora influences immune system function, and the immune system in turn modulates microbial diversity. Ideally, an effective vaccine preparation will elicit an immune response against the agent administered. We suggest that microbiota composition and diversity modulate the immune response to vaccines.

microbiome composition in these types of trials should no longer be an impediment, although interpreting the results remains a challenge.

Concluding remarks and outstanding questions

We have come to appreciate that the microbiome and host immune responses (especially early in life) are tightly linked. Given that vaccines require immune responses, one may infer that the microbiota is likely to influence vaccine responses. Moreover, studies suggest that the diversity and composition of the gut microbiota may influence the efficacy of oral vaccines. Given all this, the failure to develop protective immunity to vaccines in particular geographical areas could be due to microbial composition (Figure 2). Unfortunately, only a very small number of studies have directly addressed vaccine responses and microbiota composition. There is a real need for a mechanistic understanding of the role of microbiota in immune function, and to relate the composition of microbiota and/or specific microbial communities to responses to oral and parenteral vaccines. Hopefully, ongoing large-scale vaccination campaigns that are already in place can be exploited to evaluate the link between microbiome composition and vaccine effectiveness. For instance, the influenza vaccine could provide an excellent research model for this type of analysis. As in the studies with probiotic supplementation (although the composition of the microbial diversity post-supplementation was not evaluated), yearly flu vaccine campaigns could provide useful yearly data. Similarly, studies correlating the use of antibiotics (that cause a major effect on microbial diversity) before vaccination, followed by analysis of vaccine-specific responses, would provide major insights into changes in the microbiota and vaccine responses.

It is well known that oral vaccines are not as effective in regions of the world where malnutrition is prevalent, and there is a broad push to improve vaccine responses in these populations. The low responses could reflect prior exposure to pathogens or be due to differences in microbiota composition as a result of environmental and nutritional differences. Dietary interventions alone are unlikely to improve vaccine responses in malnourished populations to an acceptable level for efficacy. Therefore, further understanding the impact of the intestinal microbiota on vaccine effectiveness in situations of malnutrition would be first step towards improving the efficacy of vaccines worldwide. Likewise, in individuals with dysbiosis or 'leaky gut', such as celiac disease or type I diabetes, nature is providing us with natural hosts with altered gut immune status that could be exploited to measure vaccine responses. Given the central role that microbiota have on immune system development, it is a natural extension that the microbiota will impact upon vaccine efficacy. However, there is little actual data thus far. Moreover, the few studies carried out have been limited to general changes in microbiota communities. What is needed is a detailed analysis of the role of particular species within communities and their correlation with vaccine responses. Given the difficulty in defining a healthy microbiome, it makes it all the more challenging to define an optimal microbiome for a particular vaccine to generate its desired effects. However, the tools are now readily available (community sequencing, metagenomics, metabolomics, bioinformatics, etc.), and they will begin to provide the necessary answers. It is not hard to conceive of a future where microbiota composition is analyzed before vaccine administration and, if necessary, altered or a particular microbiota molecule added to ensure an optimal vaccine response. Similarly, adjuvants could include particular microbiota-derived immune-modulating molecules. The ever-expanding information on how the microbiota shapes the immune system will have a profound impact on the future of vaccines, requiring a major shift in our current approach to vaccine development.

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References

1 Brown, E.M. *et al.* (2013) The role of the immune system in governing host–microbe interactions in the intestine. *Nat. Immunol.* 14, 660–667
2 Strober, W. (2013) Impact of the gut microbiome on mucosal inflammation. *Trends Immunol.* 34, 423–430
3 Hooper, L.V. *et al.* (2012) Interactions between the microbiota and the immune system. *Science* 336, 1268–1273
4 Smith, K. *et al.* (2007) Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin. Immunol.* 19, 59–69
5 Bouskra, D. *et al.* (2008) Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* 456, 507–510
6 Hansen, C.H. *et al.* (2012) Patterns of early gut colonization shape future immune responses of the host. *PLoS ONE* 7, e34043
7 Atarashi, K. *et al.* (2011) Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 331, 337–341
8 Round, J.L. and Mazmanian, S.K. (2010) Inducible Foxp3$^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl. Acad. Sci. U.S.A.* 107, 12204–12209
9 Chung, H. *et al.* (2012) Gut immune maturation depends on colonization with a host-specific microbiota. *Cell* 149, 1578–1593
10 Ivanov, I.I. *et al.* (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498
11 Atarashi, K. *et al.* (2013) Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 500, 232–236
12 Kamada, N. *et al.* (2013) Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* 13, 321–335
13 Amendola, A. *et al.* (2014) Nod2 deficiency is associated with an increased mucosal immunoregulatory response to commensal microorganisms. *Mucosal Immunol.* 7, 391–404
14 Rakoff-Nahoum, S. *et al.* (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118, 229–241
15 Slack, E. *et al.* (2009) Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* 325, 617–620
16 Couturier-Maillard, A. *et al.* (2013) NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J. Clin. Invest.* 123, 700–711
17 Macpherson, A.J. *et al.* (2012) The habitat, double life, citizenship, and forgetfulness of IgA. *Immunol. Rev.* 245, 132–146
18 Suzuki, K. *et al.* (2004) Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proc. Natl. Acad. Sci. U.S.A.* 101, 1981–1986

19 Ichinohe, T. *et al.* (2011) Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5354–5359
20 Yatsunenko, T. *et al.* (2012) Human gut microbiome viewed across age and geography. *Nature* 486, 222–227
21 Palmer, C. *et al.* (2007) Development of the human infant intestinal microbiota. *PLoS Biol.* 5, e177
22 Penders, J. *et al.* (2006) Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 118, 511–521
23 Guaraldi, F. and Salvatori, G. (2012) Effect of breast and formula feeding on gut microbiota shaping in newborns. *Front. Cell. Infect. Microbiol.* 2, 94
24 Jost, T. *et al.* (2012) New insights in gut microbiota establishment in healthy breast fed neonates. *PLoS ONE* 7, e44595
25 Hallander, H.O. *et al.* (2002) Calibrated serological techniques demonstrate significant different serum response rates to an oral killed cholera vaccine between Swedish and Nicaraguan children. *Vaccine* 21, 138–145
26 Levine, M.M. (2010) Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. *BMC Biol.* 8, 129
27 Jiang, V. *et al.* (2010) Performance of rotavirus vaccines in developed and developing countries. *Hum. Vaccin.* 6, 532–542
28 Goveia Michelle, G. *et al.* (2010) RotaTeq: progress toward developing world access. *J. Infect. Dis.* 202, S87–S92
29 Lopman, B.A. *et al.* (2012) Understanding reduced rotavirus vaccine efficacy in low socio-economic settings. *PLoS ONE* 7, e41720
30 Grassly, N.C. *et al.* (2009) Mucosal immunity after vaccination with monovalent and trivalent oral poliovirus vaccine in India. *J. Infect. Dis.* 200, 794–801
31 Johnson, R.A. *et al.* (2012) Comparison of immune responses to the O-specific polysaccharide and lipopolysaccharide of *Vibrio cholerae* O1 in Bangladeshi adult patients with cholera. *Clin. Vaccine Immunol.* 19, 1712–1721
32 Leung, D.T. *et al.* (2011) Comparison of memory B Cell, antibody-secreting cell, and plasma antibody responses in young children, older children, and adults with infection caused by *Vibrio cholerae* O1 El Tor Ogawa in Bangladesh. *Clin. Vaccine Immunol.* 18, 1317–1325
33 Korpe, P.S. and Petri, W.A. (2012) Environmental enteropathy: critical implications of a poorly understood condition. *Trends Mol. Med.* 18, 328–336
34 Campbell, D.I. *et al.* (2003) Chronic T cell-mediated enteropathy in rural west african children: relationship with nutritional status and small bowel function. *Pediatr. Res.* 54, 306–311
35 Ghoshal, U.C. *et al.* (2003) Tropical sprue is associated with contamination of small bowel with aerobic bacteria and reversible prolongation of orocecal transit time. *J. Gastroenterol. Hepatol.* 18, 540–547
36 Humphrey, J.H. (2009) Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet* 374, 1032–1035
37 Reikie, B.A. *et al.* (2012) Ontogeny of Toll-like receptor mediated cytokine responses of South African infants throughout the first year of life. *PLoS ONE* 7, e44763
38 Smolen, K.K. *et al.* (2014) Pattern recognition receptor-mediated cytokine response in infants across 4 continents. *J. Allergy Clin. Immunol.* 133, 818–826
39 Khan, Y. and Bhutta, Z.A. (2010) Nutritional deficiencies in the developing world: current status and opportunities for intervention. *Pediatr. Clin. North Am.* 57, 1409–1441
40 Savy, M. *et al.* (2009) Landscape analysis of interactions between nutrition and vaccine responses in children. *J. Nutr.* 139, 2154S–2218S
41 Mora, J.R. *et al.* (2008) Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat. Rev. Immunol.* 8, 685–698
42 Ibs, K.H. and Rink, L. (2003) Zinc-altered immune function. *J. Nutr.* 133, 1452S–1456S
43 Kaufman, D.R. *et al.* (2011) Vitamin A deficiency impairs vaccine-elicited gastrointestinal immunity. *J. Immunol.* 187, 1877–1883
44 Klebanoff, C.A. *et al.* (2013) Retinoic acid controls the homeostasis of pre-cDC-derived splenic and intestinal dendritic cells. *J. Exp. Med.* 210, 1961–1976
45 Beijer, M.R. *et al.* (2013) A crucial role for retinoic acid in the development of Notch-dependent murine splenic $CD8^-$ $CD4^-$ and $CD4^+$ dendritic cells. *Eur. J. Immunol.* 43, 1608–1616
46 Mora, J.R. *et al.* (2006) Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 314, 1157–1160
47 Spencer, S.P. *et al.* (2014) Adaptation of Innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. *Science* 343, 432–437
48 Zhao, N. *et al.* (2013) Gestational zinc deficiency impairs humoral and cellular immune responses to hepatitis B vaccination in offspring mice. *PLoS ONE* 8, e73461
49 David, L.A. *et al.* (2013) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559–563
50 Nemes, E. *et al.* (2008) Gluten intake interferes with the humoral immune response to recombinant hepatitis B vaccine in patients with celiac disease. *Pediatrics* 121, e1570–e1576
51 Smith, M.I. *et al.* (2013) Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* 339, 548–554
52 Hashimoto, T. *et al.* (2012) ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 487, 477–481
53 Hardy, H. *et al.* (2013) Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. *Nutrients* 5, 1869–1912
54 Maidens, C. *et al.* (2013) Modulation of vaccine response by concomitant probiotic administration. *Br. J. Clin. Pharmacol.* 75, 663–670
55 Licciardi, P.V. and Tang, M.L. (2011) Vaccine adjuvant properties of probiotic bacteria. *Discov. Med.* 12, 525–533
56 MacDonald, T.T. and Bell, I. (2010) Probiotics and the immune response to vaccines. *Proc. Nutr. Soc.* 69, 442–446
57 Davidson, L.E. *et al.* (2011) Lactobacillus GG as an immune adjuvant for live-attenuated influenza vaccine in healthy adults: a randomized double-blind placebo-controlled trial. *Eur. J. Clin. Nutr.* 65, 501–507
58 de Vrese, M. *et al.* (2005) Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination. *Eur. J. Nutr.* 44, 406–413
59 Paineau, D. *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
60 Rizzardini, G. *et al.* (2012) Evaluation of the immune benefits of two probiotic strains *Bifidobacterium animalis* ssp. lactis, BB-12(R) and *Lactobacillus paracasei* ssp. paracasei, *L. casei* 431(R) in an influenza vaccination model: a randomised, double-blind, placebo-controlled study. *Br. J. Nutr.* 107, 876–884
61 Kukkonen, K. *et al.* (2006) Effect of probiotics on vaccine antibody responses in infancy – a randomized placebo-controlled double-blind trial. *Pediatr. Allergy Immunol.* 17, 416–421
62 Matsuda, F. *et al.* (2011) Evaluation of a probiotics, *Bifidobacterium breve* 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 West, N.P. and Cripps, A.W. (2013) Are vaccination models suitable to determine whether probiotics have beneficial health effects in the general population? *Hum. Vaccin. Immunother.* 9, 621–624
64 Youngster, I. *et al.* (2011) Probiotics and the immunological response to infant vaccinations: a prospective, placebo controlled pilot study. *Arch. Dis. Child.* 96, 345–349
65 Licciardi, P.V. *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
66 Soh, S.E. *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
67 Chattha, K.S. *et al.* (2013) Divergent immunomodulating effects of probiotics on T cell responses to oral attenuated human rotavirus vaccine and virulent human rotavirus infection in a neonatal gnotobiotic piglet disease model. *J. Immunol.* 191, 2446–2456
68 Wen, K. *et al.* (2012) High dose and low dose Lactobacillus acidophilus exerted differential immune modulating effects on T cell immune responses induced by an oral human rotavirus vaccine in gnotobiotic pigs. *Vaccine* 30, 1198–1207
69 Bosch, M. *et al.* (2012) *Lactobacillus plantarum* CECT7315 and CECT7316 stimulate immunoglobulin production after influenza vaccination in elderly. *Nutr. Hosp.* 27, 504–509

70 Benyacoub, J. *et al.* (2008) Feeding a diet containing a fructooligosaccharide mix can enhance *Salmonella* vaccine efficacy in mice. *J. Nutr.* 138, 123–129
71 Vos, A.P. *et al.* (2010) Specific prebiotic oligosaccharides modulate the early phase of a murine vaccination response. *Int. Immunopharmacol.* 10, 619–625
72 Stam, J. *et al.* (2011) A mixture of three prebiotics does not affect vaccine specific antibody responses in healthy term infants in the first year of life. *Vaccine* 29, 7766–7772
73 Duggan, C. *et al.* (2003) Oligofructose-supplemented infant cereal: two randomized, blinded, community-based trials in Peruvian infants. *Am. J. Clin. Nutr.* 77, 937–942
74 van den Berg, J.P. *et al.* (2013) Neutral and acidic oligosaccharides supplementation does not increase the vaccine antibody response in preterm infants in a randomized clinical trial. *PLoS ONE* 8, e70904
75 van Hoffen, E. *et al.* (2009) A specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides induces a beneficial immunoglobulin profile in infants at high risk for allergy. *Allergy* 64, 484–487
76 Arslanoglu, S. *et al.* (2007) Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J. Nutr.* 137, 2420–2424
77 Arslanoglu, S. *et al.* (2012) Early neutral prebiotic oligosaccharide supplementation reduces the incidence of some allergic manifestations in the first 5 years of life. *J. Biol. Regul. Homeost. Agents* 26, 49–59
78 Arslanoglu, S. *et al.* (2008) Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *J. Nutr.* 138, 1091–1095
79 Klein, S.L. *et al.* (2010) The Xs and Y of immune responses to viral vaccines. *Lancet Infect. Dis.* 10, 338–349
80 Benn, C.S. *et al.* (2013) A small jab – a big effect: nonspecific immunomodulation by vaccines. *Trends Immunol.* 34, 431–439
81 Markle, J.G. and Fish, E.N. (2014) SeXX matters in immunity. *Trends Immunol.* 35, 97–104
82 Ferreira, R.B. *et al.* (2010) Should the human microbiome be considered when developing vaccines? *PLoS Pathog.* 6, e1001190
83 Eloe-Fadrosh, E.A. *et al.* (2013) Impact of oral typhoid vaccination on the human gut microbiota and correlations with *S.* Typhi-specific immunological responses. *PLoS ONE* 8, e62026
84 Seekatz, A.M. *et al.* (2013) Differential response of the cynomolgus macaque gut microbiota to *Shigella* infection. *PLoS ONE* 8, e64212
85 Lupp, C. *et al.* (2007) Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Microbe* 2, 204
86 Ivanov, I.I. *et al.* (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 4, 337–349
87 Liu, C.H. *et al.* (2008) Regulation of surface architecture by symbiotic bacteria mediates host colonization. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3951–3956
88 Mazmanian, S.K. *et al.* (2005) An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122, 107–118
89 Mazmanian, S.K. *et al.* (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453, 620–625
90 Round, J.L. *et al.* (2011) The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 332, 974–977
91 Wingender, G. *et al.* (2012) Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. *Gastroenterology* 143, 418–428
92 Fang, H. *et al.* (2000) Modulation of humoral immune response through probiotic intake. *FEMS Immunol. Med. Microbiol.* 29, 47–52
93 Akatsu, H. *et al.* (2013) Lactobacillus in jelly enhances the effect of influenza vaccination in elderly individuals. *J. Am. Geriatr. Soc.* 61, 1828–1830