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Probiotics, antibiotics and the immune responses to vaccines

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Orally delivered vaccines have been shown to perform poorly in developing countries. There are marked differences in the structure and the luminal environment of the gut in developing countries resulting in changes in immune and barrier function. Recent studies using newly developed technology and analytic methods have made it increasingly clear that the intestinal microbiota activate a multitude of pathways that control innate and adaptive immunity in the gut. Several hypotheses have been proposed for the underperformance of oral vaccines in developing countries, and modulation of the intestinal microbiota is now being tested in human clinical trials. Supplementation with specific strains of probiotics has been shown to have modulatory effects on intestinal and systemic immune responses in animal models and forms the basis for human studies with vaccines. However, most studies published so far that have evaluated the immune response to vaccines in children and adults have been small and results have varied by age, antigen, type of antibody response and probiotic strain. Use of anthelminthic drugs in children has been shown to possibly increase immunogenicity following oral cholera vaccination, lending further support to the rationale for modulation of the immune response to oral vaccination through the intestinal microbiome.

1. Introduction

Over the past five decades since oral vaccines were first introduced, experience in developing countries has shown that immune responses may be lower and less consistent than in more industrialized countries. Live, oral vaccines, including oral rotavirus vaccines and oral polio vaccines (OPVs), have historically performed poorly in the developing world. Several candidate rotavirus vaccines (RIT4237, WC3, RRV and RRV-TV) had lower or no measureable efficacy in clinical trials in South America and Africa compared with Europe and North America [1]. More recent studies with new licenced vaccines, Rotarix and Rotateq, have shown lower efficacy and effectiveness in developing countries in Asia, Africa and Latin America [2–6]. The OPV has also faced challenges in the developing world. As OPV is less immunogenic in developing compared with developed countries, more doses of OPV appear to be necessary to protect children in developing countries [7–9]. This diminished performance of oral vaccines in the developing world has been postulated to be due to host factors such as maternal antibody, age at vaccination, malnutrition and micronutrient deficiencies, persistent exposure to pathogens in the environment, an altered gut microbiota and higher prevalence of medical conditions such as tuberculosis and HIV infection, or virus factors such as substantial strain heterogeneity [10–12].

Most hypotheses that propose the role of host or environmental factors in determining immune response to oral vaccines are based on observational studies. Proving a causal role for any of the factors considered responsible for the poor performance of oral vaccines is challenging. Enteric immune responses are evoked, maintained and regulated in complex relationships in which host, environment, enteric commensals, pathogens and vaccines interact, and where there is a limited understanding of these interactions [13]. Recently, a number of studies have been designed as clinical trials that have aimed to dissect the role of a limited number of factors affecting the performance of oral vaccines in developing countries.

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Table 1. Comparison of the flora of the upper small intestine of children in developed and developing countries.

	UK, duodenum ($n = 13$) [29]	Australia, duodenum ($n = 20$) [30]	India, jejunum ($n = 10$) [31]
sterile	54%	80%	0
total count $\log_{10}$/ml	3.6 (in +ves)	3 (in +ves)	4.7
coliforms	0	4	4
staphylococci	6		2
streptococci	2		9
Neisseria	2		3
Pseudomonas	0	2	2
anaerobic streptococci	1	1	4
yeasts	0	1	4
Veillonella	1		5
lactobacilli	1		5
fusobacteria			4
bifidobacteria			5
Bacteroides	0	0	0

Examples of such hypothesis-testing exploratory clinical trials are recent studies on breastfeeding and rotavirus vaccine performance in developing countries. With OPV, it had been shown that breast milk antibodies could neutralize OPV [14], and this observation was supported by studies from Africa [15]. Subsequent studies in the USA and India did not find an association between breastfeeding and vaccine immunogenicity [16,17], and a recommendation to withhold breastfeeding prior to and after oral polio vaccination that had appeared in the Red Book of the American Academy of Pediatrics in 1970 [18] was subsequently withdrawn. A similar interference by breast milk antibodies with the 'take' of oral rotavirus vaccine was proposed based on the findings that mothers in developing countries have much higher levels of neutralizing antibodies [19,20] and that lower seroconversion rates were reported in children of mothers with high anti-rotavirus IgG [21]. It was hypothesized that immunogenicity of oral rotavirus vaccines in developing countries could be improved by restriction of breastfeeding. Studies were designed and conducted in South Africa, India and Pakistan, which withheld or did not withhold breastfeeding around the time of immunization, and demonstrated that restriction of breastfeeding did not affect the IgA immune response to oral rotavirus vaccines [22,23]. Although serum IgA immune responses are not a correlate of protection, the results are reassuring to the public health community, in that there is no evidence to suggest a change in breastfeeding practices at the time of immunization. Nonetheless, given the low immunogenicity of enteric vaccines in developing countries, the identification of strategies that might potentially be feasible in communities and enhance the response to vaccines clearly needs to be explored.

2. The gut environment in developing countries

As a physical interface with the external environment, the gut epithelium has multiple functions of a physical and biological barrier that is also responsible for secretion and absorption. In health, homeostasis is maintained at intestinal surfaces between luminal microorganisms and host tissues to differentiate responses appropriate to native commensals versus potential pathogens. The protective mucous layer in the intestine controls unchecked bacterial proliferation and also serves as a feedback system to regulate both innate and adaptive immune responses [24,25]. However, it has been clear for several decades that there are distinct differences in the morphology and function of the gut in developed and developing countries.

Studies on biopsies of the small bowel during the 1960s and 1970s identified differences in the mucosa between healthy adults living in industrialized countries and adults living in developing countries. The predominant finger-like villus structure of the gut in people from Western countries was not seen in those from developing countries; these individuals had flatter leaf-like and blunt villus forms [26,27], resulting in a significant reduction in the villus-to-crypt ratio due to villus shortening and a concomitant reduction in the surface area of mature absorptive intestinal epithelial cells (IECs). Histological examination of the biopsies also showed increased numbers of inflammatory cells in the lamina propria, including lymphocytes and plasma cells [28]. Studies on the upper gastrointestinal flora in apparently healthy children in developed and developing countries also revealed marked differences, with colonization of the upper small bowel with a diverse aerobic and anaerobic flora in a large proportion of children in India while children in the UK and Australia either had no organisms, or limited numbers and species (table 1) [29–31]. Although there are no recent studies on the small bowel flora in children in developing countries, several studies have demonstrated that the microbiota in the stool of children living in low-income countries is distinct in its composition, and more variable over time, when compared with the microbiota of children living in high-income countries [32,33]. Mode of delivery and feeding practices have also been shown to independently modify microbial composition in infancy [34].

In addition to the differences in the commensal flora, it is also known that in the developing world, both with diarrhoea and in its absence, children carry multiple pathogens [35–38]. It has been hypothesized that the constant activation

of inflammatory signalling by pathogen-associated molecular patterns (PAMPs) and other danger molecules results in vaccine antigens failing to generate productive responses [39]. These data also suggest the possibility that combinations of known and unknown bacteria, viruses and parasites may contribute to the dysregulated gut inflammation seen in environmental enteropathy.

Environmental enteropathy or tropical enteropathy or environmental enteric dysfunction is a sub-clinical condition characterized by histological and functional abnormalities in the small intestine and which appears common in children living in resource-poor settings [40]. The condition is thought to reflect constant exposure to faecal pathogens in the environment, resulting in chronic intestinal inflammation [41]. The inflammation is likely to be mediated through the stimulation of innate pattern-recognition receptors by conserved bacterial components, such as lipopolysaccharide from Gram-negative bacteria. This is reflected initially in an increase in the release of pro-inflammatory cytokines and by inflammatory cell infiltration of the gut, which is followed by enteric T-cell stimulation and a cell-mediated enteropathy [42,43]. This pathology is believed to lead to an increase in intestinal permeability and malabsorption resulting in linear growth faltering and chronic malnutrition, and may also affect the immune response [40,44].

Since direct access to the gut environment is not possible, indirect methods of studying intestinal inflammation and function have received a great deal of attention in the recent past. Most inflammatory markers reflect by-products of immune cell activation while the gut barrier and absorptive function is mainly evaluated using indicators of bacterial translocation or carbohydrate absorption tests. Studies in Africa have shown that, in Gambian infants, increases in antibodies to the core lipopolysaccharide have been associated with gut translocation of Gram-negative bacteria and altered intestinal permeability measured by the lactose–mannitol urinary excretion test [45]. A chronic T-cell enteropathy has also been reported in Gambian infants resulting in a pro-inflammatory environment [42]. Although the importance of environmental enteropathy in undernutrition and poor growth is increasingly well described and the immunological effects of the condition are recognized, its role in the reduced efficacy of oral vaccines in a low-income setting is not defined.

3. Cross-talk between the intestinal microbiota and the intestinal immune system

Colonization of the intestinal tract by microbes begins shortly after birth, with rapid changes affected by diet, age and the environment. Although most currently available data that describe the role of the microbiota on mammalian physiology are derived from animal models, the influence of the intestinal microbiota on the immune system is increasingly being considered in human physiology [46,47].

In murine models, the nature of the colonizing species influences the quality of innate immune responses as well as the development of lymphoid structures, with germ-free animals having immature gut-associated lymphoid tissues (GALT), decreased numbers of intestinal lymphocytes, and diminished levels of antimicrobial peptides and immunoglobulin (Ig) A, all of which are reversed upon colonization with commensal bacteria [48].

Commensal bacteria influence the epithelial barrier in many ways; structural components and metabolites of the intestinal microbiota act on IECs and local innate leucocytes to maintain barrier integrity and regulate immune homeostasis [49]. Microbiota-derived short-chain fatty acids (SCFA), a by-product of metabolism, are important mediators in the cross-talk between the microbiota and IEC, and studies in animals indicate a role in prevention of inflammation [50] that is also reflected in the finding of fewer butyrate-producing bacteria in patients with inflammatory bowel disease [51]. Interactions of the microbiota with the mucous layer appear to play a role in colitis, colon cancer and possibly in oral tolerance to some antigens [52]. Commensal-derived PAMPs regulate the secretion of some antimicrobial peptides, such as Reg3γ, which maintains the physical separation between the microbiota and the host epithelium [53].

The intestinal microbiota directly and indirectly influence immune function. Bacteria that have been well studied include segmented filamentous bacteria (SFB), which induce accumulation of Th17 cells in the terminal ileum, possibly through induction of serum amyloid A production that could act on lamina propria dendritic cells (DCs), to stimulate a Th17 cell-inducing environment [54]. Polysaccharide A (PSA) produced by *Bacteroides fragilis* directly promotes regulatory T cell (Treg) cell differentiation via Toll-like receptor (TLR) 2 or indirectly by conditioning DCs [55]. *Clostridium* species belonging to clusters IV, XIVa and XVIII induce transforming growth factor β (TGF-β) production in IECs, which promotes Treg cell differentiation in the colon, suggesting that modification of the microbiota may allow for therapeutic manipulation of human immune disorders [56].

Commensal bacteria in germ-free mice induce dimeric secretory IgA, the most abundant Ig in mucosal secretions. Signals from commensal bacteria induce production of B- cell activating factor of the TNF family (BAFF), a proliferation-inducing ligand (APRIL) and TGF-β in the IECs and DCs, which in turn promotes the differentiation of B cells into IgA+ plasma cells. After activation by commensal bacteria, follicular dendritic cells (FDCs) also promote the differentiation of B cells into IgA+ plasma cells. B cells undergo class switch recombination to IgA in the mucosa and traffic from the intestinal lymphoid tissue structures, through the lymphatics to the bloodstream, and return to the intestinal mucosa as IgA-secreting plasma cells. Once induced, anti-bacterial IgA can be extremely long-lived but is replaced if there is induction of additional IgA specificities by other microbes as shown in a germ-free mouse model [57]. DCs sample intestinal bacteria and induce B cells to switch to IgA, while intestinal macrophages kill extracellular bacteria and thus clear bacteria that have crossed the mucous and epithelial barriers. The compartmentalization of the mucosal immune system from systemic immunity with mesenteric lymph nodes representing an anatomical and physiologic barrier functions to preserve host microbial mutualism while maintaining a continuum between innate and adaptive immunity [58].

Although intestinal microbiota promote GALT development, and induction of lymphocyte subsets including Th17 cells and Tregs, both of which play important roles in mucosal immune responses, not all members of the intestinal microbiota are beneficial; some may act as opportunistic pathogens, and an increase in certain commensals may predispose to pathogenic infection [59]. Interactions with intestinal

microbiota may even facilitate infection by other enteric pathogens, as has been demonstrated for certain viruses [60–62]. Interestingly, in parasitic infection tolerance to commensals is lost and microbiota-specific T cells are activated and differentiate to inflammatory effector cells [63].

Thus, although the mechanisms to maintain homeostasis are not fully understood, it is clear that the intestinal microbiota actively modulate the immune system to maintain a mutually beneficial relationship. Microbiota-derived factors activate a multitude of pathways that control innate and adaptive immunity in the gut, by influencing barrier function, conditioning of intestinal mononuclear phagocytes, cross-regulating innate lymphoid cells, promoting IgA secretion and regulating the balance between effector and regulatory T cells [64].

4. Probiotics and response to vaccines

Probiotics are exogenous or indigenous bacterial species that interact with various cellular components within the intestinal environment by many mechanisms. Intact, viable bacteria may be essential for probiotic effects, or these effects may be mediated by a cell wall component or structural moieties of the bacteria or metabolites. Based on evidence from *in vitro* systems, animal studies and humans with allergic disease treated with probiotics, several studies have been designed to assess the role of probiotics in modulating the response to vaccines, particularly vaccines that are used for mucosal immunization.

(a) Basis for use of probiotics

The basis for the use of probiotics as modulators of vaccination is based on hypothesized, but as yet unproven, direct or indirect actions that influence immune function. Direct effects include changes to the gut microbiota and alteration of the PAMPs presented to the GALT. Indirect effects could arise from microbial products such as SCFAs [65]. Probiotics communicate with many types of cells. Probiotics also affect IECs in multiple ways, including by enhancing barrier function by modulation of tight junctions, increasing mucin production, inducing antimicrobial and heat shock protein production, interfering with pathogenic organisms, and modulating signalling pathways and cell survival [66]. Probiotics increase β defensin secretion from IECs and directly block the signalling pathways hijacked by pathogens. Commensal or probiotic bacteria induce production of cytoprotective heat shock proteins in the intestine. Transcriptionally regulated expression of hsp25 and hsp72 was induced by transient exposure of IECs to *Lactobacillus rhamnosus* GG (LGG) cell-free conditioned media [67]. Other probiotics also induce heat shock proteins by different mechanisms, which help maintain tight junctions between IECs and promote barrier function [68]. Cytokine secretion by IECs, macrophages and DCs is regulated by probiotics through modulation of key signalling pathways such as NFκB and MAPKs, PI3K/Akt, and transcriptional regulators such as heat shock transcription factor 1 and PPARγ [69]. Changes in these pathways can also affect proliferation and survival of target cells, including IECs and macrophages, through apoptotic and anti-apoptotic mechanisms, which may be useful in limiting gut damage due to enteric pathogens [70]. Probiotics induce IgA secretion from plasma cells by increasing the number of IgA producing cells in a strain-dependent manner [71]. Through interactions with DCs, probiotics can influence T-cell subpopulations and skew them towards a Th1, Th2 or Treg response [72].

(b) Probiotics and vaccine response in infants

There are several published studies that assess the impact of concomitant probiotic administration on the response to vaccination in humans, but the studies are small, and have different interventions and outcome measurements making it difficult to directly compare results. Although there have been an excellent series of studies on the effect of probiotic administration on oral rotavirus vaccine response in a pig model with and without challenge that aim to dissect mechanisms of both humoral and cell-mediated immunity [73–77], this review is restricted to human studies.

The results from the studies on infants are variable (table 2) [78–86], and depend on the antigen, and the strain of probiotic and the location of the individuals being supplemented. Oral rotavirus vaccines have been studied in Finnish and Indian infants, both with the same probiotic, LGG ([78], R Lazarus 2014, personal communication). In 29 Finnish children supplemented with 10^{10} LGG on the day of immunization and twice daily for 5 days, there was an increase in IgA seroconversion rates ($p = 0.05$) and a higher proportion of children with IgM antibody secreting cells ($p = 0.02$). An Indian study, which supplemented children once daily with 10^{10} LGG from one week prior to the first immunization with the monovalent Rotarix vaccine at 6 weeks of age through to one week after the second dose of vaccine, showed a trend ($p = 0.066$) to increased IgA in children who were supplemented with LGG ($n = 273$) compared with those who received placebo ($n = 278$). The latter study was designed as a factorial trial which also evaluated zinc, and in the group supplemented with both zinc and probiotics ($n = 137$) compared to no supplement ($n = 135$) there was a significant increase in IgA seroconversion ($p = 0.04$), although overall seroconversion rates were very low (27.4% and 39.4% seroconversion in non-supplemented and dually supplemented children in India compared to 74% and 93% in not supplemented and LGG supplemented children in Finland).

The oral cholera vaccine Dukoral has been evaluated in children in Bangladesh given *Bifidobacterium breve* ($n = 64$) or placebo ($n = 62$), supplemented for four weeks, and vaccinated with two doses given two weeks apart [79]. Interestingly, among Bangladeshi children given placebo, cholera toxin B (CTB)-specific IgA levels were higher in serum, while in stool children on probiotics had higher CTB-specific IgA.

A range of parenteral antigens has been evaluated in small numbers of children in many settings in the industrialized world. In general, there appear to be no marked improvements in vaccine immunogenicity with probiotic administration, but this may be due to the excellent immunogenicity of most routine childhood vaccines, other than those directed at polysaccharide or conjugated polysaccharide antigens. There were variable results with respect to antibodies induced by *Haemophilus influenzae* type b (Hib) vaccination, with some studies demonstrating higher antibodies while others did not show this effect (table 2) [81,82].

(c) Probiotics and vaccine response in adults

Although there are many efforts evaluating the effect of probiotics on immune response to vaccines in adults, they share

Table 2. Effect of probiotics on vaccine immunogenicity in children.

type of vaccine(s)	probiotic(s) and vaccine, location	study design	outcomes	references
oral rotavirus vaccine	*Lactobacillus rhamnosus* GG, rhesus reassortant rotavirus vaccine, Finland	infants (2–5 months) given probiotic ($n = 29$) or placebo ($n = 28$) after vaccination and for next 5 days	increase in IgM antibody secreting cells in LGG ($p = 0.02$) trend for higher IgA in LGG group ($p = 0.05$)	[78]
	Lactobacillus rhamnosus GG, Rotarix®, India	infants ($n = 551$) given probiotic and/or zinc or placebo from 5 to 11 weeks of age, given vaccine at 6 and 10 weeks	increase in proportion of infants supplemented with both LGG and zinc seroconverting ($p = 0.04$) but not LGG alone ($p = 0.066$)	(Lazarus, personal communication)
oral cholera vaccine	*Bifidobacterium breve* (BBG-01),Dukoral®, Bangladesh	infants (2–5 years) given probiotic ($n = 64$) or placebo ($n = 60$) for four weeks, vaccination on days 21 and 35	significantly lower responders in the probiotic group ($p = 0.016$ for CTB-specific IgA) no difference in vibriocidal antibodies, probiotic group had higher serum-LPS specific IgA	[79]
hepatitis B vaccine	*Bifidobacterium longum* BL999 and *Lactobacillus rhamnosus* LPR to HepB vaccine at dose 1 and 2, DTaP/HepB (A) or three HepB doses (B), Singapore	infants ($n = 253$) given probiotic or placebo for six months; vaccinations A (probiotic $n = 29$, placebo $n = 28$) or B (probiotic $n = 77$, placebo $n = 68$) at 0, 1 and 6 months	probiotic group showed trend towards increased antiHbsAg in infants with schedule A ($p = 0.069$) no difference in probiotic versus placebo group among infants with schedule B	[80]
DTwP vaccine/Hib conjugate	*Lactobacillus rhamnosus* GG, *L. rhamnosus* LG705, *Bifidobacterium breve* Bbi99, *Propionibacterium freundenreichii,* DTwP vaccine/Hib conjugate, Finland	pregnant mothers given probiotics or placebo for last month; same to newborns for six months ($n = 47$ probiotic, $n = 40$ placebo group). DTwP vaccines given at 3, 4 and 5 months, Hib vaccine at 4 months	higher frequency of Hib-specific IgG antibody response, $p = 0.023$, trend for higher Hib-specific IgG GMT ($p < 0.064$)	[81]
DTaP, Hib, PCV7	*Lactobacillus rhamnosus GG,* DTaP, Hib, PCV7, Australia	mothers given probiotic/placebo during last month of pregnancy	decreased TT response in infants, decreased PCV response for some, no change in Hib/ Treg	[82]
parenteral tetanus vaccine	*Lactobacillus acidophilus,* LAVRI-A1, Australia	newborn infants given probiotic ($n = 58$) or placebo ($n = 60$) for six months, vaccines at 2, 4 and 6 months	lower IL-10 responses to tetanus antigen in probiotic versus placebo group ($p = 0.03$)	[83]
DTaP, polio and Hib vaccines	*Lactobacillus paracasei* ssp. *Paracasei* F19, Sweden	infants (4 months) given probiotic ($n = 89$) or placebo ($n = 90$) for nine months; vaccines administered at 3, 5.5 and 12 months	probiotic enhanced anti-diphtheria antibody titres in infants breastfed for less than six months ($p = 0.024$) and tetanus ($p = 0.035$)	[84]
MMRV vaccine	*L. acidophilus, B. bifidum, B. longum, B. infantis,* Israel	infants (8–10 months) given probiotics ($n = 25$) or placebo ($n = 22$) for five months, starting two months prior to vaccination	no difference in vaccine-specific IgG antibody titres	[85]

(*Continued.*)

Table 2. (*Continued.*)

type of vaccine(s)	probiotic(s) and vaccine, location	study design	outcomes	references
DTP-Hib/ *Pneumococcus* polysaccharide vaccine	low-fat milk fermented with *Streptococcus thermophilus*, *Lactobacillus casei* CRL431, *L. acidophilus* CRL730, Argentina	children 9 months to 10 years ($n = 162$) according to age, fourth dose of DTP-Hib vaccine at 18 months, 23 valent pneumococcal vaccine after 18 months age	pre- and post-vaccination anti-tetanus and anti-*Pneumococcus* antibody levels measured no significant difference between probiotic and placebo groups in post-vaccination antibody levels	[86]

with the studies in children the problems of variability in antigens, probiotics strains and populations, as well as the limitations of power imposed by the small numbers of participants in most studies.

Mucosal vaccines that have been evaluated in adults are the oral live-attenuated *Salmonella* Typhi Ty21a vaccine, the trivalent OPV, the oral cholera vaccine Dukoral and nasally administered attenuated trivalent influenza vaccine for 2007/2008, all of which were tested in small numbers of healthy volunteers with several different probiotics in Europe or North America (table 3) [87–90]. The studies indicate higher antibody levels (IgA or IgG) with the probiotics, but considerable variation by vaccine antigen and probiotic.

The oral cholera vaccine Dukoral has been evaluated in adults in France who were supplemented with one of seven probiotics, either *Bifidobacterium* or *Lactobacillus* ($n = 9$ in each group) or placebo ($n = 20$) for three weeks and vaccinated with two doses given one week apart [87]. In French adults, although there appeared to be differences between probiotic strains in the ability to induce antibody production, none of those tested induced a significant increase in response to the cholera toxin [79,87].

Parenteral influenza vaccines, tested in adults and in the elderly in Europe and South America (table 4), demonstrate an increase in antibody responses or no change [91–96]. It is difficult to draw any conclusions from these studies given the wide variation in antigens, the populations studied and immunization schedules, but there is weak evidence of benefit and no evidence of risk with these studies.

5. Antibiotics and response to vaccines

The skewing of the cellular immune responses by helminthic infections led to the hypothesis that these infections could affect vaccine immune response, well before T-regulatory cells were defined. A few studies in children and adults have attempted to modulate the immune response to vaccination by treatment of helminths (table 5) [97–100]. In two studies in Ecuador, oral cholera vaccine CVD 103 HgR was given to younger children and teenagers after heavy *Ascaris* infections were treated with albendazole [97,98]. In the 6–13-year-old children, the proportion of children seroconverting was greater in the albendazole-treated group ($p = 0.06$), and in a small study on teenagers, while there was no effect on antibody titres, albendazole-treated children had greater interferon (IFN)-γ and IL-2 levels. These studies have not been repeated in other settings, with other helminths or with other antigens, but a larger study in a birth cohort in Ecuador will explore vaccine responses in children with and without helminth infections [101]. In a different cohort design, the Entebbe Mother and Baby Study treated pregnant women with anthelminthics antenatally and then children quarterly after 15 months, and has investigated responses to measles, tetanus and BCG in addition to investigating tuberculosis in BCG vaccinated children [99,100]. The maternal–fetal imprinting of regulation has been previously proposed [102] and requires further study in carefully designed observational or interventional studies.

Although interference by concomitant bacterial and viral infections in the gut has been proposed as a cause of poor immune response, there are no studies in humans that have directly examined the effects of antibiotic administration

Table 3. Effect of probiotics on immune response to mucosally administered vaccines in adults.

type of vaccine(s)	probiotic(s), location	study design	outcomes	references
oral cholera vaccine Dukoral®	five *Lactobacillus* strains, two *Bifidobacterium* strains, France	healthy adults assigned to one of seven probiotics ($n = 9$ for each) or placebo ($n = 20$) for 21 days; vaccination on days 7 and 14	significantly higher vaccine-specific serum IgG antibodies with *B. lactis* Bl-04 and *L. acidophilus* La-14 ($p = 0.01$)	[87]
oral *Salmonella* Typhi Ty21a vaccine	*Lactobacillus* GG or *Lactococcus lactis*, Finland	healthy adult volunteers receiving LGG ($n = 10$), *L. lactis* ($n = 10$) or placebo ($n = 9$) for seven days; vaccination on days 1, 3 and 5	no difference in vaccine-specific IgA, IgG or IgM antibody secreting cells, Trend for higher vaccine-specific IgA	[88]
OPV 1–3	*Lactobacillus* GG, *Lactobacillus paracasei*, Germany	healthy males given LGG ($n = 21$), *L. paracasei* ($n = 21$) or no ($n = 22$) for five weeks; vaccination on day 8	significant increase in neutralizing antibodies and poliovirus-specific IgA titre ($p < 0.036$)	[89]
nasal attenuated trivalent influenza vaccine for 2007/2008	LGG and inulin, USA	healthy adults given probiotic ($n = 21$) or placebo ($n = 21$) for 28 days after vaccination	increased seroprotection rate to the H3N2 strain at day 28 ($p = 0.048$), but not to the H1N1 or B strain no effect on seroconversion at day 56	[90]

on vaccination. A number of new investigations in animal models of enteric infections, particularly rotavirus, have raised intriguing questions about the potential effects of antibiotic treatment on infection and response to vaccination. Recently, Uchiyama *et al*. [103] showed that ampicillin and neomycin, given for two to eight weeks, reduce murine rotavirus infections and symptoms and enhance rotavirus-specific IgA responses [103]. They also used a colitis model to demonstrate impairment of the late faecal and serum IgA responses to rotavirus infections. Kuss *et al*. [60] had previously shown that antibiotic-treated mice were less susceptible to poliovirus disease and supported minimal viral replication in the intestine. Exposure to bacteria or their *N*-acetylglucosamine-containing surface polysaccharides, including lipopolysaccharide and peptidoglycan, enhanced the infectivity of poliovirus, suggesting that antibiotic-mediated microbiota depletion might diminish enteric virus infection and that enteric viruses exploit intestinal microbes for replication and transmission. These studies raise interesting questions that need further exploration. How do different antibiotics modify the gut environment both in terms of the microbiota and in affecting host cells? What are the short- and long-term effects of antibiotics on wild-type infection and vaccine response? Will different host populations by age or geography respond differently to antibiotics?

Although the study did not use probiotics or antibiotics to modulate the intestinal microbiota, a recent report presented the results of a small clinical trial where the oral live-attenuated typhoid vaccine Ty21a was given to 13 volunteers with four controls and the alterations of the microbiota were investigated in parallel with the immune response [104]. The fecal microbiota composition and temporal dynamics showed considerable inter- and intraindividual variability, but there appeared to be no change in bacterial assemblage. With multi-phasic response defined as IFN-γ production more than 1% over baseline before day 14 post-immunization followed by an additional peak or peaks in IFN-γ production 14 days or later post-immunization, evaluation of *S*. Typhi-specific cell-mediated immune responses showed that individuals with multi-phasic responses had more diverse and complex communities, with 200 operational taxonomic units, mainly Clostridiales, able to distinguish late and multi-phasic responders. No changes were seen in those who mounted a humoral immune response evaluated by measurement of serum anti-lipopolysaccharide IgA and IgG. Although the study was small, it demonstrated that complex and detailed investigations of human response to vaccines could now be undertaken.

6. Conclusion

Recent studies in several animal models have significantly enhanced our understanding of mechanisms by which the gut environment and intestinal microbiota affect response to vaccines, but well-designed studies in humans have been limited. There is also a lack of studies on the human virome and the impact of viruses or bacteriophages on gut function and the immune response. A recent study using murine noroviruses showed that an enteric virus can replace the beneficial function of commensal bacteria [105], furthering understanding on the interactions between commensals and viruses [60–62], but the bulk of such studies continue

Table 4. Effect of probiotics on immune response on parenterally administered vaccines in adults.

vaccine(s)	probiotics, location	study design	outcomes	references
parenteral inactivated trivalent influenza vaccine for 2004/2005	*Lactobacillus fermentum*, Spain	healthy adults given probiotic ($n = 25$) or placebo ($n = 25$) for four weeks; vaccination on day 14	probiotic increased vaccine-specific IgA antibodies post-vaccination ($p < 0.05$), influenza-like illnesses lower for five months	[91]
parenteral attenuated trivalent influenza vaccine for 2008/2009	*Bifidobacterium lactis* (BB-12®) or *Lactobacillus paracasei*, Italy	healthy adults given probiotic ($n = 53$ for BB-12®, $n = 56$ for *L. casei* 431®) or placebo ($n = 102$) for six weeks; vaccination at week 2	increase in vaccine-specific IgG antibodies ($p < 0.001$), vaccine-specific secretory IgA antibody in saliva in BB-12® $p = 0.035$ and *L. casei* $p = 0.017$	[92]
parenteral trivalent influenza vaccine and PCV-23	*Lactobacillus paracasei* and prebiotic, Chile	elderly subjects (greater than or equal to 70 years) given either probiotic and prebiotic ($n = 30$) for six months or no supplement ($n = 30$); vaccination after four months	no effect on antibody response to vaccines, NK activity increases lower incidence of infection after 12 months, in particular respiratory illnesses ($p = 0.034$)	[93]
parenteral trivalent influenza vaccine 2004–2005, 2006–2007	*Lactobacillus paracasei*, France	pilot study: probiotic ($n = 44$) or placebo ($n = 42$) for seven weeks main study: probiotic ($n = 113$) or placebo ($n = 109$) for 13 weeks. vaccination after four weeks	higher seroconversion rate for B strain in main study at three, six and nine weeks post-vaccination in probiotic versus placebo group ($p = 0.02$)	[94]
parenteral trivalent influenza vaccine 2006–2007	*Lactobacillus plantarum*, Spain	elderly (65–85 years) given two doses of probiotic or placebo ($n = 20$ each) three months AFTER vaccination for three months	increased influenza-specific IgA ($p = 0.008$) and IgG ($p = 0.023$) antibodies	[95]
parenteral trivalent influenza vaccine	MoLac, heat-killed *Lactobacillus casei*, Japan	elderly given probiotic ($n = 8$) or placebo ($n = 7$) for 12 weeks	no difference	[96]

Table 5. Effect of anthelminthics on vaccine response.

anthelminthic	vaccine, location	study design	outcome	references
albendazole	oral cholera vaccine CVD103-HgR (5×10^8 cfu)	6–13 year olds with more than 1000 *Ascaris* eggs per gram stool twice treated with two doses albendazole ($n = 75$) or placebo ($n = 64$) one month apart with low-dose cholera vaccine one week after the second dose	10 days after vaccination, GMT vibriocidal antibodies increased in both groups ($p < 0.01$), proportion of subjects seroconverting greater in albendazole group (29.3% versus 15.6%; $p = 0.06$)	[97]
albendazole	Oral cholera vaccine CVD103-HgR (5×10^8 cfu)	13–17 year olds with more than 1000 *Ascaris* eggs per gram stool twice treated with two doses albendazole ($n = 15$) or placebo ($n = 13$) one month apart with low-dose cholera vaccine one week after second dose	post-vaccination increases in IFN-γ significant only in the albendazole-treated ($p = 0.008$), post-vaccination IL-2 significantly greater in the albendazole-treated group ($p = 0.03$).	[98]
albendazole or praziquantel		antenatal women randomized to receive albendazole (400 mg) and/or praziquantel (40 mg kg^{-1}) or placebo.	no difference in antibody responses to tetanus or measles, significant decrease in IL-5 and IL-13 in response to tetanus toxoid at 1 year	[99]
albendazole or praziquantel	BCG-Russia, BCG-Bulgaria BCG-Danish	antenatal women randomized to receive albendazole (400 mg) and/or praziquantel (40 mg kg^{-1}) or placebo. At 15 months, their children were randomized to receive albendazole or placebo quarterly until age 5 years. cytokine responses assessed in BCG vaccinated at 5 years ($n = 886$)	no consistent associations with maternal helminths or with maternal anthelminthic treatment, quarterly albendazole treatment during childhood associated with reduced IFN-γ and IL-13 responses to M.tb-crude culture filtrate protein	[100]

to be in animal models, which are not necessarily known to be directly relevant to humans.

The complex immune system of the human gastrointestinal tract is made up of cells, tissues, and immune effector molecules constantly and efficiently communicating with each other. The availability of tools to finely dissect the microbiota and conduct longitudinal immune phenotyping studies finally permit the conduction of studies that can evaluate in more detail the mechanisms by which individual and geographical differences in response to vaccines can be measured.

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