Composition of gut microbiota and its influence on the immunogenicity of oral rotavirus vaccines

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Abstract

The introduction of oral rotavirus vaccines (ORVVs) has led to a reduction in number of hospitalisations and deaths due to rotavirus (RV) infection. However, the efficacy of the vaccines has been varied with low-income countries showing significantly lower efficacy as compared to high-income countries. The reasons for the disparity are not fully understood but are thought to be multi-factorial. In this review article, we discuss the concept that the disparity in the efficacy of oral rotavirus vaccines between the higher and lower socio-economical countries could be due the nature of the bacteria that colonises and establishes in the gut early in life. We further discuss recent studies that has demonstrated significant correlations between the composition of the gut bacteria and the immunogenicity of oral vaccines, and their implications in the development of novel oral RV vaccines or redesigning the current ones for maximum impact.

Keywords

Introduction

Rotaviruses (RV) are the leading cause of diarrhoeal disease in young children worldwide. The infection is mostly mild but can be severe, leading to hospitalisation and death especially in low income settings. An estimated 450,000 RV deaths occur annually in children under the age of five and over 90% of these occur in sub-Saharan Africa and South-East Asian countries [1]. The introduction of the oral RV vaccines (ORVVs) has seen the drop in number of hospitalisations and deaths globally [2,3]. However, the efficacy of the vaccines have been varied, with low income countries showing significantly lower efficacy [4,5,6] as compared to efficacy of over 90% in higher

income countries in America and Europe [7,8,9,10]. Reasons for the disparity in vaccine efficacy between the two-income resource settings are not fully understood but are thought to be multi-factorial [11]. They include the inhibitory effect of titres of maternal antibodies acquired through placenta or breast milk i.e. breast milk-derived RV-specific IgA [12,13], interference from co-administered oral vaccines such as polio vaccine [14], HLA blood group antigen type [15]. Poor nutrition, environmental entropy, chronic and co-infections that may suppress immune response to the RV vaccination has also been suggested (not known if infants can develop micronutrition deficiencies, environmental entropy and coinfections as older children) [reviewed in 11]. Recent evidence (discussed later) suggests that the composition and diversity of the gut microbiota, especially bacteria, could have an impact on the immunogenicity of the oral RV and other oral vaccines.

The human gastrointestinal tract harbours a variety of commensal bacteria that co-evolved with the host in a symbiotic relationship in which the host provide the microbiota with a nurturing niche for growth and survival, while the commensal bacteria influence many physiological processes beneficial to the host [16]. Some of these processes include nutrient acquisition, energy metabolism and outcompeting invading exogenous and pathogenic bacteria by occupying the luminal niches [16,17,18]. Another beneficial effect of the gut microbiota that has recently been recognised is the aiding of the development, maturation and function of the mucosal immune system [19]. This is best demonstrated in germ-free mice, which possesses an underdeveloped immune system. Introduction of commensal bacteria restores the characteristics of a mature immune system [20,21,22]. As the commensal bacteria affects the development, maturation and function of the mucosal immune system, it is logical to consider it can also affect the host's response to oral vaccines. Indeed colonisation of gnotobiotic mice by segmented filamentous bacteria (non-culturable *Clostridium*-related species) has been shown to induce intestinal T cell adaptive functions [23]. In this review, we summarise the events that occur during early bacterial colonisation of the gut and how its outcome can profoundly affects the wellbeing of the individual, including the

development and maturation of mucosal immunity. We further discuss the concept that the disparity in the efficacy of oral RV vaccines between the rich and poor countries could be due the nature of the bacteria that colonises and establishes in the gut early in life. Understanding the reasons for the underperformance of the ORVs in poor countries is important so that the right interventions are designed to maximise the impact of the current vaccines or guide the development of new ones.

Early colonisation shapes the composition of gut microbiota and future immune response of the host

The composition of the gut microbiota in adults is shaped early during the first three years of life. During this period, the composition of gut microbiota is said to be highly variable and unstable [24,25]. Due to its positive oxidation/reduction potential at birth, the sterile intestinal tract of the infant is first colonised by facultative anaerobes such as lactobacilli, enterobacteria and enterococci. As the environment in the gut changes to a more reduced one due to depletion of oxygen by the first colonisers, strict anaerobes such *Bacteroides* (phylum Bacteroidetes), *Bifidobacterium* (phylum Actinobacteria), *Clostridium* species (phylum Firmicutes) subsequently flourishes [26,27]. The composition of bacteria colonising the gut of the infants during early days of life is influenced by several factors including the method of child's birth delivery (vaginal vs caesarean section), infant feeding habits (breast-fed vs formula fed) and sanitation (hygienic vs unhygienic) [24,28]. Formula-fed infants have significantly lower counts of probiotic bacteria such as *Bifidobacteria*, *Lactobacillus* and *Streptococcus* than breast-fed children do. Conversely, these infants have elevated levels of *Clostridium difficile*, *Escherichia coli* and *Klebsiella* species as compared to their breast-fed counterparts [29,30,31]. Infants born naturally through the vagina are likely to be colonized early by vaginal or faecal bacteria from their mothers whereas caesarean-section born infants are likely to be colonised by bacteria from the skin of health care workers and the hospital environment [26,32].

The composition of gut microbiota of infants exposed to different sanitary conditions are said to be different, with those exposed to rampant poor sanitation reported be distinct, more diverse and variable than that of their counterparts from good sanitation background [33,34]. Other important determinants of bacterial composition of the infants' gut are gestation age, infant hospitalisation and antibiotic treatment [24].

By the age of two, the mixture of gut bacteria in infants become generally similar to those found in adults [25] and by three, its composition and diversity fully resembles that of adults [35,36] and remains relatively stable over time. Analysis of the human gut microbiota using the small subunit 16S ribosomal RNA gene sequences reveals that Firmicutes and Bacteroides are the dominant phyla [37]. At phylum level, the composition of gut bacteria is similar among individuals but varies at species and strain level [38]. The type of bacteria that colonises and establishes in the luminal niches of the gastrointestinal track of infants can have profound effects early and later in life [39]. Early colonisation of the gut by appropriate proportions of commensal bacteria promotes the health of the infant through several mechanisms, including appropriate development and maturity of the mucosal immunity [40,19] that may later enhance response to oral vaccines. There are reports that suggest the existence of a window period immediately after birth in which the consortium of bacteria acquired plays a crucial role in directing future host immune response profile. For example, germ-free mice colonised with caecal contents at week three postnatal had an increased proinflammatory immune responses than those colonised during the first week or later [22]. In human studies, infants colonised by certain members of gut microbiota during the first 8 weeks of life had significantly higher counts of CD27$^+$ memory B cells than those colonised at other periods [41]. These immunomodulatory effects are not confined to mucosal immunity only but to the systemic immunity as well [42].

Composition of gut microbiota affects the development and function of the host immune system

Comparative studies have shown that germ-free mice possess underdeveloped mucosal immune tissue characteristics. However, these characteristics, which include induction of secretory IgA (sIgA), increase in CD4$^+$ T cell numbers as well as a well-developed and organised gut-associated lymphoid tissue (GALT), are restored when commensal bacteria are introduced in the gut [20,21,22]. Recent studies have revealed the existence of several members of the gut microbiota possessing specific immunomodulatory capabilities that differentially regulate the development of various immune cell groups [42] (Table 1). This suggests that variations in the composition and diversity of the gut bacteria may contribute to individual differences in immune response to infection or oral live attenuated vaccines. For example, *Bacteroides fragilis*, a Gram-negative bacteria belonging to phylum Bacteroidetes, affect mucosal T cell homeostasis by promoting not only Th1 systematic development [43] but also regulatory T cell function [44]. A bacterial polysaccharide (PSA) produced by *B. flagilis* during early colonisation of the gut has been shown to promote the cellular and physical maturation of the developing immune system [43]. Colonisation of the gut of germ-free animals with PSA producing *B. flagilis* restored the systemic T cell insufficiencies and Th1/Th2 imbalances as well as directing lymphoid organ development as compared to animals inoculated with PSA mutant *B. fragilis* that did not restore any of the immunomodulatory functions [43]. In another study, colonisation of germ-free animals with PSA secreting *B. fragilis* modulated the mucosal T cell homeostasis by inducing the production of IL-10 by Foxp3+ Tregs [44]. IL-10 prevents further proliferation of Th17 cells and damage to the mucosal barrier [45]. In contrast, gnotobiotic mice colonised with mutant PSA *B. fragilis* did not induce the production of IL-10 and instead induced proinflammation.

Table 1: Examples of subsets of the gut bacteria with specific immunomodulatory effects

Bacterial group/species	Specific immunomodulatory effects	Reference
Bacteroides fragilis	promotes Th1 systematic development and regulatory T cell functions	Mazmanian et al. 2005; Round and Mazmanian. 2010
Clostridium cluster IV and XIVa	promotes constitutive accumulation and differentiation of CD4^{+} T regulatory cells	Atarashi et al. 2011
Shingomonas bacteria	affect iNKT cell phenotypes and functions	Wingender et al. 2012
Segmented filamentous bacteria	Induction of Th17 cells, promotion of IgA production and general CD4 T cell accumulation	Ivanon et al. 2009 Umesaki et al. 1995

Another example of the member of the gut bacteria with specific immunomodulatory effects are the *Clostridia* species. Clostridia are a highly heterogeneous group belonging to phylum Firmicutes. They are made up of more than 19 clusters [46], of which cluster IV and XIVa are indigenous and constitute up to 40% of total gut bacteria [47]. They include the genera *Clostridium*, *Eubacterium*, *Ruminococcus*, *Coprococcus*, and *Roseburia* (Cluster XIVa group) while the cluster IV group is a collection of species belonging to the *Clostridium*, *Faecalibacterium*, and *Ruminococcus* genera. They are one of the early colonizers of the intestines of breast-fed infants and occupy a specific region in the intestinal mucosa (the mucus layers in vicinity of the epithelium). This positioning enables them to engage in modulating physiologic, metabolic and immune processes that are crucial to the host [48]. Indeed, presence of *Clostridia* species, especially those from Cluster IV and XIVa in the colon, has been shown to promote 'constitutively' an accumulation and differentiation of CD4^{+} T regulatory cells [49]. CD4^{+}T regulatory cells express a Foxp3 transcription factor and are important in the immune cell homeostasis.

The segmented filamentous bacteria (SFB) are another member of the gut microbiota with unique immunomodulatory role. Recent evidence suggests that induction of Th17 cells is only achieved in the presence of SFB. Th17 cells, which secrete interleukin-17 (IL-17), IL-17F and IL-22

and belong to a subset of T helper cells, mediates the host efficient immune response to mucosal pathogens such viruses, bacteria or fungi. One study [50] showed that Th17 cells were absent in gnotobiotic mice but were induced upon introduction of a full complement of gut bacteria collected from specific pathogen free mouse. Interestingly, colonisation with *B. flagilis* or Cluster IV and XIVa Clostridia, both Treg inducing bacterial members, did not induce Th17 induction [51,45] suggesting that an unknown member of the gut bacteria was involved in Th17 induction. Colonisation of the germ-free mice with SFB or mice lacking SFB led to the induction of these immune cells [51,23] implicating SFB as Th17 cells inducing bacteria. In addition to improving protection against enteric pathogens such as Enteropathogenic *E. coli* in rabbits and *Citrobacter rodentium* in mice, [52,50]. SFB possess other immunomodulatory roles such as promotion of IgA production and general CD4 T cell accumulation [53], although it is not yet known if these effects are mediated by Th17 cells.

In a study to determine whether the nature of bacteria that colonise the gut early in infants are associated with B cell maturation and activation, children colonised with *E. coli* and/or Bifidobacteria during the first weeks of life showed a significantly higher counts of CD27^{+} memory B cells at 18 month of age as compared to those not colonised by same type of bacteria [41]. Early colonisation of the infants gut with *Staphylococcus aureus*, on the other hand, correlated with lower counts of the circulating memory B cells at four months of age. A similar exploratory study in Sweden reported that early colonisation of the gut with a higher diversity of Bifidobacterial species enhanced the maturation of the mucosal secretory IgA system as compared to those who were not [54]. Collectively, these studies indicate that specific members of the gut bacteria directly induce particular subsets of the host immune response, suggesting that the relative abundance of these bacteria in the gut can affect readiness of the immune response in the host to infection or oral vaccines.

Table 2: Summary of studies showing correlation between certain members of the gut microbiota and oral rotavirus and other vaccine immunogenicity.

Name of bacteria	Name of oral vaccine	Subjects	Correlation Coefficient (*p*)	Reference
Streptococcus bovis	Rotavirus vaccine	Humans	$p = 0.008$	Harris et al. 2017
Serratia	Rotavirus vaccine	Humans	$p = 0.01$	Harris et al. 2017
Escherichia coli	Rotavirus vaccine	Humans	$p = 0.00$	Harris et al. 2017
Bifidobacterium longum	Polio, BCG and Tetanus	Humans	$p = 0.05$	Huda et al. 2014
Clostridiales	Salmonella Typhi	Humans	$p < 001$	Eloe-Fadrosh et al. 2013
Collinsella	Rotavirus vaccine	Pigs	$p = 0.001$	Twitchell et al. 2016
Lactobacillus LGG	Rotavirus vaccine	Pigs	$p = 0.07$	Chattha et al. 2013
Bifidobacterium Bb12	Rotavirus vaccine	Pigs	$p = 0.07$	Chattha et al. 2013
Oscillospira	Shigella dysenteriae	Macaque	$p < 001$	Seekatz et al. 2013

Composition of certain members of gut bacteria correlates with ORV vaccine immunogenicity

Evidence from human studies is starting to emerge associating the relative abundance of particular members of the gut bacteria with oral RV vaccine (ORVV) immunogenicity (Table 2). A recent study in rural Ghana, for example, reported differences in prevaccination composition of the gut microbiota between 6- week old matched ORVV and non-ORVV responders [55]. The study found that ORVV responders had abundant members of Bacilli phylum, especially *Streptococcus bovis* while non-ORVV responders had abundant Bacteroidetes phylum, in particular *Bacteroides* and *Prevotella* species. Furthermore, the study reported that enterobacteriace: bacteroidetes ratio was significantly higher in the vaccine responders as compared to the non-responders. A similar study in Pakistan by the same group reported that relative abundance of Gram-negative bacteria such as *Serratia* and *E. coli* correlated positively with RV vaccine response as compared to non-responders [56]. The increased abundance of *Bacteroides* in non-ORVV responders was interesting, as certain members of this phylum possess lipopolysaccharides (LPS) that are different in both function and

structure to the LPS present in Enterobacteriace. Lipopolysaccharides, present in most Gram-negative bacteria, are a strong immunogenic inducer of the innate immune system [57]. However, the immunostimulatory capacity is influenced by differences in LPS structure, especially the lipid A core [58]. Lipopolysaccharides from *Bacteroides* species have been reported to impair or inhibit the stimulation of inflammatory cytokines in vitro as compared to those derived from Enterobacteriace such as *Streptococcus bovis* [59]. The authors suggested that early gut colonisation with bacteria expressing less toxigenic or inhibitory types of LPS could dampen innate immune responses to the live attenuated RV contained in the vaccine. Alternatively, the authors suggest that a more immunogenic LPS (a relative abundance of bacteria possessing stronger immunogenic LPS) would have an adjuvant effect on the oral RV vaccine response in the vaccine responders (see Fig. 1). Similarly, a relative abundance of flagellin-producing bacteria as in most Gram-negative bacteria may supplement innate and later adaptive immune response to RV vaccine. Bacterial flagella has been shown to prevent RV infections through TLR5/NLRC4-mediated production of IL-22 and IL-18 [60].
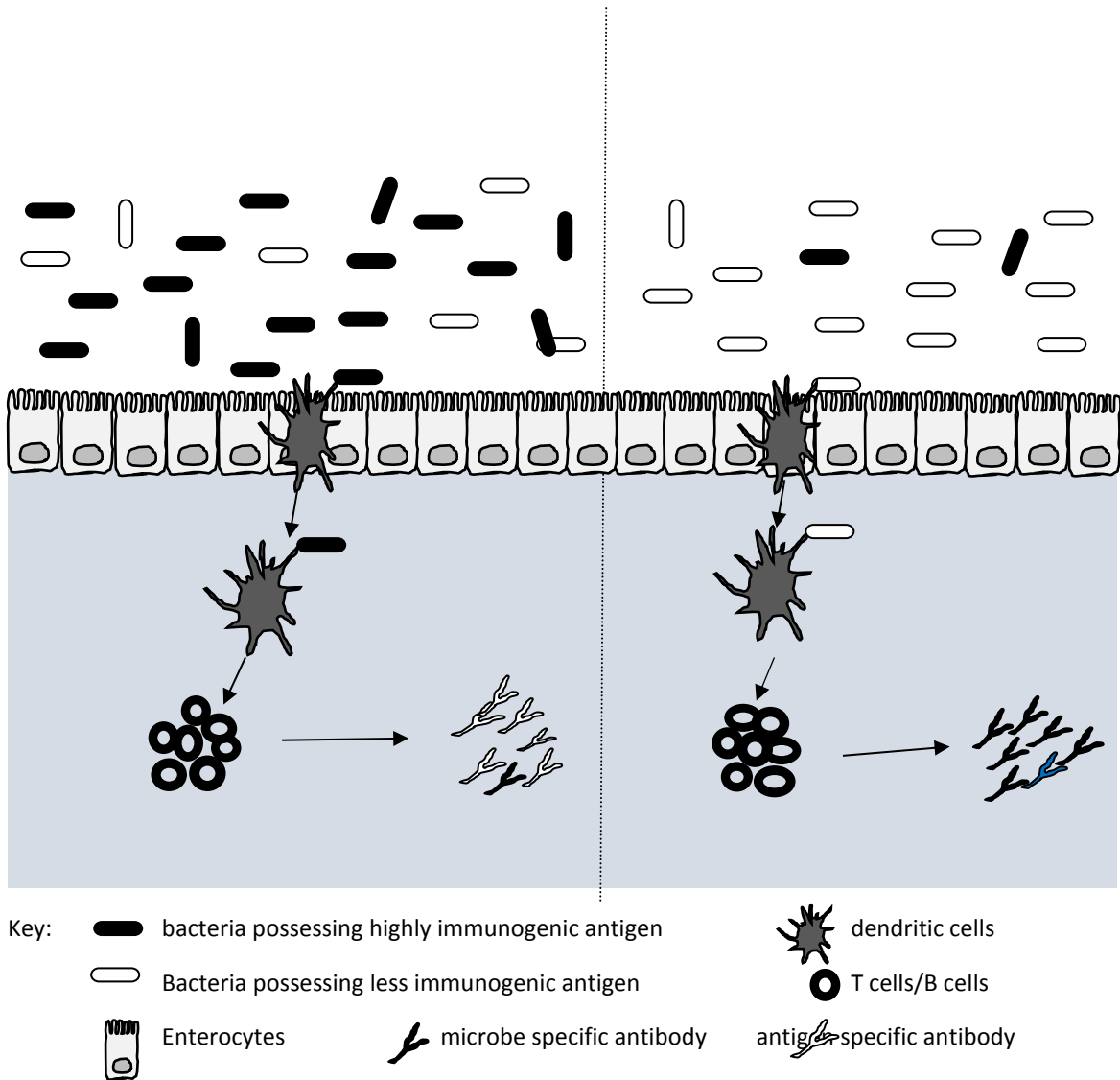
Figure 1: Illustration of how relative abundance of certain gut bacteria can affect oral vaccine immunogenicity. More immunogenic (a relative abundance of bacteria possessing stronger immunogenic antigens such as LPS or flagellin) would have an adjuvant effect on the oral RV vaccine response whereas early gut colonisation with bacteria expressing less toxigenic or inhibitory types of antigens could dampen innate immune responses to the live attenuated RV contained in the vaccine.

Another hypothesis is that since the oral vaccine contains live attenuated viruses, the gut bacteria could themselves be facilitators of viral replication through possession and expression of blood group antigens or glycan, as revealed by recent studies [61,62]. For example, infection of B cells by human norovirus required a commensal bacteria cofactor, specifically bacteria expressing an appropriate histo-blood group antigen (HBGA) glycans [63,61]. It has also been shown that RV may

recognize the HBGAs as ligands or receptors and bind HBGAs in a type-dependent manner when infecting the cells [63,62].
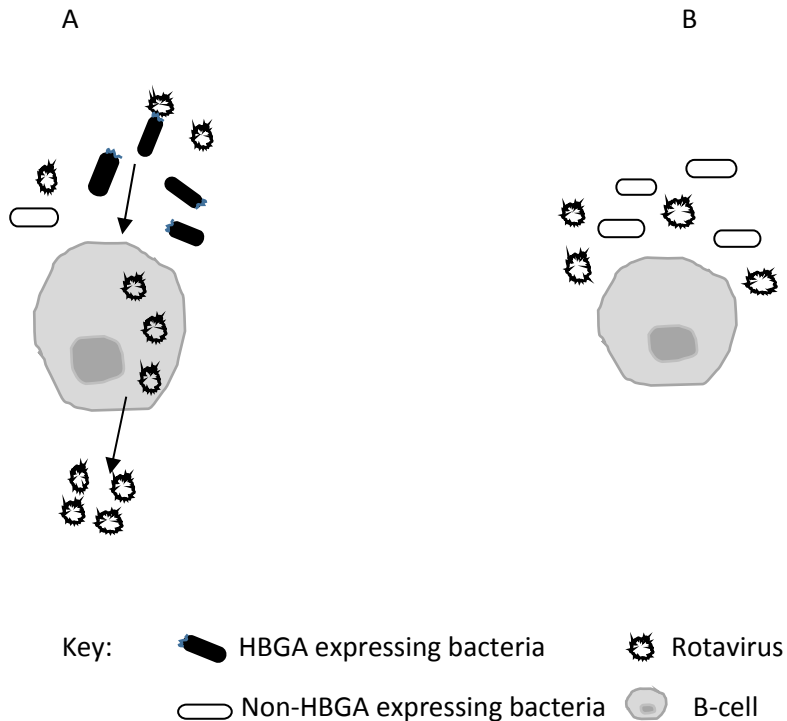

Figure 2: Illustration of how bacteria expressing blood group antigens or glycan can facilitate viral replication and enhance vaccine immunogenicity. (A) Infection of B-cells by human rotavirus and norovirus is stimulated by commensal bacteria that express appropriate histo-blood group antigens (HBGA). (B) No B-cell viral infection in absence of HBGA-expressing bacteria

Significant correlations have also been reported between composition of certain members of the gut bacteria and immunogenicity of other oral vaccines. For instance, a high abundance of Actinobacteria especially *Bifidobacterium longum* sub species in stool microbiota in infants in Bangladesh positively correlated with T cell responses to oral polio (OP), Bacillus Calmette-Guérin (BCG) and tetanus toxoid (TT) vaccines [64]. Conversely, poor vaccine responses and neutrophilia were associated with a high abundance of Clostridiales, Enterobacteriace and Pseudomonadales. When human volunteers were administered with oral live attenuated *Salmonella typhi* (Ty21a) vaccine, the majority of the vaccinated individuals with greater bacterial richness and bacterial

diversity had a higher CD8^{+} IFN-y response to the vaccine as compared to the control group [64]. Further bacterial analysis revealed that abundance of Clostridiales correlated significantly with higher CD8^{+} IFN-y in individuals producing them. IFN-y producing CD8^{+} T cells are one of the suggested correlates of protection against RV diarrhoea [65].

Work in animal models also demonstrate an association between relative abundance of certain members of the gut bacteria and oral vaccine immunogenicity. A recent study showed that gnotobiotic pigs inoculated with unhealthy gut microbiota obtained from children with impaired immune response to RV vaccine as well as gut inflammation and gut permeability had a significantly lower effector T cell immune response to ORV vaccine [66]. In contrast, gnotobiotic pigs colonised with healthy microbiota obtained from children with low enteropathy had a robust immune response to ORV vaccine. Furthermore, certain members of the gut microbiota, especially *Collinsella* (a member of Coriobacteriace) correlated significantly with RV specific IFN-y producing CD8+ T cell responses. A similar study in gnotobiotic piglets investigating the effects of early colonisation with probiotic bacteria on T cell responses to ORV vaccine showed that oral administration with *Lactobacillus* LGG and *Bifidobacterium* Bb12 enhanced ORVV efficacy by elevating systemic Th1 and innate immune responses [67]. A study of macaques from different geographical locations administered with two live attenuated *Shigella dysenteriae* vaccine candidates and challenged with wild type *S. dysenteriae* showed that vaccinated macaques with distinct and higher diversity in intestinal bacterial composition had an improved protection against virulent *S. dysenteriae* [68]. In addition, there was a positive correlation between some bacterial genera and vaccine-specific IgG and IgA antibody levels. Specifically, the study found that *Oscillospira* positively correlated with antibody levels whereas *Streptococcus* had a negative correlation with the protective responses. Together, these studies suggest that relative abundance of certain immunomodulatory members of the gut bacteria can influence the immunogenicity of oral RV vaccines and other oral vaccine response.

Altering composition of gut bacteria enhances ORVV response

Probiotics are exogenous/indigenous bacteria introduced orally into the gastrointestinal track with the aim of conferring beneficial health effects on the host. Although most of them are transient in nature, their introduction can briefly change the composition of the gut bacteria and affect the immune response to immunisation. For example, colonisation of gnotobiotic pigs with probiotic *L. rhamnosus* strain GG (LGG) and *B. lactis* Bb12 (Bb12) induced systemic Th1 immunostimulatory effects to oral attenuated human RV vaccination and enhanced immunoregulatory responses to the RV infection [69]. Similarly, colonisation of AttHRV vaccinated neonatal pigs with *L. rhamnosus GG (LGG)* and *B. lactis Bb12*(Bb12) together with colostrum and milk resulted in higher mean serum IgA HRV antibody titres and intestinal IgA antibody secreting cell (ASC) numbers compared to colostrum/milk fed, non-colonized vaccinated pigs [69]. Likewise, vaccination of neonatal gnotobiotic pigs with or without *L. acidophilus* (LA) significantly induced higher levels of HRV-specific IFN-γ producing CD8$^+$ T cell responses in ileum and spleen, as well as IgA and IgG antibody-secreting cell responses in ileum, serum IgM, IgA and IgG antibody in the AttHRV vaccinated and LA-fed pigs as compared to the AttHRV vaccinated pigs without LA colonization [70]. Variation in abundance of particular bacteria appears to have a differential effect on ORVV responses. For instance, gnotobiotic piglets administered daily with a high dose (1 x 10^9), low dose (1 x 10^6), or no probiotics of *L. acidophilus* and then vaccinated with oral attenuated human RV vaccine at days 5 and 15 after probiotic treatment induced different immune responses [71]. Piglets with low doses of the probiotic bacterium induced higher IFN-γ production by CD4$^+$ and CD8$^+$ T cells in the intestine, in systemic sites as well as in the blood, whereas piglets inoculated with high doses of the same bacterium enhanced mucosal and systemic Tregs.

Administration of probiotics in human subjects have had similar findings on oral RV vaccine response. For example, although not overwhelmingly significant, Finish children given *L. rhamnosus* (LGG) on the day of oral RV vaccination and thereafter twice daily for five days had an increased IgA seroconversion rates (p = 0.05). They also had a higher numbers of IgM antibody secreting cells (p =

0.02) than those only given the vaccine [72]. Similarly, Indian children supplemented with LGG a week before the Rotarix vaccination at week 6 had an increased IgA (p = 0.066) as compared to those given a placebo [72]. Alteration to the composition and diversity of the microbiota due to antibiotic therapy can also affect oral vaccine responsiveness. For instance, treating mice for two to eight weeks with ampicillin and neomycin induced more durable rotavirus specific IgA responses as well as reduction in rotavirus infection and symptoms [73]. Similarly, mice treated with doxycycline or clarithromycin showed a reduced antibody response to hepatitis B virus vaccine [74]. However, mice treated with the same antibiotics responded well to a live attenuated *Salmonella enterica* serovar typhi (Ty21) vaccine [74]. Presuming that these animal studies are translatable to humans, the implication is that the administration of oral RV vaccine and possibly other oral vaccines could be timed after antibiotic therapy to raise low seroconversion rates associated with the vaccine's inefficacy in low-income countries. However, since healthy participants would need to take antibiotics prior to vaccine administration, strict human subject regulations would make such experiments impossible to conduct. Nethertheless, the above examples collectively suggest that changes to the composition and diversity of the gut bacteria has an effect on how an individual respond to infection or oral vaccines.

Implications for vaccine design and administration

Oral RV vaccines are admistered early in childhood when both the gut microbiome and immune system are still developing. As the growing evidence strongly points to the link between the composition of the gut microbiota and oral RV vaccines immunogenicity, the current approach in vaccine development and its administration needs a major shift. For example, future vaccines could be designed to include specific immunomodulatory probiotics to compensate vaccine recipients whose composition of gut bacteria lacks the necessary immunostimulatory bacteria. One such probiotic could be the HBGA-expressing bacteria that would allow replication of the attenuated RV contained in the vaccine and later improve vaccine immunogenicity. They could also include

bacterial-derived immunostimulatory molecules that would enhance the vaccines immunogenicity. Since oral RV vaccines rely on the replication of the attenuated RV contained in the vaccine, the next generation RV vaccines could be designed to circumvent this requirement by developing non-replicating vaccines or changing the route of vaccine administration (e.g. parenterally administered vaccines). Such non-replicating and parenterally administered vaccines are currently in various stages of pre-clinical and clinical development [75]. Furthermore, as the ablation of the gut bacteria by antibiotic treatment affects the RV replication and induces more durable rotavirus specific IgA responses, the current oral RV vaccines could be administered immediately after antibiotic therapy to enhance the vaccine responsiveness.

Summary

Several hypotheses have been put forward to explain the existing disparity in ORVV effectiveness between higher and lower socio-economic countries, including the composition of gut microbiota. Certain members of the gut bacteria possesses specific immunomodulatory capabilities that differentially regulate the development and function of various immune cell groups, suggesting that the relative abundance of these bacteria in the gut can affect readiness of the immune response in the host to infection or oral vaccines. Evidence from animal and human studies is starting to emerge directly associating the relative abundance of particular subset of the gut bacteria with ORVV immunogenicity. These bacteria either possess antigens that are more immunogenic and would have an adjuvant effect on the oral RV vaccine response or facilitate the replication of the attenuated viruses contained in the oral vaccines through possession and expression of blood group antigens or glycans. Studies linking the gut bacteria to oral vaccine immunogenicity (and efficacy) are still in their infancy and more evidence of its association is going to be discovered. Exciting as it is, this emerging field has challenges researchers have to overcome. Most of the studies thus far have been associative, focussing on general changes in the gut microbiota and vaccine

immunogenicity. It is imperative that future studies concentrate on identifying specific bacterial strains or bacterial-derived molecules with immunomodulatory capabilities that can influence viral infection and enhance oral vaccine responsiveness so that right interventions are designed to maximise the impact of the current vaccines or guide the development of novel ones.

Contributors

CM conceptualised the paper, searched the literature, designed the tables and figures, and wrote the first draft of the article. MBT reviewed and revised the manuscript.

Declaration of interest

We declare no competing interest

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