

## Role of the Gut Microbiota as a Natural Adjuvant for Vaccine

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### Abstract

Vaccines have provided the most beneficial contribution to public health. Generating antigen specific antibody responses and long lasting memory are crucial for the protective immunity offered by vaccination. Unfortunately, not all individuals respond in the same manner to vaccine formulations. The microbiota is established during postnatal development and remains relatively stable for long periods. Our understanding that the microbiota can have beneficial effects on human health has led immunologists to investigate how these organisms may shape the innate and adaptive immune responses of the host. In this review we examine the impact of the microbiota on the host immune responses to vaccines and explore the possibility of how the commensal bacteria may act as natural adjuvants to enhance systemic immune responses to vaccines.

**Keywords:** Microbiota; Vaccines; B cells; Innate immunity; Adaptive immunity; Adjuvants; LPS

### Introduction

Vaccination remains one of the most beneficial contributions of biomedical science to public health where they have reduced the burden of infectious disease by protecting the most vulnerable, young infants and the aged [1]. Bacteria were once viewed as the nemesis of human health as they were the cause of harmful and insidious infectious diseases. However, it has only been in recent years that the influence of the gut microbiota on human health has come to be truly appreciated [2]. Disruption of the balance of microbial species within human tissues can actually lead to inflammatory responses and disease [3]. While the importance of commensal microorganisms in preventing or alleviating certain diseases has been quite firmly established, their potential role as an adjuvant for vaccination is currently being explored and this topic will form the focus of the review.

### Compartmentalization of the microbiota

The “microbiota” refers to the wide variety of microorganisms which colonise the human body as their natural habitat [4]. The microbiota is established during postnatal development and a range of factors influence the composition of the microflora including gestational age, route of birth, infant diet, maternal diet and weight as well as exposure to antibiotics early in life [4-6]. Antibiotics which are used to treat infections caused by bacterial pathogens directly impact on the diversity and function of the microbiota [6].

Mucosal surfaces of the skin, gut, respiratory and reproductive tissues provide a physical barrier that limits access of microorganisms to the underlying tissues. Each surface is colonised by a diverse range of microbes and the stability of these niches will be influenced by a continuous dialogue of communication between the microbes and the

host structural, immunological, hormonal and nervous systems networks [7-9]. It is evident that a primary role of the healthy microbiota on the immune system is to reinforce barrier immunity and to contain the microbes to the external mucosal surface. In the gut, respiratory tract and reproductive system this is achieved through the secretion of mucous and antimicrobial peptides by epithelial cells and secretion of IgA (sIgA) across the epithelial barrier to the luminal surface [7]. The gastrointestinal tract was the first tissue where compartmentalisation of the microbiota was described [10]. The stratification of microbes in the mucous layer overlying the intestinal epithelial cells prevents contact between microbes and host cells. Further, the production and secretion of antimicrobial peptides by epithelial cells seeks to further control those organisms which reside in the inner mucous layer, so as to ensure the integrity of the epithelial barrier and tissue homeostasis [11].

Studies by Naik et al. revealed that the resident microflora of the skin is compartmentalised in a manner which is independent of the gut microflora. The skin microflora can control the balance of effector and regulatory T cells in the skin tissue mediated by Myd88 and IL-1 receptor signalling [12]. Spontaneous skin inflammatory diseases in humans such as atopic dermatitis, rosacea, and psoriasis have been associated with dysbiosis of the skin microbiota. This study highlighted that microbial products derived from the microflora may play a crucial role in regulating immune responses at this site.

DNA sequencing of bacterial samples collected from various human tissues has enabled scientists to identify the key resident species that occupy tissue specific niches. Colonisation of these niches will be influenced by environmental and genetic factors and the makeup of the resident flora in various tissue niches may vary between groups of people living in different areas of the world and their varied health outcomes [13]. In the gut the dominant bacterial species include *Bacteroides*, *Prevotella* and *Ruminococcus* and the two phyla which form a majority of the human gut microbiota are Bacteroidetes and Firmicutes [14]. The dominant bacterial taxa that colonize the skin

include Actinobacteria, Firmicutes and Proteobacteria. The vaginal mucosa is dominated by *Lactobacillus* spp, while *Fusobacteria* spp, Firmicutes and Proteobacteria are predominant within the oral cavity [9].

### Impact of the commensal flora on the immune system

The microbiome has a crucial role in the development of the immune system in the neonatal period that is necessary to establish lifelong host-microbial and immune homeostasis [15]. The prenatal and perinatal developmental period is linked to the effects of environmental factors (e.g. microbial exposure) that influence immune and tissue maturation of the host [16]. Indeed early life exposures can have important outcomes on susceptibility to disease in adult life and this topic has been recently reviewed by Torow et al. [17]. Short chain fatty acids produced by microbes can shape the adult immune system [18,19]. Gomez de Augero et al. [15] identified that bacterial derived metabolites produced by maternal gut derived commensal organisms were transferred to the offspring via the breast milk and could be detected in tissues of the offspring. The bacterial metabolites included a range of ligands bound by the aryl hydrocarbon receptor (Ahr) which is expressed by group 3 innate lymphoid cells (ILC3). These Ahr ligands could induce expansion of the ILC3 cells that secrete IL-17 and IL-22 which are key cytokines that play a crucial role in maintaining barrier function within the intestine, by inducing the secretion of antimicrobial peptides and to promote epithelial repair respectively.

Germ Free Mice (GFM) that develop without an intestinal flora display defects in adaptive immune function. Colonization of GFM with intestinal bacteria can promote maturation of mucosal and systemic secondary lymphoid tissues [20]. In addition the development of CD4<sup>+</sup> regulatory T cells, Th17 cells and TCR $\gamma\delta$ <sup>+</sup> cells are deficient in GFM indicating that the development of a range of adaptive immune cell types rely on successful colonisation and maintenance of a stable and diverse intestinal microflora [18-22].

### Vaccination

The main aim of vaccination is to stimulate innate and adaptive immune responses to microbial antigens to induce long lived memory T and B cells. CD4<sup>+</sup> Th helper cells elicited to the vaccine antigen can provide help to antigen specific memory B cells and CD8<sup>+</sup> T cells [23-25]. The CD4<sup>+</sup> Th cells provide help to B cells to generate neutralizing antibodies that can efficiently bind and neutralise the target pathogen to facilitate its removal. It was noted by Janeway that a foreign antigen on its own was insufficient to elicit an adaptive immune response [26]. Adjuvants have been used extensively with vaccines to improve the potentially poor immune response experienced when the vaccine is administered on its own [23]. The main components of adjuvants were molecules derived from the bacterial cells. The discovery of Pathogen Associated Molecular Patterns (PAMPs) as the conserved elements of microbes and their ability to bind to Pattern Recognition Receptors (PRRs) helped to consolidate the view that PAMPs were a key element responsible for the beneficial effects of adjuvants [27]. The PRRs can be located on the cell surface or within the cytosol or endosomes of Antigen Presenting Cells (APCs) to enable the innate immune system to distinguish between extracellular versus intracellular pathogens [27,28]. APCs activated by PAMPs undergo maturation for efficient antigen processing and presentation; they can express co-stimulatory molecules and secrete cytokines to help drive the adaptive immune response [27]. Despite knowledge that adjuvants improve

immunogenicity, the differential responses by groups of people around the world to the same vaccine makes it difficult to identify ways to ensure everyone mounts an effective response. A number of potential explanations have been offered regarding the reason for the variability to vaccine responses include: genetics, socioeconomic status and nutritional intake [29]. The consideration of the gut microbiome as a natural adjuvant, however, is one that is gaining traction in research, and is offering relatively promising results.

The potential for adjuvants to exist naturally in the human body in the context of the commensal organisms that constitute the microbiota is exciting especially considering the benefits that vaccination has provided to improvements in public health worldwide. To produce a vaccine which has enhanced effects upon exposure to the natural tissue environment of the host could potentially reduce production costs of vaccines by eliminating the need to include an adjuvant, but the vaccine would still retain the benefits of an improved health outcome for the population. The generation of antigen specific antibodies provide a link between the adaptive and innate immune response as antibodies have a multifaceted role in effector activities including neutralizing pathogens, to promote opsonisation and phagocytosis by phagocytic cells such as macrophages, they function in complement-mediated lysis of pathogens, or to direct antibody dependent cellular cytotoxicity mediated by NK cells. Since many of the bacterial antigens are conserved between symbiotic and commensal bacteria Zeng et al. hypothesized that antibodies directed to symbiotic bacteria may be beneficial in controlling systemic infections of pathogens [10]. In this study the commensal gut bacteria were used to incite the production of immunoglobulin G (IgG) antibodies, which, through opsonisation, could eliminate pathogenic Gram-negative bacteria. One antigen identified as a key inducer of an antibody response to bacteria was Murein Lipoprotein (MLP). Using a mouse model it was shown that recognition of MLP derived from commensal bacteria was dependent on CD4<sup>+</sup> T cells and Toll-like Receptor 4 (TLR4) expressions on B cells to produce IgG that enabled the control of systemic *Escherichia coli* and *Salmonella* infections [10]. The outcomes of this study highlighted how bacteria in a localised area "the gut" are able to influence systemic immune responses throughout the body. If commensal bacteria could promote antibody production under homeostatic conditions, then utilising this natural response generated to commensal bacteria could in turn enhance antibody responses to vaccine antigens.

To further explore the relationship between the intestinal microflora and the response to vaccination with a model antigen ovalbumin in infant mice, Lamouse et al. treated mothers with antibiotics during pregnancy and breastfeeding stages, and the effects on their offspring to an immunization response was measured [30]. Pups derived from antibiotic treated mothers showed changes in the relative abundance of three main gut bacteria- Bacteroidetes, Enterobacteriaceae and Firmicutes when compared to the control pups derived from non-treated mothers. Notably, antibiotic affected mice were reported to harbour a higher prevalence of Enterobacteriaceae which can cause inflammatory responses. Furthermore, while MyD88 expression was consistent between the test and control population of adult mice, the expression of key effector molecules important for gut homeostasis was affected such as antimicrobial peptides (e.g. REGIII $\gamma$ , cryptidins), the pattern recognition receptor NOD2 and downstream signalling components (RIP2, RelM $\beta$ ) [30]. These results suggest that without the "normal microbiota", immune regulation in the gut would be altered, that could lead to dysbiosis or disease. Furthermore, the 7 day old mice derived from antibiotic treated mothers when immunised with ovalbumin (OVA) subcutaneously, displayed significantly reduced

antibody titres to OVA, compared to the immunisation response of aged matched pups derived from control mice. However, immunisation of pups at 14 days of age saw no significant difference between the two populations; potentially implicating a developmental factor in the vaccine response. It was important to note that antibiotic treatment per se had no demonstrable effect on total serum IgG levels in the pups immunised at one week old, suggesting the reduced antibody response to OVA was not due to any impact of antibiotics on B cell development or function. Furthermore, GFM immunised with the same vaccine at different ages had a minor but reproducible decrease in OVA antibody titres, which were not accounted for by developmental age. Colonisation of the GFM with intestinal bacteria was able to reverse this deficit to OVA immunization [30]. While these results do not prove that the microbiota actively acts as an adjuvant, they certainly support the notion that a complete lack of, as well as an alteration in, the normal gut microbiota can compromise vaccine responses in mice.

A more comprehensive study by Oh et al. [31] supported the positive association between the gut microbiota and the immune response following vaccination with the Trivalent Influenza Virus (TIV) vaccine. The study utilised wild type, germ-free and antibiotic-treated mice to establish the fact that the gut microbiota does in fact act as a natural adjuvant for the TIV and oral polio vaccines. Further testing indicated that flagellin of the gut bacteria could activate the Toll-like receptor 5 (TLR5) to promote an effective humoral response. Following primary vaccination, the titre of vaccine-specific antibodies and the frequency of plasma B cells was increased in wild-type mice as compared to germ-free and antibiotic-treated mice after 7 days [31]. It was also discovered that inoculation of flagellated, and not aflagellated, *E. coli* restored the antibody response to flagellin experienced by antibiotic-treated and germ-free mice. Furthermore, in terms of the humoral response, a positive association was identified between the presence of "normal" gut microbiota and improved memory B cell response [31]. Interestingly, the same study observed that flagellin could enhance the antibody response to the inactivated polio vaccine that was delivered in the absence of an adjuvant. In contrast antibody responses to other adjuvanted or live vaccinations such as yellow fever 17D were not affected in these mice [31]. Recognition of the gut microbiota by the PRR nucleotide binding oligomerization domain containing (NOD) 2 was identified to be important for the adjuvant properties of the mucosal adjuvant cholera toxin when co-administered with the protein antigen Human Serum Albumin (HSA) [32]. Mice carrying mutations in *Nod2* or the downstream signalling adaptor protein *Ripk2* displayed impaired antibody responses to HSA. Reconstitution of GFM mice with muramyl dipeptide a *Nod2* ligand or colonization with bacteria with *Nod2* stimulatory activity was sufficient to restore anti-HSA responses in vivo [32].

While the knowledge gained from this line of research is important to elucidate the specific role of the microbiota in vaccine response, it is necessary to consider whether similar results are observed in humans. Rotavirus (RV) is largely implicated in the gastroenterological-related mortality of children, particularly in locations where the efficacy of the Rotavirus vaccination is reduced. Researchers were motivated to identify if changes in the microbiome was a relevant contributing factor to these associations to Rotavirus vaccine responses [33]. A 2017 study by Harris et al. compared the immune response to the Rotavirus vaccine of a cohort of infants from Ghana and compared them to infants from Netherlands. The results obtained by Harris et al. [33], showed that the microbiome (as identified through faecal analysis) of vaccine responders and non-responders were significantly different. It

was also noted that the microbiome of responder children from both Dutch and Ghanaian cohorts proved significantly more similar, in terms of diversity, compared to non-responders. Analysis of bacterial species revealed an increased prevalence of Bacteroidetes among vaccine non-responders, while in responder children an increased prevalence of *Streptococcus bovis* was observed. Conversely, a parallel study conducted in Pakistan [34] noted that vaccine responders to the Rotavirus vaccine (anti-RV IgA  $\geq 20$  IU/mL) harboured a significantly higher Gram-negative to Gram-positive bacterial ratio, particularly *Serratia* and *E. coli* species, compared to non-responders. This may be related to what was identified by Zeng et al. [10] who noted that Gram-negative commensal gut bacteria enabled control of infection by *E. coli* and other Gram-negative pathogens. Thus, if these Gram-negative bacteria have a higher abundance in the gut of vaccine responders, the immune system may have been primed through systemic action of commensal bacteria and hence, mounted a better response to the vaccination. It remains to be seen if this is anyway related to exposure to flagellin or LPS as potential PAMPs that act as adjuvants to stimulate vaccine mediated antibody responses. It is important to note that LPS can vary from species to species of Gram negative bacteria [35]. LPS derived from bacteroidetes species was demonstrated to have an inhibitory capacity to stimulate inflammatory cytokines when compared to LPS derived from *E. coli* [36].

Furthermore, when Harris et al. [34] compared with Dutch age-matched counterparts (who experienced higher immunogenicity of the vaccine) the Pakistani infants had lower abundances of Proteobacteria. In addition, results from a study of Indian infants who responded to the oral Rotavirus vaccine found that they were more likely than non-responders to have more than one Enteropathogen as part of their microbiota [37]. This trend of enteric and Gram-negative bacteria specifically being associated with relatively improved immunogenicity seems to support the fact that the composition of the microbiota may impact the vaccine response in humans. As Harris et al. [33] found the abundance of Bacteroidetes positively correlated with non-responder status to the Rotavirus vaccine, one may hypothesise that the immunogenic effects of Gram-negative bacteria occur only at a certain degree of microbial abundance. These results will surely have interesting implications as research develops to elucidate how specific bacterial species may act to hinder or improve the systemic immunological response to vaccines.

The specific mechanism of action regarding bacterial improvement of vaccine responses has been addressed by Zhang et al. [38]. The study sought to investigate whether mice could be protected against Rotavirus and whether those with chronic infections could be cured. In concordance with the results of the study by Oh et al. [31] it was found that flagellin and its receptors were vital in the prevention and cure of Rotavirus-infected mice. Moreover, TLR5 and the NOD-like receptor C4, were important in responding to flagellin triggering the production of IL-22 and 18, for each receptor respectively [38]. These receptors, pathways and molecules enabled the immune system to destroy cells that had been infected with Rotavirus and rescue mice with chronic infections. The implication of these findings is that, in addition to strengthening the argument that the microbial components are important in the systemic immune response, it also provides an alternative, potentially more effective, treatment for viral infections.

*Shigella* species are Gram negative bacteria that invade mucosal tissues and cause bacillary dysentery or shigellosis in both humans and non-human primates such as macaques. These animals are widely used in pathogenesis and vaccine research. Seekatz et al. [39] observed that

macaques from different geographical areas had distinct composition of the faecal microflora. They observed that the Mauritian macaques had a genetically distinct and highly diverse bacterial community in the faecal microflora compared to non-Mauritian macaques. They also observed that the two different macaque populations responded differently to *Shigella* immunization. Both macaque populations could generate antibody responses to *Shigella* but clinical shigellosis was only observed among the non-Mauritian macaques [39]. This is further evidence that the microbiota should be considered when developing vaccinations.

## Conclusion

In light of the discussion presented here the gut microbiota appears to have the potential to act as an adjuvant for vaccination in some contexts, but also may impair the immune response depending on the specific composition and abundance of microbes present. Influenza virus and Rotavirus are pathogens that can cause significant harm in human populations. Yet there is hope in studying components of the commensal bacteria (e.g. flagellin, LPS etc), through innate receptor signalling and T and B cell activation, they could in future improve responses to vaccines against these other pathogens. Notwithstanding that there are many factors which can influence the response to vaccination and that the role of the gut microbiota in such a response is not easily elucidated, the studies discussed still provide relevant insight into future directions of research. Furthermore, they enable one to develop an appreciation for the microbiota; an entity which, when truly understood, may improve the health outcomes of many individuals around the world who have been disadvantaged simply due to their place of birth and diet.

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