



# Microbiota Influences Vaccine and Mucosal Adjuvant Efficacy

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A symbiotic relationship between humans and the microbiota is critical for the maintenance of our health, including development of the immune system, enhancement of the epithelial barrier, and acquisition of nutrients. Recent research has shown that the microbiota impacts immune cell development and differentiation. These findings suggest that the microbiota may also influence adjuvant and vaccine efficacy. Indeed, several factors such as malnutrition and poor sanitation, which affect gut microbiota composition, impair the efficacy of vaccines. Although there is little evidence that microbiota alters vaccine efficacy, further understanding of human immune system-microbiota interactions may lead to the effective development of adjuvants and vaccines for the treatment of diseases.

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## INTRODUCTION

The human gut harbors trillions of bacteria that contribute to host physiological and immunological functions such as nutrient acquisition, maintenance of the gut immune system, and protection against exogenous pathogens (1-3). One of the recent notable findings is the contribution of gut microbiota to the development and homeostasis of the immune system (1,3). The effects of microbiota on the host immune system have been determined by studies in germ-free (GF) animals (4,5). GF mice showed reduced numbers and size of Peyer's patches (5), less lamina propria CD4<sup>+</sup> T cells (6), reduced levels of the class-switched antibodies IgA and IgG (7,8), and they lacked a developed gut-associated lymphoid tissue (9,10). Thus, maturation of the gut immune system generally takes place following the colonization of gut microbiota

suggesting a mutual relationship between the immune system and the microbiota.

The gut microbiota consists of bacterial components and metabolites, both of which are important for the development and maintenance of the host immune system. Resident microbiota contains pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS), peptidoglycan, flagellin, and bacterial DNA and RNA that are recognized by pattern recognition receptors (PRRs). PRRs are expressed on immune cells, including antigen-presenting cells (APCs), and they play crucial roles in the activation of innate immunity. Toll-like receptors (TLRs) (11) and NOD-like receptors (NLRs) (12) are the main PRRs that recognize bacterial components. While the importance of microbial sensing and activation by the innate immune system has been well recognized, recent work has also uncovered an important

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Abbreviations: GF, germ-free; PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors; MDP, muramyl dipeptide; NLRs, Nod-like receptors; CLRs, C-type lectin receptors; SCFAs, short chain fatty acids; SIBO, small intestinal bacterial overgrowth; CT, cholera toxin

role of bacterial metabolites in the gut immune response. Short-chain fatty acids (SCFAs) are the end products of fermentation of dietary fibers by the anaerobic gut microbiota and have been shown to exert multiple beneficial effects on host immune homeostasis (13). For example, butyrate-induced signaling inhibits histone deacetylases (14), increases the size, and enhances the function of the colonic regulatory T cell pool (15). Collectively, microbiota-derived molecules, including both bacterial components and metabolites, influence the host immune function.

Based on the role of gut microbiota in development of the immune system, it is logically expected that the microbiota may influence vaccine efficacy. Although there is little evidence in this regard so far, several reports have indicated that the microbiota could influence vaccine efficacy and adjuvant activity. This review explores the possible role of the microbiota in determining vaccine effectiveness.

## MICROBIOTA INFLUENCES VACCINE EFFICACY

Vaccines display a protective effect against a broad range of diseases including infection, cancer, and allergy. However, vaccine efficacy varies at an individual level. Multiple reports have shown that people in developing countries exhibit reduced responses to oral vaccines. The efficacy of rotavirus (16), poliovirus (17,18), and cholera (19,20) vaccines in developing countries with poor sanitation is lower than that in industrialized nations. Nicaraguan children were shown to have blunted antibody responses to oral cholera vaccine as compared to Swedish children (21). Similarly, children in poorer regions of North India displayed reduced mucosal immune responses compared to children in other parts of the country (22). Furthermore, growing evidence suggests that the gut microbiota, which is known to be different in children from developed and developing countries (23), plays an important role in determining vaccine efficacy. For example, an abundance of stool Actinobacteria including *Bifidobacterium* was shown to be positively correlated with higher responses to oral and parenteral vaccines and a larger thymus in Bangladeshi infants. Conversely, a high abundance of Clostridiales, Enterobacteriales, and Pseudomonadales was associated with neutrophilia and lower vaccine responses (24). Furthermore, small intestinal bacterial overgrowth (SIBO), which is defined as excessive bacteria in the small intestine and is common in children in developing countries but rare in industrialized settings, contributed

to lower antibodies to cholera toxin after immunization (25). Since SIBO presents malabsorption and systemic symptoms, competition between the host and microbiota for nutrients and defects in the intestinal barrier may cause reduced vaccine responses. Indeed, whole blood cells derived from South African infants aged 2 years were shown to be severely hyporesponsive to TLR ligands including LPS and secreted lower levels of cytokines and chemokines in response to stimulation of TLRs than the age-matched children from North America, South America, and Europe (26,27). In summary, factors that affect gut microbial communities, such as malnutrition, poor sanitation, increased antigen exposure, antibiotic treatment, nutritional status, and lack of breast milk antibodies, could influence vaccine efficacy.

Animal studies have also indicated that gut microbiota composition affects vaccine efficacy. Mice treated with clarithromycin or doxycycline showed reduced antibody induction against the hepatitis B virus surface antigen (HBsAg) vaccine. In contrast, mice treated with the same antibiotics exhibited increased antibody responses to live attenuated *Salmonella enterica serovar Typhi* (Ty21a) vaccine (28). Macaques, showing a high level of diversity in the intestinal microbial composition, had improved protection upon challenge with virulent *Shigella dysenteriae* after immunization with live attenuated *S. dysenteriae* vaccine. In addition, *Oscillospira* and *Streptococcus* were found to be positively and negatively correlated, respectively, with vaccine-specific IgG and IgA induction and protective responses (29). These findings suggest that intestinal microbial diversity and particular microbiome compositions can influence antibody responses.

It has also been suggested that prebiotics (compounds that increase the number of certain commensals) could enhance the efficacy of oral vaccines. Prebiotic fructo-oligosaccharide (FOS) has been shown to improve the efficacy of a vaccine against *Salmonella* infection. Treatment with FOS prior to vaccination resulted in improved host responses and enhanced protection against infection (30). Another study showed that a prebiotic mixture containing galacto- and fructo-oligosaccharides (GOS and FOS) enhanced systemic immune responses to influenza vaccine. In addition, the prebiotic mixture increased proportions of certain members of the microbiota, suggesting a role for the specific microbial community in the increased host immune responses (31).

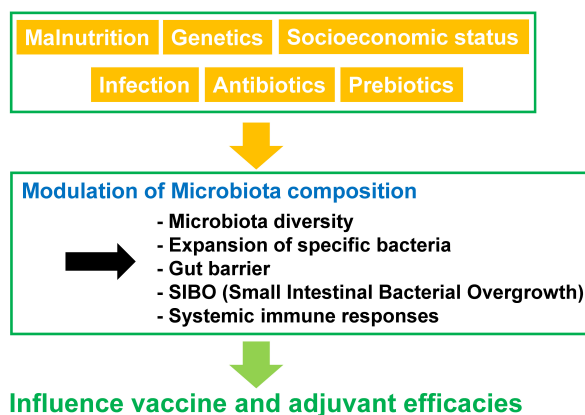
## GUT MICROBIOTA PROVIDES A SOURCE OF NATURAL ADJUVANTS

Since bacteria-derived components have potent immunostimulatory capacity, they constitute a major potential source of adjuvants. For example, lipopolysaccharide (LPS), peptidoglycan (PGN), CpG DNA, and trehalose dimycolate (TDM) are known to enhance the immune response against co-administered antigens (32). This adjuvant activity is mediated through the activation of TLRs, NLRs, and CLR (C-type lectin receptors) that mediate signals responsible for activation of the host innate immune system (11,12,33). A systems biology approach to study the innate and adaptive response induced by vaccination of humans with non-adjuvanted influenza subunit vaccine (Trivalent inactivated vaccine, TIV) revealed that TLR5 levels within three days of receiving the vaccine were positively correlated to the strength of the antibody response against the virus (34). TLR5 is a cell-surface receptor specific for flagellin, the monomeric component of bacterial flagellum used for cell motility, and has not been associated with viral recognition. Oh et al. found that knockout mice lacking the TLR5 protein produced significantly lower levels of antibodies after receiving TIV than wild-type mice (35). Because TIV does not interact directly with TLR5, the authors turned their focus to the microbiome in order to clarify the role of TLR5 signaling in vaccine efficacy. Indeed, GF mice and antibiotic-treated mice showed impaired responses to TIV. Furthermore, colonization of GF mice with flagellin-expressing, but not flagellin-lacking, bacteria was sufficient to enhance the vaccine efficacy. Results of this study indicate that flagellin from the gut microbiota acts as a natural adjuvant to enhance protective immune responses to the TIV vaccine.

## NASAL MICROBIOTA-DERIVED COMPONENT ENHANCES MUCOSAL ADJUVANT EFFICACY

Cholera toxin (CT), derived from *Vibrio cholerae*, is widely used as a mucosal adjuvant in animal immunology. CT is composed of pentameric B subunits that bind to the cell surface GM1 ganglioside receptor, and a monomeric A catalytic subunit that activates the heterotrimeric guanine nucleotide binding protein G $\alpha$ , which in turn stimulates cAMP production by adenylate cyclase. This mechanism is thought to be responsible for the adjuvant effect of CT (36,37). Although the toxicity of CT prevents its clinical application, elucidation of the

mechanisms of its potent adjuvant activity may lead to the development of nontoxic and effective mucosal adjuvants for vaccination. We have shown that the mucosal adjuvant activity of CT relies on Nod2 signaling induced by endogenous microbiota (38). Nasal or oral immunization of GF and antibiotic-treated mice with human serum albumin (HSA) + CT exhibited reduced levels of antigen-specific IgG1, recall-stimulated cytokine responses, and reduced follicular helper T (T<sub>FH</sub>) and plasma cells. These results indicate that the resident microbiota enhances the adjuvant effect of CT. Furthermore, the effect of the endogenous microbiota on the adjuvant activity of CT was impaired in Ripk2 (the adaptor molecule required for Nod1 and Nod2 signaling)-deficient or Nod2 (recognizes peptidoglycan molecules, muramyl dipeptide (MDP))-deficient mice, but not in MyD88 (the adaptor molecule required for TLR signaling)-KO or Nod1 (recognizes peptidoglycan molecules, diaminopimelic acid (DAP))-KO mice. GF mice immunized with a combination of HSA + CT and MDP produced high levels of antigen-specific IgG1, suggesting that Nod2 signaling promotes the adjuvanticity of CT. Furthermore, several members of the MDP-rich endogenous microbiota such as *Staphylococcus sciuri* promoted the adjuvant activity of CT in GF mice in a Nod2-dependent manner. This study indicates that the adjuvanticity of CT relies on Nod2 signaling triggered by the MDP-rich endogenous microbiota. Thus, it can be suggested that environmental factors that affect the composition of the microbiota and stimulatory activity that bacterial components exert on host PRRs may be important to determine the capacity of mucosal vaccines to provide protective immunity.



**Figure 1.** Microbiota influences vaccine responses. Nutritional conditions, host genetics, socioeconomic status, infection, treatment with antibiotics, and prebiotics modulate gut microbial communities, which in turn, influence the vaccine and adjuvant efficacies.

## CONCLUDING REMARKS

It is well known that the efficacy of oral vaccines depends on several factors such as genetic background, prior exposure to antigen via natural infection or vaccination, and nutritional status. Current findings suggest that the low responses to oral vaccines could be due to differences in the microbiota composition as a result of genetic, environmental, and nutritional variation. Indeed, growing evidence indicates that the microbiota influences vaccine and mucosal adjuvant efficacy (Fig. 1). However, investigations of the relationship between the microbiota and the efficacy of vaccines are at an initial stage, and data accumulated so far are limited. Therefore, further understanding of the impact of the microbiota on vaccine effectiveness is necessary. Detailed analysis of the role of particular species within communities in vaccine responses will provide novel insights into the development of strategies for improved vaccine efficacy.

## CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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