



Contents lists available at ScienceDirect

## Vaccine

journal homepage: [www.elsevier.com/locate/vaccine](http://www.elsevier.com/locate/vaccine)

## Review

## Impact of the intestinal environment on the immune responses to vaccination

Koji Hosomi<sup>a</sup>, Jun Kunisawa<sup>a,b,c,d,e,\*</sup><sup>a</sup> Laboratory of Vaccine Materials, Center for Vaccine and Adjuvant Research, and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health, and Nutrition (NIBIOHN), Japan<sup>b</sup> International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Japan<sup>c</sup> Graduate School of Medicine, Graduate School of Pharmaceutical Sciences, Graduate School of Dentistry, Osaka University, Japan<sup>d</sup> Graduate School of Medicine, Kobe University, Japan<sup>e</sup> Research Organization for Nano & Life Innovation, Waseda University, Japan

## ARTICLE INFO

## Article history:

Received 3 June 2020

Received in revised form 14 August 2020

Accepted 31 August 2020

Available online xxxx

## Keywords:

Gut microbiota

Lymphoid-tissue resident commensal bacteria

*Alcaligenes*

Fatty acid

Vitamin

IgA antibody

## ABSTRACT

Vaccination has contributed greatly to the control of infectious diseases; however, regional and individual differences are occasionally observed in the efficacy of vaccination. As one explanation for these differences, much attention has focused on the intestinal environment constructed by the interaction of diet and the gut microbiota. The intestinal environment has several physiological effects on the host immune system, both locally and systemically, and consequently influences the efficacy of vaccination. In this review, we discuss the impact of the gut microbiota and dietary nutrients on systemic and oral vaccination as well as their applications in various strategies for immunoregulation, including use as vaccine adjuvants. This information could contribute to establishing methods of personalized vaccination that would optimize host immunity by changing the gut environment to maximize vaccine effects.

© 2020 Published by Elsevier Ltd.

## Contents

1. Introduction	00
2. Role of gut commensal bacteria and probiotics in systemic vaccination	00
3. Gut commensal bacteria and oral vaccination	00
4. Lymphoid-tissue-resident commensal bacteria in the regulation of host immunity	00
5. Application of <i>Alcaligenes</i> as a vaccine adjuvant	00
6. Roles of vitamins in intestinal immune responses to oral vaccination	00
7. Roles of dietary lipids in the intestinal IgA production and oral vaccination	00
8. Conclusion	00
Declaration of Competing Interest	00
Acknowledgment	00
References	00

\* Corresponding author at: Saito-Asagi, Ibaraki City, Osaka 567-0085, Japan. Tel.: 81-72-641-9871; Fax.: 81-72-641-9872/7-6-8.

E-mail addresses: [hosomi@nibiohn.go.jp](mailto:hosomi@nibiohn.go.jp) (K. Hosomi), [kunisawa@nibiohn.go.jp](mailto:kunisawa@nibiohn.go.jp) (J. Kunisawa).

## 1. Introduction

Although various types of vaccines have contributed greatly to the control of infectious diseases, infectious diseases are still a serious public health problem, and the development of additional effective vaccines is required. When considering the development of new vaccines, the identification or design of good antigens is essential. Several approaches have been established to increase the immunogenicity of antigens, such as bioinformatics-based searches for epitopes and protein engineering technology [1,2]. Adjuvants have also been developed to promote and/or modulate immune responses to vaccine antigens [3]. Adjuvants mainly target the activation and modulation of innate immunity as a bridge to the acquired immunity conferred by antibody production and T cell responses against vaccines.

As is important to the design of vaccine antigens and development of adjuvants, host environmental factors, such as the gut microbiota and dietary nutrition, are considered to be involved in the efficacy of vaccination. Indeed, regional and individual differences are occasionally observed in the efficacy of vaccination. For instance, the efficacy of the oral rotavirus vaccines is lower in low-income countries than in higher income countries, including the United States, European nations, and Japan, where efficacy exceeds 90% [4–8]. Although the reasons for the differences in vaccine efficacy are not fully understood, nutrition, intestinal microbiota, environmental enteropathy, gastrointestinal infections, and genetic backgrounds have been suggested as determinants of vaccine-induced protection [9].

The intestine contains many microorganisms, including bacteria, archaea, and viruses. Accumulating evidence has revealed that their diverse functions contribute to the development, maintenance, and regulation of the host immune system [10]. In regard to activation of immune responses, segmented filamentous bacteria (SFB) induce the production of intestinal IgA antibodies and Th17 cell differentiation through the activation of macrophages [11,12]. A consortium of 11 bacterial strains isolated from human faeces is capable of robustly inducing interferon- $\gamma$ -producing CD8 T cells in the intestine [13]. In contrast, some *Clostridium* species and lactate-producing bacteria such as *Lactobacillus* show anti-inflammatory properties through the induction of interleukin 10 (IL-10) and transforming growth factor  $\beta$  (TGF- $\beta$ ) and the differentiation of regulatory T cells [14–16]. Thus, the gut microbiota is involved in both activation and suppression of immune responses and could contribute to vaccine effects.

In addition to the microbiota, nutrients are essential for the induction and maintenance of a suitable host immune system, and deficient or inappropriate intake of nutrients is therefore frequently associated with increased risk of infection [17,18]. For instance, micronutrients, often referred to as vitamins and minerals, are vital to healthy development and disease prevention. Indeed, several human trials reported that supplementation with vitamin A reduces mortality due to diarrhoea [19]. The World Health Organization recommends routine vitamin A supplementation in infants and children 6–59 months of age for the reduction of morbidity and mortality, as well as combining the administration of vitamin A supplements with vaccination [19].

These facts collectively suggest that components of the intestinal environment could be manipulated to maximize the efficacy of vaccination, provide biomarkers for personalized vaccination strategies, or act as vaccine adjuvants. In this review, we discuss recent findings on the molecular and holistic aspects of the intestinal environment in the context of vaccine development.

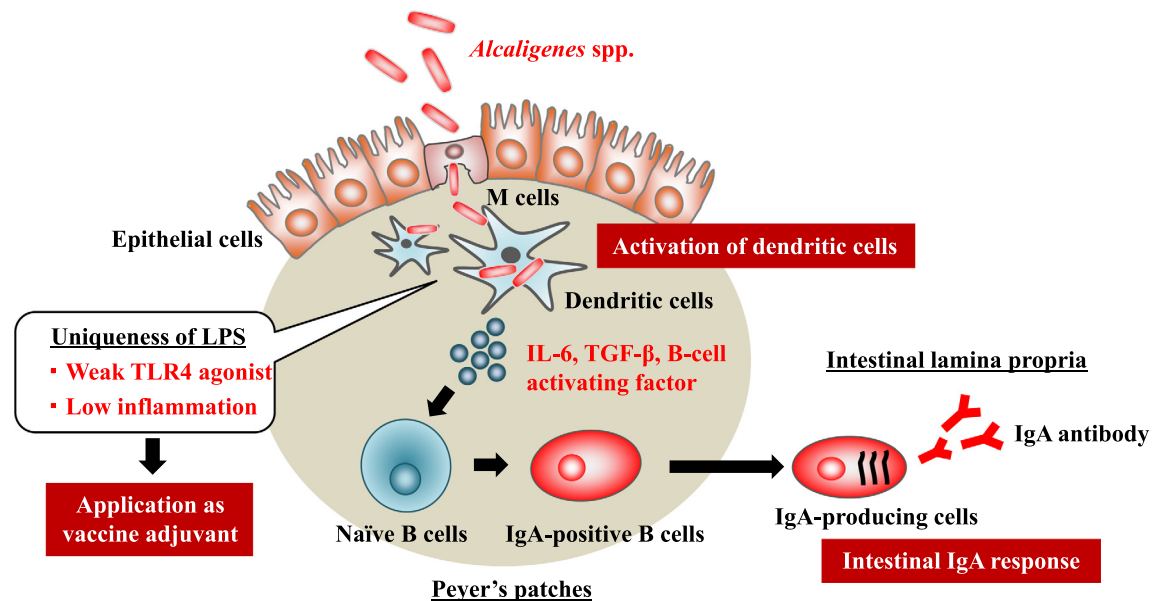
## 2. Role of gut commensal bacteria and probiotics in systemic vaccination

The gut microbiota is involved in the development and regulation of the host immune system and affects responses to vaccination. Antibody production against the seasonal influenza vaccine is attenuated in germ-free (GF) mice and mice treated with antibiotics, indicating that commensal bacteria are necessary for appropriate immune responses to vaccination [20]. Similarly, decreased antibody production in response to seasonal influenza vaccination was observed in Toll-like receptor 5 (TLR5)-deficient mice, and the administration of TLR5 ligand to antibiotic-treated mice restored the antibody production to the same level as in wild-type mice, indicating that commensal bacteria-initiated stimulation of innate immunity by TLR5 influences the effects of the seasonal influenza vaccine [20]. Several possibilities can be considered as mechanisms underlying TLR5-mediated activation of systemic immunity. First, since TLR5 is expressed on the basolateral surfaces of the gut epithelial cells [21], translocation of flagellin into the basolateral sites of the intestinal epithelium may result in the activation of epithelial cells and the consequent production of several cytokines [e.g., APRIL (A Proliferation-Inducing Ligand) and IL-6] to activate both local and systemic immune system [22]. As the other possibility, it was reported that macrophages and B cells also express TLR5 in the peripheral lymph nodes, translocated flagellin may enhance the production of similar cytokines (e.g., APRIL and IL-6) and the expression of CD86 from macrophages and also directly promotes the plasma cell differentiation [20]. Furthermore, some kinds of immune cells can traffic from the intestine into the lymph nodes [23], it is also possible that locally activated cells may move to the draining lymph nodes from the intestine and affected systemic immunity.

Because commensal bacteria enhance vaccine effects, the potential for probiotics and prebiotics to modulate vaccine responses is attracting attention. For example, probiotics such as *Lactobacillus* and *Bifidobacterium* and prebiotics such as oligosaccharides modulate innate and adaptive immunity. A systematic review summarized the effects of several probiotics and prebiotics in influenza vaccination [24]. In clinical trials, both probiotics and prebiotics enhanced immune responses against seasonal influenza vaccines (e.g., H1N1, H3N2, and B strains), resulting in reductions in the incidence of influenza-like symptoms and severe illness [24]. For instance, a combination of long-chain inulin and oligofructose enhanced serum antibody titres in healthy middle-aged humans after seasonal influenza vaccination [25]. In enterally fed elderly Japanese persons, supplementation with lactic acid bacteria-fermented milk products affected the gut microbial composition and maintained the enhanced immune responses against the H1N1, H3N2, and B antigens for a longer period after vaccination [26]. Thus, supplementation with probiotics or prebiotics enhances the immune responses to systemic vaccination through the alteration of gut microbial composition, function, or both.

## 3. Gut commensal bacteria and oral vaccination

Although many currently available vaccines are administered by injection, many pathogens invade through mucosal tissues, such as the respiratory and gastrointestinal tracts. Therefore, mucosal immune responses are needed to effectively prevent the invasion of pathogens at mucosal tissues [27]. Oral and nasal vaccines are attracting attention as prospective mucosal vaccines. For



**Fig. 1.** Regulation of the intestinal immune system by *Alcaligenes* through the unique properties of its LPS. *Alcaligenes* is the predominant symbiotic bacterial genus residing in the dendritic cells (DCs) in the Peyer's patches (PPs) and shows unique properties in modulating the intestinal immune system. *Alcaligenes* organisms activate DCs to promote the production of IL-6, TGF- $\beta$ , and B-cell activating factor, key cytokines in the differentiation of IgA-positive B cells and the enhancement of IgA production. *Alcaligenes*-derived LPS can activate DCs to produce IL-6 without causing excessive inflammation, making it a good candidate for a vaccine adjuvant.

example, oral vaccines for rotavirus are clinically used worldwide and protect against rotavirus gastroenteritis [28,29].

The efficacy of intestinal immune responses to oral vaccination is attenuated in GF mice and antibiotic-treated mice in comparison with the efficacy in specific-pathogen-free mice [30,31]. Several human studies have also shown an association between response to oral vaccination and the gut microbiota [32]. For example, a cohort study of children in Ghana determined that the composition of the gut microbiota, and in particular the proportion of *Streptococcus bovis*, differed between high and low responders to the oral rotavirus vaccine [33]. Thus, the bacterial composition in the gut could affect the efficacy of vaccination with oral vaccines.

#### 4. Lymphoid-tissue-resident commensal bacteria in the regulation of host immunity

The induction of antigen-specific IgA antibody responses is important for intestinal defence by oral vaccination. Gut-associated lymphoid tissues such as Peyer's patches (PPs) play a pivotal role in the induction of antigen-specific IgA antibody responses [27]. Several lines of evidence have indicated that maturation of PPs requires microbial stimulation. Indeed, PPs in GF mice are abnormally small and lack germinal centres, resulting in impaired induction of antigen-specific IgA responses. Recent studies have shown the specific functions of several bacteria in the activation of PPs. For example, SFB, which are present in the epithelial cell layer of the intestine that includes PPs, induce germinal centre formation in the PPs and promote IgA antibody production in the intestine [11,34]. Several species of *Lactobacillus* were reported to promote IgA antibody production and host defence against pathogens, leading to their use as probiotics. *Lactobacillus crispatus* activates innate immune cells such as CD11b<sup>+</sup> dendritic cells (DCs) in the PPs, leading to increased IL-6 and IgA antibody production in the intestine [35]. *L. pentosus* and *L. plantarum* also activate DCs in the PPs through TLR2, promoting IgA antibody production [36,37].

Although many studies have focused on the gut microbiota present in the intestinal lumen or associated with the intestinal

epithelium, we previously identified *Alcaligenes* spp., including *A. faecalis*, as a dominant genus within the PPs of mice and humans, and termed them lymphoid-tissue-resident commensal bacteria (LRCs) [38,39]. A subsequent study showed that *A. faecalis* is captured by DCs in the PPs after being taken up by M cells, which are epithelial cells specialized for antigen uptake [40] (Fig. 1). *A. faecalis* activates DCs to promote the production of IL-6, TGF- $\beta$ , and B-cell activating factor, key cytokines in the enhancement of IgA production [38]. An additional study showed that *A. faecalis*-produced lipopolysaccharide (LPS) appropriately mediates production of IL-6 from DCs through its weak agonistic activity against TLR4, allowing *Alcaligenes* spp. to maintain their homeostatic relationship with host immunity without inducing excessive inflammation [41]. Collectively, the evidence highlights that LRCs, including *A. faecalis*, interact with DCs in the PPs and regulate host immune functions, including IgA antibody production.

We further investigated bacteria that persistently colonize colonic macrophages and revealed the presence of *Stenotrophomonas maltophilia* in murine colonic macrophages [42]. *S. maltophilia* induces IL-10 production from macrophages through a 25-kDa hypothetical protein encoded by the *smlt2713* gene and annotated as a bacterial protein exported by the type II secretion machinery [42].

Collectively, specific bacteria such as *A. faecalis* and *S. maltophilia* establish a symbiotic relationship within the intestinal immune cells and modulate their functions, including production of IgA antibodies and dampening of inflammatory conditions, possibly affecting host immune responses to oral vaccination.

#### 5. Application of *Alcaligenes* as a vaccine adjuvant

Adjuvants activate innate immunity, thus providing stronger vaccine responses. Therefore, microbial components such as LPS, which can activate innate immunity, likely can be used as adjuvants. LPS activates innate immunity through TLR4. The structure of LPS, which varies among bacterial species, determines its ligand activity. Because *A. faecalis* has a unique function as an LRC, we focused on its LPS activity and found that *A. faecalis*-derived LPS

has weaker TLR4 agonistic activity than *Escherichia coli*-derived LPS [41]. Of note, as with *A. faecalis* itself, the activity of *A. faecalis*-derived LPS can activate DCs sufficiently to produce IL-6 without causing excessive inflammation, leading us to hypothesize that the unique activity of *A. faecalis*-derived LPS makes it suitable as a vaccine adjuvant (Fig. 1). Indeed, *A. faecalis*-derived LPS enhances antigen-specific immune responses without excessive inflammation when it is used as an adjuvant in a murine model [41]. For instance, mice subcutaneously immunized with ovalbumin (OVA) together with *A. faecalis* LPS showed increased levels of OVA-specific antibody production compared with mice immunized with OVA alone. In addition, *A. faecalis* LPS induced OVA-specific T cells, especially ones producing IL-17 [41].

LPS consists of an O antigen, core, and lipid A, and the activity of LPS at least partly depends on the lipid A structure, such as the number of phosphate groups and the number of acyl groups [43,44]. For example, monophosphoryl lipid A, which is chemically modified from lipid A of *Salmonella* by removing one phosphate group, has lower activity than the parent lipid A containing two phosphate groups [45]. Also, the structure of lipid A differs among bacterial species. For example, lipid A of *E. coli* has six acyl groups, but lipid A of *Bacteroides dorei*, a commensal bacterial species of the gut, has four acyl groups [46]. *B. dorei*-derived lipid A acts as an antagonist of TLR4 and inhibits the activation of innate immunity by *E. coli*-derived lipid A [46]. Thus, the unique immunoregulatory functions of intestinal bacteria, including those of *Alcaligenes*, can be artificially modified and applied to the development of safe and effective vaccine adjuvants.

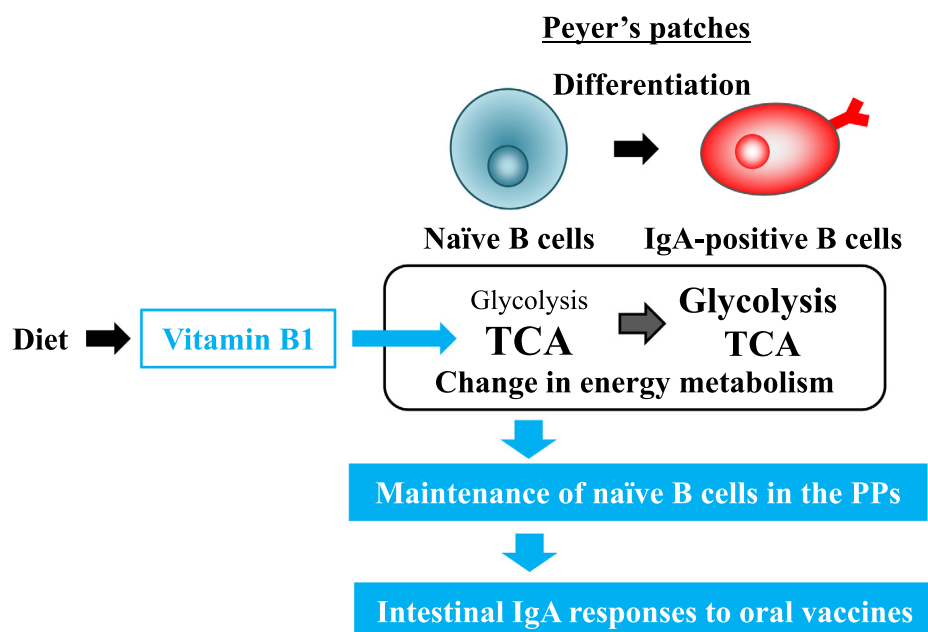
## 6. Roles of vitamins in intestinal immune responses to oral vaccination

Another important factor involved in the control of intestinal immune responses is the nutritional condition. Mice in a fasting state show decreased numbers of B cells in the PPs, which consequently reduces production of antigen-specific IgA antibodies

against oral vaccine antigens [47]. The underlying mechanism is an increase in the expression of CXCL13 in bone marrow stromal cells through metabolic reprogramming in response to fasting, leading to the recruitment of naive B cells from the PPs to the bone marrow. In addition, the numbers of activated germinal-centre B cells in the PPs are reduced by the apoptosis induced by starvation [47].

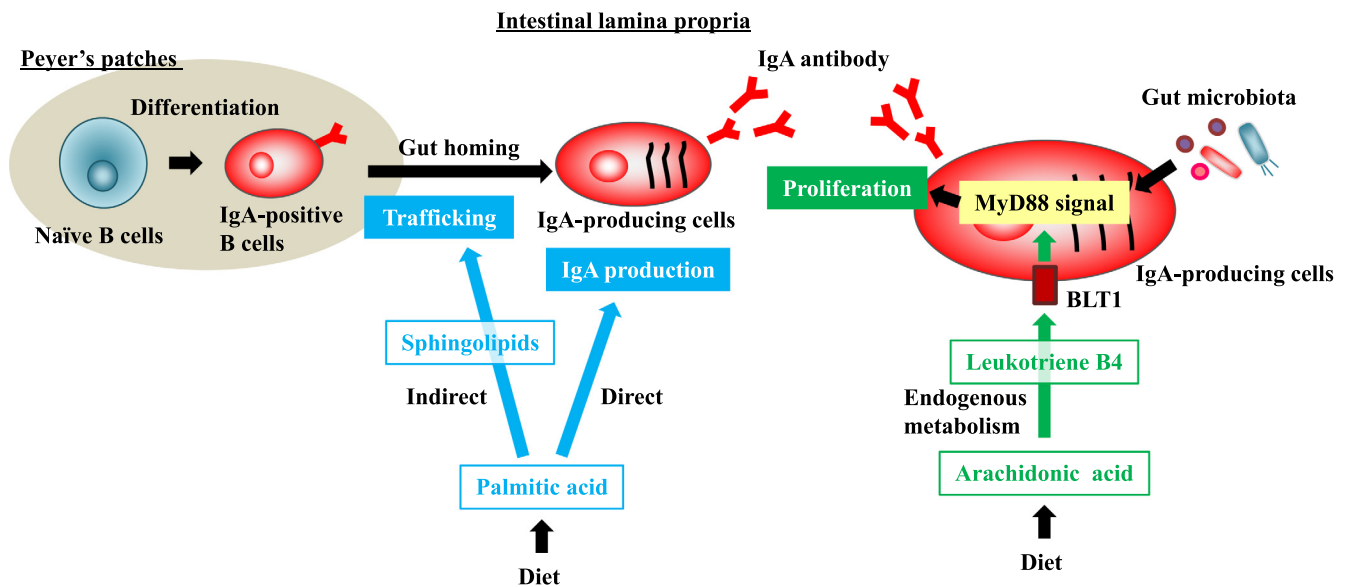
With regard to specific dietary nutrients, dietary vitamin A deficiency increases the risk of gastrointestinal infection and reduces the efficacy of oral vaccination. Several lines of evidence indicate that the molecular mechanism includes intestinal DCs expressing retinaldehyde dehydrogenase (RALDH), which can convert vitamin A into retinoic acid (RA) [48,49]. RA induces class-switching recombination of immunoglobulin from IgM to IgA and coincident differentiation of naive B cells to IgA-positive B cells via RA receptors in the PPs [48]. Moreover, RA induces the expression of the gut-homing receptors  $\alpha 4\beta 7$  integrin and chemokine receptor CCR9, promoting the migration of IgA-positive B cells into the intestinal lamina propria (iLP) [48]. Therefore, vitamin A deficiency reduces IgA-producing plasma cells in the iLP, which leads to the attenuation of the intestinal immune response, including IgA antibody production.

In addition to vitamin A-mediated immune regulation, we reported that the immune response to oral vaccination is attenuated by a deficiency of dietary vitamin B1 [50]. Mice deficient in dietary vitamin B1 showed smaller PPs, with reduced numbers of naive B cells [50]. Consequently, the mice exhibited impaired IgA antibody responses to oral vaccination. We focused on energy metabolism as an underlying mechanism because vitamin B1 acts as an essential cofactor in TCA cycles for energy generation, and we noted that B cells depend on vitamin B1 most during their early differentiation in the intestine [50] (Fig. 2). In the intestine, naive B cells differentiate into IgA-positive B cells in the PPs and then migrate into the iLP and become IgA-producing plasma cells. A metabolomics analysis revealed that naive B cells depend on TCA cycles for energy generation, whereas IgA-producing plasma cells show low dependence on TCA cycles and increased dependence



**Fig. 2.** Roles of vitamin B1 in intestinal immune responses to oral vaccination through regulation of energy metabolism. Vitamin B1 is essential for energy metabolism, especially maintenance of the TCA cycle, and therefore is associated with maintenance of naive B cells, which predominantly use the TCA cycle for energy generation. Consequently, vitamin B1 is involved in intestinal IgA production and immune responses to oral vaccination.





**Fig. 3.** Enhancement of IgA antibody production by palmitic acid, arachidonic acid, and their metabolites. Palmitic acid enhances IgA antibody production in the intestine through two pathways. One is a direct effect of palmitic acid on IgA-producing cells to enhance IgA production. The other is the endogenous generation of sphingolipids from palmitic acid, which promotes the trafficking of IgA-positive B cells. Arachidonic acid is converted to leukotriene B4 in vivo and its receptor, BLT1, is expressed on IgA-positive B cells and IgA-producing cells. The leukotriene B4–BLT1 axis promotes the proliferation of these cells through the enhancement of gut microbiota–derived stimuli that increase the expression of MyD88.

on glycolysis. Vitamin B1 deficiency leads to impaired TCA-dependent energy metabolism and thus results in reduced numbers of naïve B cells and consequent regression of PPs and attenuated intestinal IgA responses to oral vaccines [50].

## 7. Roles of dietary lipids in the intestinal IgA production and oral vaccination

We reported that dietary fatty acids also affect intestinal immune functions, including antigen-specific IgA antibody production in response to oral vaccines. For example, we previously reported that dietary palmitic acid can promote intestinal IgA antibody production [51]. The amount of faecal IgA antibody was increased in mice fed with palm oil containing abundant amounts of palmitic acid, a saturated fatty acid, and the palmitic-acid-rich diet was associated with increased numbers of IgA-producing plasma cells in the intestine [51]. We found two pathways by which dietary palm oil promotes IgA antibody production (Fig. 3). In a direct effect, palmitic acid stimulates IgA-producing plasma cells to enhance IgA antibody production. In an indirect effect, palmitic acid is converted into sphingolipids, which may promote cell trafficking and proliferation. Indeed, we previously reported that sphingosine-1-phosphate, one metabolite of sphingolipids, regulated trafficking of IgA-positive B cells from the PPs into the ILP [52].

Our recent findings indicate that dietary fatty acids and the gut microbiota cooperatively control the intestinal immune system [53]. Leukotriene B4 is a metabolite of arachidonic acid, which originates from dietary omega-6 linoleic acid and acts as a ligand of leukotriene B4 receptor 1 (BLT1). During B-cell differentiation in the intestine, B cells start to express BLT1 upon class switching to IgA from IgM and maintain its expression after their differentiation into IgA-producing plasma cells. Consistent with these findings, leukotriene B4 enhances antigen-specific IgA antibody production in response to oral vaccines, which promotes the proliferation of IgA-producing plasma cells [53].

The mechanisms of how dietary fatty acids control the intestinal immune system include harmonized communication with

commensal bacteria. Stimulation by commensal bacteria through TLRs activates MyD88 signalling in immune cells, which enhances immune responses to vaccines [53]. We found that the leukotriene B4–BLT1 signal induces the expression of MyD88 in B cells and thereby promotes the proliferation of IgA-producing plasma cells (Fig. 3) and consequently antigen-specific IgA antibody production in response to oral vaccines [53]. Thus, metabolites derived from dietary oils control immunological signalling from gut microbiota, affecting oral vaccine efficacy.

## 8. Conclusion

Vaccination has contributed greatly to the control of infectious diseases since Edward Jenner developed the smallpox vaccine. However, emerging and re-emerging infectious diseases including influenza, Ebola, and Covid-19 are still big issues in public health worldwide, and new vaccines need to be developed. Even when vaccines are available, regional and individual differences may be observed in the efficacy of vaccination. This review article describes the involvement of the intestinal environment as a key factor influencing the efficacy of vaccination. In the future, a person's intestinal environment might be modified through nutritional guidance as well as the application of gut microbes, to maximize vaccine efficacy. We hope that the fundamental research we describe herein will help the field progress and will contribute to saving people worldwide from infectious diseases.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

The work related to this review article was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Japan Society for the Promotion of Science under grant num-

bers JP18H02150 (J.K.), JP18H02674 (J.K.), JP17K09604 (J.K.) and JP18K17997 (K.H.); the Japan Agency for Medical Research and Development (AMED) under grant numbers JP17fk0108223h0002 (J.K.), JP17ek0410032s0102 (J.K.), JP17fk0108207h0002 (J.K.), JP17ek0210078h0002 (J.K.), JP17ak0101068h0001 (J.K.), JP17g-m1010006s0101 (J.K.), JP18ck0106243h0003 (J.K.) and JP19ek0410062h0001 (J.K.); the Ministry of Health, Labour, and Welfare of Japan under grant number JP19KA3001 (J.K. and K.H.); the Terumo Foundation for Life Sciences and Arts (J.K.); the ONO Medical Research Foundation (J.K.); the Canon Foundation (J.K.); and Cross-ministerial Strategic Innovation Promotion Program (SIP; to J.K.); the Joint Research Project of the Institute of Medical Science, the University of Tokyo (J.K.) and Public/Private R&D Investment Strategic Expansion Program (PRISM; to J.K.).

## References

- [1] Lan H, Hosomi K, Kunisawa J. Clostridium perfringens enterotoxin-based protein engineering for the vaccine design and delivery system. *Vaccine* 2019;37:6232–9. <https://doi.org/10.1016/j.vaccine.2019.08.032>.
- [2] Hu Z, Ott PA, Wu CJ. Towards personalized, tumour-specific, therapeutic vaccines for cancer. *Nat Rev Immunol* 2018;18:168–82. <https://doi.org/10.1038/nri.2017.131>.
- [3] Tetsutani K, Ishii KJ. Adjuvants in influenza vaccines. *Vaccine* 2012;30:7658–61. <https://doi.org/10.1016/j.vaccine.2012.10.007>.
- [4] Zaman K, Anh DD, Victor JC, Shin S, Yunus M, Dallas MJ, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *The Lancet* 2010;376:615–23. [https://doi.org/10.1016/S0140-6736\(10\)60755-6](https://doi.org/10.1016/S0140-6736(10)60755-6).
- [5] Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of human rotavirus vaccine on severe diarrhoea in African infants. *N Engl J Med* 2010;362:289–98. <https://doi.org/10.1056/NEJMoa0904797>.
- [6] Linhares AC, Velázquez FR, Pérez-Schael I, Sáez-Llorens X, Abate H, Espinoza F, et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *The Lancet* 2008;371:1181–9. [https://doi.org/10.1016/S0140-6736\(08\)60524-3](https://doi.org/10.1016/S0140-6736(08)60524-3).
- [7] Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor J, Cohen R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *The Lancet* 2007;370:1757–63. [https://doi.org/10.1016/S0140-6736\(07\)61744-9](https://doi.org/10.1016/S0140-6736(07)61744-9).
- [8] Kawamura N, Tokoeda Y, Oshima M, Okahata H, Tsutsumi H, Van Doorn LJ, et al. Efficacy, safety and immunogenicity of RIX4414 in Japanese infants during the first two years of life. *Vaccine* 2011;29:6335–41. <https://doi.org/10.1016/j.vaccine.2011.05.017>.
- [9] Clarke E, Desselberger U. Correlates of protection against human rotavirus disease and the factors influencing protection in low-income settings. *Mucosal Immunol* 2015;8:1–17. <https://doi.org/10.1038/mi.2014.114>.
- [10] Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol* 2010;10:159–69. <https://doi.org/10.1038/nri2710>.
- [11] Goto Y, Panea C, Nakato G, Cebula A, Lee C, Diez MG, et al. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity* 2014;40:594–607. <https://doi.org/10.1016/j.immuni.2014.03.005>.
- [12] Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009;139:485–98. <https://doi.org/10.1016/j.cell.2009.09.033>.
- [13] Tanoue T, Morita S, Plichta DR, Skelly AN, Suda W, Sugiura Y, et al. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* 2019;565:600–5. <https://doi.org/10.1038/s41586-019-0878-z>.
- [14] Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013;504:446–50. <https://doi.org/10.1038/nature12721>.
- [15] Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013;500:232–6. <https://doi.org/10.1038/nature12331>.
- [16] Hayashi A, Sato T, Kamada N, Mikami Y, Matsuoka K, Hisamatsu T, et al. A single strain of Clostridium butyricum induces intestinal IL-10-producing macrophages to suppress acute experimental colitis in mice. *Cell Host Microbe* 2013;13:711–22. <https://doi.org/10.1016/j.chom.2013.05.013>.
- [17] Hosomi K, Kunisawa J. The Specific Roles of Vitamins in the Regulation of Immunosurveillance and Maintenance of Immunologic Homeostasis in the Gut. *Immune Netw* 2017;17:13–9. <https://doi.org/10.4141/in.2017.17.1.13>.
- [18] Lamichhane A, Kiyono H, Kunisawa J. Nutritional components regulate the gut immune system and its association with intestinal immune disease development. *J Gastroenterol Hepatol* 2013;28(Suppl 4):18–24. <https://doi.org/10.1111/jgh.12259>.
- [19] Imdad A, Mayo-Wilson E, Herzer K, Bhutta ZA. Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age. *Cochrane Database of Systematic Reviews* 2017. <https://doi.org/10.1002/14651858.CD008524.pub3>.
- [20] Oh JZ, Ravindran R, Chassaing B, Carvalho FA, Maddur MS, Bower M, et al. TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. *Immunity* 2014;41:478–92. <https://doi.org/10.1016/j.immuni.2014.08.009>.
- [21] Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. *Nat Rev Immunol* 2010;10:131–44. <https://doi.org/10.1038/nri2707>.
- [22] Chassaing B, Ley RE, Gewirtz AT. Intestinal epithelial cell toll-like receptor 5 regulates the intestinal microbiota to prevent low-grade inflammation and metabolic syndrome in mice. *Gastroenterology* 2014;147(1363–1377):. <https://doi.org/10.1053/j.gastro.2014.08.033>.
- [23] Nakanishi Y, Ikebuchi R, Chtanova T, Kusumoto Y, Okuyama H, Moriya T, et al. Regulatory T cells with superior immunosuppressive capacity emigrate from the inflamed colon to draining lymph nodes. *Mucosal Immunol* 2018;11:437–48. <https://doi.org/10.1038/mi.2017.64>.
- [24] Lei W-T, Shih P-C, Liu S-J, Lin C-Y, Yeh T-L. Effect of Probiotics and Prebiotics on Immune Response to Influenza Vaccination in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients* 2017;9:.. <https://doi.org/10.3390/nu9111175>.
- [25] Lomax AR, Cheung LVY, Noakes PS, Miles EA, Calder PC. Inulin-Type  $\beta$ -2 Fructans have Some Effect on the Antibody Response to Seasonal Influenza Vaccination in Healthy Middle-Aged Humans. *Front Immunol* 2015;6:490. <https://doi.org/10.3389/fimmu.2015.00490>.
- [26] Akatsu H, Nagafuchi S, Kurihara R, Okuda K, Kanesaka T, Ogawa N, et al. Enhanced vaccination effect against influenza by prebiotics in elderly patients receiving enteral nutrition. *Geriatr Gerontol Int* 2016;16:205–13. <https://doi.org/10.1111/ggi.12454>.
- [27] Kunisawa J, Kurashima Y, Kiyono H. Gut-associated lymphoid tissues for the development of oral vaccines. *Adv Drug Deliv Rev* 2012;64:523–30. <https://doi.org/10.1016/j.addr.2011.07.003>.
- [28] Badur S, Öztürk S, Pereira P, AbdelGhany M, Khalaf M, Lagoubi Y, et al. Systematic review of the rotavirus infection burden in the WHO-EMRO region. *Hum Vaccin Immunother* 2019;15:2754–68. <https://doi.org/10.1080/21645515.2019.1603984>.
- [29] Yoshikawa T, Matsuki T, Sato K, Mizuno M, Shibata M, Hasegawa S, et al. Impact of rotavirus vaccination on the burden of acute gastroenteritis in Nagoya city. *Japan. Vaccine* 2018;36:527–34. <https://doi.org/10.1016/j.vaccine.2017.12.006>.
- [30] Crean TI, John M, Calderwood SB, Ryan ET. Optimizing the germfree mouse model for in vivo evaluation of oral Vibrio cholerae vaccine and vector strains. *Infect Immun* 2000;68:977–81. <https://doi.org/10.1128/iai.68.2.977-981.2000>.
- [31] Lamoué-Smith ES, Tzeng A, Starnbach MN. The intestinal flora is required to support antibody responses to systemic immunization in infant and germ free mice. *PLoS ONE* 2011;6:.. <https://doi.org/10.1371/journal.pone.0027662>.
- [32] Magwira CA, Taylor MB. Composition of gut microbiota and its influence on the immunogenicity of oral rotavirus vaccines. *Vaccine* 2018;36:3427–33. <https://doi.org/10.1016/j.vaccine.2018.04.091>.
- [33] Harris VC, Armah G, Fuentes S, Korpela KE, Parashar U, Victor JC, et al. Significant Correlation Between the Infant Gut Microbiome and Rotavirus Vaccine Response in Rural Ghana. *J Infect Dis* 2017;215:34–41. <https://doi.org/10.1093/infdis/jiw518>.
- [34] Talham GL, Jiang HQ, Bos NA, Cebra JJ. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect Immun* 1999;67:1992–2000.
- [35] Tobita K, Yanaka H, Otani H. Lactobacillus crispatus KT-11 enhances intestinal immune functions in C3H/HeN mice. *J Nutr Sci Vitaminol* 2010;56:441–5. <https://doi.org/10.3177/jnsv.56.441>.
- [36] Kotani Y, Kunisawa J, Suzuki Y, Sato I, Saito T, Toba M, et al. Role of Lactobacillus pentosus Strain b240 and the Toll-like receptor 2 axis in Peyer's patch dendritic cell-mediated immunoglobulin A enhancement. *PLoS ONE* 2014;9:.. <https://doi.org/10.1371/journal.pone.0091857>.
- [37] Kikuchi Y, Yoshida H, Ogita T, Okita K, Fukudome S-I, Suzuki T, et al. In vivo dose response and in vitro mechanistic analysis of enhanced immunoglobulin A production by Lactobacillus plantarum AYA. *Biosci Microbiota Food Health* 2015;34:53–8. <https://doi.org/10.12938/bmfb.2014-016>.
- [38] Obata T, Goto Y, Kunisawa J, Sato S, Sakamoto M, Setoyama H, et al. Indigenous opportunistic bacteria inhibit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *Proc Natl Acad Sci USA* 2010;107:7419–24. <https://doi.org/10.1073/pnas.1001061107>.
- [39] Fung TC, Bessman NJ, Hepworth MR, Kumar N, Shibata N, Kobuley D, et al. Lymphoid-Tissue-Resident Commensal Bacteria Promote Members of the IL-10 Cytokine Family to Establish Mutualism. *Immunity* 2016;44:634–46. <https://doi.org/10.1016/j.immuni.2016.02.019>.
- [40] Sato S, Kaneto S, Shibata N, Takahashi Y, Okura H, Yuki Y, et al. Transcription factor Spi-B-dependent and -independent pathways for the development of Peyer's patch M cells. *Mucosal Immunol* 2013;6:838–46. <https://doi.org/10.1038/mi.2012.122>.
- [41] Shibata N, Kunisawa J, Hosomi K, Fujimoto Y, Mizote K, Kitayama N, et al. Lymphoid tissue-resident Alcaligenes LPS induces IgA production without

- excessive inflammatory responses via weak TLR4 agonist activity. *Mucosal Immunol* 2018;11:693–702. <https://doi.org/10.1038/mi.2017.103>.
- [42] Takahashi I, Hosomi K, Nagatake T, Toubou H, Yamamoto D, Hayashi I, et al. Persistent colonization of non-lymphoid tissue-resident macrophages by *Stenotrophomonas maltophilia*. *Int Immunol* 2020;32:133–41. <https://doi.org/10.1093/intimm/dxz071>.
- [43] Park BS, Song DH, Kim HM, Choi B-S, Lee H, Lee J-O. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 2009;458:1191–5. <https://doi.org/10.1038/nature07830>.
- [44] Raetz CRH, Reynolds CM, Trent MS, Bishop RE. Lipid A modification systems in gram-negative bacteria. *Annu Rev Biochem* 2007;76:295–329. <https://doi.org/10.1146/annurev.biochem.76.010307.145803>.
- [45] Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TC. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. *Science* 2007;316:1628–32. <https://doi.org/10.1126/science.1138963>.
- [46] Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, et al. Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. *Cell* 2016;165:842–53. <https://doi.org/10.1016/j.cell.2016.04.007>.
- [47] Nagai M, Noguchi R, Takahashi D, Morikawa T, Koshida K, Komiyama S, et al. Fasting-Refeeding Impacts Immune Cell Dynamics and Mucosal Immune Responses. *Cell* 2019;178(1072–1087):. <https://doi.org/10.1016/j.cell.2019.07.047>e14.
- [48] Mora JR, Iwata M, Eksteen B, Song S-Y, Junt T, Senman B, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 2006;314:1157–60. <https://doi.org/10.1126/science.1132742>.
- [49] Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song S-Y. Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 2004;21:527–38. <https://doi.org/10.1016/j.immuni.2004.08.011>.
- [50] Kunisawa J, Sugiura Y, Wake T, Nagatake T, Suzuki H, Nagasawa R, et al. Mode of Bioenergetic Metabolism during B Cell Differentiation in the Intestine Determines the Distinct Requirement for Vitamin B1. *Cell Rep* 2015;13:122–31. <https://doi.org/10.1016/j.celrep.2015.08.063>.
- [51] Kunisawa J, Hashimoto E, Inoue A, Nagasawa R, Suzuki Y, Ishikawa I, et al. Regulation of intestinal IgA responses by dietary palmitic acid and its metabolism. *J Immunol* 2014;193:1666–71. <https://doi.org/10.4049/jimmunol.1302944>.
- [52] Gohda M, Kunisawa J, Miura F, Kagiya Y, Kurashima Y, Higuchi M, et al. Sphingosine 1-phosphate regulates the egress of IgA plasmablasts from Peyer's patches for intestinal IgA responses. *J Immunol* 2008;180:5335–43.
- [53] Nagatake T, Hirata S-I, Koga T, Kuroda E, Kobari S, Suzuki H, et al. BLT1 mediates commensal bacteria-dependent innate immune signals to enhance antigen-specific intestinal IgA responses. *Mucosal Immunol* 2019. <https://doi.org/10.1038/s41385-019-0175-z>.