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Invited Review Article

A comprehensive understanding of the gut mucosal immune system in allergic inflammation

Daisuke Tokuhara^{a, b, c, d, e}, Yosuke Kurashima^{a, b, c, f, g, h}, Mariko Kamioka^{a, b, c}, Toshinori Nakayamaⁱ, Peter Ernst^{c, d, j}, Hiroshi Kiyono^{a, b, c, d, *}^a Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan^b International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan^c Division of Gastroenterology, Department of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California San Diego, San Diego, CA, USA^d Division of Comparative Pathology and Medicine, Department of Pathology, University of California San Diego, San Diego, CA, USA^e Department of Pediatrics, Osaka City University Graduate School of Medicine, Osaka, Japan^f Department of Mucosal Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan^g Department of Innovative Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan^h Laboratory of Vaccine Materials, National Institute of Biomedical Innovation, Health and Nutrition, Osaka, Japanⁱ Department of Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan^j Center for Veterinary Sciences and Comparative Medicine, University of California, San Diego, CA, USA

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Abbreviations:

DC, dendritic cell; Foxp3, forkhead box P3;

GALT, gut-associated lymphoid tissue;

IDO, indoleamine 2,3-dioxygenase;

LP, lamina propria; LTi, lymphoid tissue

inducer; MLN, mesenteric lymph node;

OVA, ovalbumin; pIgR, polymeric Ig

receptor; PP, Peyer's patch; RA, retinoic

acid; ROR γ t, retinoic acid receptor-relatedorphan receptor γ t; SIgA, secretory IgA;TGF- β , transforming growth factor β ;

S1P, sphingosine 1-phosphate;

MALT, mucosa-associated lymphoid tissues;

NALT, nasopharyngeal-associated lymphoid

tissue; ILF, isolated lymphoid follicle;

MAdCAM-1, mucosal vascular addressin cell

adhesion molecule 1

ABSTRACT

Despite its direct exposure to huge amounts of microorganisms and foreign and dietary antigens, the gut mucosa maintains intestinal homeostasis by utilizing the mucosal immune system. The gut mucosal immune system protects the host from the invasion of infectious pathogens and eliminates harmful non-self antigens, but it allows the cohabitation of commensal bacteria in the gut and the entry of dietary non-self antigens into the body via the mucosal surface. These physiological and immunological activities are regulated by the ingenious gut mucosal immune network, comprising such features as gut-associated lymphoid tissue, mucosal immune cells, cytokines, chemokines, antimicrobial peptides, secretory IgA, and commensal bacteria. The gut mucosal immune network keeps a fine tuned balance between active immunity (against pathogens and harmful non-self antigens) and immune tolerance (to commensal microbiota and dietary antigens), thus maintaining intestinal healthy homeostasis. Disruption of gut homeostasis results in persistent or severe gastrointestinal infection, inflammatory bowel disease, or allergic inflammation. In this review, we comprehensively introduce current knowledge of the gut mucosal immune system, focusing on its interaction with allergic inflammation.

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* Corresponding author. Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.

E-mail address: kiyono@ims.u-tokyo.ac.jp (H. Kiyono).

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Introduction

The gastrointestinal tract—the tube-like structure covered by mucosa from the oral cavity to the anus—is continuously and directly exposed to inputs from the external environment and entry of dietary antigens and pathogens. A major physiological function of the gastrointestinal tract is the digestion of food, absorption of nutrients and water, and elimination of unnecessary wasted products; the intestine is a major site of entry for many bacterial and viral pathogens and has a vast and diverse microbial community.^{1,2} Despite the complexity of the outer environment, intestinal homeostasis is ingeniously maintained under the control of the unique gut mucosal immune network, which is characterized by such features as gut-associated lymphoid tissues (GALT or Peyer's patches; PPs), secretory IgA (SIgA), antimicrobial peptides (e.g. defensins), mucosal immune cells (e.g. Th1, Th2, Th17, Tfh and Treg cells), cytokines (e.g. IL-10), chemokines (e.g. CCR9), and commensal bacteria.^{3–8}

Food allergy, which generally occurs in early childhood, is triggered by the ingestion of a dietary antigen via the gut mucosal membrane; it causes clinical signs such as gastrointestinal disorders, urticarial, and distant airway inflammation, ranging in severity from mild to life-threatening.⁹ Because the prevalence of food allergy seems to be increasing in developed countries,^{9,10} the development of effective clinical methods for the prevention and treatment of the allergic disease would be of substantial social and financial values, but we first need to acquire a better understanding of the underlying gut mucosal immune system for the regulation of allergic immune response. The healthy mucosal immune system is inducing and sustaining the unresponsiveness status to dietary antigens—that is known as “oral tolerance” characterized by the depletion or anergy of reactive (or pathogenic) T cells,^{11–13} and/or the induction of Tregs cells at mucosal sites.^{14,15} Disruption of the gut mucosal immune system can lead to failure to induce protective immunity and/or tolerance, thus causing severe gastrointestinal infection, inflammatory bowel disease, or food allergy.^{16,17} Here, we highlight current knowledge regarding the role of the gut mucosal immune system in shaping and maintaining intestinal homeostasis, and we discuss the interactive gut mucosal immune system against food allergy, focusing particularly on the regulatory pathways leading to immune tolerance and its disruption causing undesired allergic response.

Development of the gut mucosal immune system

Food allergy tends to occur in early childhood (<2 years old), and immediate-type food allergy has the highest incidence in infants (<1 year old).^{9,10} Therefore, the interaction between this early food allergy and the development of the gut mucosal immune system is of particular interest. The gut mucosal immune system develops both anatomically and functionally with age dependent manner.^{18–20} Of the mucosa-associated lymphoid tissues (MALT), PPs and the mesenteric lymph nodes (MLNs), family of GALT begin developing during the fetal life, whereas isolated lymphoid follicles (ILFs) and nasopharyngeal-associated lymphoid tissue (NALT) develop and mature after the birth.^{3,21,22} In humans, aggregates of T lymphocytes that contain small numbers of B cells are the initial structures (anlagen) of PPs and are seen in the fetus from 14 weeks of gestational age.¹⁹ The PPs increase in number and size with age until adolescence.¹⁸ In murine models, underdevelopment of MLNs or PPs, or both coincides the reduction of SIgA and oral tolerance and thus increases susceptibility to infection and food allergy.^{23–25} MLN- and PP-deficient mice show strong reductions in IgA-producing B-cell counts and increased susceptibility to *Listeria monocytogenes* infection.²³ Oral tolerance fails to be induced in

both MLN- and PP-deficient mice.²⁴ In an ovalbumin (OVA)-induced allergy model, deficiency of PPs alone resulted in failed induction of oral tolerance to OVA and the development of severe allergic signs upon OVA challenge, unlike in wild-type mice.^{25,26} Moreover, the absence of MLNs alone is sufficient to impair the induction of oral tolerance to OVA.²⁴ These findings suggested that the presence and maturation of GALT play one of key roles for the establishment of balanced gut mucosal immune system contributed by the SIgA and oral tolerance induction.

The cellular and molecular mechanisms of PP and MLN development have been clarified in murine studies. Although the development of PP and MLN structures is guided by different signals to some extent, in both cases development requires the interaction between lymphoid tissue inducer (LTi) cells (or currently termed as type 3 innate lymphoid cells: ILC3)²⁷ and lymphoid tissue organizer cells (Fig. 1).^{3,28} In addition, the differentiation of LTi cells from IL-7R α ⁺ lymphoid progenitors requires the expression of inhibitor of DNA binding 2 (ID2) and the retinoic acid receptor-related orphan receptor γ (ROR γ t) in developing PPs and MLNs.^{29,30} Signaling via IL-7R α or TNF-related activation-induced cytokine receptor, or both, leads to the expression of LT α 1 β 2 by LTi cells, which promote lymphoid tissue formation.³¹

Development of the gut mucosal immune system is influenced by the outer environment in the form of nutrition and commensal bacteria.^{27,32–35} As an example of nutritional involvement, retinoic acid (RA), a metabolite of vitamin A, plays a crucial role in induction of the chemokine CXCL13 leading to the formation of lymphoid cell clusters (Fig. 1).³² Inhibition of RA synthesis reduces the formation of LTi cell-mediated lymphoid cell clusters; therefore, maternal levels of dietary retinoids control the size of secondary lymphoid organs in the gut.²⁷ Commensal bacteria promote gut development. Compared with conventionally raised animals, germ-free animals have fewer goblet cells, a thinner mucus layer, reduced villus thickness, and impaired brush border differentiation.³³ In addition, PPs and MLNs are underdeveloped in germ-free mice.³⁴ In contrast, postnatal microbial colonization promotes the maturation of GALT including their size and the formation of germinal center in PPs.³⁵ Bacterial products such as LPS and peptidoglycan have been shown to stimulate to establish conventional mucus properties as well as the maturation of GALT in germ-free mice.^{36,37}

Gut mucosal barrier as the site of entry of dietary antigens and pathogens

Intestinal homeostasis is maintained largely by the harmonized physical, chemical and immunological defense system against pathogens (e.g., bacteria, viruses and fungus) and by immune tolerance to dietary antigens and commensal bacteria.^{38,39} In terms of the defense mechanism, mucosal surfaces in the small intestine are both specifically and non-specifically protected against pathogen invasion and unfavorable antigen entry into the body through both anatomical and immunologic defense mechanisms. As non-specific mechanisms, physical and chemical barrier comprising of intestinal epithelial cells covered with mucus layer and antimicrobial peptides (e.g., defensins and lysozymes) protects against pathogen invasion as a first line of defense.³⁹ Intestinal epithelial cells, in association with fucose, the production of which is induced by commensal bacteria and ILC3, inhibit pathogenic bacterial infections (e.g., *Salmonella typhimurium*).^{8,40} Further, these fucosylation products are nutrients for some of commensal bacteria (e.g., *Bacteroides* and *Lactobacillus*).⁸ As an example of specific mechanisms, intestinal epithelial cells produce pro-inflammatory chemokines (e.g., MCP-1 and IL-8) and cytokines (e.g., IL-15) in response to luminal stimuli and induce the recruitment of diverse immune cells in the lamina propria (LP) to inflamed regions. In this

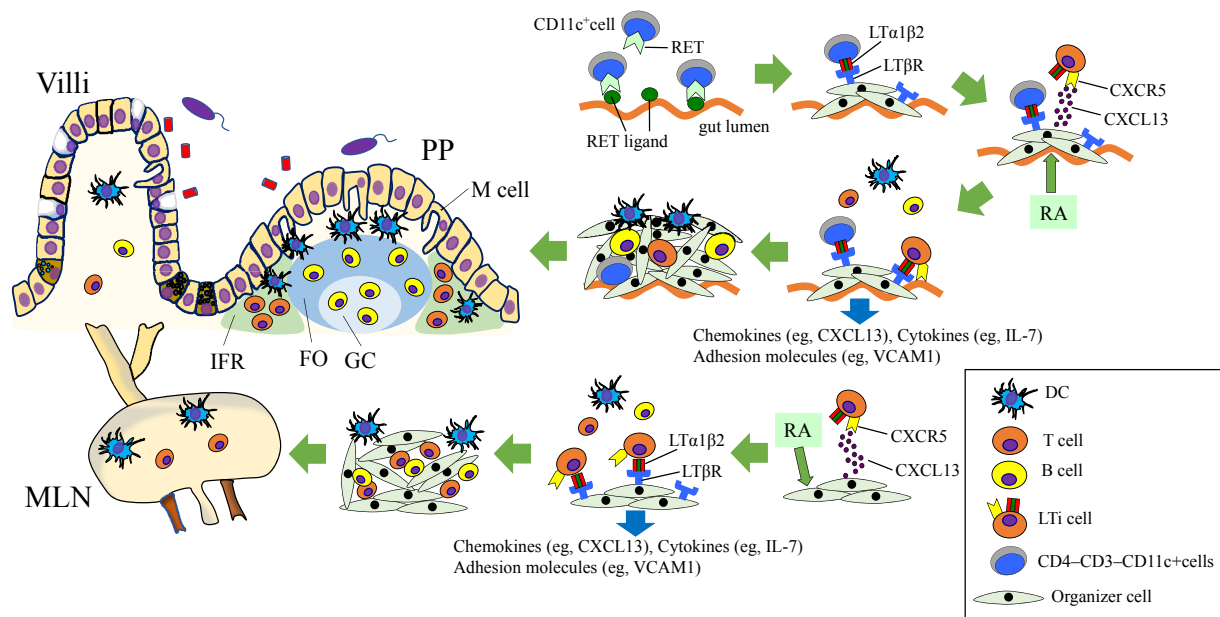


Fig. 1. Overview of the structure and organogenesis of gut-associated lymphoid tissue. In the development of PPs, $CD45^{+}IL-7R\alpha^{+}CD4^{+}CD3^{-}CD11c^{+}$ cells expressing RET are stimulated by the RET ligand expressed on the developing gut. This is followed by the upregulation of $LT\alpha1\beta2$ on the $CD11c^{+}RET^{+}$ cells. $LT\alpha1\beta2$ binds to $LT\beta R$ expressed on lymphoid tissue organizer cells and recruits $CD45^{+}IL-7R\alpha^{+}CD4^{+}CD3^{-}$ LTi cells. $LT\beta R$ stimulation enhances the production of ICAM-1, VCAM-1, and mucosal vascular addressin cell adhesion molecule 1 on—and the release of CXCL13, CXCL12, CCL19, CCL21, and IL-7 from—the organizer cells. These adhesion molecules and chemokines promote the adhesion and attraction of LTi cells and the organizer cells, leading to the formation of the PP anlagen. In the development of MLNs, the organizer cells secrete CXCL13, recruit LTi cells expressing CXCR5 (a receptor for CXCL13), and initiate the formation of cell clusters. LTi cells express $LT\alpha1\beta2$ that is capable of binding to $LT\beta R$ expressed on the organizer cells, thus enhancing the close interaction between LTi cells and the organizer cells. This close interaction upregulates the expression of adhesion molecules (ICAM-1, VCAM-1, and MAdCAM-1) on, and the release of chemokines (CCL19, CCL21, and CXCL13) from, the organizer cells, further recruiting LTi cells and enhancing the adhesion of migrated LTi cells and other immune cells (B and T cells and DCs). This is followed by the formation of the MLN anlagen. In the development of PPs and MLNs, RA induces the expression of CXCL13 by mesenchymal lymphoid organizer cells. CXCL13 then attracts LTi precursor cells from the blood to form the first lymphoid cell clusters. DC, dendritic cell; ICAM-1, intercellular adhesion molecule 1; LTi, lymphoid tissue inducer; MAdCAM-1, mucosal vascular addressin cell adhesion molecule 1; MLN, mesenteric lymph node; PP, Peyer's patch; RA, retinoic acid; VCAM-1, vascular cell adhesion molecule 1; RET, Tyrosine receptor kinase.

regard, our recent study revealed that microRNAs, which are small noncoding RNA molecules such as miR-1224, miR-3473a, and miR-5128 that regulate post-transcription, are involved in homeostasis and disruption of the epithelial barrier via modulation of the expression of genes (e.g., *AQP8* and *ABCG2*) in epithelial cells in the colon, rather than in the small intestine.⁴¹ Among these epithelial cells, goblet cells secrete mucins, which are major components of the mucus layer.³⁹ Goblet cells also secrete other biologically active products contributing to innate immunity, such as trefoil peptides, resistin-like molecule β , and Fc- γ binding protein, which stabilize the mucus layer.⁴² Another type of epithelial cells, Paneth cells, located at the base of the intestinal glands, produce defensins, which kill or inactivate bacteria by attacking their basic cell-wall structures.⁴³ Lactoferrin, a protein generally derived from breast milk, inhibits bacterial growth through iron-dependent and -independent mechanisms.⁴⁴ Commensal bacteria indirectly affect the barrier functions of epithelial cells and help to inhibit allergic sensitization. Clostridial colonization induces IL-22 production by both $ROR\gamma t^{+}$ ILCs and T cells in the LP and reduces the uptake of orally administered dietary antigens into the systemic circulation by the stabilization of intestinal epithelial permeability and thus contribute in the prevention of allergic diseases.⁴⁵

Secretory IgA

SIgA is a unique immunological feature of the gut mucosal immune system.^{46,47} Its primary function is specific defense against pathogens and toxins.^{46,47} Further SIgA antibodies have shown to establish and maintain healthy gut microflora.^{48,49} In relation to allergic condition, there is evidence that a reduction in SIgA levels is

involved in allergy development. Low levels of salivary and intestinal SIgA are associated with an increased risk of allergic manifestations during early life.^{50–52} In mice, allergen sensitization using bovine lactoglobulin reduces intestinal allergen-specific SIgA levels and the number of IgA-producing cells in PPs compared with those in actively tolerized mice.⁵³ SIgA may contribute to immune exclusion to reduce allergen uptake,⁵⁴ but the mechanism by which SIgA protects against food allergy needs further study. Because commensal bacteria are critical mediators of oral tolerance,^{55,56} the interaction between SIgA and commensal bacteria is an issue of interest given its association with a reduction in allergy development. Children developing allergic manifestations—particularly asthma—during childhood have a lower proportion of IgA bound to fecal bacteria at 12 months of age than do healthy children.⁵⁷ SIgA may limit the overgrowth of selected or undesired species, thus enabling an increase in microbial diversity in infancy followed by the induction of oral tolerance.

As an initial step to evoke the production of specific SIgA in the gut, pathogens, commensal bacteria, and antigens must be transported across the epithelial barrier into the GALT, including PPs, the center court for the initiation of antigen-specific immune response in the gut, are seen as dome-like structures located in the small intestine opposite the mesentery (Fig. 1).^{18,38} Histologically, they consist of the follicular B-cell-rich areas and interfollicular T-cell-rich areas covered with follicle-associated epithelium containing M cells specialized for antigen sampling (Fig. 1).³⁸ Antigen-presenting cells (APCs) such as dendritic cells (DCs) are present in the sub-epithelium and in the interfollicular zones in the PPs.³⁸ In follicle-associated epithelium, pathogens or antigens are taken up by the M cells, which form intraepithelial pockets specialized for the

transepithelial transport of antigens by macromolecular transcytosis to APCs.⁴ M cells can sample microorganisms and/or macromolecular antigens non-specifically (or engulfing capacity)^{58,59}; they can also take up specific bacteria possessing FimH adhesin, such as *Escherichia coli* and *Salmonella enterica*, via glycoprotein 2 expressed on their apical membranes.⁶⁰

Following antigen uptake by the M cells, APCs such as DCs beneath the M cells capture the antigens, which are then processed and presented peptide-MHC complex to naive CD4⁺ T cells. PP DCs produce IL-10 and induce the differentiation of Th2 cells.⁶¹ Activated T cells secrete the IgA inducing cytokines such as transforming growth factor β (TGF- β) and IL-4 to promote the class-switching of B cells to produce IgA.⁶² Cytokines secreted by mucosal T cells (IL-4 and TGF- β) and epithelial cells (TGF- β) cooperate to promote the maturation of IgA-producing B cells.^{63,64} Further, IL-5 and IL-6 produced by Th cells assist IgA committed B cells to differentiate to IgA producing plasma cells.^{3,65,66} In a T-cell-independent pathway, PP DCs directly induce IgA production in B cells via the stimulation of PP DC-derived RA and IL-6.⁶⁷ Furthermore, intestinal DC-derived RA enhances the expression of $\alpha\beta$ 7 integrin and CCR9 on T and B cells upon activation and imprints the cells with “gut-homing” specificity.⁶⁷ Through gut-homing, the activated antigen-specific T and B cells migrate from PPs through the efferent lymphatics to the regional MLNs and then to the intestinal LP by way of the lymphatic ducts and blood circulation.⁶⁷

For the gut imprinting system, this trafficking of activated antigen-specific B and T cells from the blood to the intestinal LP is regulated by interactions of gut-homing molecules involving $\alpha\beta$ 7 integrin and CCR9.^{68,69} Small intestinal microvascular endothelial cells express mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), which binds to $\alpha\beta$ 7 integrin.⁶⁸ In addition, the endothelial cells release CCL25, which binds to its receptor, CCR9⁶⁹; in this way, activated antigen-specific B and T cells expressing $\alpha\beta$ 7 integrin and CCR9 are attracted to the LP. In addition, epithelial cells produce CCL25, further attracting these immune cells to the LP region below the villous epithelium. In the presence of various cytokines, including IL-5 and IL-6 produced by Th2 cells, IgA-committed B cells differentiate into IgA-producing plasma cells in intestinal LP.

Dimeric or polymeric IgA released from the plasma cells attaches to the polymeric Ig receptor (pIgR) located on the basolateral surfaces of mucosal epithelial cells; it is then transcytosed to the apical surface and secreted into the mucus layer as SIgA.⁷⁰ During transcytosis, a portion of the pIgR is cleaved off; the remaining portion (called the secretory component) stays attached to the IgA.⁷⁰ Therefore, IgA, rather than IgG or IgM, is the predominant immunoglobulin isotype in most mucosal secretions.^{46,70} SIgA attaches to pathogenic microorganisms and toxins and blocks their access to the mucosal epithelial cells. Furthermore, M-cell-mediated reverse transcytosis of antigen–SIgA complex contributes to presentation and processing by antigen-presenting cells, leading to an adaptive immune response against the antigen.⁷¹ The above mechanisms inducing antigen-specific SIgA production in the gut have been used to develop mucosal vaccines against gastrointestinal infectious diseases.^{74,69,72,73} Injectable vaccines induce antigen-specific IgG serum antibodies, but they are less effective in stimulating the gut mucosal immune system and eliciting a pathogen-specific SIgA response in the gut lumen.⁴⁶ In contrast, the delivery of vaccines across mucosal surfaces—such as by the oral route—effectively provides pathogen-specific mucosal immune responses in addition to systemic immune responses.^{74,69,72,73}

Although M cells and DCs in the PPs mainly play a crucial role in the induction of acquired immunity, alternatively, antigens can be taken up by intestinal villous M cells or epithelial DCs located in the small intestine.^{74,75} TLR2, -4, and -9-mediated MyD88-dependent

signaling in epithelial cells promotes transepithelial extension of the cellular processes of epithelial DCs to directly sample luminal antigens into the LP.⁷⁶ LP DCs also contribute to IgA production by B cells: In the LP, CD11b^{hi}CD11c^{hi} DCs expressing TLR5 induce the differentiation of naive B cells into IgA-producing plasma cells in response to flagellin stimulation.⁷⁷ Commensal bacteria, including the 100 trillion bacteria in the gut, are also key components in shaping up SIgA induction system. Generally, commensal bacteria reside on the surface of the gut lumen or the intestinal epithelium,³³ but our recent studies have revealed that some commensal bacteria (e.g., *Alcaligenes faecalis*) inhabit the inside of the DCs in PPs and produce necessary signals for the activation of IgA production system via the unique LPS.^{78–80}

Induction of oral immune tolerance

Immune tolerance is sustained immune unresponsiveness to beneficial antigens and commensal bacteria. A healthy immune system is generally situated under tolerant or quiescent condition sustaining unresponsiveness to self-antigen, dietary antigens and commensal bacteria. Based on numerous laboratory findings in humans and animals, oral tolerance is identified as reductions or unresponsiveness in systemic delayed-type hypersensitivity, T-cell proliferation, and Th2-type cytokine production.^{25,26,81} Serum antibody responses—particularly IgE production and Th1-dependent IgG2a production—can also be suppressed.⁸¹ The mechanisms of tolerance induction involve multiple pathways, including 1) deletion of reactive T cells,¹² 2) anergy of reactive T cells,^{11,13} and 3) induction of regulatory T cells at mucosal sites.^{14,15} The contribution of each suppression pathway to oral tolerance induction depends on the animal model and the type of antigen used.

Among these inhibitory pathways, Treg-mediated oral tolerance is well studied and established. Three groups of immune cells such as, CX3CR1⁺ macrophages, CD103⁺ DCs, and Treg cells have been shown to play a crucial role, and villous LP and MLNs are the main inductive sites for oral tolerance (Fig. 2).^{82–86} First, CX3CR1⁺ macrophages directly take up small or soluble antigens from the intestinal lumen by sending their cellular processes out into the lumen across the epithelial barrier. Then, the CX3CR1⁺ macrophages transfer the antigens to CD103⁺ DCs in the LP via gap junctions, which are intercellular communication processes expressed on CD103⁺ DCs and formed by membrane proteins such as connexin 43.⁸² To a lesser extent, goblet cells can serve as epithelial gateways for the uptake of small antigens; this uptake is driven by the interaction of acetylcholine and muscarinic acetylcholine receptors.^{87,88} These epithelial antigen sampling systems are in close interaction with CD103⁺ DCs for the processing and presentation of the captured antigens. After transfer of the antigens from the CX3CR1⁺ macrophages or epithelial cells, CD103⁺ DCs move from the LP to the MLNs in a CCR7-corresponding chemokine CCL21 -dependent process.^{83,86} The CD103⁺ DCs then stimulate naive CD4⁺ T cells to differentiate into forkhead box P3 (Foxp3)-expressing antigen-specific Treg cells via the release of RA, TGF- β , and indoleamine 2,3-dioxygenase (IDO) (Fig. 2).^{84,89–91} In this process, RA is capable of directly inducing Treg-cell differentiation, but TGF- β -mediated Foxp3 upregulation in Treg differentiation requires RA as well. Sustained IDO expression on CD103⁺ DCs is regulated by TGF- β .⁹² Another type of Treg cells, Foxp3-negative IL-10-producing Treg cells (Tr1 cells), is also induced from naive CD4 T cells and involve in the establishment of oral tolerance.⁹³ In addition to stimulating Treg-cell differentiation, CD103⁺ DCs producing RA induce Treg-cell production of the gut-homing molecules $\alpha\beta$ 7 integrin and CCR9 (Fig. 2)⁸⁵; these molecules respectively bind to MAdCAM-1 and CCL25,^{68,69} which are expressed on, and released

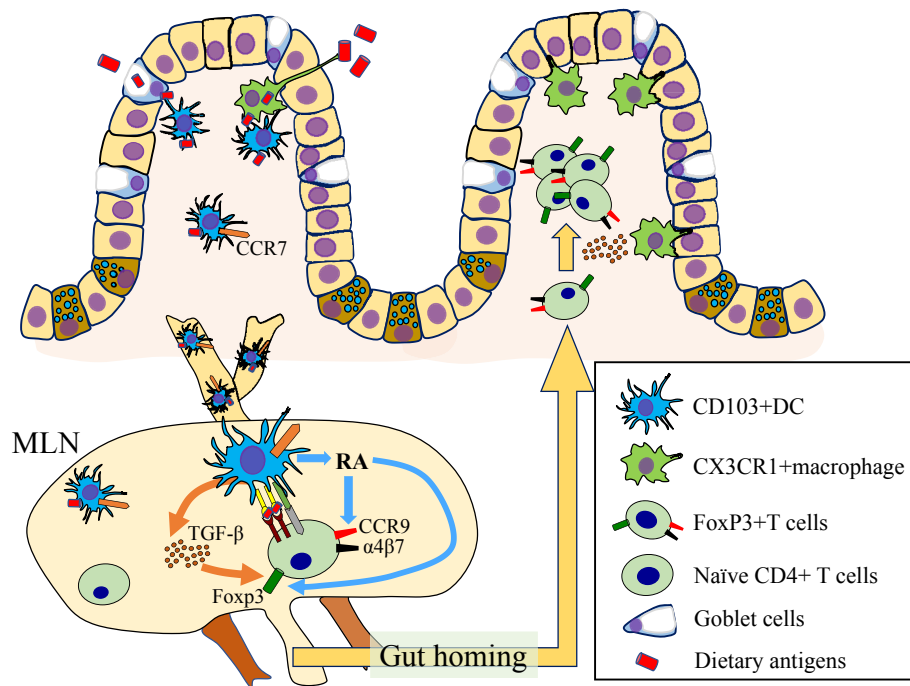


Fig. 2. Mechanisms of oral tolerance induction. Luminal dietary antigens are taken up directly by CX3CR1⁺ macrophages or goblet cells and transferred to CD103⁺ DCs in the LP. CD103⁺ DCs migrate to the MLN via the expression of CCR7. In the MLN, CD103⁺ DCs differentiate naive CD4 T cells into FoxP3⁺ Treg cells via stimulation by RA and TGF- β released from CD103⁺ DCs. IDO also promotes Treg differentiation. In addition, RA induces the production of the gut-homing molecules CCR9 and α 4 β 7 integrin on activated Treg cells. Activated Tregs enter the systemic circulation through the efferent lymphatics and blood vessels and migrate to the lamina propria in the small intestine. Antigen-specific Treg cells proliferate in the lamina propria in response to IL-10 released from CX3CR1⁺ macrophages. DC, dendritic cell; Foxp3, forkhead box P3; IDO, indoleamine 2,3-dioxygenase; LP, lamina propria; MLN, mesenteric lymph node; RA, retinoic acid; TGF- β , transforming growth factor β .

from, microvascular endothelial cells in the small intestinal LP. This results in the homing of effector Treg cells to the small intestinal LP, where they create downregulatory condition in order to avoid unnecessary active immune status. Further, antigen-specific Treg cells are expanded in the LP in response to IL-10 released from CX3CR1^{high} macrophages (Fig. 2).⁸⁵ Vitamin B is a factor that promotes the survival of gut Treg cells via the vitamin B9 receptor (folate receptor 4).⁹⁴

Antigen uptake by mucosal DCs axis of villus epithelium-LP-MLN cascade is important for the induction of oral tolerance to soluble antigens in the small intestine.⁸⁶ Further, PPs have been shown to involve in oral tolerance induction in the small intestine. M-cell-targeted antigen delivery has been thus reported to induce oral tolerance.⁹⁵ However, oral tolerance is inducible even in the absence of PPs. Using PP-null mice model, it was shown that oral tolerance to hapten was induced and maintained.²⁴ While oral tolerance against OVA protein was altered with exacerbated elevation of Th2 cytokines after OVA challenge.²⁵ A current concept is that the amount or nature of the antigen (e.g. soluble, small, or particle), or both, determines its route of uptake into the different parts of mucosal tissue (LP or PPs) for the induction of oral tolerance.^{24,25,82} Particulate materials and microbiota mostly enter into the GALT by M-cell-mediated transcytosis, whereas soluble or small antigens induce oral tolerance after being absorbed by epithelial cells and/or taken up epithelium associated macrophages, DCs or goblet cells, and to a lesser extent, the GALT.^{24,25,82} Therefore, PPs might play a subordinate role in oral tolerance to proteins.

Evidence is accumulating that dietary antigen is critical in the induction of Treg cells in the small intestine but not in the colon,⁹⁶ whereas commensal microbiota play a more important role in the induction of Treg cells in the colon than in the small intestine.^{55,56} In antigen-free mice that experience no exposure to any antigens, the number of Treg cells in the small intestine is significantly lower

than in germ-free mice. However there is no significant difference in the number of Treg cells in the colonic mucosa between antigen-free and germ-free mice.⁹⁶ On the other hand, when dietary antigens are ingested during weaning, Treg cells are produced in dramatic numbers in the small intestine to positively suppress the immune response to ingested dietary antigens.⁹⁶ In germ-free mice, the numbers of Treg cells are reduced in the LP of the colonic mucosa, but when these mice are colonized with *Clostridium* species—commensal bacteria—Treg cells are produced in the colonic mucosa. This production is mediated by butanoic acid, a product of anaerobic fermentation.⁵⁵ Polysaccharide A on the outer membrane of *Bacteroides fragilis* is recognized by TLR2 on CD4⁺ T cells and activates a signaling cascade involving myeloid differentiation primary response protein 88 and the induction of Treg-cell differentiation in the colon (Fig. 3).⁵⁶

Disruption of gut mucosal immune system leads to allergic inflammation

Disruption of a passage of the induction and regulation machinery necessary for oral tolerance induction discussed above leads to mucosal inflammation. CD103⁺ DCs producing IDO drive Foxp3⁺ Treg development, whereas inhibition or genetic deletion of IDO accelerates Th1 and Th17 differentiation and exacerbates colitis.⁸⁴ RA, a metabolite of vitamin A, is crucial for the induction of Treg differentiation and gut homing receptors (e.g., CCR9 and α 4 β 7); therefore, when mice are deficient in vitamin A, the numbers of α 4 β 7-positive T cells in PPs and MLNs are reduced.^{90,91} As a consequence, antigen-specific T cells are unable to migrate from mucosal inductive sites (e.g., PPs) to effector site (e.g., LP), leading to an absence of Treg cells and effector T cells in the effector tissues. In addition, oral tolerance is abrogated by disruption of the gut-homing properties of Treg cells in β 7-integrin- and MadCAM1-

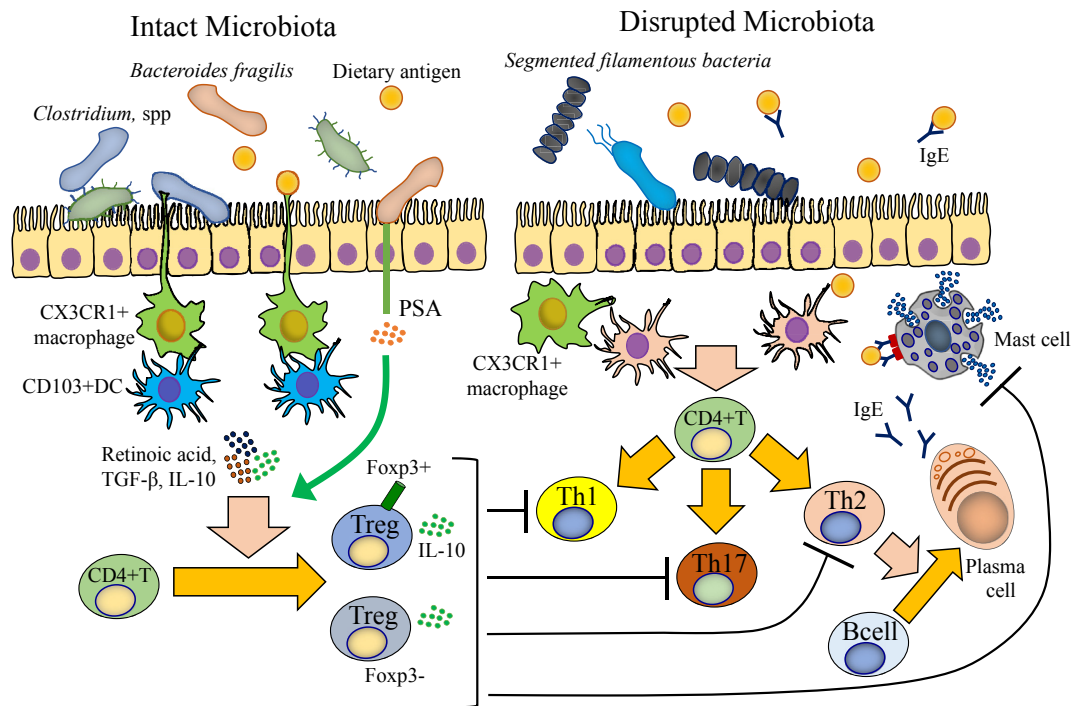


Fig. 3. Interaction between gut microbiota and induction or disruption of oral tolerance. *Clostridium* spp., *Bacteroides fragilis*, and other commensal bacteria stimulate epithelial cells, T cells, macrophages, and DCs and promote the differentiation of naive CD4⁺ T cells into Tregs. *Bacteroides fragilis*-producing PSA promotes the production of not Foxp3⁻Tregs but Foxp3⁺ Tregs. Segmented filamentous bacteria and other microbiota activate DCs and macrophages and induce the production of pathogen- or antigen-specific inflammatory Th1 and Th17 cells via IL-1 β , IL6, and IL23. Antigen-stimulated DCs promote Th2-cell differentiation, followed by the induction of antigen-specific IgE-producing plasma cells. Dietary antigen–IgE complex-mediated degranulation of mast cells promotes IgE-mediated allergic inflammation. Healthy Treg induction is capable of inhibiting the inflammatory process of Th1, Th2, and Th17 differentiation and mast cell activation. DC, dendritic cell; Foxp3, forkhead box P3; PSA, polysaccharide A.

deficient mice.⁸⁵ Mice deficient in CCR7, which is required for CD103⁺ DC trafficking to MLNs, have impaired oral tolerance induction.⁸⁶ CX3CR1 is a key molecule on CX3CR1-expressing macrophages in the transfer of antigens from the lumen to CD103⁺ DCs and for Treg proliferation in the intestinal LP via IL-10 production; deficiency of CX3CR1 results in reduced production of IL-10 in those macrophages, leading to impaired local Treg-cell proliferation.⁸⁵ Loss of function in mucosal Treg cells results in severe inflammatory phenotypes in the lungs in addition to intestine.⁹⁷

Destruction of commensal bacteria is also a crucial factor in the failure of oral tolerance induction and in the development of allergic inflammation.^{45,98} Depletion of commensal bacteria by oral antibiotic treatment increases serum IgE concentrations; it also increases steady-state circulating basophil populations and exacerbates basophil-mediated Th2-cell responses and allergic inflammation.⁴⁵ In conditions of continuous good health, microbial symbiosis limits naive CD4⁺ T cell differentiation into the effector T cells including Th1 and Th17 cells while promotes the induction of IL-10 production of CX3CR1⁺ macrophages and Treg cells. However, when the dysbiosis is occurred, CX3CR1⁺ mononuclear phagocytes shift the T-cell differentiation to pathogen-specific Th1-cell proliferation, thus inducing mucosal inflammation (Fig. 3).¹⁶ Goblet cells have been shown to possess a capacity of antigen passage from the lumen to LP (Goblet cell-mediated antigen passage system),⁸⁷ however its activity is suppressed in the colon by the colonic microbiota.⁸⁸ On the other hand, antibiotic depletion of the microbiota leads to the activation of goblet-cell-mediated antigen passage, which accelerates the delivery of luminal antigens to both CD103⁺ and CD103⁻ APC in the colonic LPs. This results in the production of inflammatory cytokines (e.g., IL-6 and IL-17) and chemokines (e.g., CXCL1) and an influx of leukocytes, including neutrophils, reflecting a state of acute inflammation.⁸⁸

In IgE-dependent food allergy, intestinal mast cells play a critical role in exacerbating allergic inflammation and symptoms (e.g., diarrhea) via chemical mediators such as histamine released by mast cells bound with the allergen-IgE complex (Fig. 3). In a model of OVA-induced allergic diarrhea,^{26,99} our previous study showed that sphingosine 1-phosphate (S1P) is responsible for the trafficking of pathogenic Th2 and mast cells.¹⁰⁰ Our recent study further extended our knowledge of the mechanisms of mast-cell-mediated intestinal inflammation: Mast cells promote intestinal inflammation via the interaction of extracellular ATP and P2X7 purinoceptors; this pathway is effectively inhibited by P2X7 purinoceptor-specific antibody.¹⁰¹ Accumulated evidence has revealed that ATP-P2X7 pathways enhance IgE-mediated degranulation of mast cells in an autocrine and paracrine manner.¹⁰²

Preventive and therapeutic approaches to allergic inflammation

Studies are now using accumulated evidence on the gut mucosal immune system to develop preventive and therapeutic approaches to allergy. In a model of OVA-induced allergic diarrhea that have been established in our group, Th2-dominant environment accelerates diarrhea^{26,99}; therefore, an immunotherapy aimed at inhibiting the Th2-type response has been considered.¹⁰³ In our previous study, intranasal administration of IL-12p70 naked DNA expression plasmids resulted in the synthesis of corresponding cytokines in large intestinal CD11c⁺ DCs; this inhibited the antigen-specific Th2-type response, preventing OVA-induced allergic diarrhea and suppressing clinical signs and symptoms, including OVA-specific IgE synthesis.¹⁰³ The murine model of OVA-induced allergic diarrhea is associated with infiltration by mast cells and pathogenic CD4⁺ T cells; trafficking of both of these types of cells is promoted

by S1P.¹⁰⁰ On the basis of this finding, we further demonstrated that FTY720, a modulator of the S1P receptor, protects against allergic diarrhea by inhibiting the migration of systemically primed pathogenic CD4⁺ T cells and mast cells in the colon.¹⁰⁰

The concept of oral tolerance induction is also being applied to the prevention of pollinosis. Oral administration of genetically modified edible rice seeds, containing the major T-cell epitopes derived from cedar pollen allergens, induces oral immune tolerance to pollen protein and thus reduced allergen-specific IgE levels, T-cell proliferative reactions, histamine production, and allergy symptoms.⁸¹

Nutritional approaches have been explored to treat allergic diarrhea. A metabolite of dietary ω 3 polyunsaturated fatty acids, 17,18-epoxyeicosatetraenoic acid, reduces OVA-induced allergic diarrhea by inhibiting mast cell degranulation in the colon via an IgE-independent pathway.¹⁰⁴ The importance of vitamins A and B in inducing and maintaining Treg cell implicates the utility of vitamin supplementation in children against food allergy; however a recent study showed that early vitamin use in children (within the first 6 months) was associated with a higher risk for food allergies in an exclusively formula-fed population.¹⁰⁵ Therefore, further study is needed to determine the usefulness, doses, and timing of vitamin use in terms of food allergy onset.

Commensal bacteria are critical in inducing oral tolerance and maintaining gut homeostasis, as described in murine models.⁴² This suggests that the use of probiotics or prebiotics, or both, may reduce the incidence of allergic diseases in children,^{106,107} but currently the evidence is insufficient for recommending their use during pregnancy or infancy for this purpose.^{9,108} Another aspect of commensal bacteria is their destruction of symbiotic condition by the excess use of antibiotic has been suggested in association with allergic diseases in children.^{109,110} Antibiotic use during early life—especially multiple antibiotic uses—increases the risk of allergic diseases, including food allergy, in children,^{109,110} possibly because of the pathological disturbance of the induction and maintenance of healthy commensal bacteria leading to the reduction of Treg cells which result in the enhanced allergic sensitization.⁴⁵ This evidence suggests that appropriate use of antibiotics is desirable for preventing allergic diseases in children. Verification by further studies in humans will influence the guidelines for allergy prevention.

Conclusions

The gut mucosal immune system maintains homeostasis through an ingenious network including such factors as GALT, immune cells of innate and acquired types, chemokines, and cytokines. External environmental factors such as commensal bacteria, vitamin A and metabolites participate in the construction of this unique gut mucosal homeostatic network. Disruption or deficiency of the components of the gut mucosal immune and homeostatic network lead to the impairment of oral tolerance, SIgA production, and mucosal barrier formation, eventually promoting allergen sensitization and allergic inflammation. Elucidation of the mechanisms creating and disrupting the intestinal mucosal immune and homeostatic network is key to the development of preventive and therapeutic approaches to allergy. Further studies exploring these mechanisms are critical.

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Conflict of interest

The authors have no conflict of interest to declare.

References

- Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003;**361**: 512–9.
- Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *Plos Biol* 2007;**5**:e177.
- Kiyono H, Fukuyama S. NALT- versus Peyer's-patch-mediated mucosal immunity. *Nat Rev Immunol* 2004;**4**:699–710.
- McGhee JR, Mestecky J, Dertzbaugh MT, Eldridge JH, Hirasawa M, Kiyono H. The mucosal immune system: from fundamental concepts to vaccine development. *Vaccine* 1992;**10**:75–88.
- McGhee JR, Kunisawa J, Kiyono H. Gut lymphocyte migration: we are halfway 'home'. *Trends Immunol* 2007;**28**:150–3.
- Kurashima Y, Kiyono H. Mucosal ecological network of epithelium and immune cells for gut homeostasis and tissue healing. *Annu Rev Immunol* 2017;**35**:119–47.
- Tokuhara D. Challenges in developing mucosal vaccines and antibodies against infectious diarrhea in children. *Pediatr Int* 2018;**60**:214–23.
- Goto Y, Uematsu S, Kiyono H. Epithelial glycosylation in gut homeostasis and inflammation. *Nat Immunol* 2016;**17**:1244–51.
- Ebisawa M, Ito K, Fujisawa T, Committee for Japanese Pediatric Guideline for Food Allergy. The Japanese Society of Pediatric Allergy and Clinical Immunology: The Japanese Society of Allergy. Japanese guidelines for food allergy 2017. *Allergol Int* 2017;**66**:248–64.
- Koplin JJ, Mills EN, Allen KJ. Epidemiology of food allergy and food-induced anaphylaxis: is there really a Western world epidemic? *Curr Opin Allergy Clin Immunol* 2015;**15**:409–16.
- Schwartz RH. A cell culture model for T lymphocyte clonal anergy. *Science* 1990;**248**:1349–56.
- Rocha B, von Boehmer H. Peripheral selection of the T cell repertoire. *Science* 1991;**251**:1225–8.
- Takahashi I, Nakagawa I, Kiyono H, McGhee JR, Clements JD, Hamada S. Mucosal T cells induce systemic anergy for oral tolerance. *Biochem Biophys Res Commun* 1995;**206**:414–20.
- Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3⁺ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity* 2008;**29**:114–26.
- Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 2012;**482**:395–9.
- Kim M, Galan C, Hill AA, Wu WJ, Fehlner-Peach H, Song HW, et al. Critical role for the microbiota in CX3CR1⁺ intestinal mononuclear phagocyte regulation of intestinal T cell responses. *Immunity* 2018;**49**:151–63.
- Antoni L, Nuding S, Wehkamp J, Stange EF. Intestinal barrier in inflammatory bowel disease. *World J Gastroenterol* 2014;**20**:1165–79.
- Cornes JS. Number, size, and distribution of Peyer's patches in the human small intestine. *Gut* 1965;**6**:225–9.
- Spencer JO, Macdonald TT, Fin T, Isaacson PG. The development of gut associated lymphoid tissue in the terminal ileum of fetal human intestine. *Clin Exp Immunol* 1986;**64**:536–43.
- Tokuhara D, Nochi T, Matsumura A, Mejima M, Takahashi Y, Kurokawa S, et al. Specific expression of apolipoprotein A-IV in the follicle-associated epithelium of the small intestine. *Dig Dis Sci* 2014;**59**:2682–92.
- Hamada H, Hiroi T, Nishiyama Y, Takahashi H, Masunaga Y, Hachimura S, et al. Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J Immunol* 2002;**168**:57–64.
- Rennett PD, Browning JL, Mebius R, Mackay F, Hochman PS. Surface lymphotoxin α/β complex is required for the development of peripheral lymphoid organs. *J Exp Med* 1996;**184**:1999–2006.
- Eugster HP, Muller M, Karrer U, Car BD, Schnyder B, Eng VM, et al. Multiple immune abnormalities in tumor necrosis factor and lymphotoxin- α double-deficient mice. *Int Immunol* 1996;**8**:23–36.

24. Spahn TW, Fontana A, Faria AM, Slavin AJ, Eugster HP, Zhang X, et al. Induction of oral tolerance to cellular immune responses in the absence of Peyer's patches. *Eur J Immunol* 2001;**31**:1278–87.
25. Fujihashi K, Dohi T, Rennert PD, Yamamoto M, Koga T, Kiyono H, et al. Peyer's patches are required for oral tolerance to proteins. *Proc Natl Acad Sci U S A* 2001;**98**:3310–5.
26. Takayama N, Igarashi O, Kweon MN, Kiyono H. Regulatory role of Peyer's patches for the inhibition of OVA-induced allergic diarrhea. *Clin Immunol* 2007;**123**:199–208.
27. van de Pavert SA, Ferreira M, Domingues RG, Ribeiro H, Molenaar R, Moreira-Santos L, et al. Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity. *Nature* 2014;**508**:123–7.
28. Honda K, Nakano H, Yoshida H, Nishikawa S, Rennert P, Ikuta K, et al. Molecular basis for hematopoietic/mesenchymal interaction during initiation of Peyer's patch organogenesis. *J Exp Med* 2001;**193**:621–30.
29. Eberl G, Marmion S, Sunshine MJ, Rennert PD, Choi Y, Littman DR. An essential function for the nuclear receptor ROR γ t in the generation of fetal lymphoid tissue inducer cells. *Nat Immunol* 2004;**5**:64–73.
30. Boos MD, Yokota Y, Eberl G, Kee BL. Mature natural killer cell and lymphoid tissue-inducing cell development requires Id2-mediated suppression of E protein activity. *J Exp Med* 2007;**204**:1119–30.
31. Vondenhoff MF, Greuter M, Goverse G, Elewaut D, Dewint P, Ware CF, et al. LT β signaling induces cytokine expression and up-regulates lymphangiogenic factors in lymph node anlagen. *J Immunol* 2009;**182**:5439–45.
32. van de Pavert SA, Olivier BJ, Goverse G, Vondenhoff MF, Greuter M, Beke P, et al. Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. *Nat Immunol* 2009;**10**:1193–9.
33. Sommer F, Bäckhed F. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol* 2013;**11**:227–38.
34. Pollard M, Sharon N. Responses of the peyer's patches in germ-free mice to antigenic stimulation. *Infect Immun* 1970;**2**:96–100.
35. Yamanaka T, Helgeland L, Farstad IN, Fukushima H, Midtvedt T, Brandtzaeg P. Microbial colonization drives lymphocyte accumulation and differentiation in the follicle-associated epithelium of Peyer's patches. *J Immunol* 2003;**170**:816–22.
36. Petersson J, Schreiber O, Hansson GC, Gendler SJ, Velcich A, Lundberg JO, et al. Importance and regulation of the colonic mucus barrier in a mouse model of colitis. *Am J Physiol Gastrointest Liver Physiol* 2011;**300**:G327–33.
37. Kiyono H, McGhee JR, Michalek SM. Lipopolysaccharide regulation of the immune response: comparison of responses to LPS in germfree, *Escherichia coli*-monoassociated and conventional mice. *J Immunol* 1980;**124**:36–41.
38. Kunisawa J, Nochi T, Kiyono H. Immunological commonalities and distinctions between airway and digestive immunity. *Trends Immunol* 2008;**29**:505–13.
39. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009;**9**:799–809.
40. Goto Y, Obata T, Kunisawa J, Sato S, Ivanov II, Lamichane A, et al. Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science* 2014;**345**:1254009.
41. Lee J, Park EJ, Yuki Y, Ahmad S, Mizuguchi K, Ishii KJ, et al. Profiles of microRNA networks in intestinal epithelial cells in a mouse model of colitis. *Sci Rep* 2015;**5**:18174.
42. Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr Gastroenterol Rep* 2010;**12**:319–30.
43. Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ. Secretion of microbicidal α -defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol* 2000;**1**:113–8.
44. Ward PP, Conneely OM. Lactoferrin: role in iron homeostasis and host defense against microbial infection. *Biomolecules* 2004;**17**:203–8.
45. Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A* 2014;**111**:13145–50.
46. Tokuwara D, Yuki Y, Nochi T, Kodama T, Mejima M, Kurokawa S, et al. Secretory IgA-mediated protection against *V. cholerae* and heat-labile enterotoxin-producing enterotoxigenic *Escherichia coli* by rice-based vaccine. *Proc Natl Acad Sci U S A* 2010;**107**:8794–9.
47. Boullier S, Tanguy M, Kadaoui KA, Caubet C, Sansonetti P, Corthésy B, et al. Secretory IgA-mediated neutralization of *Shigella flexneri* prevents intestinal tissue destruction by down-regulating inflammatory circuits. *J Immunol* 2009;**183**:5879–85.
48. Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, et al. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proc Natl Acad Sci U S A* 2004;**101**:1981–6.
49. Mathias A, Pais B, Favre L, Benyacoub J, Corthésy B. Role of secretory IgA in the mucosal sensing of commensal bacteria. *Gut Microbes* 2014;**5**:688–95.
50. Fagerås M, Tomicic S, Voor T, Björkstén B, Jenmalm MC. Slow salivary secretory IgA maturation may relate to low microbial pressure and allergic symptoms in sensitized children. *Pediatr Res* 2011;**70**:572–7.
51. Kukkonen K, Kuitunen M, Haahela T, Korpela R, Poussa T, Savilahti E. High intestinal IgA associates with reduced risk of IgE-associated allergic diseases. *Pediatr Allergy Immunol* 2010;**21**:67–73.
52. Sandin A, Björkstén B, Böttcher MF, Englund E, Jenmalm MC, Braback L. High salivary secretory IgA antibody levels are associated with less late-onset wheezing in IgE sensitized infants. *Pediatr Allergy Immunol* 2011;**22**:477–81.
53. Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. *J Allergy Clin Immunol* 2004;**114**:377–82.
54. Mantis NJ, Rol N, Corthésy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol* 2011;**4**:603–11.
55. 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.
56. Round JL, Mazmanian SK. Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A* 2010;**107**:12204–9.
57. Dzidic M, Abrahamsson TR, Artacho A, Björkstén B, Collado MC, Mira A, et al. Aberrant IgA responses to the gut microbiota during infancy precede asthma and allergy development. *J Allergy Clin Immunol* 2017;**139**:1017–25.
58. 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.
59. Nochi T, Takagi H, Yuki Y, Yang L, Masumura T, Mejima M, et al. Rice-based mucosal vaccine as a global strategy for cold-chain- and needle-free vaccination. *Proc Natl Acad Sci U S A* 2007;**104**:10986–91.
60. Hase K, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M, et al. Uptake through glycoprotein 2 of FimH⁺ bacteria by M cells initiates mucosal immune response. *Nature* 2009;**462**:226–30.
61. Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 1999;**190**:229–39.
62. Sato A, Hashiguchi M, Toda E, Iwasaki A, Hachimura S, Kaminogawa S. CD11b⁺ Peyer's patch dendritic cells secrete IL-6 and induce IgA secretion from naive B cells. *J Immunol* 2003;**171**:3684–90.
63. Goodrich ME, McGee DW. Regulation of mucosal B cell immunoglobulin secretion by intestinal epithelial cell-derived cytokines. *Cytokine* 1998;**10**:948–55.
64. Asano T, Kaneko H, Terada T, Kasahara Y, Fukao T, Kasahara K, et al. Molecular analysis of B-cell differentiation in selective or partial IgA deficiency. *Clin Exp Immunol* 2004;**136**:284–90.
65. Beagley KW, Eldridge JH, Kiyono H, Everson MP, Koopman WJ, Honjo T, et al. Recombinant murine IL-5 induces high rate IgA synthesis in cycling IgA-positive Peyer's patch B cells. *J Immunol* 1988;**141**:2035–42.
66. Beagley KW, Eldridge JH, Lee F, Kiyono H, Everson MP, Koopman WJ, et al. Interleukins and IgA synthesis. Human and murine interleukin 6 induce high rate IgA secretion in IgA-committed B cells. *J Exp Med* 1989;**169**:2133–48.
67. Mora JR, Iwata M, Eksteen B, Song SY, Junt T, Senman B, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 2006;**314**:1157–60.
68. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, et al. α 4 β 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* 1993;**74**:185–95.
69. Wurbel MA, Philippe JM, Nguyen C, Victorero G, Freeman T, Wooding P, et al. The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9. *Eur J Immunol* 2000;**30**:262–71.
70. Shimada S, Kawaguchi-Miyashita M, Kushi A, Sato T, Nanno M, Sako T, et al. Generation of polymeric immunoglobulin receptor-deficient mouse with marked reduction of secretory IgA. *J Immunol* 1999;**163**:5367–73.
71. Rochereau N, Drocourt D, Perouzel E, Pavot V, Redelingshuys P, Brown GD, et al. Dectin-1 is essential for reverse transcytosis of glycosylated SIgA-antigen complexes by intestinal M cells. *Plos Biol* 2013;**11**:e1001658.
72. Nochi T, Yuki Y, Matsumura A, Mejima M, Terahara K, Kim DY, et al. A novel M cell-specific carbohydrate-targeted mucosal vaccine effectively induces antigen-specific immune responses. *J Exp Med* 2007;**204**:2789–96.
73. Takeyama N, Yuki Y, Tokuwara D, Oroku K, Mejima M, Kurokawa S, et al. Oral rice-based vaccine induces passive and active immunity against enterotoxigenic *E. coli*-mediated diarrhea in pigs. *Vaccine* 2015;**33**:5204–11.
74. Jang MH, Kweon MN, Iwatani K, Yamamoto M, Terahara K, Sasakawa C, et al. Intestinal villous M cells: an antigen entry site in the mucosal epithelium. *Proc Natl Acad Sci U S A* 2004;**101**:6110–5.
75. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001;**2**:361–7.
76. Chieppa M, Rescigno M, Huang AY, Germain RN. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J Exp Med* 2006;**203**:2841–52.
77. Uematsu S, Fujimoto K, Jang MH, Yang BG, Jung YJ, Nishiyama M, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat Immunol* 2008;**9**:769–76.
78. Obata T, Goto Y, Kunisawa J, Sato S, Sakamoto M, Setoyama H, et al. Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *Proc Natl Acad Sci U S A* 2010;**107**:7419–24.
79. 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.
80. 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.
81. Takagi H, Hiroi T, Yang L, Tada Y, Yuki Y, Takamura K, et al. A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for

- inhibition of Th2-mediated IgE responses. *Proc Natl Acad Sci U S A* 2005;**102**: 17525–30.
82. Mazzini E, Massimiliano L, Penna G, Rescigno M. Oral tolerance can be established via gap junction transfer of fed antigens from CX3CR1⁺ macrophages to CD103⁺ dendritic cells. *Immunity* 2014;**40**:248–61.
 83. Jang MH, Sougawa N, Tanaka T, Hirata T, Hiroi T, Tohya K, et al. CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes. *J Immunol* 2006;**176**:803–10.
 84. Matteoli G, Mazzini E, Iliev ID, Mileti E, Fallarino F, Puccetti P, et al. Gut CD103⁺ dendritic cells express indoleamine 2,3 dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction. *Gut* 2010;**59**: 595–604.
 85. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, et al. Intestinal tolerance requires gut homing and expansion of FoxP3⁺ regulatory T cells in the lamina propria. *Immunity* 2011;**34**:237–46.
 86. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 2006;**203**:519–27.
 87. Knoop KA, McDonald KG, McCrate S, McDole JR, Newberry RD. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. *Mucosal Immunol* 2015;**8**:198–210.
 89. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 2004;**21**:527–38.
 90. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3⁺ T reg cells via retinoic acid. *J Exp Med* 2007;**204**:1775–85.
 91. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103⁺ DCs induces Foxp3⁺ regulatory T cells via a TGF- β and retinoic acid-dependent mechanism. *J Exp Med* 2007;**204**:1757–64.
 92. Belladonna ML, Volpi C, Bianchi R, Vacca C, Orabona C, Pallotta MT, et al. Cutting edge: autocrine TGF- β sustains default tolerogenesis by Idocompetent dendritic cells. *J Immunol* 2008;**181**:5194–8.
 93. Battaglia M, Gianfrani C, Gregori S, Roncarolo MG. IL-10-producing T regulatory type 1 cells and oral tolerance. *Ann N Y Acad Sci* 2004;**1029**: 142–53.
 94. Kunisawa J, Hashimoto E, Ishikawa I, Kiyono H. A pivotal role of vitamin B9 in the maintenance of regulatory T cells *in vitro* and *in vivo*. *PLoS One* 2012;**7**: e32094.
 95. Suzuki H, Sekine S, Kataoka K, Pascual DW, Maddaloni M, Kobayashi R, et al. Ovalbumin-protein sigma 1 M-cell targeting facilitates oral tolerance with reduction of antigen-specific CD4⁺ T cells. *Gastroenterology* 2008;**135**: 917–25.
 96. Kim KS, Hong SW, Han D, Yi J, Jung J, Yang BG, et al. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* 2016;**351**:858–63.
 97. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat Immunol* 2003;**4**:330–6.
 98. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med* 2012;**18**:538–46.
 99. Kweon MN, Yamamoto M, Kajiki M, Takahashi I, Kiyono H. Systemically derived large intestinal CD4⁺ Th2 cells play a central role in STAT6-mediated allergic diarrhea. *J Clin Invest* 2000;**106**:199–206.
 100. Kurashima Y, Kunisawa J, Higuchi M, Gohda M, Ishikawa I, Takayama N, et al. Sphingosine 1-phosphate-mediated trafficking of pathogenic Th2 and mast cells for the control of food allergy. *J Immunol* 2007;**179**:1577–85.
 101. Kurashima Y, Amiya T, Nochi T, Fujisawa K, Haraguchi T, Iba H, et al. Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoceptors. *Nat Commun* 2012;**3**:1034.
 102. Tsai SH, Kinoshita M, Kusu T, Kayama H, Okumura R, Ikeda K, et al. The ectoenzyme E-NPP3 negatively regulates ATP-dependent chronic allergic responses by basophils and mast cells. *Immunity* 2015;**42**:279–93.
 103. Hino A, Fukuyama S, Kataoka K, Kweon MN, Fujihashi K, Kiyono H. Nasal IL-12p70 DNA prevents and treats intestinal allergic diarrhea. *J Immunol* 2005;**174**:7423–32.
 104. Kunisawa J, Arita M, Hayasaka T, Harada T, Iwamoto R, Nagasawa R, et al. Dietary ω 3 fatty acid exerts anti-allergic effect through the conversion to 17,18-epoxyeicosatetraenoic acid in the gut. *Sci Rep* 2015;**5**:9750.
 105. Milner JD, Stein DM, McCarter R, Moon RY. Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma. *Pediatrics* 2004;**114**:27–32.
 106. Kuitunen M, Kukkunen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009;**123**:335–41.
 107. Kukkunen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, et al. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007;**119**:192–8.
 108. Muraro A, Halken S, Arshad SH, Beyer K, Dubois AE, Du Toit G, et al. EAACI Food Allergy and Anaphylaxis Guidelines Group. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy* 2014;**69**: 590–601.
 109. Hirsch AG, Pollak J, Glass TA, Poulsen MN, Bailey-Davis L, Mowery J, et al. Early-life antibiotic use and subsequent diagnosis of food allergy and allergic diseases. *Clin Exp Allergy* 2017;**47**:236–44.
 110. Love BL, Mann JR, Hardin JW, Lu ZK, Cox C, Amrol DJ. Antibiotic prescription and food allergy in young children. *Allergy Asthma Clin Immunol* 2016;**12**:41.