REVIEW ARTICLE

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Beyond Hygiene: Commensal Microbiota and Allergic Diseases

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Complex communities of microorganisms, termed commensal microbiota, inhabit mucosal surfaces and profoundly influence host physiology as well as occurrence of allergic diseases. Perturbing factors such as the mode of delivery, dietary fibers and antibiotics can influence allergic diseases by altering commensal microbiota in affected tissues as well as in intestine. Here, we review current findings on the relationship between commensal microbiota and allergic diseases, and discuss the underlying mechanisms that contribute to the regulation of allergic responses by commensal microbiota.

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INTRODUCTION

Metazoans have co-evolved with microorganisms by establishing a trans-kingdom symbiotic relationship that influences many physiological functions of the host, including nutrient absorption, resistance to pathogenic infection, immune defense, and tissue repair (1). Microbial inhabitants, collectively called as commensal microbiota (See Box 1) (2), are estimated to outnumber our host cells (3) and colonize nearly all the mucosal surfaces including respiratory tracts, skin, vagina and gastrointestinal tracts (4).

Although the importance of commensal microbiota in the development of host immune system had long been predicted (5) and germ-free (GF) mice, an experimental model critical for studying host-microbiota interaction, had been developed in 1959 (6), the details of how commensal microbiota influences many of the host's physiological functions is beginning to be realized only

Box 1. Classification of bacterial inhabitants

Commensalism refers to functional relationships between two organisms where one organism benefits and the other remains unaffected. This is in contrast to mutualism where each organism benefits or parasitism where one benefits and the other is harmed. As many species of commensal microbiota turned out to be actually mutualistic and some commensal microbes can be pathogenic in certain circumstances influenced by genetic and environmental factors or affected by host immune status (pathobionts), the better terms to describe "commensal" microbiota and its relationship with host are "symbiotic" microbiota and symbiosis, respectively.

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Abbreviations: GF, germ-free; Ig, Immunoglobulin; TH, T helper; PAF, platelet-activating factor; AD, Atopic dermatitis; SCFAs, Short-chain fatty acids; CRS, Chronic rhinosinusitis; Treg cells, Regulatory CD4 T cells; CMA, Cow's milk allergy; IECs, Intestinal epithelial cells; pTreg, peripheral Treg; ROR, RAR-related orphan Receptor; ILCs, Innate lymphoid cells; SPF, Specific pathogen-free; DCs, Dendritic cells; MNP, Mononuclear phagocytic; tTreg, thymus-derived Treg; HDM, House dust mite

in recent years. In addition to their role in nutrient absorption and development of the immune system in the mucosal sites, emerging evidence strongly suggests that commensal microbiota affects metabolic disorders such as obesity and diabetes, behavioral or neurological disorders like autism, Alzheimer diseases, and immune disorders such as autoimmune/lymphoproliferative diseases and allergy (7).

The term 'allergy' is derived from Greek words; allos (other) and ergon (reaction), and means different reaction to antigens. Currently, the definition of allergy is immunological hypersensitivity to benign antigens that do not typically induce any immune responses (8). Studies of allergic patients and animal models showed that many types of allergic diseases are mediated by allergen-specific immunoglobulin E (IgE) and characterized by allergic sensitization and hypersensitivity reactions upon re-exposure. During sensitization, allergens can induce T helper (T_H) 2 cells and subsequent production of allergen-specific IgE. Upon the re-exposure of allergen, allergen-bound IgEs result in degranulation of mast cells and basophils to release many mediators, such as histamine and platelet-activating factor (PAF), that induce symptoms of allergic diseases (9). The prevalence of allergic diseases is relatively low in developing or undeveloped country and allergic patients tend to have low T_H1, but elevated T_H2 immune response (10). Hence, 'hygiene hypothesis' has been introduced to propose that the imbalance towards T_H2 responses, due to reduced infectious burdens and subsequent reduction of T_H1 responses, causes allergic diseases (11).

However, many allergic diseases occur in the mucosal tissues harboring numerous species of the commensal microbiota and are closely associated with the compositional changes of the commensal microbiota at affected mucosal tissues or in the intestine, suggesting that commensal microbiota plays an important role in the pathogenesis of allergic diseases. Accordingly, alteration of commensal microbiota by genetic and environmental factors such as the mode of delivery (natural vs. surgical), dietary intervention during ontogeny (breast- vs. formula-feeding), westernized diet (high in fat and low in fiber), antibiotics and indoor environment, are all associated with the incidence of allergic diseases (12-15). In this review, we summarize current understanding of the relationship between commensal microbiota and allergy, and revise 'hygiene hypothesis' by incorporating commensal microbiota as a pivotal contributing factor for allergic diseases.

SKIN AND AIRWAY ALLERGIC DISEASES AND DYSBIOSIS OF MICROBIOTA

With recent advances in next generation sequencing technologies, metagenomic analyses including a milestone study by the Human Microbiome Project Consortium have revealed that distinct communities of commensal microbiota exist at different mucosal tissues or even at different sites of same tissue depending on the environmental features (4,16) (Fig. 1A). Furthermore, allergic diseases are associated with microbial dysbiosis that

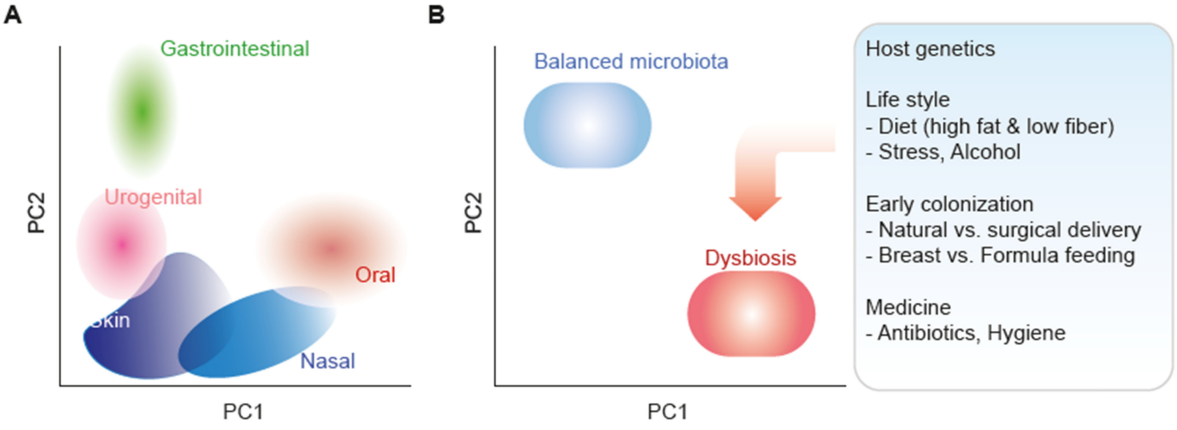

Figure 1. Differences between microbial communities based on anatomic sites and factors influencing dysbiosis of commensal microbiota. (A) Each mucosal site harbors distinct microbial community as indicated by principal coordinates plot (reproduced from (4)). Microbial populations within skin are more variable than those at other mucosal sites. Inhabitants within nasal cavity resembles those in skin. Lung microbiota was reported to be similar with those in oral cavity. (B) Several factors can cause perturbations of commensal microbiota, leading to the states of microbial dysbiosis, which is associated with allergic diseases.

is characterized by changes in microbial composition, distribution of individual species and metabolic alterations relative to normal microbiota in healthy individuals. Increasing evidence suggests that allergic diseases in skin and airways, such as atopic dermatitis and asthma, are not only associated with dysbiosis of commensal microbiota at affected mucosal tissues but also with dysbiosis of intestinal microbiota (Fig. 1B).

Atopic dermatitis

Skin, the largest organ in our body, is constantly exposed to microorganisms from external environment. Despite the presence of both physical and chemical barrier functions such as thick keratinized epithelial cell layers and antimicrobial molecules, respectively, skin can harbor specific commensal microbes beneficial for protection from opportunistic pathogen infections. In normal skin, Firmicutes, Actinobacteria and Proteobacteria are the predominant phyla relative to other mucosal tissues; e.g., Bacteriodetes predominate in the gut, but is a minor component of skin microbiota (17). In addition, different communities of microbes can preferentially colonize different topographic sites of the skin characterized by distinct environmental specifications like hair, moisture and sebum (18).

Atoptic dermatitis (AD), or atopic eczema, is a chronic allergic skin disease with a typical symptom of pruritic rash. The disease often develops in infants prior to the 1 year age, affecting approximately 20% of children (19). The role of skin microbiota in AD is better understood than other allergic diseases, as *Staphylococcus aureus* has been found to be closely associated with AD. *S. aureus* is a part of normal microbiota as a pathobiont in nasal passages: ~20% of individuals are estimated to be long-term carriers and ~60% are intermittent carriers (20). Metagenomic analyses of skin microbiota in AD patients have revealed that *S. aureus* become predominant in about 90% of AD patients, resulting in a dramatic decrease of skin microbiota diversity (21). It was shown that *Staphylococcal* products such as *Staphylococcal* α-hemolysin (22) and extracellular vesicles form *S. aureus* can modulate skin barrier functions and induce atopic dermatitis-like skin inflammation in animal models (22-27). *Staphylococcal* enterotoxins, acting as super-antigens, can induce allergic skin inflammation by inducing strong T cell activation (28). *Staphlylococcal* δ-toxin can also induce allergic skin disease by activating mast cells (29).

These studies suggest that dysbiosis caused by predominant colonization of *S. aureus* are important exacerbating factor for the pathogenesis of AD. However, it is doubtful whether *S. aureus* is the initiating factor of atopic dermatitis. A recent study reported that infant AD patients, unlike adult patients, do not show increased abundance of *S. aureus* in affected skin lesions. However, commensal *Staphylococcus* species other than *S. aureus* were observed to be less predominant (30). It is interesting that colonization of *S. epidermidis*, the most frequently isolated species in human epithelial cell layers, can impair establishment of *S. aureus* by producing antimicrobial molecules (31).

Dysbiosis in intestinal microbiota has also been suggested as an important contributing factor for AD pathogenesis. Probiotic treatment, for example, has a positive effect in ameliorating AD (32). Furthermore, several prospective (or longitudinal) cohort studies showed that reduced diversity of intestinal microbiota at early life is closely associated with the increased risk of AD (33). Abundance of specific gut microbes at species or subspecies level, including *Clostridium Perfringens*, *Clostridium Difficile* and *Faecalibacterium prausnitzii* subspecies, which have low capacity of producing short-chain fatty acids (SCFAs), was known to be associated with AD. *Lactobacillus paracasei* on the other hand appears to have a beneficial role, since its abundance was associated with lower susceptibility to AD (34-36).

Allergic rhinitis

Respiratory tract is divided into upper airway and lower airway. Upper airway includes nasal and oropharynx area, and lower airway includes trachea, bronchi, bronchioles and lungs. In human nasal cavity, Firmicutes and Actinobacteria are the major phyla, while the major phyla in oropharynx are Proteobacteria, Firmicutes and Bacteriodetes (37-39). It is interesting that microbiota on the nasal cavity resembles microbiota on skin while microbiota on oropharynx resembles microbiota in oral cavity. In the lung, which is often mistakenly considered as a sterile site, Bacteriodetes, Firmicutes and Proteobacteria phyla are identified as the major phyla (40-42). Lung microbiota is similar in composition to those in oropharynx despite of reduced biomass (43). It is considered that oral microbiota are the primary source of lung microbiota.

Human studies demonstrate that microbiota in the upper airway may differ in composition among individuals depending on the type and status of the disease. Allergic rhinitis is a representative disease occurring in the upper airway. Interestingly, it was observed that unlike other allergic diseases, microbial diversity in seasonal allergic

rhinitis (hay fever) is not reduced, but rather increased during allergy season (44). As in AD, *S. aureus* is one of the candidate microbes associated with perennial (non-seasonal) allergic rhinitis (45). Chronic rhinosinusitis (CRS) was also associated with altered microbiota in the nasal cavity with an increased abundance of *Staphylococcus*, *Haemophilus*, and *Moraxella* (46-49). As *S. aureus* is observed commonly in both allergic rhinitis and AD, it is possible that the underlying mechanisms by which *S. aureus* contribute to the occurrence of diseases in upper airway might be similar to those contributing to the pathogenesis of AD.

Asthma

Asthma is a chronic allergic disorder in airways leading to variable and recurring airflow obstructions. Most asthma begins in childhood with sensitization to aeroallergens such as house dust mites, cockroaches, animal dander, fungi and pollens (50). Alteration of microbial composition in both upper and lower airway was also observed in asthma patients. Although microbial compositions are different between upper and lower airway, there appears to be distinctive outgrowth of Proteobacteria including *Haemophilus*, *Moraxella* and *Neisseria* spp. within both sites of asthma patients (41,51). Prevalence of Proteobacteria was found in mild patients without regular inhaled corticosteroid therapy as well as in severe asthma patients. On the other hand, *Klebsiella* species of Proteobacteria phylum were enriched with increased severity of the disease (52). It was previously reported that there were differences in lung microbiota between corticosteroids sensitive and resistant group, suggesting that heterogeneity of asthma can affect lung microbiota as well (53,54).

Animal models for studying the role of airway microbiota in allergic airway diseases are limited especially for allergic rhinitis or CRS, but several studies by using asthma animal models revealed that the establishment of lung microbiota at early life is important for the prevention of asthma. Increases in bacterial load in the lungs and shifts from a predominance of Gammaproteobacteria and Firmicutes towards Bacteriodetes during ontogeny are important for tolerance against allergens exposed in airway (55). Furthermore, early microbial colonization is required in order to control the invariant natural killer T cells in lung, which are capable of producing IL-4 and IL-13 and contribute to T_H2-mediated asthma (56).

In comparison with allergic rhinitis, the relationship between intestinal microbiota, especially at early life, and asthma was relatively well defined. A recent prospective study showed that infants with lower abundance of *Bifidobacterium*, *Akkermansia* and *Faecalibacterium* incurred a higher relative risk of asthma by promoting adaptive immune dysfunctions, featured by increased proportion of IL-4^+ T_H2 cells and reduced percentage of $CD25^+$ $Foxp3^+$ Regulatory $CD4^+$ T (Treg) cells (57). Another study also showed that a higher risk of asthma is associated with the lower abundance of genera such as *Faecalibacterium*, *Lachnospira*, *Veillonella* and *Rothia* in infants despite of similar abundance of dominant *Bifidobacterium* (58). Furthermore, supplementing with microbes abundant in healthy controls into GF mice previously colonized with fecal microbiota from infants of high risk for asthma reduced severity of allergic airway inflammation (58). It was also reported that abundance of *Bifidobacterium* is decreased in adult asthma patients (59). It seems that *Faecalibacterium*, especially subspecies producing SCFAs, and *Bifidobacterium* provides beneficial effect for preventing asthma.

In line with human studies, animal model deficient of intestinal microbiota, like GF mice (60) or mice with altered intestinal microbiota by feeding diet containing less dietary fiber contents (61,62), could exacerbate allergic airway inflammation.

FOOD ALLERGY AND DYSBIOSIS OF INTESTINAL MICROBIOTA

Food allergies, hypersensitivity responses to dietary antigens, have been increased, especially in developed or westernized countries over recent decades (63) and have become major health issues. While delayed type non-IgE mediated food allergy contributes to some of the cases, the most common and well defined is IgE-mediated food allergy against antigens from milk, egg, peanut, soybean, wheat, tree nut, fish and shellfish. The etiology of increased prevalence of food allergy is largely unknown but data from human studies showed that changes in microbiota at early life due to mode of delivery (natural vs. surgical) or exposure to antibiotics even at low dose can contribute to the increased incidence of food allergy (Fig. 1) (64-66).

However, compared to the studies on the relationship between intestinal microbiota and extra-intestinal allergic diseases, human studies on gut microbiota and food allergy are limited. A few studies on cow's milk allergy (CMA) infants showed that patients have higher total bacteria and anaerobic bacteria (67) and also have higher proportion of butyrate-producing *Clostridium*

spp. than healthy control (68). A recent study based on metagenomic analysis of 16S rRNA sequences reported that CMA infants had more diverse microbiota than control group and had a significant reduction in *Bifidobacterium* whereas butyrate-producing microbes such as *Ruminococcus* and *Faecalibacterium* were enriched (69). Such butyrate-producing microbes might be beneficial to achieve tolerance to food allergens or ameliorate the symptoms of food allergy, since outgrowing infants from CMA by the treatment of hydrolyzed casein formula supplemented with *Lactobacillus rhamnosus* GG had higher fecal butyrate concentrations than allergic infants.

Despite the ill-defined relationships between the intestinal microbiota and food allergy in human studies, experimental IgE-mediated food allergy animal models could help to elucidate the role of intestinal microbiota on the pathogenesis of food allergy and reveal several mechanistic insights.

Induction of intestinal peripheral Treg cells

Colonization of GF mice with *Clostridium* species can induce *de novo* differentiation of Treg cells in the laminar propria (LP) of colon but not in the small intestine. Such colonic peripheral Treg (pTreg) cells were proposed to suppress allergen-specific IgE responses and subsequently prevent allergen-induced diarrhea (70,71). *Clostridium* species can contribute to the generation of colonic pTreg cells by inducing transforming growth factor-β (TGF-β) from intestinal epithelial cells (IECs) (70) or dendritic cells (72).

More importantly, *Clostridium* species can induce the production of SCFAs such as acetate, butyrate and propionate by degrading dietary fibers such as starch and cellulose (73). SCFAs produced by intestinal microbes can enhance the generation of colonic pTreg cells and stabilize post-translational acetylation of Foxp3 through the inhibition of histone deacetylase by butyrate, resulting in increased stability and suppressive functions of Treg cells (74,75). Although it is unclear whether *Clostridium*-induced pTregs in the colon directly prevent allergic responses to dietary antigens, a subset of pTreg cells induced by intestinal microbiota is also present in the small intestine. These pTreg cells express RAR-related orphan Receptor (ROR)γt and are known to inhibit mucosal T_H2 cell responses (76), which are important for the initiation of IgE-mediated food allergy by promoting B cell differentiation into IgE-producing plasma cells (Fig. 2) (77).

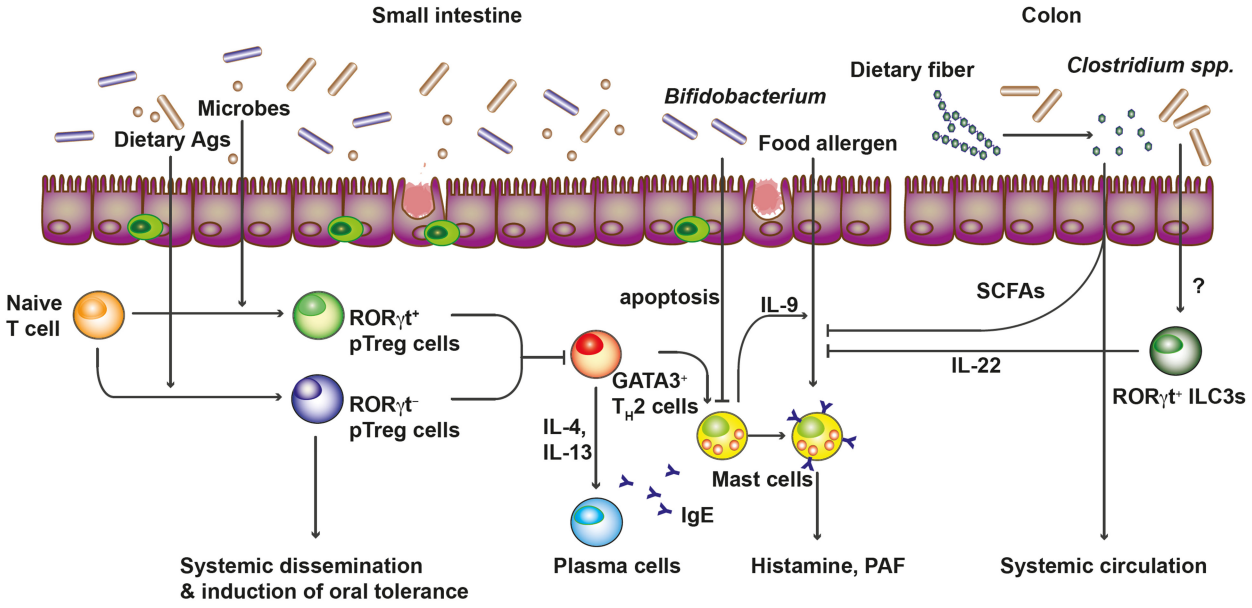

Figure 2. Interplay between intestinal microbiota and immune cells in the context of IgE-mediated food allergy. pTreg cells are abundant in the small intestine. Dietary antigens- or intestinal microbes-induced pTreg cells, distinguished from each other by RORγt expression, can suppress mucosal TH2 cells generated against food allergens. TH2 cells can induce IgE-producing plasma cells and increase mucosal mast cells expressing IL-9 or mediating food allergic symptoms through the degranulation of histamine, PAF and so on. IL-13 or IL-9 can increase intestinal permeability to food allergens, while SCFAs or IL-22 induced by intestinal microbiota in colon can promote intestinal barrier functions. Certain species of intestinal microbiota such as Bifidobacterium can induce apoptosis of mast cells, thus preventing food allergy.

Regulation of intestinal permeability to food allergens

In addition to the capacity of intestinal microbiota to promote intestinal pTreg cells, *Clostridium* species can lead to the production of IL-22 from RORγt$^+$ type 3 innate lymphoid cells (ILC3s) and CD4$^+$ T cells in colonic LP. IL-22 not only ensures protection of microbial translocation from the lumen to peripheral organs by mediating production of anti-microbial peptides and mucus from IECs (78) but also regulates epithelial layer permeability to food allergen (Fig. 2) (79). Hence, colonization of GF or antibiotics-treated mice with *Clostridium* spp. can inhibit oral sensitization of allergen and allergen-induced IgE responses, thereby preventing food allergy.

Interestingly, SCFAs can increase epithelial barrier functions, measured by fluorescein isothiocyanate (FITC)-dextran permeability assay, in a GPR43-dependent manner (80) or through the stabilization of HIF-α particularly by butyrate (81). Therefore, it is also possible that SCFAs can promote barrier functions of the intestine (Fig. 2).

Modulation of hypersensitivity reactions during effector phase

Degranulation of mast cells by allergen-mediated cross-linking of IgE bound to FcεR is necessary to provoke hypersensitivity reactions in IgE-mediated food allergy (77). Thus, as in the case of atopic dermatitis (29), microbial modulation of mast cell activation has been shown to regulate IgE-mediated food allergy as well. Treatment of Prebiotic galacto- and fructo-oligosaccharides supplemented with *Bifidobacterium breve* can reduce whey-induced food allergy by promoting the production of Galectin-9 from IECs that subsequently neutralize allergen/IgE binding on mast cells (82). A recent study also showed that *Bifidobacterium* species, particularly B. longum isolated from infants, could induce the apoptosis of mast cells in the small intestine and ameliorate food allergen-induced diarrhea (Fig. 2) (83).

Due to the absence of commensal microbiota, GF mice or antibiotics-treated mice are skewed towards T_H2 cells and possess high level of IgG1 and IgE in serum (84). Oral sensitization of peanut allergen with cholera toxin can lead to increased peanut-specific IgE responses in GF mice relative to specific pathogen-free (SPF) mice (79). Underlying mechanisms leading to skewed T_H2 responses and hyper-IgE syndrome in GF mice remain elusive, however, the involvement of intestinal microbiota is evident since colonization of diverse intestinal microbiota specifically at early life has been demonstrated to prevent the generation of IgE responses (85). Role of dietary antigens on the generation of IgE responses in GF mice is still controversial but dietary antigens appear to contribute to hyper-IgE syndrome in GF mice, since antigen-free mice, GF mice raised in a condition devoid of dietary antigens, possess much reduced level of IgE relative to GF mice (unpublished data).

INTESTINAL IMMUNE SYSTEM: KEEPING ALLERGIC RESPONSES UNDER CONTROL

So far, we discussed evidence suggesting that intestinal microbial dysbiosis is tightly associated with various types of allergic diseases. It is evident therefore that an intricate functional interplay between intestinal immune system and intestinal microbiota is important for shaping the physiological outcome of individual's allergic responses. Several mechanistic issues for keeping allergic responses under control by intestinal microbiota still remains to be addressed but intestinal microbiota-mediated control of allergic diseases seems to be relevant to the capacity of intestinal immune system to generate tolerance, rather than immunity, and promote intestinal barrier functions.

Intestine is the largest lymphoid organ where about 70% of immune cells in our body reside largely due to enormous antigenic burdens, mostly derived from food and intestinal microbiota. Under normal conditions, dietary antigens are rendered less immunogenic through oral tolerance mechanisms (as reviewed in (86)). Oral administration of nominal antigen can induce pTreg cells in mesenteric lymph nodes, which migrate into the small intestine for further expansion and conditioning to exert fully suppressive activity. Such pTreg cells can be dispersed systemically to prevent hypersensitivity responses against cognate antigen at other local tissues.

Typically, the intestinal immune system is skewed to generate tolerance against enteric antigens through multilayered mechanisms. Mucosal antigen-presenting cells, especially CD103$^+$ dendritic cells (DCs) and CX_3CR1^{hi} mononuclear phagocytic (MNP) cells that are populated through small intestinal lamina propria, can integrate intestinal environmental cues to exert tolerogenic functions. Mucosal CD103$^+$ CD11b$^+$ DCs are known to express more anti-inflammatory mediators, TGF-β, IL-10, retinoic acids and indoleamine 2,3-dioxygenase, but less pro-inflammatory cytokines, IL-6, IL-12 and TNF-α relative to their counterparts in secondary lymphoid tissues. These tolerogenic mucosal DCs can sample luminal antigens, migrate into mLNs, where they promote the generation of pTreg cells.

Indeed, pTreg cells characterized by lower expression

of Neuropilin-1 (Nrp-1) relative to thymus-derived Treg (tTreg) cells are abundantly found in the small intestine as well as in colon. Both dietary antigens- and intestinal microbiota-induced pTreg cells (Nrp-1^{lo} RORγt^{-} and Nrp-1^{lo} RORγt^{+}, respectively) are present in the small intestine (76,87). Prodigious mucosal immunity against newly administrated oral antigens could be generated in antigen-free mice depleted of both dietary antigens- and intestinal microbiota-induced pTreg cells, suggesting that mucosal pTreg cells can further condition mucosal environment to be more tolerogenic and promote oral tolerance (87).

Incidence of food allergy as well as other allergic diseases is higher in infants than adults despite of the dominance of beneficial *Bifidobacterium* species (88). Intestinal microbiota in infants are less diverse due to the limited energy source in breast- and formula-milk (89) and vulnerable to exogenous perturbations such as exposure to antibiotics. While it is controversial whether other allergic diseases can be outgrown, most children suffering from food allergy can outgrow their allergies by the unknown mechanisms (90). It is possible that the exposure to diverse dietary antigens and the surge of intestinal microbiota caused by the change of diets can induce pTreg cells in the small intestine that can subsequently promote tolerogenic intestinal environment and facilitate tolerance to food allergens.

Interestingly, intestinal microbiota-induced Nrp-1^{lo} RORγt^{+} pTreg cells in the small intestine can express suppressive mediators such as IL-10 and CTLA4 at higher levels compared to Nrp-1^{hi} tTreg cells or dietary antigens-induced Nrp-1^{lo} RORγt^{-} pTreg cells (unpublished data). Furthermore, individual species of intestinal microbiota differs its capacity to generate RORγt^{+} pTreg cells (91), which can possibly control mucosal T_H2 responses (76). Under normal conditions, small intestinal T_H2 cells are much lower than T_H1 cells expressing Tbet and IFN-γ even in GF condition (87). However, the role of dietary antigens- or intestinal microbiota-induced pTreg cells on the control of intestinal T_H2 cells against food antigens still remains elusive.

Epithelial barrier functions are important for preventing allergic diseases. Increasing evidence suggests that defective intestinal barrier functions, known as "leaky gut syndrome", are implicated in food allergy as well as other allergic diseases (92). Intestinal T_H2 responses are not only important for the induction of food allergy by increasing IgE-producing plasma cells but are also known to influence epithelial barrier functions. During the onset of food allergy, T_H2 cells can promote the generation of IL-9 producing mast cells in the small intestine (93). Cytokines such as IL-13 and IL-9 are known to increase the intestinal permeability while IL-10 can promote barrier integrity of IECs (94). As mentioned before, increase in IL-22 by *Clostridium* species in the colon can also enhance epithelial barrier integrity in the small intestine (79). However, further investigation is required as the production of IL-22 by RORγt^{+} ILC3s in the small intestine of GF mice with increased susceptibility to food allergy, are actually higher than those in SPF mice (95).

As mentioned earlier, *Clostridium* species can promote the production of SCFAs (74), which can circulate via bloodstream and lymph to exert immune modulation within small intestine where sensitization of food allergen occurs or at distal mucosal sites such as the skin and the lung. Therefore, SCFAs-mediated immune regulation can serve as a critical mechanistic link between intestinal microbiota and allergic diseases associated with distal mucosal sites. In accordance to these findings, feeding of high-fiber diet can lead to protection against peanut allergy induced by oral sensitization of peanut with cholera toxin (80) and can ameliorate allergic airway diseases induced by house dust mite (HDM) (61). Interestingly, as SCFAs can exert regulatory effects on multiple cell types including epithelial cells, T cells and DCs, SCFAs-mediated regulatory mechanisms can differ, depending on the kind of SCFAs and type of allergic diseases. Acetate and butyrate, rather than propionate, are important for controlling peanut-induced allergy (80) while propionate are responsible for the protection of HDM-induced allergic airway disease by enhancing lung DCs with impaired capacity to mediate Th2 polarization (61).

It was previously shown that circulatory antigens could be taken up by small intestinal CX_3CR1^{hi} MNP cells (96), which can transfer peptide-MHCII complex to CD103^{+} DCs through a gap junction-mediated mechanism to mediate oral tolerance (97). In this regard, intestinal tolerogenic DCs might sample circulating allergens exposed through other mucosal sites to limit allergic responses by the generation of allergen-specific pTreg cells, which can be regulated by intestinal environment and intestinal microbiota. However, this possibility has yet to be addressed.

CONCLUSION

Role of commensal microbiota has been revisited recently and abundant evidence suggests that the host-microbiota interactions can actually determine the status of health and disease. Disrupted host-microbiota interactions can

not only influence immune disorders directed towards self- and foreign- antigens but also affect the pathogenesis of metabolic or neurological disorders.

Based on the advance of metagenomic analyses, human and animal studies clearly demonstrated that allergic diseases are associated with the dysbiosis of commensal microbiota. However, causal relationships between commensal microbiota and allergic disease have rarely been elucidated. In this regard, functional metagenomic analyses including metatranscriptomics, metaproteomics and metabolomics can be promising for understanding the etiology and increasing prevalence of allergic disease over the last decades. Furthermore, identification of novel microbial genes and molecular pathways capable of modulating mucosal T helper responses might be immensely helpful for in-depth understanding of the process. Additionally, comparative metagenomics during the onset of allergic disease can reveal inter-relationships among microbiota at different anatomic sites.

As intestinal microbiota influences the pathogenesis of most allergic diseases, studies on mutualistic interactions between host immune system and intestinal microbiota could reveal important regulatory mechanisms such as SCFAs, which are an important links between host immune system, diet and intestinal microbiota. However, despite of advances in mucosal immunology, several aspects of these tripartite interactions, such as mechanisms for increased susceptibility to allergic disease in GF mice with skewed Th2 responses and hyper-IgE syndrome, need to be studied for better understanding of microbiota-mediated immune regulation as well as for developing effective therapeutic ways for allergic diseases.

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CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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