Gut matters: Microbe-host interactions in allergic diseases

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The human body can be considered a metaorganism made up of its own eukaryotic cells and trillions of microbes that colonize superficial body sites, such as the skin, airways, and gastrointestinal tract. The coevolution of host and microbes brought about a variety of molecular mechanisms, which ensure a peaceful relationship. The mammalian barrier and immune functions warrant simultaneous protection of the host against deleterious infections, as well as tolerance toward harmless commensals. Because these pivotal host functions evolved under high microbial pressure, they obviously depend on a complex network of microbe-host interactions. The rapid spread of immune-mediated disorders, such as autoimmune diseases, inflammatory bowel diseases, and allergies, in westernized countries is thus thought to be due to environmentally mediated disturbances of this microbe-host interaction network. The aim of the present review is to highlight the importance of the intestinal microbiota in shaping host immune mechanisms, with particular emphasis on allergic diseases and possible intervention strategies. (J Allergy Clin Immunol 2012;129:1452-9.)

Key words: *Intestinal microbiota, bacteria, barrier function, allergy, asthma, eczema, immune responses, oral tolerance, functional food, probiotic*

Abbreviations used
DC: Dendritic cell
MAMP: Microbe-associated molecular pattern
MLN: Mesenteric lymph node
PP: Peyer patch
SCFA: Short-chain fatty acid
Treg: Regulatory T

Allergies, including asthma, dermatitis, rhinitis, and food allergies, are chronic inflammatory diseases driven by deregulated immune responses toward minute amounts of harmless antigens (allergens). Epidemiologic and clinical studies indicate that the increased incidence of autoimmune and allergic diseases in developed countries is associated with reduced microbial exposure and alteration of microbial communities in various body sites.[1] Recent advances in high-throughput molecular technologies in the field of metagenomic and metabolomic analysis have opened up new ways to identify core microbial communities linked to the onset of pathologies.[2-5] Nevertheless, it is still challenging to reach consensus in the definition of a "normal healthy" microbiota of the human gut, airway, and skin at the functional level. Moreover, the molecular mechanisms underlying microbe-host interactions that shape host immune functions are still elusive. In the present review we intend to discuss up-to-date knowledge of how the host microbiota is involved in the regulation of immune responses and the development of allergic disorders. We will provide a brief summary of clinically relevant evidence of the role of microorganisms in allergies, highlight the central role of the gut and its microbiota in regulating peripheral immune functions, and finally discuss data on the use of prebiotics and probiotics for the treatment or prevention of allergic symptoms.

IMPORTANCE OF MICROBE-HOST INTERACTIONS FOR ALLERGIC DISEASES: EVIDENCE FROM HUMAN STUDIES

Our gut harbors the majority of mammalian-associated microbes (10^8 to 10^{12} colony-forming units/g intestinal content). The intestinal microbiota, which is defined as the highly complex and dynamic assemblage of the thousands of microorganisms living in our gut,[5] has therefore been proposed as a major non–self-factor affecting the development of allergies. Changes in gut microbial composition have been reported in patients with allergic diseases at distant body sites, such as rhinitis and atopic eczema.[6-9] Alterations of the intestinal microbiota might actually precede the development of allergic manifestations in children, supporting the hypothesis that microbial dysbiosis is not only a consequence but also a cause of allergy.[10,11] Beyond the gut, the microbiota of the skin and bronchi was also altered in patients with allergic diseases at the respective sites (eczema or asthma).[12-15]

Potential reasons for microbial dysbiosis in allergic subjects lie in complex individual-specific interactions between genetic predispositions[16] and environmental factors, such as birth delivery mode, diet, hygiene, and medication. Birth delivery mode, for instance, markedly influences initial microbial colonization of newborns.[17,18] Natural birth, which results in immediate exposure of the child to the mother's vaginal and fecal microbiota, is associated with a reduced incidence of allergies compared with that seen in children born by means of cesarean section.[19] The revised hygiene hypothesis states that reduced microbial exposure in early childhood results in an increased T_H2/T_H1 response ratio and in defective regulatory immune mechanisms that contribute to a

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higher incidence of immune-mediated diseases, such as allergies, in developed countries.[20] In line with the hygiene hypothesis, growing up in a farming environment, including close contact with cows and consumption of raw milk, is strongly associated with reduced incidence of allergic diseases.[21] Although the farming environment seems to be protective through exposure to increased numbers and varieties of microorganisms,[22] its effect on the intestinal and bronchial microbiota, as well as potentially protective microbes, remain to be characterized. Exposure to pathogens, such as *Helicobacter pylori*, parasites, or *Mycoplasma tuberculosis*, have also been associated with reduced incidence of allergic diseases.[23-25] However, protective effects are pathogen specific, and early viral infections of the airways are a risk factor for allergic asthma.[26] Finally, breast-feeding and the use of antibiotics are 2 additional factors that markedly influence the intestinal and extraintestinal microbiota.[27-31] However, clinical results concerning their effect on allergy development are still conflicting.[32-35]

In summary, clinical data strongly suggest that microbial inhabitants of the human body, especially the intestinal microbiota, influence the development and severity of allergic diseases. Characterization of microbiota dysbiosis beyond phylogenetic diversity analysis is now essential to identify alterations of core microbial functions that contribute to early immune disturbances in allergic patients.

THE INTESTINE AS GATEKEEPER OF IMMUNITY

The intestine emerged as an important target in the prevention and therapy of allergies because the intestinal immune system has unique regulatory functions that affect local and systemic immune responses.[36] In the following paragraphs, we focus on 2 intestinal functions of relevance for systemic immune reactions: gut barrier and oral tolerance.

The intestinal mucosa provides the border between inner tissues and tremendous amounts of mostly harmless food- and bacteria-derived antigens. It is usually very efficient in preventing translocation of microorganisms and reducing the amount of permeating microbe-associated molecular patterns (MAMPs) and antigens.[37] However, increased gut permeability in pediatric and adult patients with asthma and eczema was reported in several clinical and *ex vivo* studies.[38-41] Both acute and chronic barrier disruption caused by mechanical damage, infection, dysbiosis (eg, resulting in overexpression of bacterial proteases), or dietary components can enhance translocation of microbial triggers that can act in distant body sites, such as the lung or skin.[42-44] For instance, diet-induced barrier alterations can result in increased occurrence of LPSs in the blood, which was in turn found to promote the development of diabetes.[43,45] Recent studies in mice provided additional evidence linking gut barrier function to inflammatory responses in distant body sites.[46,47] The authors demonstrated that intestinal epithelial barrier breakdown (eg, enhanced translocation of fluorescein isothiocyanate–dextran and reduced occludin protein levels) contributes to acute lung inflammation after skin burn. By means of surgery (abdominal vagotomy and stimulation of the right cervical nerve), they concluded that the neuroenteric axis is essential for gut barrier–mediated prevention of secondary acute lung injury. All these findings indicate that gut permeability is a promising function worth further investigation in the context of allergy development.

Because inflammatory immune responses toward low levels of penetrating MAMPs and antigens would be detrimental to the host, the intestinal immune system evolved to be highly tolerant toward these structures. An array of innate and adaptive tolerance mechanisms ensures prevention of inflammatory reactions toward harmless MAMPs and antigens.[48] Anti-inflammatory microenvironments in the intestinal mucosa, the gut-associated lymphoid tissue, and the mesenteric lymph nodes (MLNs) favor the development of IgA-secreting B cells and antigen-specific regulatory T (Treg) cells, 2 major antigen-specific tolerance mechanisms.[36] In contrast to other immunoglobulin-driven reactions, antigen binding by IgA results in efficient antigen neutralization without induction of proinflammatory signaling cascades. In addition, the export of high amounts of dimeric IgA toward the intestinal lumen through intestinal epithelial cells results in efficient immune exclusion of the respective antigen and reinforces the intestinal barrier.[49] Low levels of fecal IgA have been associated with increased development of IgE-mediated allergic diseases in children, supporting the relevance of IgA for systemic immune homeostasis.[50] Importantly, the induction of antigen-specific Treg cells on oral antigen exposure not only confers gastrointestinal but also peripheral tolerance toward the specific antigen (oral tolerance, Fig 1). Antigen-specific Treg cells exert potent anti-inflammatory activities, either through suppression of cell-cell contacts or secretion of anti-inflammatory cytokines, such as IL-10 and TGF-β.[51] Noticeably, the capacity to induce tolerogenic mechanisms toward antigens can also be referred to as mucosal tolerance because it is not exclusive to the gastrointestinal tract but rather a general feature of mucosal body surfaces, including nasal and bronchial mucosa.

The induction of mucosal tolerance is an important and extensively investigated therapeutic goal in patients with a wide range of chronic inflammatory diseases.[52-55] In the context of allergies, sublingual immunotherapies have been successfully developed to reduce systemic reactivity toward the respective allergen through induction of oral tolerance.[56] However, deregulated intestinal immune responses, such as those in patients with food allergies, in which the immune system is overreacting toward minute amounts of food antigens, or inflammatory bowel diseases, in which an overreaction toward microbial antigens triggers chronic inflammation, were found to be associated with strongly reduced or loss of oral tolerance.[57,58] These findings indicate that oral tolerance is dependent on the proper development and function of the intestinal barrier and immune system, which in turn are shaped by microbe-host interactions. In the next section we will provide details on the role of microbe-host interactions in the development of specific immune cell populations and immune mechanisms that are thought to underlie the development of allergies.

EFFECT OF MICROBE-HOST INTERACTIONS ON INTESTINAL AND SYSTEMIC IMMUNE FUNCTIONS

The major role of microbe-host interactions for host health has been underlined by abundant experimental studies using gnotobiotic animal models. The morphology and functions of the immune system in germ-free animals differ from those in colonized animals. Organized immune structures, such as Peyer patches (PPs) and MLNs, are smaller in germ-free animals, contain lower numbers of B and T cells, and lack germinal centers. Furthermore, germ-free animals exhibit decreased

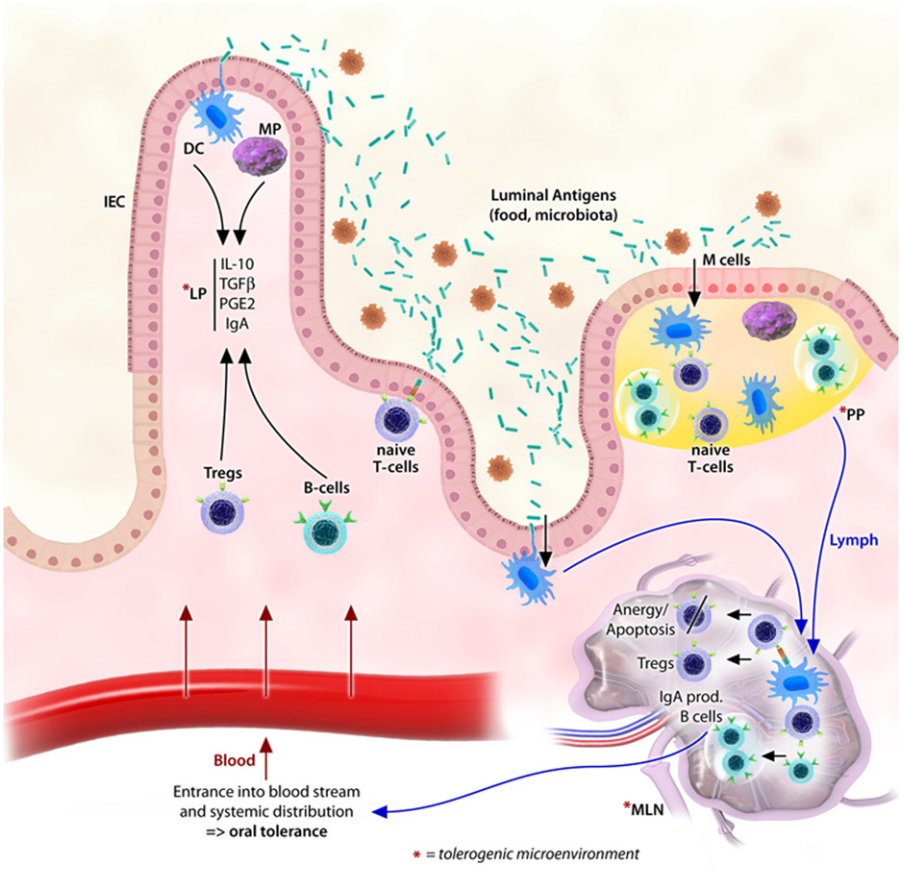

FIG 1. Oral tolerance mechanisms. Innocuous antigens that enter mucosal tissues through organized antigen sampling of M cells in PPs or lamina propria DCs induce tolerogenic mechanisms on antigen presentation. The tolerogenic microenvironment in the mucosal tissue and PPs primes antigen-presenting cells toward reduced antigen responsiveness, resulting either in clonal deletion of antigen-specific T cells through induction of T-cell anergy or apoptosis or the induction of antigen-specific Treg cells on antigen presentation in PPs or MLNs. These Treg cells are subsequently systemically distributed through the bloodstream and enter both mucosal and peripheral tissues, where they can exert anti-inflammatory activities on antigen contact. The induction of IgA-secreting plasma cells contributes to the induction of oral tolerance because IgA not only plays an important role in immune exclusion of antigens but also contributes to mucosal antigen sampling and anti-inflammatory antigen neutralization. *IEC,* Intestinal epithelial cells; *LP,* lamina propria; *MP,* macrophage; *PGE2,* prostaglandin E_2.

numbers of dendritic cells (DCs), $CD4^+$ T cells, and IgA-producing B cells in the lamina propria, as well as lower numbers of intraepithelial T cells.[59-61] In addition, the spleen was found to be smaller in germ-free animals and to contain fewer lymphocytes, especially $CD4^+$ T cells.[62] These findings demonstrate that microbial triggers are necessary for the development and maturation of the immune system. In line with these morphologic changes, several immune functions are compromised in germ-free animals, resulting in aberrant immune responses. Germ-free animals produce less IgA[63] and are characterized by T_H2-skewed immune responses, indicating that microbial triggers are necessary for counterbalancing T_H2 responses through the induction of T_H1, T_H17, and/or Treg cells.[64-66] Colonization of germ-free mice with microbial communities or single bacterial strains or even oral application of specific microbial components, such as polysaccharide A from *Bacteroides fragilis*, is sufficient to normalize the morphology of the immune system and the T_H1/T_H2 balance.[67,68] However, the highly complex nature of microbe-host interactions that shape host immune responses is demonstrated by recent findings in the context of T_H17 responses. The induction of T_H17 cells in the lamina propria of germ-free mice was found to be dependent on a specific population of microbes, the segmented filamentous bacteria.[66] In addition, microbial ATP was shown to induce differentiation of T_H17 cells,[69] whereas polysaccharide A from *B fragilis* prevents T_H17 responses through induction of Treg cells.[68] With regard to the development and function of Treg cells, the effect of microbe-host interactions is still controversial.[70,71] Although germ-free mice do have $CD25^+CD4^+$ regulatory cells in PPs and MLNs, their number and suppressive activity are reduced, resulting in failure to induce oral tolerance in germ-free mice.[72] Interestingly, colonization with specific bacterial species (eg, *Bifidobacterium infantis*) resulted in normalization of oral tolerance induction, whereas colonization with other species (eg, *Clostridium perfringens*) did not, indicating that species-specific yet unknown bacterial structures or products are crucial for the induction of regulatory mechanisms.[73]

Consistent with a decreased T_H1/T_H2 ratio, germ-free mice were recently shown to mount an exaggerated allergic airway reaction compared with that seen in colonized mice, indicating the important role of microbe-host interactions in the development of allergic diseases. Ovalbumin challenge in sensitized germ-free mice resulted in increased infiltration of lymphocytes and eosinophils into the airways and increased local levels of ovalbumin-specific IgE and typical T_H2 cytokines compared with those seen in colonized mice. Whereas increased allergic reaction correlated with reduced secretion of bronchial IgA, reduced numbers of plasmacytoid DCs and alveolar macrophages, and increased numbers of basophils, numbers of Treg cells and levels of regulatory cytokines were unchanged.[74] However, the molecular mechanisms and responsible microbial structures underlying the protective effect of the microbiota on the development of the allergic airway reaction remain to be elucidated. Furthermore, it would be highly interesting to clarify to what extent the observed protection is mediated through the intestinal or bronchial microbiota. In addition to exaggerated allergic airway reaction, germ-free mice seem to be more susceptible to IgE-mediated cow's milk allergy.[75] Germ-free mice were more responsive to oral sensitization (increased IL-4 secretion by stimulated splenocytes *ex vivo*) and were characterized by greater reduction in body temperature (clinical cow's milk allergy symptom) and higher blood levels of mast cell protease 1 and β-lactoglobulin–specific IgG_1 after oral challenge with β-lactoglobulin compared with colonized mice. Interestingly, whereas sensitization had no significant effect on dominant intestinal bacterial groups (denaturating gradient gel electrophoresis analysis), severe allergic disease was associated with low counts of cecal staphylococci (culture analysis), suggesting that subdominant species might play protective roles. With regard to specific active microbial molecules, short-chain fatty acids (SCFAs) can exert an array of anti-inflammatory effects in experimental models of inflammation. The increased severity of dextran sodium sulfate–induced colitis in germ-free mice compared with that seen in colonized mice can be prevented by giving acetate in drinking water.[76] Importantly, the protective effect of acetate is dependent on the presence of the SCFA receptor GPR43. Knockout of GPR43 resulted in worsened dextran sodium sulfate–induced acute colitis, 2,4,6-trinitrobenzene sulfonic acid–induced colitis (T_H1 mediated), arthritis, and allergic asthma in colonized mice, indicating that bacterial SCFAs are able to mediate potent anti-inflammatory effects through this pathway.

In summary, the use of gnotobiotic mice and experimental allergy models has been very useful in testing the causative role of microbiota in shaping regulatory immune responses in the gut and the development of allergic responses. However, microbial functions must be further investigated. For instance, the role of specific microbial features (eg, adhesion and enzymatic activities), and microbial structures (eg, MAMPs, antigens, and metabolites) remain to be elucidated at the level of the entire ecosystem. In spite of this lack of knowledge, targeted modulation of the intestinal microbiota is a major goal of basic and clinical research aiming at the development or re-establishment of immune homeostasis.

INTERVENTION STRATEGIES TARGETING THE INTESTINAL MICROBIOTA IN PATIENTS WITH ALLERGIC DISEASES

Although attempts to modulate the microbiota through antibiotics, such as amoxicillin or clarithromycin, have been of no to low protective relevance in adult asthmatic patients,[77,78] the application of prebiotics and probiotics is hypothesized to be a safe and efficient approach for the prevention and treatment of allergic diseases.

Prebiotics are food components that cannot be digested by the host but are metabolized by and promote the growth of specific subgroups of endogenous gut bacteria thought to be beneficial for the host.[79] An array of experimental data indicate that prebiotic-mediated compositional changes in murine intestinal microbiota protect the host against the development of allergic diseases.[80-82] Mechanistically, a study in mice showed protective effects of prebiotic uptake on cow's milk allergy through increased oral tolerance induction after oral wheat sensitization.[83] In contrast, results from clinical interventions based on the use of prebiotics are heterogeneous. Some studies showed significantly reduced allergic manifestations in prebiotic-treated children, whereas others did not detect any protective effect.[84-87] Furthermore, there is still no proof that potential protective effects are due to prebiotic-induced compositional or functional changes in the intestinal microbiota.

Probiotics are live microorganisms that confer a health benefit to the host when administered in adequate amounts (World Health Organization/Food and Agriculture Organization 2001). They are proposed to exert either direct protective effects on the host or to modulate the intestinal microbiota in a protective way. Many different bacterial strains or mixtures thereof, as well as synbiotics (combination of prebiotics and probiotics), have been used in clinical trials to assess protective effects in the context of allergic sensitization and allergic diseases, but results have been conflicting. Several studies reported a significant reduction in the incidence and severity of allergic diseases after probiotic treatment,[88,89] whereas others did not observe protective effects.[90] Meta-analysis of data from clinical studies concluded that probiotics cannot be generally recommended for the treatment of eczema[91] or the prevention of allergies in general (eczema, rhinitis, asthma, and food allergy).[92] These results stress the importance of selecting suitable bacterial strains and application schemes for different patient subgroups.

Microorganisms need to be screened *in vitro* and in experimental studies for protective microbe-microbe or microbe-host interactions to preselect potentially effective probiotic bacteria for targeted application in clinical studies. Until now, the selection of probiotic strains has mostly been performed on a hypothesis-free basis. In addition, the reported effects of probiotic therapy in clinical studies remain mostly descriptive, resulting in a lack of understanding of protective mechanisms. Clinical studies showed that probiotics can improve mucosal barrier function,[93] increase allergen-specific IgA levels,[94] and positively affect an array of other immune-modulatory effects affecting the T_H1/T_H2 balance.[95-97] Moreover, in experimental studies the protective effect of probiotics on allergic diseases was mediated by the induction of regulatory mechanisms, such as generation, proliferation, and activity of tolerogenic DCs and T cells.[94,98,99] However, it is unclear whether probiotic bacteria directly induce these effects or whether protection is conferred through a probiotic-mediated stabilization or modulation of the intestinal microbiota. Studies addressing this question provided contradictory results. Whereas uptake of 2 probiotic strains (*Lactobacillus acidophilus* ATCC 700396 and *Bifidobacterium animalis subsp. lactis* ATCC SD5220) affected neither disease severity (Scoring Atopic Dermatitis score) nor the diversity and composition of

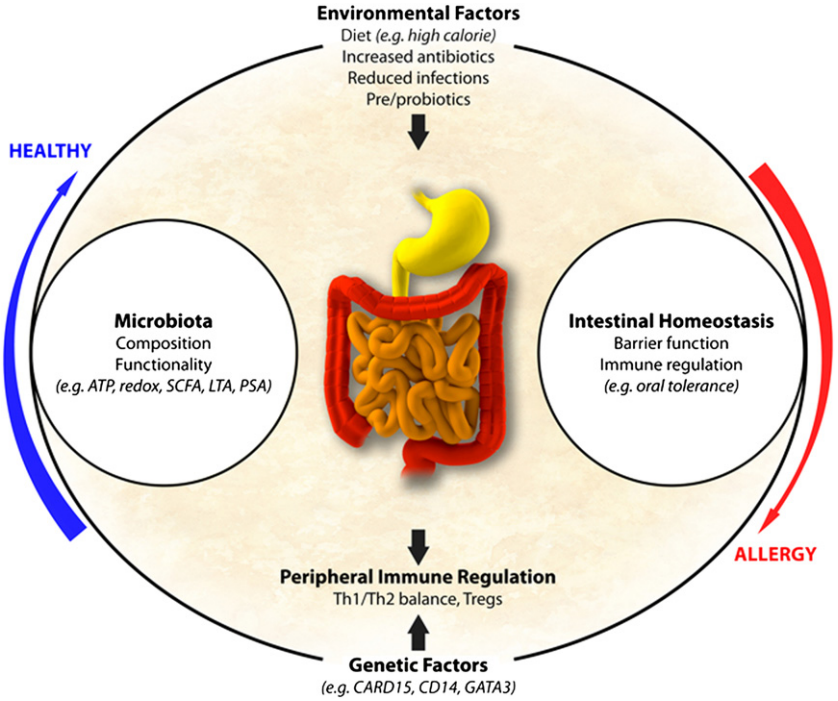

FIG 2. Systemic immune homeostasis depends on intestinal microbe-host interactions. Under the influence of genetic and environmental factors, the activity and antigenic potential of gut microorganisms have a strong influence on barrier maintenance, the development of gut-associated lymphoid tissues, and eventually systemic immune responses. *LTA*, Lipoteichoic acid; *PSA*, polysaccharide A; *redox*, redox potential.

TABLE I. Current knowledge and future research needs

Consensus	Perspective
Alterations of the gut microbiota are associated with the development and severity of allergies.	Dysbiosis of microbial ecosystems in allergic patients must be described at functional levels (metagenome, proteome, or metabolome).
	Dysbiosis in early-life periods must be further investigated to reach consensus in the definition of "healthy" infant microbiota and to mine microbial parameters involved in the onset of allergic symptoms.
	The causative role of changes in gut microbial communities (dysbiosis) in patients with allergic diseases must be clearly demonstrated, such as through microbial transfer experiments in germ-free murine models of allergy.
The intestinal mucosa is a highly efficient barrier, which prevents translocation of microorganisms, as well as dietary and microbial molecules.	Intestinal barrier dysfunctions must be further investigated in homogeneous populations of allergic patients. The occurrence of specific bacterial molecules (eg, LPS, heat shock proteins, fatty acids, and DNA) in the blood of allergic patients must be analyzed.
There is inconsistent support for the efficacy of probiotics and prebiotics in preventing allergies.	Probiotic structure-function relationships with regard to probiotic-microbiota or probiotic-host interactions need to be elucidated to enable targeted application of probiotics.
	The clinical relevance of selected prebiotics and probiotics needs to be investigated in a sufficient number of state-of-the-art clinical studies.
	The effect of prebiotics and probiotics on gut microbial ecosystems must be analyzed beyond the sole level of bacterial diversity.

the main bacterial genera in the intestines of atopic children,[100] uptake of *Lactobacillus rhamnosus* GG and *Lactobacillus gasseri* TMC0356 resulted in reduced allergic rash–induced alterations of the fecal microbiota in Japanese patients with cedar pollinosis,[101] suggesting that the effect of probiotics on the intestinal microbiota is strain specific and dependent on the resident microbiota. A synbiotic mixture (*B breve* M-16V and Immunofortis; Nutricia Cuijk BV, Cuijk, The Netherlands) had a strong effect on the intestinal microbiota (increased proportion of bifidobacteria vs clostridia and eubacteria), which did not translate into improvement in disease severity (Scoring Atopic Dermatitis score).[102] In contrast, uptake of *L rhamnosus* GG affected humoral parameters, such as blood levels of IgA- or IgM-secreting cells but not the number of specific microbial species in intestinal and skin samples from infants with atopic dermatitis.[103] Furthermore, a combination of *L acidophilus* ATCC 700396 and *B lactis* ATCC

SD5219 did not prevent allergic rash–induced changes in intestinal microbiota but had protective effects with regard to eosinophil infiltration in patients with allergic rhinitis,[6] which speaks in favor of the direct effects of the ingested strains.

In summary, additional state-of-the-art clinical intervention trials are needed before any recommendation can be made concerning the use of prebiotics and probiotics for the prevention or therapy of allergic diseases. Direct or microbiota-mediated protective effects of probiotic strains on host immune functions are far from being understood. In this respect analyzing changes in gut microbiota only at the level of bacterial diversity is not likely to be sufficient to measure the effect of probiotic microorganisms on intestinal ecosystems.[104]

CONCLUSIONS

Fig 2 provides a schematic overview of key issues highlighted in the present review with respect to the role of microbe-host interactions in allergy development. Future research needs are summarized in Table I. Ecologic approaches dedicated to the characterization of ecosystem functions and alteration thereof (dysbiosis) in early-life periods will be essential for characterizing the establishment of a "normal" microbiota (as in the sense of diverse and stable microbial consortia) expressing key functions that affect intestinal and systemic immune homeostasis. Intervention trials could then be designed with the aim of stabilizing or re-establishing these key functions. With regard to the use of specific microorganisms for the prevention or reduction of allergic diseases, experimental studies are required to preselect candidate strains with a high protective potential and to dissect protective molecular mechanisms. The clinical relevance of candidate strains must then be determined in high-quality clinical studies.

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