

CME Review

The role of the microbiome in immune cell development



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At the conclusion of this activity, participants should be able to:

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- Discuss the implications of altered microbial colonization on the development of asthma and allergic disease

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Introduction

Humans and other mammals maintain a symbiotic relation with their indigenous microbiota, which is a vast and complex microbial ecosystem, most of which resides in the gastrointestinal (GI) tract. This ecosystem and its entire genetic content are

collectively referred to as the *microbiome*. The microbiome is primarily bacterial, but also contains fungi, yeast, viruses, and archaea. Accumulating evidence suggests that the microbiome plays several roles in host physiology, including nutrition, metabolism, and immune response.¹ In 2013, the World Allergy Organization published a position statement regarding the biodiversity hypothesis and allergic disease,² in which they noted that “Metagenomic and other studies of healthy and diseased individuals reveal that reduced biodiversity and alterations in the composition of the gut and skin microbiota are associated with various inflammatory conditions, including asthma, allergic and inflammatory bowel diseases (IBD), type 1 diabetes, and obesity. Altered indigenous microbiota and the general microbial deprivation characterizing the lifestyle of urban people in affluent countries appear to be risk factors for immune dysregulation and impaired tolerance.” The focus of this CME article is on the role of the intestinal microbiome in immune cell development and in directing host immune responses in the GI tract and at distant sites, with a final discussion on the role of the intestinal microbiome in the development of allergy and asthma.

The Human Intestinal Microbiome

The GI tract harbors most of the human microbiome.³ A schematic of the GI tract is shown in Figure 1. The composition of the gut microbiome varies along the length of the GI tract based on the specific microenvironment. The small intestine harbors far fewer bacterial numbers than the more distal cecum and large intestine and contains larger numbers of aerobes and facultative anaerobes, such as *Lactobacillus*, *Streptococcus*, and *Enterococcus* species. Although facultative anaerobes persist, obligate anaerobes dominate the populations in the cecum and large intestine, with *Bacteroides* species making up the majority of gram-negative organisms and *Clostridia* species as the dominant gram-positive organisms.⁴ Overall, there are more than 1,000 different bacterial species that have been identified as contributing to the colonic microbiome, although any individual appears to harbor consortia of

400 to 500 species. Although individuals vary with respect to bacterial species, the functional capacity of the microbiota (total gene content) seems to show significant similarities, suggesting common microbial functions contribute to colonization and host physiology.⁵

Postnatal Microbiota Development in Human Neonates

Human colonization is believed to begin at birth; however, recent studies may be challenging that presumption. Recently published work has identified a unique placental microbiome, which shares greatest similarity to the oral microbiome, and suggests that the in utero environment is not as sterile as assumed.⁶ Intestinal colonization fluctuates during early colonization, with significant increases in diversity during the first 3 years of life.⁷ Changes in colonization continue throughout life but at a slower pace. Several factors influence initial colonization, including mode of delivery (vaginal vs caesarian) and breast vs formula feeding. Infants who are delivered vaginally are initially colonized by the maternal microbiota of the birth canal, whereas those delivered by caesarian section are initially colonized by the microbiota from skin and other environmental sources.⁸ Breast milk contains bacteria and numerous immune and nutritional factors that influence microbial colonization. These include maternal IgA, antimicrobial peptides and proteins, and human milk oligosaccharides, which promote the growth of specific bacterial species.⁹ The neonate has a relatively underdeveloped immune system that is skewed toward regulatory responses, including decreased cytokine production, enhanced regulatory T- and B-cell responses, and an immune-dampening response to microbial products by intestinal epithelial Toll-like receptors (TLRs).¹ These factors likely combine to allow noninflammatory colonization of the neonatal GI tract. Intestinal microbial composition and diversity can be altered throughout life by environmental exposures, such as living on a farm vs in a city, diet, antibiotic use, and disease. It appears that early alterations in the intestinal microbiota can have a profound influence on the development of later immune-mediated diseases, such as allergy and asthma.

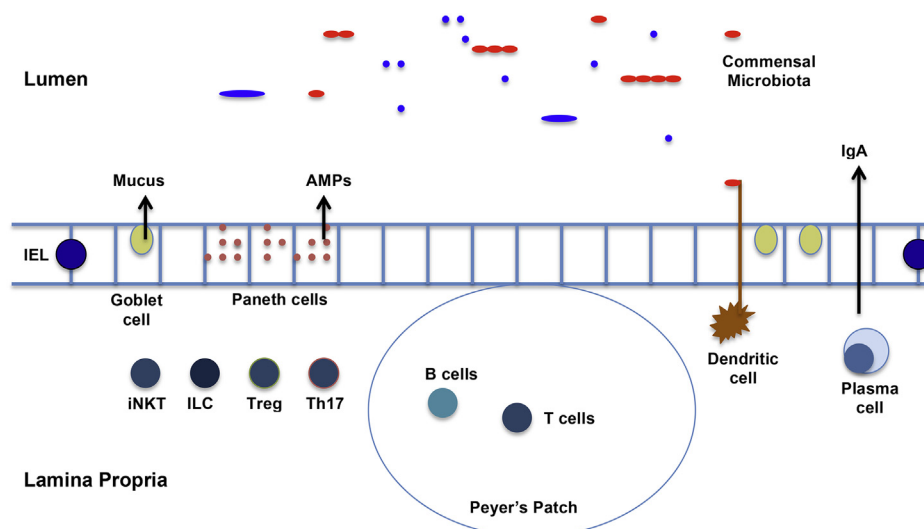


Figure 1. Schematic model of the small intestine. A single layer of epithelial cells separates the contents of the intestinal lumen from the mammalian host. The epithelial cells include absorptive enterocytes, goblet cells, and Paneth cells. Intraepithelial lymphocytes (IELs) percolate through the epithelial layer. The epithelial surface is covered by mucus, which is secreted by goblet cells. Paneth cells secrete antimicrobial peptides (AMPs) and proteins, which diffuse into the mucus layer. IgA is produced and secreted by plasma cells in the lamina propria. The IgA is transported by transcytosis and released into the lumen, where it is contained in the mucus layer. The commensal microbiota lives predominantly in the outer mucus layer and generally does not interact directly with the epithelial surface. Dendritic cells in the lamina propria extend dendrites into the lumen to sample luminal bacteria. Lymphoid structures, such as Peyer patches, underlie the epithelial surface and contain B and T cells. Other immune cells contained within the lamina propria include T-helper cell type 17 (Th17), regulatory T cells, innate lymphoid cells (ILCs), and invariant natural killer T cells (iNKT). The abundance, differentiation, and function of these cells are modulated by the commensal microbiota.

Postnatal Immune Development

The study of germ-free mice and their microbial reconstitution has contributed greatly to understanding the role of the microbiome in immune development. Germ-free animals have an underdeveloped immune system, at mucosal surfaces and systemically. Immune structures, such as Peyer patches, lymph nodes, and spleen, are smaller and lack germinal centers. The intestinal epithelial architecture also is immature, and there is decreased or absent expression of different epithelial antimicrobial peptides and proteins and decreased mucosal IgA production.¹⁰ Reconstitution with a complete complex microbiota completely restores immune development. Interestingly, reconstitution with single species of bacteria, such as segmented filamentous bacteria (SFB) or even with the *Bacteroides fragilis* surface capsule polysaccharide, can restore most aspects of immune function.^{11–13} Certain bacteria or groups of bacteria also have been identified that are associated with the differentiation of specific adaptive immune subsets, as will be discussed further.¹⁴

Impact of Microbiota on Innate Immunity

Antimicrobial peptides, innate immune effectors produced by intestinal epithelial cells, most notably by Paneth cells in the small intestinal crypt, play an important role in host protection from enteric pathogens and in the containment and composition regulation of the intestinal microbiota.¹⁵ Studies in neonatal and germ-free mice have shown that some of these peptides, including lysozyme and α -defensins, are constitutively produced in the absence of bacterial colonization. Others, such as angiogenins and Reg3 γ , are induced in response to bacterial colonization. The direct signaling of bacterial products in the lumen of the GI tract through TLRs and the myeloid differentiation primary response 88 (MyD88) pathway result in the induction of these epithelial antimicrobials.¹⁶ However, Reg3 γ induction is not limited to direct TLR interaction. Interleukin (IL)-22 production by innate and adaptive lymphoid cells in response to the microbiota and/or microbial antigens also drives Reg3 γ expression in intestinal epithelial cells. The presence of Reg3 γ is essential for spatial separation of commensal bacteria from the epithelial surface.¹⁷ In Reg3 γ -deficient mice, the microbiota is found in close contact with the epithelium, resulting in increased expression of inflammatory genes.^{17,18}

Lymphoid cells localized to mucosal surfaces are involved in innate and adaptive immune processes, and many engage with the intestinal microbiota to drive immune development and host response. Innate lymphoid cells (ILCs) have been identified abundantly in the GI tract. These cells are lymphoid in origin, but “innate” in function, lack T- or B-cell receptors (TCRs or BCRs), and appear not to engage in antigen-specific immune responses. They are classified into 3 groups based on their transcription factor profiles and cytokine production.¹⁹ Group 1 ILCs express T-bet transcription factor and produce T-helper cell type (T_H) 1 cytokines. Group 2 ILCs express GATA-binding protein-3 transcription factor and produce T_H2 cytokines, and group 3 ILCs express the RAR-related orphan receptor- γ T transcription factor and produce IL-17a and IL-22. Group 3 ILCs are abundant at mucosal surfaces, particularly in the GI tract. There is evidence that the development and function of some subsets of group 3 ILCs are induced in response to microbial colonization and are involved in enhancing B-cell IgA production.²⁰ In addition, there is evidence that the production of IL-22 by ILCs plays an important role in containing and shaping the composition of the intestinal microbiota.^{21,22}

On the spectrum from innate to adaptive lymphoid cells are T cells containing invariant TCRs or limited TCR gene rearrangements. These include the $\gamma\delta$ T cells and invariant natural killer T cells, which behave in an “innate-like” manner. Invariant natural killer T cells respond to CD1d-restricted presentation of microbial

lipid antigens but also can be activated in response to other inflammatory cytokines.²³ These cells are abundant at the GI mucosal surface, and their abundance and specific phenotype seem to be regulated by the intestinal microbiota and appear to be associated with inflammatory immune responses in the intestine and the lung.^{23,24} The $\gamma\delta$ T cells make up a small percentage of total T cells but are enriched in the GI tract mucosa among the intraepithelial lymphocytes and show decreased T-cell receptor diversity. Like group 3 ILCs, they have an important role in the regulation of the microbiota. For example, Reg3 γ production by intraepithelial and lamina propria $\gamma\delta$ T cells is induced in response to the commensal microbiota^{25,26} and contributes to the containment and composition of the microbiota and the containment of enteric pathogens.^{25,26}

Most interactions of the commensal microbiota with the innate immune system are focused on microbial containment at mucosal surfaces and prevention of infection, striking a delicate balance between regulation and inflammation to achieve homeostasis. The influence of this microbial ecosystem on the acquired or adaptive immune system reflects a similar process, driving a combination of barrier protection by B-cell production of IgA and the balanced differentiation of regulatory T cells and T_H17 cells.

B Cells and IgA

Immunoglobulin A is abundantly produced and secreted at all mucosal surfaces, where it contributes profoundly to the noninflammatory containment and clearance of bacteria and viruses. Commensal bacteria are taken up by intestinal dendritic cells, which extend their dendrites into the intestinal lumen.²⁷ Mucosal B cells interact with the intestinal dendritic cells, ultimately triggering the development of IgA-secreting plasma cells, which home to the mucosal lamina propria.²⁸ Enterocytes deliver the IgA into the gut lumen by transcytosis. The IgA accumulates in the intestinal mucus, where it interacts with the commensal microbiota to contain it within the gut lumen. This is a dynamic process, in which B cells are constantly responding to the shifting microbial populations at the mucosal surfaces.²⁹ Animals lacking IgA show significant changes in their commensal microbial composition, showing a complex reciprocal interaction between microbes and B cells contributing to mucosal homeostasis.³⁰

Regulatory T Cells

Regulatory T cells (Treg cells) are a subset of CD4⁺ T cells, abundant in the intestinal tract in the presence of the commensal microbiota, but significantly decreased in germ-free animals. Treg cell numbers are normalized when the microbiota is reconstituted in germ-free mice, and their development appears to be driven by a mixture of clostridial species (cluster IV and XIVa strains).¹⁴ Treg numbers also can be re-established by reconstitution of the microbiota using the altered Schaedler flora, a defined group of 8 bacterial strains that are common colonizers of the mouse intestines (including a combination of *Lactobacillus*, *Bacteroides*, *Flexistipes*, and *Clostridium* species). Interestingly, the monoassociation with *B. fragilis* or the *B. fragilis* capsular polysaccharide, acting along the TLR2 pathway, also drives Treg cell differentiation.^{31,32} The loss or absence of Treg cells in the intestine results in the development of colitis,³³ which can be ameliorated by the adoptive transfer of natural or induced Treg cells.³⁴

T_H17 Cells

The T_H17 cells contribute to host defense by the production of proinflammatory cytokines, but T_H17 cells also produce IL-22, which enhances the antibacterial barrier by induction of mucosal antimicrobial peptides. Mice that are germ free or have an antibiotic-depleted microbiota have significantly decreased or

absent intestinal T_H17 cell development. One specific bacterial species, SFB, has been shown to induce T_H17 development. The presence of SFB has long been observed to be protective against enteric pathogen infection,³⁵ possibly through its ability to induce T_H17 host responses.^{12,13} Interestingly, attempts to block the production of IL-17 in patients with Crohn disease resulted in worsening of intestinal inflammation,³⁶ possibly because of the multiple mechanisms of IL-17 action in strengthening the antimicrobial mucosal barrier and containing the microbiota.³⁷

Impact of Microbiota on Immune Response at Local and Distant Sites

Clearly, many immune responses induced by intestinal microbial colonization function to contain the microbiota and prevent bacterial infection and excessive local inflammation. In combination, the immune responses strengthen the mucosal barrier and dampen the host inflammatory response. Therefore, as would be expected, significant disruption of immune homeostasis at the intestinal mucosal surface results in colitis and dysbiosis, which is dependent on the presence of colonizing bacteria.³⁸ Perhaps less expected, it has become clear that microbiota-immune interactions in the GI tract are important drivers of immune responses at distant mucosal sites and systemically. Early evidence for this was noted in genetically susceptible animals, in which the presence of SFB and induction of T_H17 cells resulted in systemic autoimmunity and inflammation in mouse models of autoimmune arthritis and experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis.^{39,40} In addition, the intestinal microbiota has been implicated in several additional inflammatory and immune disease processes, including obesity, nonalcoholic fatty liver disease, allergy, asthma, and even neurologic and psychiatric diseases such as anxiety and autism.⁴¹

Intestinal Microbiota and Allergy and Asthma

Allergies are driven by abnormal immune responses to otherwise harmless antigenic triggers or allergens, resulting in chronic inflammation. The incidence of allergic disease has increased profoundly in developed countries in the past several decades, too rapidly for genetic shifts and therefore likely owing to environmental influences or gene–environment interactions. Recent studies have noted strong associations between the development of atopic disease with changes in host-associated microbiota composition and diversity, suggesting a role for changing environmental microbial exposure in driving these diseases. The hygiene hypothesis has suggested that the lack of environmental microbial exposure causes immune dysregulation, resulting in increased T_H2 responses. The hygiene hypothesis has been amended and built on to develop several related concepts, such as the “microflora hypothesis,” which suggests that alterations in the intestinal microbiota disrupt immune tolerance.⁴² The “old friends” hypothesis posits that the coevolution of the human host with its microbiome has established essential regulatory interactions between microbiota and host and when disrupted leads to immune dysregulation (the “vanishing microbiota hypothesis”), all ultimately relating changes in bacterial colonization to increases in allergic and immune disease.^{43,44} This has been supported by epidemiologic studies in humans showing that individuals who have exposure to a greater diversity of bacteria at birth (vaginal vs caesarian) have a lower incidence of asthma and atopy.^{45,46} Some studies have suggested that breast feeding also is protective.⁴⁷ Children who grow up on farms are exposed to a wider variety and abundance of microbes and thus appear to be protected from allergic disease.⁴⁸ The vanishing microbiota hypothesis suggests that humans are “hardwired” to interact with specific bacterial species, and that the loss of these species through decreased

microbial exposure or through antibiotic use renders the immune system overly responsive to environmental antigens. A large body of research on the pathogen *Helicobacter pylori* exemplifies this hypothesis. Although *H. pylori* is an important cause of peptic ulcer disease, the loss of *H. pylori* in the human population has been strongly associated with an increased incidence of asthma and allergy.⁴⁹ In human studies of eczema, there is evidence for changes in the microbiome in the gut and at the skin surface.⁵⁰ Although it may be difficult to distinguish between cause and effect at the site of allergic manifestation, there is evidence that changes in the intestinal microbiome occur before the development of allergy, strongly suggesting a causative role for the GI microbiome in driving allergic disease.⁵¹

Approaches for intervention, by administering probiotics to alter intestinal microbial composition as a means to treat allergy, have yielded mixed results in humans. Probiotics are defined as live microorganisms that confer a health benefit to the host. They can be administered as single or complex mixtures of organisms and sometimes are given as synbiotics or probiotics in combination with prebiotics (nutritional substrates to enhance probiotic growth). Some studies have shown efficacy in ameliorating allergy symptoms, whereas other studies have shown no effect. This may be due to differences in the specific bacterial strains and strain combinations used and to the probiotic interactions with the recipients’ indigenous microbiota. It is not clear whether probiotics act directly on the host to provide health benefits or whether they act through modulating the host colonization. Clearly, more rigorous and carefully controlled studies are needed before therapeutic recommendations are possible, and it is likely that a “one size fits all” approach may not be achievable. There is evidence that probiotics are having an impact on measurable parameters of immune function, with beneficial effects on the T_H1–T_H2 balance, improving regulatory responses, and enhancing barrier function.⁵²

Several different mechanisms for the microbiota–immune interaction have been implicated in the development of allergy through the use of mouse models. Early studies using mouse models of asthma showed that the immune systems of germ-free mice and antibiotic-treated mice are skewed toward T_H2 responses, which are important drivers of allergic responses. These animals develop worse asthma, which is ameliorated after reconstitution of the microbiota.⁵³ Recent studies in neonatal mice have shown that antibiotic treatment with vancomycin in the perinatal period alters the intestinal microbiota, results in smaller numbers of Treg cells, elevated serum IgE, and increased susceptibility to allergic asthma in adulthood.^{54,55} Interestingly, there appears to be a limited window of opportunity for this effect early in life, because treatment of adult mice with vancomycin does not increase asthma susceptibility. In addition, this effect appears to be related to specific bacterial composition, because treatment with another nonabsorbable broad-spectrum antibiotic, streptomycin, does not enhance the development of asthma. This supports the earlier findings in humans about the importance of bacterial diversity and environmental exposures early in life in the risk for developing asthma and atopy. There is accumulating evidence for the importance of Treg cells in dampening the development of atopy, through their control of mucosal T_H2 inflammatory responses.⁵⁶

Additional support for the importance of microbiota-mediated T_H2 responses in the development of allergy comes from recent work showing that antibiotic depletion of the microbiota in mice results in increased serum IgE, essential for mediating increased numbers of circulating basophils, basophil-driven T_H2 responses, and exacerbated allergic lung inflammation. Modulation of IgE production requires MyD88 (TLR) expression and signaling on B cells.⁵⁷ The interaction of microbes and microbial products with TLRs has been implicated in numerous aspects of immune regulation, including IgA production and Treg cell proliferation.^{10,28,32}

The intestinal microbiota also interacts with the host through its fermentation of dietary fiber, which has nutritional and immune implications. The byproducts of bacterial fermentation, most abundantly the production of short-chain fatty acids (lactate, butyrate, and propionate), can supply energy to intestinal epithelial cells, alter the intestinal microbial milieu, and signal the host by binding to G-protein coupled receptors. Butyrate produced by the microbiota has been shown to induce differentiation of colonic Treg cells.⁵⁸ Mice fed high fermentable fiber diets showed changes in their intestinal microbiota, increased circulation of short-chain fatty acids, and protection from allergic airway inflammation compared with mice fed low-fiber diets. Administration of propionate to mice influenced bone marrow hematopoiesis, resulting in increases in populations of lung macrophage and dendritic cells, which had a decreased ability to generate T_H2 responses.⁵⁹ The ability to influence allergic responses through dietary influence on the microbiome may have enormous implications for future interventions in the treatment and prevention of allergic disease.

Conclusions

Data that have accumulated over the past several decades strongly suggest an important positive correlation between changes in human indigenous microbial colonization and the development of allergic and immune-related diseases. Changes in intestinal microbial diversity and composition have been driven by urbanization, diet, antibiotic use and other medical interventions, environmental influences, and other unknown factors. Because the intestinal microbiota is an essential force in the appropriate development of mucosal and systemic innate and adaptive immunity, changes in diversity and composition have serious implications for human health and disease, as have been seen. Ongoing research, which is beginning to allow a mechanistic understanding of microbiota–host interactions, should provide useful direction for future approaches for prevention or therapeutic intervention in the treatment of allergy and immune-mediated disease, perhaps through the use of probiotics, diet, or specific targeting of essential biochemical pathways involving bacterial metabolites that drive dysregulated immune responses.

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References

- [1] Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014;157:121–141.
- [2] Haahtela T, Holgate S, Pawankar R, et al. The biodiversity hypothesis and allergic disease: world allergy organization position statement. *World Allergy Organ J*. 2013;6:3.
- [3] Gill SR, Pop M, Deboy RT, et al. Metagenomic analysis of the human distal gut microbiome. *Science*. 2006;312:1355–1359.
- [4] Brown EM, Sadarangani M, Finlay BB. The role of the immune system in governing host–microbe interactions in the intestine. *Nat Immunol*. 2013;14:660–667.
- [5] Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464:59–65.
- [6] Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Science Transl Med*. 2014;6:237ra265.
- [7] Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486:222–227.
- [8] Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A*. 2010;107:11971–11975.
- [9] Marcobal A, Sonnenburg JL. Human milk oligosaccharide consumption by intestinal microbiota. *Clin Microbiol Infect*. 2012;18(suppl 4):12–15.
- [10] Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009;9:313–323.
- [11] Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*. 2005;122:107–118.
- [12] Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity*. 2009;31:677–689.
- [13] Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009;139:485–498.
- [14] Atarashi K, Tanoue T, Oshima K, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*. 2013;500:232–236.
- [15] Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat Rev Microbiol*. 2011;9:356–368.
- [16] Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science*. 2006;313:1126–1130.
- [17] Vaishnava S, Yamamoto M, Severson KM, et al. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science*. 2011;334:255–258.
- [18] Loonen LM, Stolte EH, Jaklofsky MT, et al. REG3gamma-deficient mice have altered mucus distribution and increased mucosal inflammatory responses to the microbiota and enteric pathogens in the ileum. *Mucosal Immunol*. 2014;7:939–947.
- [19] Hepworth MR, Sonnenberg GF. Regulation of the adaptive immune system by innate lymphoid cells. *Curr Opin Immunol*. 2014;27:75–82.
- [20] Magri G, Miyajima M, Bascones S, et al. Innate lymphoid cells integrate stromal and immunological signals to enhance antibody production by splenic marginal zone B cells. *Nat Immunol*. 2014;15:354–364.
- [21] Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat Immunol*. 2011;12:383–390.
- [22] Sonnenberg GF, Monticelli LA, Alenghat T, et al. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science*. 2012;336:1321–1325.
- [23] Zeissig S, Blumberg RS. Commensal microbiota and NKT cells in the control of inflammatory diseases at mucosal surfaces. *Curr Opin Immunol*. 2013;25:690–696.
- [24] Olszak T, An D, Zeissig S, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science*. 2012;336:489–493.
- [25] Ismail AS, Behrendt CL, Hooper LV. Reciprocal interactions between commensal bacteria and gamma delta intraepithelial lymphocytes during mucosal injury. *J Immunol*. 2009;182:3047–3054.
- [26] Duan J, Chung H, Troy E, Kasper DL. Microbial colonization drives expansion of IL-1 receptor 1-expressing and IL-17–producing gamma/delta T cells. *Cell Host Microbe*. 2010;7:140–150.
- [27] Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol*. 2001;2:361–367.
- [28] Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol*. 2010;10:159–169.
- [29] Hapfelmeier S, Lawson MA, Slack E, et al. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science*. 2010;328:1705–1709.
- [30] Suzuki K, Meek B, Doi Y, et al. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proc Natl Acad Sci U S A*. 2004;101:1981–1986.
- [31] 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;107:12204–12209.
- [32] Round JL, Lee SM, Li J, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science*. 2011;332:974–977.
- [33] Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. *Annu Rev Immunol*. 2009;27:313–338.
- [34] Haribhai D, Lin W, Edwards B, et al. A central role for induced regulatory T cells in tolerance induction in experimental colitis. *J Immunol*. 2009;182:3461–3468.
- [35] Heczko U, Abe A, Finlay BB. Segmented filamentous bacteria prevent colonization of enteropathogenic *Escherichia coli* O103 in rabbits. *J Infect Dis*. 2000;181:1027–1033.
- [36] Hueber W, Sands BE, Lewitzky S, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut*. 2012;61:1693–1700.
- [37] O'Connor W Jr, Zenewicz LA, Flavell RA. The dual nature of T(H)17 cells: shifting the focus to function. *Nat Immunol*. 2010;11:471–476.
- [38] Uhlig HH, Powrie F. Mouse models of intestinal inflammation as tools to understand the pathogenesis of inflammatory bowel disease. *Eur J Immunol*. 2009;39:2021–2026.
- [39] Wu HJ, Ivanov II, Darce J, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity*. 2010;32:815–827.
- [40] Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A*. 2010;108(suppl 1):4615–4622.
- [41] Sommer F, Backhed F. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol*. 2013;11:227–238.
- [42] Noverr MC, Huffnagle GB. The 'microflora hypothesis' of allergic diseases. *Clin Exp Allergy*. 2005;35:1511–1520.
- [43] Rook G, Brunet L. Microbes, immunoregulation, and the gut. *Gut*. 2005;54:317–320.
- [44] Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol*. 2009;7:887–894.

- [45] Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. *Clin Exp Allergy*. 2008;38:629–633.
- [46] van Nimwegen FA, Penders J, Stobberingh EE, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *J Allergy Clin Immunol*. 2011;128:948–955. e941–e943.
- [47] Just J, Belfar S, Wanin S, Pribil C, Grimfeld A, Duru G. Impact of innate and environmental factors on wheezing persistence during childhood. *J Asthma*. 2010;47:412–416.
- [48] Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med*. 2011;364:701–709.
- [49] Blaser MJ, Chen Y, Reibman J. Does *Helicobacter pylori* protect against asthma and allergy? *Gut*. 2008;57:561–567.
- [50] Penders J, Thijs C, van den Brandt PA, et al. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut*. 2007;56:661–667.
- [51] Vael C, Vanheirstraeten L, Desager KN, Goossens H. Denaturing gradient gel electrophoresis of neonatal intestinal microbiota in relation to the development of asthma. *BMC Microbiol*. 2011;11:68.
- [52] Hormannsperger G, Clavel T, Haller D. Gut matters: microbe–host interactions in allergic diseases. *J Allergy Clin Immunol*. 2012;129:1452–1459.
- [53] Russell SL, Finlay BB. The impact of gut microbes in allergic diseases. *Curr Opin Gastroenterol*. 2012;28:563–569.
- [54] Russell SL, Gold MJ, Willing BP, Thorson L, McNagny KM, Finlay BB. Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. *Gut Microbes*. 2013;4:158–164.
- [55] Russell SL, Gold MJ, Hartmann M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep*. 2012;13:440–447.
- [56] Josefowicz SZ, Niec RE, Kim HY, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature*. 2012;482:395–399.
- [57] Hill DA, Siracusa MC, Abt MC, et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med*. 2012;18:538–546.
- [58] Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013;504:446–450.
- [59] Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med*. 2014;20:159–166.