

Correlation between early-life regulation of the immune system by microbiota and allergy development



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Early postnatal life is a key time for development of the immune system and colonization of the host by microbiota. Recent studies have shown that specific limbs of the immune system can be regulated by microbiota in a time-restricted period during early life. Studies in mouse models have shown that perturbations of the microbiota during early life can cause immune effects that can persist into adulthood and create increased host susceptibility to certain diseases. Here we discuss the role of early-life regulation of the immune system by the microbiota and how it can be related to allergy development. (*J Allergy Clin Immunol* 2017;139:1084-91.)

Key words: Allergy, microbiota, early-life, window of opportunity, neonate

Human subjects are colonized by trillions of microorganisms that include fungi, *archaea*, viruses, *protozoans*, and bacteria. The greater numbers of these microbes can be found in the intestinal tract and in the colon in particular. The past decades have shown a growing interest in studying the phenotype and function of the microbiota in the gut and its roles in immunity and health outcomes. Analyses of microbiota composition in adult human subjects have revealed numerous associations between specific bacterial phyla and disease and elucidated a number of pathways through which this can occur. Perhaps the earliest example is the relationship described between the gut microbiome and the phyla-level changes associated with metabolic pathways and obesity.^{1,2} Since that time, there have been numerous other examples in which alterations in microbiota composition or function, as imputed by metagenomics or direct analysis of the

Abbreviations used

GF: Germ-free
iNKT: Invariant natural killer T
SPF: Specific pathogen-free
Treg: Regulatory T

microbiota, are associated with human diseases. For example, type 2 diabetes is characterized by decreased quantities of butyrate-producing bacteria³ in comparison with cardiovascular disease, which is associated with an increased gut microbiota, driving the production of inflammatory lipid mediators.⁴ In the gut itself microbial dysbiosis is also strongly associated with inflammatory bowel disease development.⁵ This latter example has been quite instructive because it been difficult to determine whether the microbial alterations are primary and causative or secondary events that are derived from intestinal inflammation itself.⁶

Of recent interest is the possibility that many of the disease-associated microbial changes that have been observed and their immune consequences might originally develop during the earliest days of life. During the early postnatal period, the microbiota is in the process of colonizing the host, and its composition is highly unstable. This time of early-life bacterial colonization also correlates with development of the immune system and its education, which allows it to tolerate its environment to fight pathogens and avoid allergy and autoimmunity.

Increasing evidence shows that certain immune cell populations can be regulated by microbiota in a time-restricted fashion. Furthermore, perturbations of the microbiota during a specific early-life time period can have persistent effects on the immune system later in life. This has predicted the existence of a window of opportunity during the early life of the host that is amenable to environmental manipulation. This review describes the immune processes that are regulated by microbiota specifically during early life and their relation to disease development and allergy in particular.

ESTABLISHMENT OF THE MICROBIOTA DURING EARLY LIFE

Mucosal tissues are colonized by microbes that have the capacity to interact with the host. In the adult the gut microbiota can regulate the host's immune response,⁷ metabolism,⁸ and digestion,⁹ which in the latter case provides the mucosa with specific enzymes and other proteins that would not be otherwise produced.¹⁰ Four principal phyla generally comprise a healthy gut

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Terms in boldface and italics are defined in the glossary on page 1085.

microbiome: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria.

Although the fetal compartment has long been considered sterile, colonization of the host by microbes seems to be initiated before birth. Maternally derived bacteria can be isolated from umbilical cord blood of healthy neonates born by means of cesarean section.¹¹ Bacteria are detected in the meconium of pre-term human babies.¹² Labeled *Enterococcus faecium* orally inoculated to pregnant mice can be retrieved in the meconium of the fetus 1 day before birth.¹³ In addition, by using a mutant strain of *Escherichia coli*, a recent study has shown that transient intestinal colonization of a pregnant mouse can exert effects on the offspring's immune system.¹⁴

Newborns are exposed to a large **diversity** of maternal bacteria during birth. Logically, the composition of the newborn microbiota is deeply influenced by the mode of delivery and exposure to bacteria. The microbiota composition of infants born by means of vaginal birth is similar to that of the maternal vaginal and gut microbiota,¹⁵ whereas babies born by means of cesarean section harbor a microbiota resembling the human skin microbiota.¹⁶ These differences in microbiota composition between infants born by means of vaginal birth and cesarean section are persistent, with microbes associated with cesarean section still detectable 2 years after birth.¹⁷ The infant microbiota structure is highly unstable and has low diversity¹⁸ compared with the adult microbiota.¹⁹ The first major shift in intestinal microbiota composition was initially thought to be associated with the introduction of solid food.²⁰⁻²² A more recent study has shown that differences in microbial composition and function associated with solid-food introduction do not become apparent until the infant discontinues breast-feeding, suggesting that the latter rather than the transition to a solid-food diet is the major factor that determines a shift of the microbial ecology toward an adult-like configuration in 12-month-old infants.¹⁵ The human intestinal microbiota further evolves with age and stabilizes after 3 years of life.²³ However, even at 5 years after birth, the gut microbiota might still not be definitely established.²⁴ Thus the precise time

after which the microbiota switches to an individual specific adult type remains to be firmly determined. Dietary and environmental factors are important in molding the composition of the microbiota after birth. Antibiotic use²⁵ and breast-feeding/formula feeding^{26,27} are associated with modifications of microbiota composition. The infant genotype can also influence the early gut microbiota composition, as shown in infants carrying the human leukocyte antigen DQ2 haplotype.²⁸ Therefore the infant microbiota evolves with age and reflects the history of exposure to external factors, as well as host genetics, before stabilizing to an individual adult microbiota arrangement (Fig 1).

This period of microbiota evolution to an adult configuration during early life coincides with development of the immune system. Emerging evidence from rodent studies suggests that part of the immune system is educated by microbiota during early life in a time-restricted fashion. This has been recognized to occur during the neonatal period of life, especially before weaning.²⁹ However, the specific details of these temporal effects in rodents and especially in human subjects remain to be defined. It has also been shown that the immune influences induced by the microbiota during this specific window of time might be a determining factor in resistance or susceptibility to diseases, such as allergy, during infancy and potentially during adulthood.

EARLY-LIFE COLONIZATION AND ALLERGIC DISEASES

The increased incidence of allergy worldwide in the context of progressive urbanization and industrialization has led to investigations about the influence of environmental and dietary factors associated with “westernized” countries that might be involved in the development of allergies. It is now well established that early-life sensitization to allergens influences susceptibility to allergic disease development in later life.^{30,31} The hygiene hypothesis is based on the initial observation that family size and position in the household in childhood were associated with the development of hay fever, asthma, and atopic

GLOSSARY

ARCHAEA: A domain of primitive single-celled microorganisms that lack a nucleus, as well as organelles. Archaea and bacteria make up the 2 domains of Prokaryotae. Archaea are thought to have arisen from extreme settings, such as hot, salty, and/or acidic environments.

DIVERSITY: A term used in bacterial community metrics analysis that includes both richness (ie, the number of different species represented in the community) and evenness (ie, equality in abundance of the species).

ENZYME A20: A cytoplasmic zinc finger protein that inhibits nuclear factor κ B (NF- κ B) activity and TNF-mediated programmed cell death. NF- κ B signaling cascades are heavily controlled by ubiquitination, and several proteins, including A20, can interfere with these processes. Genome-wide association studies have identified A20 as a susceptibility gene in patients with inflammatory disease.

GROUP 3 INNATE LYMPHOID CELLS (ILC3s): Innate immune cells with lymphoid features that do not have antigen receptors. They can quickly respond to multiple tissue-derived factors, such as cytokines, eicosanoids, and alarmins, by producing multiple proinflammatory and immunoregulatory cytokines. ILC classification into groups based on their transcription factors and cytokines parallels helper T-cell subsets. ILC3s resemble T_H17 cells. ILC3s are defined by production of IL-17A and

IL-22 and expression of retinoic acid–related orphan receptor γ t. They have been implicated in immunity against extracellular bacteria and autoimmune diseases.

HELIOS-NEGATIVE REGULATORY T CELL: Natural regulatory T (nTreg) cells are forkhead box protein 3 (Foxp3)⁺ and usually are thymic derived. However, some Foxp3⁺ Treg cells can be peripherally induced. A subset of Treg cells express Helios, a transcription factor with a poorly understood function in Treg cells, but might represent an additional way to differentiate subsets of Treg cells.

INVARIANT NATURAL KILLER T CELLS: A subset of lymphocytes that express surface molecules characteristic of both natural killer (NK) cells (CD16) and T cells (CD3). All NKT cells recognize lipids bound to CD1, an MHC-like molecule. They are capable of rapidly secreting cytokines after stimulation. The T-cell receptor α chains in invariant NKT cells have limited diversity and are characterized by a unique V α 24-J α 18 rearrangement in humans.

PROTOZOANS: Motile single-celled organisms, such as amoeba, containing nuclei and organelles. They are often divided based on kinetic properties. Examples include *Plasmodium* species, *Entamoeba histolytica*, *Trypanosoma* species, *Leishmania* species, *Giardia lamblia*, *Cryptosporidium* species, and *Toxoplasma gondii*.

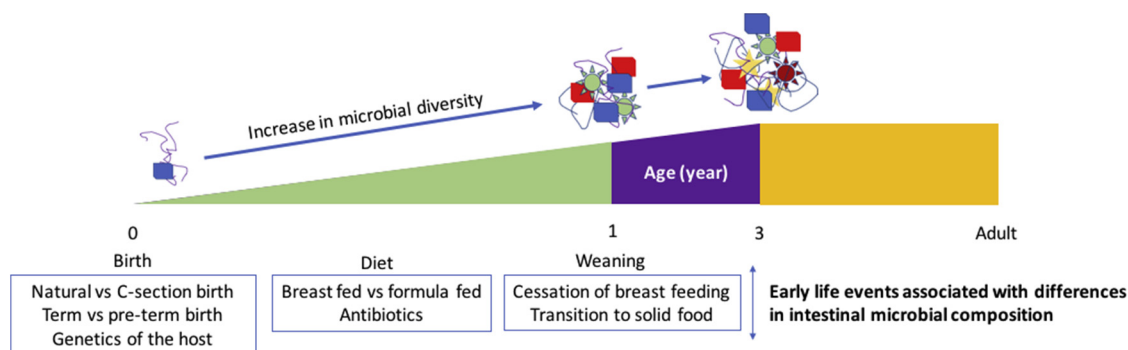


FIG 1. Evolution of microbiota from birth to adult life. Microbial diversity increases from birth to 3 years of life and stabilizes. Genetics, type of birth, diet, and environmental factors are associated with differences in intestinal microbial composition. *C-section*, Cesarean section.

dermatitis, suggesting that exposure to microbial agents during early life can protect against the development of allergic diseases.^{32,33} The implication of this hypothesis is that an absence of proper microbial exposure during early life can lead to increased susceptibility to these disorders. In a subsequent publication Bach³⁴ suggested that this hypothesis might have important implications for a broad range of autoimmune conditions by noting the inverse correlation between decreased infectious disease exposure in Western societies and the increasing incidence of numerous autoimmune conditions.

Clinical studies have provided evidence for a link between bacterial composition in early life and allergy development. The composition of the lung microbiome has been identified as being altered in patients with allergic diseases and asthma in particular. A higher bacterial burden and diversity are observed in the lower airways of asthmatic patients compared with those of healthy subjects.³⁵ The Proteobacteria phylum is particularly enriched in asthmatic patients.^{35,36} This excess of Proteobacteria is interestingly reminiscent of observations in patients with inflammatory bowel disease, raising the possibility that such changes are secondary events.⁶ A definitive association between dysbiosis in the airway and asthma is further limited by inclusion in these studies of patients treated with inhaled corticosteroids that can influence the composition of the airway microbiome. In parallel, perturbations in airway colonization have been associated with early-life asthma.³⁷ In this study colonization of the oropharynx with a diverse microbial species at 1 month of age was associated with increased risk of wheezing and asthma development by age 5 years. Colonization of the nasopharynx in early life has also been shown to influence the risk of recurrent wheezing and asthma development.³⁸ Indeed, early-life asymptomatic colonization with *Streptococcus* microbial species is identified as a strong asthma predictor.

Similar to the airway, early-life perturbations of the intestinal microbiota have also been shown to be associated with allergic diseases. An increased risk of allergic sensitization and allergic rhinitis, but not asthma, in the first 6 years of life is correlated with reduced bacterial diversity of the infant's intestinal flora.³⁹ Infants with atopic eczema exhibit a lower diversity of the gut microbiota.⁴⁰ Moreover, differences in the neonatal gut microflora might precede the development of atopy.⁴¹ Together, these studies suggest that how the host is colonized by microbes during early

life in different mucosal tissues can influence the later development of allergy.

EARLY-LIFE REGULATION OF THE IMMUNE SYSTEM BY MICROBIOTA AND SUSCEPTIBILITY TO ALLERGY

Experimental studies of germ-free (GF) compared with specific pathogen-free (SPF) mice demonstrate that the microbiota is deeply implicated in maturation of the immune system.

Regulation of the immune system by microbiota can be initiated prenatally through transplacental transfer of molecular signatures from the microbiota to the developing fetus, which in the case examined is associated with early postnatal development of *group 3 innate lymphoid cells*.¹⁴ It is interesting that this period coincides with the transplacental movement of IgG and that these observed effects were extinguished in $J_H^{-/-}$ mice, which lack B cells, suggesting they might be causally linked. Postnatally, maternal breast milk contains anti-commensal antibodies that help dampen T-cell responses toward the microbiota in suckling pups.⁴²

Adult GF mice are known to present smaller lymphoid structures, including Peyer patches, mesenteric lymph nodes, and isolated lymphoid follicles.⁴³⁻⁴⁵ These major structural defects are associated with a decrease in the activation, frequency, or both of lymphoid cells in the intestines and other organs, including, $\alpha\beta$ T cells,⁴⁶ B cells,⁴⁷ and innate lymphoid cells,^{48,49} that can be rescued by conventionalization with SPF microbiota at any age. However, the observation that transcriptional differences can be observed in the intestines of GF mice conventionalized during adult life with SPF microbiota compared with those conventionalized at birth⁵⁰ supports the notion that some immune cell abnormalities observed in GF mice cannot be normalized by reintroducing commensal bacteria in the adult but are instead regulated by microbiota only during a specific time period associated with early life. Furthermore, it is now recognized that when this early-life window of opportunity is not properly exposed to microbiota in mouse models, the effects of this inadequate exposure to the early-life immune system can persist into adult life and thus cause permanent consequences for the health of the host.²⁹ Several lines of evidence reveal an association between specific

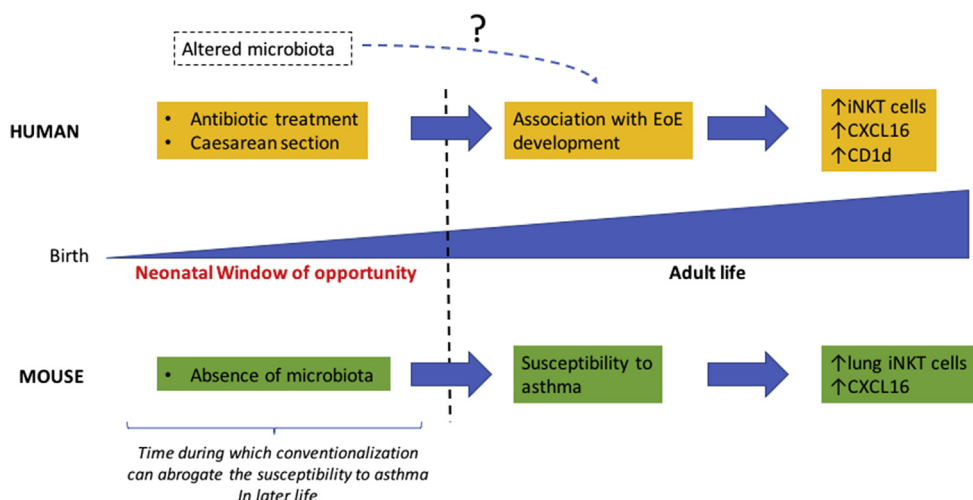


FIG 2. Role of early-life microbiota in the regulation of iNKT cells and development of allergic disease in human subjects and mice. Cesarean section and antibiotic treatment are associated with modifications of the microbiota in early life and EoE development in later life. Whether these microbial modifications are directly related to these diseases in human subjects remains to be established. EoE development is an allergic disease that depends on iNKT cell activation. iNKT cell numbers, as well as CXCL16 and CD1d expression, are increased in patients with EoE less than 5 years of age. These increases are normalized by an elimination diet. An absence of microbiota in GF mice during early life leads to an accumulation of lung iNKT cells, increased CXCL16 expression, and increased susceptibility to asthma in later life. Conventionalization with microbiota during a specific early-life timeframe, but not thereafter, can normalize the number of iNKT cells in the lung and susceptibility to colitis and asthma.

early-life dysregulation of the immune system by microbiota and development of allergic diseases.

Invariant natural killer T cells and eosinophilic esophagitis

Eosinophilic esophagitis (EoE) is a chronic allergen-mediated esophageal disease induced by T_H2 cytokines that is characterized by esophageal dysfunction and eosinophilic inflammation. The antigens that trigger the disease appear to be predominantly found in food, although their nature remains undefined. EoE is a recently recognized condition that exhibits an increasing prevalence in developed and developing countries worldwide.^{51,52} Environmental exposures seem to predispose to EoE. As an example, early-life antibiotic treatment and cesarean delivery are associated with the development of EoE, suggesting that microbiota might have a role in determining the risk for development of this disease.⁵³ EoE was recently shown to be closely associated with a minor specialized subset of T cells, the *invariant natural killer T cell* (iNKT). iNKT cells use an $\alpha\beta$ T-cell receptor heterodimer with limited diversity for the recognition of exogenous (bacterial and nonbacterial) and endogenous (self) lipid antigens specifically presented by the MHC class I-like molecule CD1d.⁵⁴ EoE artificially induced by peanut allergen exposure in mice is dependent on iNKT cells because CD1d-deficient mice that lack iNKT cells are protected from disease induction.⁵⁵ In parallel, a clinical study of children with EoE showed that transcripts for the chemokine CXCL16, the iNKT T-cell receptor marker V α 24, and Cd1d are upregulated in esophageal biopsy specimens of children with EoE, especially during early life.⁵⁶ It was also observed that successful treatment of patients with EoE with dietary elimination of allergens leads to reduction of the

transcriptional signatures associated with infiltrating iNKT cells and normalization of transcript levels for CXCL16 and CD1d. Moreover, this study showed that EoE and the aforementioned increase in CD1d-restricted iNKT cell responses is potentially associated with use of antibiotics during the first year of life based on retrospective surveys, implicating a role for the microbiota during early life in regulation of iNKT cell responses to EoE-associated allergens in later life. Interestingly, the microbiota is a known regulator of iNKT cell presence and function in mice. iNKT cells play an important role in the control of commensal and pathogenic microbiota.^{57,58} At the same time, the microbiota regulates iNKT cells. In adult GF mice, for example, iNKT cells accumulate in the colon and lung, leading to increased susceptibility to an experimental model of colitis and airway hyperresponsiveness that is associated with an increased expression of CXCL16.⁵⁹ The increased quantity of iNKT cells in these tissues and susceptibility to the associated diseases can be normalized by colonization with standard microbiota or treatment with a CXCL16-neutralizing antibody during neonatal but not adult postweaned life. This regulation of CXCL16 is associated with microbiota-mediated epigenetic regulation.⁵⁹ Therefore CXCL16 can regulate iNKT cell accumulation in the lung and colon in a microbiota-dependent but also age- and tissue-specific manner. In addition, monocolonization with the human bacterium *Bacteroides fragilis* or treatment with a *B fragilis*-derived glycosphingolipid during neonatal life can normalize the increased number of iNKT cells in the adult colon but not in the lung in a CXCL16-independent manner, supporting the idea that tissue-specific microbes can regulate iNKT cell function.⁶⁰ Furthermore, these same studies showed that mice that were not conventionalized during the neonatal period or were not monocolonized with *B fragilis* during this time were shown

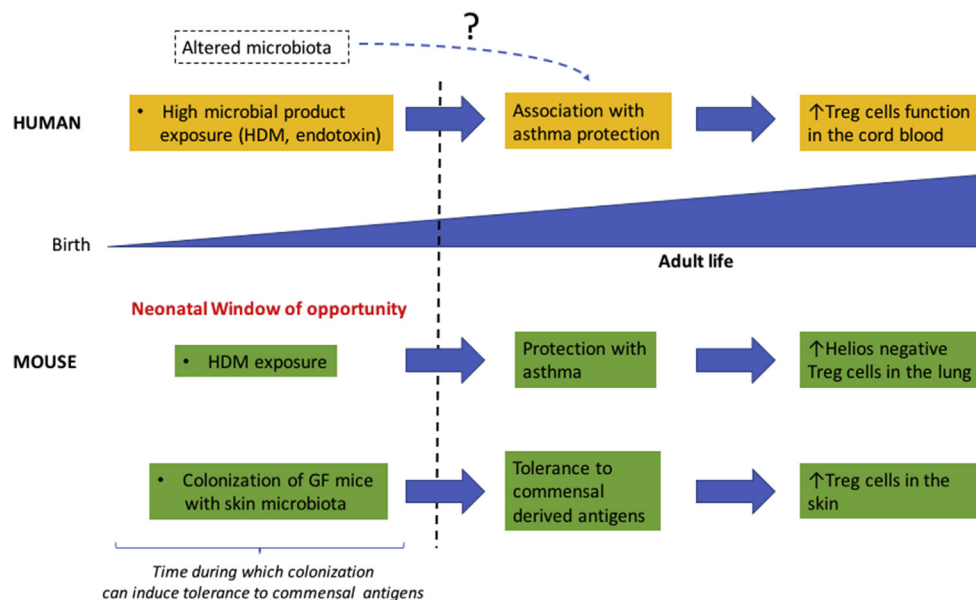


FIG 3. Role of early-life microbiota in the regulation of Treg cells and development of allergic diseases in human subjects and mice. High microbial exposure during early life is associated with asthma protection and increased Treg cell function in fetal cord blood. In mice house dust mite (HDM) exposure during early life but not thereafter protects from asthma caused by the emergence of induced (Helios-negative) Treg cells. Treg cells have the capacity to accumulate in the skin of mice in response to colonization with a skin commensal bacterial strain specifically during early life and are required to establish tolerance to commensal-derived antigens in the adult.

to be highly susceptible to later-life environmental triggers of experimental colitis by using oxazolone⁶¹ or asthma by using ovalbumin sensitization,⁶² both of which are known to be CD1d-dependent responses.^{63,64} Together, these studies show that microbiota can regulate iNKT cells during an early life-restricted window of time and influence later-life susceptibility to inflammatory diseases, such as allergy, in mice. The mouse and human studies described above suggest a prominent role of early-life microbiota in the regulation of iNKT cells and development of later-life susceptibility to allergic diseases (Fig 2).

Regulatory T cells and asthma

The type of environment to which a subject is exposed determines the range of antigens that are encountered by the immune system. Environments that are rich in microbial products have been observed to significantly decrease susceptibility to specific allergic diseases. As an example, exposure to farms during early childhood provides a protective effect against allergy development, including diseases like asthma.⁶⁵ Microbial product exposure during childhood, as measured based on endotoxin levels in mattress dust, is associated with protection against hay fever, atopic asthma, and atopic sensitization.⁶⁶ Interestingly, this protective effect has been observed in farming and nonfarming households in a rural environment.⁶⁶ Farm dust and endotoxin protect against allergy at least in part through a mechanism involving a modification of communication between epithelial cells and dendritic cells in a pathway that is dependent on the ubiquitin-modifying enzyme *A20*, which normally negatively regulates these responses.^{67,68} In parallel, maternal farm exposure is associated with increased regulatory T (Treg) cell function in cord blood of pregnant mothers and protection from asthma in

their offspring.⁶⁹ Furthermore, the environment is known to influence the composition of the microbiota. Accordingly, children with a farm lifestyle have different microbial diversity than those with less contact with farm animals and farm products.⁷⁰ Therefore diversity of the intestinal microbiota might be involved in protection from allergy observed in children exposed to a farm lifestyle, and this can be correlated to specific modifications of the immune system during early life. In fact, work from Pfefferle and Renz⁷¹ have identified a number of potential candidate microorganisms that might be involved in providing protection from asthma.

Interestingly, regulation of Treg cells by the microbiota during early life seems to play an especially important role in the development of allergic diseases in response to environmental exposure based on studies in mice. Mice exposed to house dust mite antigens during the first 2 weeks of life but not thereafter are protected from allergic airway inflammation because of the emergence of an induced *Helios-negative Treg cell* subset.⁷² Emergence of Helios-negative Treg cells after administration of house dust mite is dependent on microbial exposure and correlates with an increase in the bacterial load and Bacteroidetes phyla in the lung. Moreover, the expansion of Treg cells can be abrogated by blocking programmed death ligand protein 1 in early life, resulting in increased asthma susceptibility. In another study Treg cells have the capacity to accumulate in mouse skin in response to colonization with a skin commensal bacterial strain specifically during a window of time that consists of 2 weeks after birth.⁷³ In this study accumulation of Treg cells in the skin during neonatal life is required to establish tolerance to commensal-derived antigens in the adult.

Antibiotic use in neonatal mice drives changes in the microbiota that are associated with an alteration in

colon-associated Treg cells and an increased susceptibility to airway hyperresponsiveness.⁷⁴ Consistent with this, prenatal and postnatal exposure to antibiotics in human subjects is associated with an increased risk for asthma.⁷⁵ Together, these studies show that Treg cell function can be regulated specifically during early life by the microbiota and exposure to specific environmental factors that can regulate their numbers and function (Fig 3). Furthermore, such early-life regulation of Treg cells can have effects that persist into adult life and affect the predisposition to allergy.

IgE and anaphylactic reactions

B cells are the source of different classes of immunoglobulins with different functions. Among them, IgE antibodies can bind Fc receptors on the surfaces of mast cells on recognition of specific antigen and lead to their activation. Activation of mast cells by IgE is central to the development of allergic diseases because it can trigger the sudden release of granules into the bloodstream that contain large amounts of inflammatory mediators, such as histamine, which lead to anaphylactic reactions. GF mice are well known to have decreased levels of other antibody isotypes, such as IgA and IgG₁, which can be normalized by conventionalization by the microbiota in adult mice.⁴⁷ In contrast, GF mice and mice with low-diversity microbiota have increased IgE levels in serum that are associated with increased levels of IgE isotype switching within mucosal sites in a pathway that is dependent on CD4 T cells and IL-4.⁷⁶ These increased IgE levels in serum lead to an increase in IgE binding to the mast cell surface and exaggerated orally induced systemic anaphylaxis. Only conventionalization with standard microbiota within 4 weeks after birth can normalize IgE levels in adult mice. Interestingly, a correlation has been observed between specific fecal microbial species in the infant and IgE-mediated food allergy.⁷⁷ These studies showed that the microbiota can regulate IgE expression in an age-restricted fashion during early life in mice and that dysbiosis during infancy might be a predisposing factor to IgE-mediated allergy development.

CONCLUDING REMARKS

Among other hypotheses about the cause of allergy, the hygiene hypothesis states that exposure to microbial agents during early life can protect against the development of allergic diseases and *vice versa*. This hypothesis has been gaining acceptance because it is becoming increasingly clear that exposure to the microbiota during a specific early-life window of time is essential for the normal development of certain elements of the immune system. The perturbations that develop when appropriate microbial signals are not received during this window of opportunity can have long-lasting effects on the immune system, the duration of which is still yet to be defined, resulting in susceptibility to diseases associated with these tissue compartments, such as colitis, asthma, atopic dermatitis, anaphylaxis, and potentially others. The exact timeframe of the window of opportunity during which interventions for allergic protection might be successful still needs to be determined, as well as the relevant equivalence for translation into human studies. Because there is strong epidemiologic correlation between early-life exposures associated with removal of (eg, by antibiotics) or exposure to (eg, rural exposures) microbes during early life in human subjects and the setting of

allergy, understanding these pathways is of outmost importance to develop beneficial interventions in human subjects for protecting against allergic diseases. In the future, one of the principal challenges will be to determine the mechanisms that allow the immune system to be regulated by microbiota in a time-restricted fashion.

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