Published in final edited form as:

J Allergy Clin Immunol. 2013 June ; 131(6): 1465–1478. doi:10.1016/j.jaci.2013.04.031.

Effect of barrier microbes on organ-based inflammation

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Abstract

The prevalence and incidence of chronic inflammatory disorders, including allergies and asthma, as well as inflammatory bowel disease, remain on the increase. Microbes are among the environmental factors that play an important role in shaping normal and pathologic immune responses. Several concepts have been put forward to explain the effect of microbes on the development of these conditions, including the hygiene hypothesis and the microbiota hypothesis. Recently, the dynamics of the development of (intestinal) microbial colonization, its effect on innate and adaptive immune responses (homeostasis), and the role of environmental factors, such as nutrition and others, have been extensively investigated. Furthermore, there is now increasing evidence that a qualitative and quantitative disturbance in colonization (dysbiosis) is associated with dysfunction of immune responses and development of various chronic inflammatory disorders. In this article the recent epidemiologic, clinical, and experimental evidence for this interaction is discussed.

Keywords

Chronic inflammation; allergy; asthma; inflammatory bowel disease; hygiene hypothesis; microbiota hypothesis; biodiversity; dysbiosis; microbiome

Noncommunicable diseases (NCDs) represent an emerging health problem of global dimension. Allergies and autoimmune diseases are 2 major NCD entities that have been increasing in prevalence and incidence, particularly since the end of World War II. The highest disease burden is observed in westernized and industrialized countries; however, in the developing regions of the world, NCDs are also becoming increasingly prevalent, and

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Disclosure of potential conflict of interest: H. Garn has consultant arrangements with, is employed by, and has stock/stock options with Sterna Biologicals; has received grants from DFG, BMBF, Hessen, BMWI, and Behring/Rontgen Stiftung; and has received payment for lectures from Mead Johnson Nutrition. H. Renz is President of DGAKI and is on the Seminar Committee for the American Academy of Allergy, Asthma & Immunology; has consultant arrangements with Allergopharma, ALK-Abelló, Bencard, Sterna Biologicals, Novartis, and Boehringer Ingelheim; receives grants from DFG, BMBF, the European Union, Land Hessen, Stiftung Pathobiochemie, Ernst-Wendt-Stiftung, DAAD, and ALK-Abelló; and has received payment for lectures from Allergopharma, Novartis, Abbott, Med-Update, and cmeAkademie. The rest of the authors declare that they have no relevant conflicts of interest.

current numbers are similar to those observed a few decades ago in westernized and industrialized regions.¹

Allergies and autoimmune diseases are chronic inflammatory conditions perpetuated by inappropriate immune responses to antigens. There is now accumulating evidence for the contribution of microbes to chronic inflammatory diseases, including classical autoimmune conditions, such as rheumatic arthritis, systemic lupus erythematosus, and vasculitis. However, the largest body of evidence has been obtained in the areas of allergy and chronic inflammatory bowel disease (IBD), which will be the focus of this article. Allergies primarily manifest at the site of environmental barriers (the skin and the mucosal surfaces) as the result of an inappropriate immune response to otherwise harmless environmental antigens, such as food allergens and aeroallergens. The major clinical conditions comprise atopic eczema, allergic asthma, allergic rhinoconjunctivitis, and food allergies. A common immunologic pattern, at least during the acute phases of disease exacerbation, is the development of the T_H2-dominated immune response controlling eosinophilia, IgE production, and subsequent mast cell activation.²

IBD is a group of disorders that chronically affect the intestines in a relapsing and remitting manner. Clinically, there are 2 subtypes of IBD that are diagnosed based on clinical presentation and examination of mucosal tissues by using endoscopy, radiology, and pathology. These 2 forms of disease are ulcerative colitis (UC), which is a superficial inflammation restricted to the colon, and Crohn disease (CD), which is a transmural inflammation that can affect any area of the gastrointestinal tract. Most commonly, CD affects the colon alone in approximately one third of patients, the small intestine alone in approximately one third of patients, and both the small intestine and colon in the remainder of subjects.

There has been a great deal of progress in understanding the pathogenesis of these disorders. These studies have shown that IBD represents a central problem in the tridirectional relationship between the commensal microbiota, intestinal epithelium, and immune system that is associated with intestinal tissues.^{3,4} Each of these 3 aforementioned factors is in constant communication with the others and is regulated by genetic and environmental factors. The investigation of these relationships have supported a model in which IBD represents an inappropriate mucosal immune response that is directed against the commensal microbiota and potentially directly against the host tissues (autoimmunity) in a genetically susceptible host, which are in turn determined by a variety of yet to be defined and poorly understood environmental factors.⁵ Whether IBD can be characterized as an autoimmune disease or is an immune-mediated disease that uses similar pathogenic mechanisms but is oriented toward components of the microbiota remains an unresolved matter. To date, although autoantibodies can be identified in patients with IBD, such as antineutrophilic cytoplasmic antibodies in the case of UC,^{6,7} it has yet to be demonstrated that any of the putative autoantigens to which these autoantibodies are directed play a direct pathogenic role in patients with the disease.

It is clear that IBD has a strong genetic basis.^{5,8,9} This has been recognized since the original definition of these disorders, given the well-recognized familial associations that

characterize these clinical conditions. *Genome-wide association studies (GWASs)* have provided significant support for a genetic basis of these disorders.^{8,9,10–18} GWASs have identified more than 160 loci that confer a risk or protection from these diseases.⁹ Interestingly, approximately two thirds of these genetic risk loci are shared between CD and UC, which counts for the overlapping immunopathogenesis and response to similar treatments for both of these disorders.^{17,19} These observations in GWASs support the existence of functionally important pathogenic modules in these diseases and, more to the point, have focused further attention on the immune response of the host to microbiota as a fundamental feature of these disorders. A large number of the genetic risk factors associated with IBD involve the manner in which the intestinal epithelium, as well as the innate and adaptive immune pathways, interact with and respond to the commensal microbiota.^{5,20} Interesting in this regard are observations that the genetics of IBD overlap significantly with the genetics of other infectious disease disorders, especially mycobacterial infections.⁹ This suggests that in patients with IBD, pathways associated with the appropriate response to infectious pathogens are inappropriately operative and directed at the commensal microbiota. Alternatively, and yet to be proved, is that IBD represents the response to a yet to be defined and unidentified infectious pathogen. There has been a great deal of work over the years to identify potential pathogens as a basis for these disorders.²¹ However, none has been yet defined. This has led to the current belief that IBD represents an inappropriate immune response to the commensal microbiota.²²

What is the cause of this dramatic development, mounting an “epidemic in slow motion”?²³ Allergies and IBD develop as a consequence of complex gene-environment interactions.^{24,25} A substantial effort has been undertaken to characterize the genetic constitution of these conditions, resulting in the identification of multiple *polymorphisms*, particularly in genes regulating mucosal and barrier integrity, as well as innate and adaptive immune functions.^{5,9} Although the genetic makeup certainly contributes to disease manifestation, genetics alone cannot explain the dramatic increase in NCDs, which has been observed over only few decades. Therefore attention turns to the other side of the equation, which is the role of environmental factors on the development of inflammation or *vice versa* the protection thereof. Over the years, several hypotheses and concepts have been developed to explain the contribution of environmental factors on chronic inflammatory diseases.

THE HYGIENE HYPOTHESIS

The hygiene hypothesis was developed in the late 1980s. A starting point was the observation that children with older siblings show a consistent and significant protection, particularly against respiratory allergies.²⁶ A similar effect was observed for frequent viral infections in young children, the anthroposophical lifestyle (R. Steiner theory), the effect of early day care, and being raised under the conditions of traditional farming, as originally observed in the Alpine region of central Europe.²⁷ These observations were linked to a lack of infectious microbes, particularly in early childhood. In its original sense the hygiene hypothesis focused on the effects of environmental microbes in shaping normal immune responses closely linked to clinical and immunologic tolerance. A number of environmental microbes have been recently identified and were further functionally characterized with regard to their capacity to modulate immune responses (discussed below).

THE MICROBIOTA HYPOTHESIS

That endogenous microbes might play an important role in shaping (early) immune responses represents a rather old concept.²⁸ However, until recently, because of the lack of appropriate technologies, it was not possible to comprehensively study the human microbiome in a qualitative and quantitative fashion, as well as in terms of functional consequences for immune development. The availability of genetic techniques opened a new dimension in this area. The microbiota hypothesis starts with the assumption that a modern/industrialized lifestyle leads to altered microbial diversity. This altered diversity is closely associated with the loss of ancient and coevolved microbes that have a strong effect on immune responses.²⁹ The disappearance of these partners leads to dysbiosis and a dysfunctional immune system, which is not able to mount tolerance in an appropriate fashion, resulting in the development of chronic inflammatory disease. The microbiota hypothesis focuses on (organized) microbial colonization of the skin and the mucosal surfaces. This concept is best studied for the intestinal microbiome.

THE BIODIVERSITY HYPOTHESIS

This hypothesis is an extension of both the hygiene and microbiota hypotheses. According to this concept, environmental biodiversity is closely linked to the composition of bacterial colonization. Our biodiversity consists of all organic biomaterial directly or indirectly originated from plants and animals. Biodiversity includes but is not restricted to the contact to animals, living conditions in the home, dietary habits, use of antibiotics, and other lifestyle factors.³⁰ Therefore the biodiversity hypothesis provides a link between changes in lifestyle conditions, altered microbiota, and disease development.

Here we will discuss recent evidence for the role of the microbiota in shaping normal immune homeostasis (Fig 1). We will focus primarily on the gut as the best studied site of microbial colonization. Furthermore, recent evidence for a connection between altered colonization (dysbiosis) and inflammatory immune responses will be discussed in the context of chronic IBD and allergic conditions.

MICROBES CONTRIBUTE TO THE DEVELOPMENT OF IMMUNE RESPONSES

The gastrointestinal microbiota represents the by far best studied ecosystem of the microbiome coexisting with human subjects. This system consists of more than 100 trillion micro-organisms, most of which are bacteria. Two of about 55 known phyla (Firmicutes and Bacteroidetes) dominate the human intestinal tract. This illustrates the relative exclusivity of the human intestinal microbiota, indicating the special relationship between us and them. The individual microbiome consists of approximately 15% of the much more than 1000 thus far identified species.^{31,32} This ecosystem has coevolved with the appearance of vertebrates, which, from an immunologic point of view, are characterized by the presence of an adaptive immune system mainly defined by the presence of T and B cells.

Under normal homeostatic conditions, these microbiota establish a relatively stable pattern. It has been recently recognized that there is a very close bidirectional interaction between these bacteria and our immune system.³³ Some microbes provide us with essential nonnutrient factors, such as vitamins. We also benefit from their energy machinery, which increases our harvest of nutrients in the gut. Furthermore, of great importance is the fact that microbial triggers are necessary for the development and maturation of our immune system, as recently summarized in several important reviews.^{34,35} In this regard *germ-free (GF) mice* represent an important model system to study the effect of (controlled) colonization on the innate and adaptive immune system.^{36–39}

It is of great importance that the host organism controls the exposure to barrier microbes to benefit most from interaction with the them. Direct contact with the microbes must be minimized and the exposure to penetrating bacteria needs to be limited to keep the immune system in balance.

Dysbiosis describes the disturbed balance in the barrier microbiota, resulting in perturbed immune regulatory functions and altered metabolic activities. Dysbiosis has been described in association with many diseases, predominantly those of the gut, such as chronic IBD. However, as recognized lately, extraintestinal immune-mediated conditions show dysbiosis at various barriers.^{28,40,41}

DYSBIOSIS AND CHRONIC IBD

The commensal microbiota associated with the intestinal surfaces is a highly complex microbial ecosystem that is significantly influenced by host and environmental factors. Given the fact that IBD incidence is increasing around the world, including in regions that have previously been characterized by low rates of prevalence, such as Asia and the Middle East, it is increasingly clear that environmental factors are critically important to the pathogenesis of these disorders and that the effect of these environmental factors, especially on the commensal microbiota, are especially important.¹ It is therefore interesting that antibiotics, for example, can have profound effects on the composition of the microbiota and that these changes in the microbiota caused by antibiotic ingestion can be durable and persistent.^{29,42,43} Interestingly, early-life exposure to antibiotics (during the first year of life) has been associated with increased risk for the development of IBD in later life, as has been previously observed in the relationship between early-life antibiotic exposure and other inflammatory disorders, such as asthma.^{44–47} Such epidemiologic observations further highlight the critical role that early-life microbial exposures have for the development of the immune system and the role that this plays in either protection from or susceptibility to diseases, such as IBD, later in life. Studies in GF and antibiotic-treated mouse models have shown this to be extremely important and focused attention on the role played in the activity of specific immunologic pathways, such as *CD1d*-restricted presentation of lipid antigens to *natural killer T cells*.^{48–50} In the absence of normal microbial exposure during the earliest periods of life in animal models, the host becomes highly susceptible to environmental triggers that activate this subset of cells and induce the development of organ-based inflammation in the colon.⁵⁰ This has been shown to be important not only for the pathogenesis of IBD but also for asthma.^{50,51}

As noted, the human colon and distal small intestine represent the most dense collections of microbial communities that are associated with the human body. Recent studies have demonstrated that the phylogenetic composition of the bacterial communities associated with the intestines varies with age and assumes its adult configuration in the first 2 to 3 years of life.⁵² During this early period of time, the diversity of these microbial communities increases, and stability is generated.⁵² Interestingly, despite the fact that there is a unique microbial composition for each subject, these microbial taxa converge on a common group of metabolic pathways that are relatively consistent within a human population.^{52,53} Beyond the third year of life, the bacterial community in terms of intraindividual diversity shows a rather stable distribution, although a great degree of interindividual variation exists. On the level of phyla, the intestinal colonization starts with a predominance of Firmicutes. Shift and modification in the commensal microbiota correspond very closely with changes in diet and health, as recently indicated.⁵⁴ Interestingly, both the bacterial diversity and complexity of metabolic pathways associated with the commensal microbiota in intestinal surfaces is far less rich and diverse in Western subjects compared with subjects from nonwesternized cultures, such as in Malawi or among Amerindians.⁵² This suggests that abnormalities in the commensal microbiota might represent an important source of risk for the development of IBD in more westernized populations and thus might be antecedent to the development of disease. Thus the pressures of westernization can have major effects on the function of the microbiome and thus the risk for IBD.

There is a large quantity of information available that argues in favor of an important role of the intestinal microbiota and the pathogenesis of IBD. The observations supporting this have been extensively reviewed elsewhere.^{20,21,55,56} In addition, it is also clear that there are unique alterations of the intestinal microbiota in patients with both CD and UC that distinguish the microbiota from that of healthy unaffected subjects within the same geographic region.⁵⁷ A number of studies have shown that a common feature of these alterations among the bacterial phyla that are identifiable in the human microbiota of patients with IBD are characterized by decreased Bacteroidetes together with increased Actinobacteria and typically Proteobacteria in patients with UC.⁵⁸ In patients with CD, decreased biodiversity of the Firmicutes with disappearance of particular Firmicutes, such as *Faecalibacterium* and *Roseburia* species, together with altered composition of the *Bacteroides* species, as well as increased Proteobacteria, are observed.^{57,59} One organism of interest that has commonly been observed to be lost or decreased in patients with CD is the Firmicute *Faecalibacterium prausnitzii*.^{60,61} This Firmicute is quite interesting because it is associated with regulatory properties in mouse models, such as the trinitrobenzene sulfonic acid-induced model of colitis.⁶⁰ Human subjects who lack this organism are at increased risk for relapse of CD after surgical resection.⁶⁰ Levels of other organisms, such as enteroadherent and invasive *Escherichia coli*, are also observed to be increased in patients with CD and exhibit proinflammatory activities after binding to the intestinal epithelium in the distal small intestine.^{61,62} Together, these studies suggest that the loss of protection and the bloom of aggressive proinflammatory microorganisms might characterize IBD.

Although these comments have focused on bacteria, there is increasing reason to believe that there is an important role to be played by viruses, fungi, and potentially other life forms in

the pathogenesis of IBD and potentially other immune-mediated (autoimmune) diseases. In the case of viruses, the phenotypic Paneth cell manifestations associated with hypomorphic mutations of the autophagy gene, *Atg16l1*, requires the presence of murine norovirus in animal models.⁶³ Similarly, a significant fraction of patients with CD exhibit abnormal immune responses to the polysaccharide antigens of *Saccharomyces cerevisiae*.⁶⁴ Although the mechanistic basis for this is unknown, it is now recognized that not only do fungi normally reside within the milieu of the normal commensal microbiota but also their responses to these within-mucosal tissues are controlled by lectin receptors, such as dectin-1 and *T_H17* cell pathways, both of which are affected by genetic polymorphisms that confer a risk for the development of IBD.^{65–69} It can thus be hypothesized that aberrant host responses to viruses and fungi might play an important yet to be defined role in the pathogenesis of both forms of IBD.

A major question in IBD research is whether these changes in the microbiota are primary and occur before the development of IBD or are secondary to intestinal inflammation. This controversy remains unresolved, but there is a significant amount of information available to suggest, at the very least, that there is a meaningful secondary component to these alterations. For example, mice with a genetic deletion of *Il10*, which predisposes to the development of intestinal inflammation when infected with *Citrobacter rodentium*, a model of enteropathogenic *E coli*, or mice exposed to a chemical agent that is disruptive to the intestinal epithelium, dextran sodium sulfate, all have intestinal inflammation in association with alterations of the microbiota that are quite similar to those described in patients with IBD, namely a disruption of Firmicutes and Bacteroidetes together with a bloom of Proteobacteria.⁷⁰ However, even if the changes in patients with IBD that have been observed in at least one third of these patients are secondary to the intestinal inflammation, understanding these changes is critically important for diagnosis, prognosis, and therapeutic strategies when approaching these disorders.

In summary, it is clear that there is an important role for the commensal microbiota in both determining the risk for and defining the pathogenesis of IBD. With regard to the former, current models predict that inappropriate colonization of the mucosal tissues during critical periods of life when immune development is occurring are likely to be important determinants of risk or protection in the development of these disorders (Fig 2). Thus understanding the environmental and genetic factors that regulate the colonization and composition of the mucosal surfaces with microbiota are critically important in understanding these disorders. Similarly, studies into the genetics, immunology, and microbiology of IBD clearly demonstrate the importance of understanding the relationships among these 3 factors and the contributions of environmental agents in determining the pathogenesis of these disorders. Abnormalities in the manner in which the intestinal epithelial barrier, the innate immune system, and the adaptive immune system interact with the commensal microbiota are critical mechanisms that are associated with the development of these disorders. Given these comments and observations, it is hoped that from these experimental lessons, we will have the opportunity to not only treat these conditions through manipulation of immune response but also through engineering the composition and function of the commensal microbiota to avoid them in the first place or correct them.

DYSBIOSIS AND ALLERGIC DISEASE

There is accumulating evidence for a close association between microbial dysbiosis and several allergic phenotypes, including eczema and food allergy. Most of these studies focus on gastrointestinal colonization. However, there is also some evidence that microbial colonization at other surfaces, such as the skin or respiratory tract, might also be associated with clinical phenotypes. Starting at birth with a sterile gut compartment, the first few days and weeks set the stage for the initial colonization pattern. This is markedly influenced by several factors, including the mode of delivery, maternal (parental) colonization, disease status of the mother, and others. During infancy and early childhood, other environmental and lifestyle components further contribute to microbial diversity, including nutritional behaviors. One of the critical questions resulting from these human studies is the question about a possible cause-effect relationship between microbes and disease development. Here a number of experimental strategies involving murine models help to understand the effect of defined microbes on immune function and dysfunction. In addition, the environment might also contribute to these processes. We will discuss each of these components in the following subsections.

Intestinal colonization in early life

Many epidemiologic studies indicate that the prenatal and postnatal period represents an important window of opportunity to modulate immune responses, affecting the development of allergies later in life (Fig 3). Prominent examples are the studies conducted in the traditional farming environment, which indicate that exposures during pregnancy and within the first 3 years of life are particularly important in this regard.⁷¹

Until rupture of the amniotic sac, the fetus is essentially considered sterile. Immediately after delivery, colonization of the neonate's gut starts to take place.⁷² The mode of delivery represents an important determination, influencing the early colonization pattern in the neonate. In vaginally delivered babies the flora, at least during the first 6 months, resembles closely the microbial population of the mother's vagina.⁷³ Lactobacillae represent the dominant population.⁷² In contrast, infants delivered by means of cesarean section show an early colonization pattern that closely resembles the mother's skin.⁷³ The skin and vagina are 2 unrelated habitats, with a predominance of the phyla Actinobacteria, followed by Firmicutes, Proteobacteria, and, to a lesser extent, Bacteroidetes at the skin; the vagina is overwhelmingly dominated by the phylum Firmicutes.^{74–77}

The maternal gut represents an important source of bacteria for early colonization, particularly in vaginally delivered babies.⁷² Facultative anaerobes, such as *E coli* and *Streptococcus* species, dominate the first few days.⁷⁸ *Bifidobacterium*, *Bacteroides*, and *Clostridium* species follow by 1 to 2 weeks of age because of oxygen deprivation.⁷⁹ In contrast, babies born by means of cesarean section show an up to 12 months' delayed colonization pattern with these bacteria. In these babies *Clostridium*, *Enterobacter*, and *Klebsiella* species, originating from the (nonliving) environment, expand rapidly in the gut. The flora composition of Western infants has apparently dramatically changed over the last few decades, with a decrease in early *E coli* and *Bacteroides* species colonization paralleled by an increase in *Staphylococcus* species colonization.^{80,81}

This close relationship between the maternal microbiota at various body sites and the intestinal colonization pattern of the neonate and infant stimulated the question of whether the mode of delivery would have an effect on the risk of allergy development later in life. In 2009, a meta-analysis was published that is based on a Medline search of clinical studies published between 1966 and 2007.⁸² This meta-analysis included a total of 26 studies and calculated an increased risk for delivery by means of cesarean section and the development of food allergies (odds ratio [OR], 1.45), allergic rhinitis (OR, 1.24), asthma (OR, 1.18), and hospitalization for asthma indicative of severe asthma (OR, 1.21). However, more recent data indicate that the situation is much more complex. A recently published Finnish nested case-control study detected an increased risk of infants born by means of cesarean section for the development of cow's milk allergies within the first 2 years of life (OR, 1.18).⁸³ A Norwegian study including more than 37,000 children (Norwegian Mother and Child Cohort Study) found an increased likelihood of current asthma at 36 months of age (OR, 1.17), which was even stronger among children of nonatopic mothers (OR, 1.33).⁸⁴ A retrospective cohort analysis of approximately 250,000 records of births between 1970 and 1989, which was conducted in Oxford from the Oxford Record Linkage Study, found an increased OR of 1.2 for the hospitalization for asthma.⁸⁵ A recently published Dutch study found a decreased risk of sensitization to food allergies (OR, 0.52) and asthma (OR, 0.47) only among vaginally delivered home-born infants with atopic parents.⁸⁶ However, the data still remain inconsistent because several other studies could not detect an association with increased risk of food allergy,⁸⁷ egg allergy by 1 year of age,⁸⁸ and risk of wheezing in childhood and adolescence in the retrospective analysis of a birth cohort study in Brazil.⁸⁹ Although important possible confounders, such as atopic status of the mother, maternal age, smoking, breast-feeding, and others, have been considered in most of these studies, the possible association between delivery by means of cesarean section and the development of allergic phenotypes early in life still requires further analysis to establish a cause-effect relationship with (early) colonization of the gut and perhaps other (mucosal) surfaces. In particular, prospective studies are required that should include detailed analysis of microbial colonization of the infants, as well as functional studies on their immune responses.

The early feeding regimen represents the next important factor determining (early) colonization. Although the data are inconsistent and somewhat contradictory, as elegantly reviewed recently,⁸⁰ it appears that breast-fed babies are less colonized with *E coli*, *Clostridium difficile*, and *Bacteroides* species, whereas higher counts are detected for *Staphylococcus* species. The infant's microbiome is not only shaped directly through exposure to maternal and environmental bacteria, but in addition, maternal immune factors modulate indirectly the development of the infants' microbiome. Secretory IgA antibodies, which are abundant in breast milk, play an important role in this regard. The maternal secretory IgA repertoire is shaped under the influence of her own microbial exposure and is fully delivered to the baby through breast milk, not only providing passive protection but also favoring the development of the infant's regulatory immune network.⁹⁰ However, the (early) bacterial colonization itself also contributes to shaping the secretory IgA and IgM network. This was previously linked particularly to the colonization with *Bacteroides fragilis* (but not *Bifidobacterium* species or lactobacilli-like microbes) at the age of 1 month.⁹¹

Intestinal microbiota and allergies in infancy and childhood

Because the first few years of life have been identified as an important and vulnerable period for the establishment of a stable intestinal microbiome later in life, the question arises whether altered microbial colonization (= dysbiosis) is associated with the development of certain allergic phenotypes. This topic is of increasing interest.

Studies performed after the fall of the “iron curtain” in Eastern and Western European countries indicated significant differences in the composition of gut flora, pointing to the importance of lifestyle factors (nutrition and others) on the development of the intestinal microbiome. Furthermore, these studies also demonstrated marked differences between atopic and nonatopic infants, with lower counts of lactobacillae, bifidobacteriae, and *Bacteroides* species, together with higher counts of *C difficile* in atopic infants.^{92–94} A similar trend has also been found in a Finnish study with more clostridia and fewer bifidobacteriae at 3 weeks of age in sensitized versus nonsensitized infants (1 year of age).⁹⁵ This is supported by a Dutch prospective birth cohort study showing that the presence of *C difficile* in stool samples at 1 month of age was associated with increased risk of several atopic phenotypes at 2 years of age.⁹⁶ Furthermore, *E coli* was associated with a higher risk for eczema. In contrast, a recent study on children aged 3 to 5 years found no significant differences in lactic acid bacteria or bifidobacteriae in children with and without atopic wheeze (indicative of asthma).⁹⁷ Another study examined 11 bacterial groups and calculated the ratio of definitive anaerobic to facultative anaerobic bacteria in neonatal stool samples and found no association with the development of eczema or food allergy at the age of 18 months.⁸¹ However, a follow-up of a subgroup of these infants revealed a reduced diversity in early fecal microbiota in infants with atopic eczema later in life.⁹⁸

Important differences in the colonization pattern were not only reported on the genus level but also on the species level. A small preliminary study found in breast-fed allergic infants a predominance of *Bifidobacterium adolescentis* together with a lower colonization rate with *Bifidobacterium infantis*, *Bifidobacterium breve*, and *Bifidobacterium longum*⁹⁹ compared with that seen in breast-fed healthy control subjects. A comprehensive review on this complex situation has been published.¹⁰⁰

With the advent of modern, non-culture-based technologies, recent data now provide further insight into the temporal association between dysbiosis and allergy phenotypes. A prospective cohort was examined in Norway using repeated fecal sampling, followed by **quantitative RT-PCR** analysis of 12 different bacterial species and revealed that subjects with atopic sensitization (representative of T_H2 development with IgE production) at an age of 2 years had lower *E coli* levels at 4 months and 1 year of age, higher levels of *B longum* at 1 year of age, and lower levels of *B fragilis* at 2 years of age. For *E coli* and *B longum*, the differences were only transient and disappeared at 2 years of age. Analysis of fecal bacteria composition by means of 16srRNA gene **pyrosequencing** in a Japanese cohort revealed that allergies at the age of 2 years (food allergy, asthma, and atopic eczema determined by means of questionnaire) were associated with a reduction in the genera *Acinetobacter* and *Clostridium* at 1 month and higher abundance of *Bacteroides* species at 1 month and *Propionibacterium* and *Klebsiella* species at 2 months. Infants with a higher *Bacteroides*

species colonization, *Klebsiella* species colonization, or both showed less colonization of *Clostridium perfringens* and *Clostridium butyricum*.¹⁰¹

A study performed in Singapore examined stool microbiota in cesarean section–delivered and total formula-fed infants through the first 2 years of life. In the group of infants with atopic eczema, a marked overrepresentation of Proteobacteria and Firmicutes was found within the first year of life, whereas the noneczema group had a higher representation of Actinobacteria. These differences disappeared at the age of 2 years.¹⁰² In a Spanish study fecal samples of 2- to 12-month-old nonallergic children or children with cow's milk protein allergy were analyzed by means of *in situ* hybridization by using a panel of 10 rRNA probes. They found in the cow's milk allergy group a higher proportion of *Clostridium coccoides* and of the Atopobium cluster compared with healthy subjects.¹⁰³

The microbial diversity of the gut microbiota has been extensively studied recently in the context of allergic manifestations in early life. These studies used powerful, cultivation-free molecular methods, such as 16srRNA and PCR amplification and sequencing technologies. In a recent study¹⁰⁴ stool samples were analyzed at 1 week, 1 month, and 12 months of age and associated with atopic eczema at 2 years of age. In the eczema group a lower diversity of total microbiota of the phylum Bacteroidetes and the genus *Bacteroides* were detected at 1 month. Furthermore, the microbiota showed a high degree of diversity and interindividual variability and stabilized around 12 months of age. Proteobacteria, which comprise gram-negative strains, were more abundant in infants without allergic manifestations at the age of 12 months. Similarly, in another study¹⁰⁵ microbial diversity was found to be significantly lower at day 7 of life in infants with eczema at the end of the first year. An international study presented a similar finding.⁹⁸ These investigators collected fecal samples at 1 week of age and again found a reduced diversity in the early fecal microbiota of infants with atopic eczema during the first 1½ years of life. An elegant prospective study in Denmark¹⁰⁶ presented the data on a birth cohort of more than 400 high-risk children who were followed for 6 years for the clinical presentation of atopic phenotypes. The early intestinal flora at 1 and 12 months after birth showed a reduced diversity in infants with IgE sensitization, positive skin prick test responses, eosinophilia, or allergic rhinitis. However, no association was found for asthma and atopic eczema. The development of asthma has been investigated in another study.¹⁰⁷ These investigators also performed a prospective birth cohort study on 110 children and were able to show that fecal colonization at the age of 3 weeks with either a *B fragilis* subgroup or a *C coccoides* subcluster was an early indicator of possible asthma later in life.

Taken together, there is now ample evidence for dysbiosis in general of the intestinal microbiota in children with various allergic phenotypes within the first few years of life (Fig 4). The data indicate that the changes in gut microbiota composition precede development of the clinical manifestation. However, the cause-effect relationship between these altered microbiota and development of the clinical phenotype still remains unclear. Also, until now, no clear-cut pattern of species or genus involved in this dysbiosis can be depicted from the studies available thus far. Furthermore, there is still some inconsistency in terms of the clinical manifestation being affected. Most consistently, a relationship between IgE-dependent sensitization, atopic eczema, and food allergies with an altered intestinal

microbiota has been reported (Table I). The reason for this could be that these are also the earliest clinical and immunologic phenotypes of allergies in life. However, in terms of respiratory allergies (asthma or allergic rhinitis), the data are still conflicting. Altogether, however, a common pattern in most of the studies is a reduced diversity of the intestinal microbiota, which manifests apparently very early in life, perhaps even within the first few weeks of life.

Parental influence on infants' colonization

Parental allergies might have an effect on colonization patterns early in life. In a prospective Swedish study, it was shown that infants from nonallergic parents were more frequently colonized by lactobacilli compared with infants with allergic parents. Children who remained nonallergic at an age of 5 years were colonized with lactobacilli on several occasions during the first 2 months of life. They acquired lactobacilli more frequently during the first weeks of life than their allergic counterparts; this was irrespective of parental allergy. Furthermore, more nonallergic than allergic (at 5 years) children were colonized with *Bifidobacterium bifidum* at 1 week of age. This study shows that heredity for allergy might have an effect on the gut microbiota in infants. Early lactobacilli colonization (*Lactobacillus casei*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus*) seems to decrease the risk for allergy at 5 years of age, despite allergic heredity.¹⁰⁸ In addition to the mode of delivery, one of the maternal factors influencing the early colonization pattern of the babies is the maternal intestinal flora itself. It was recently shown that high accounts of maternal total aerobes and enterococci were associated with increased risk of infant wheeze but not with eczema or atopic wheeze.¹⁰⁹ Is it possible to increase the presence of bifidobacteriae and lactobacilli in allergic infants? One study investigated the influence of lactose (administered as a lactose-containing formula) on the composition of the gut microbiota in infants with cow's milk allergy. They found that the addition of lactose to an extensively hydrolyzed formula was able to positively modulate the composition of gut microbiota by increasing the total fecal counts of lactobacilli and bifidobacteriae, together with a decrease in *Bacteroides* species and Clostridia.¹¹⁰ Along this line, it was shown that daily supplementation of the *L casei* subspecies *Rhamnosus* (LGG) in a double-blind randomized trial had a positive effect on establishing a more stable colonization pattern of phylogenetically clustered taxa, including a number of other probiotic species. These important modifications of the intestinal flora are characteristic of communities that are more resistant to perturbation and outgrowth of pathogens. Whether this is linked to immunologic effects of such probiotic strains needs to be further elucidated.¹¹¹

Gut microbiota and allergic immune responses: Animal model insights into mechanisms

The majority of the clinical studies discussed above suggest a strong association between a disturbed intestinal colonization and the development of allergic phenotypes. However, a direct or indirect cause-effect relationship and the formal proof and elucidation of underlying mechanisms still remain to be established. This gap has recently begun to be filled in by the development of a variety of animal model systems with specific focus on the questions of whether and how intestinal microflora can regulate immune responses also outside the gut.¹¹²

Actually, at least 4 major strategies were followed to investigate the relationship of intestinal microbial colonization with development of normal and pathologic immune system functions. These included the colonization of specific pathogen-free (SPF) but otherwise normofloric mice, investigation of mice after antibiotic treatment, analysis of GF mice with and without specific recolonization, and examination of mice with impaired recognition of microbial components, subsequent signaling pathways, or both.

It is meanwhile well recognized from GF and *Toll-like receptor (TLR)* knockout mice that the presence and recognition of commensal intestinal microflora is required for establishing and maintaining intestinal homeostasis, including development of an appropriate immune status. Already in 1998, Sellon et al¹¹³ realized that enteric bacteria are necessary for the activation of the adaptive immune system, such as CD4 cell activation or IgA production, because GF IL-10-deficient mice did not have colitis like their SPF counterparts. This mechanism was later shown to be dependent on intact TLR signaling pathways.¹¹⁴ Furthermore, it was demonstrated that not only pathogens but also commensal bacteria are recognized by TLRs under steady-state conditions and that this interaction is critical for protection against gut injury and associated mortality.¹¹⁵ Especially TLR2 seems to play an important role in the regulation of epithelial barrier function because both TLR2 and *myeloid differentiation primary response gene-88 (MyD88)* knockout mice exhibit impaired tight junction-associated epithelial integrity.¹¹⁶ This not only affects the susceptibility to mucosal injury and its consequences but also the development of oral tolerance and immunity and thus the development of allergic and autoimmune diseases. In this regard Sudo et al¹¹⁷ discovered that GF mice were not capable of developing oral tolerance against the model allergen ovalbumin because of abrogation of T_H1-mediated responses. In consequence, GF mice had stronger T_H2 responses, as demonstrated by increased production of IL-4. Reconstitution of the intestinal flora with the predominant intestinal bacterium *B. infantis* completely restored oral tolerance induction to a T_H2-promoting allergen. Interestingly, this mechanism was only effective when the reconstitution was performed in neonates but not in adult mice. This points to a restricted window of opportunity early in life that is probably associated with the development of gut-associated lymphoid tissue at the neonatal stage.¹¹⁷ Similarly, enforced sensitization to cow's milk proteins resulted in stronger antibody responses in GF compared with conventional mice, which were abrogated after inoculation of conventional mouse flora to GF animals.¹¹⁸

Interestingly, missing or altered intestinal colonization not only affects intestinal immune responses but also influences immunologic mechanisms at distal mucosal sites, such as the lung. For example, the expression of the short variant spleen tyrosine kinase, which is expressed in a variety of hematopoietic and nonhematopoietic cells, was found to be significantly increased in lungs and spleens of GF animals.¹¹⁹ Furthermore, Herbst et al¹²⁰ could demonstrate that GF mice exhibited exaggerated allergic immune responses in the lung after intranasal allergen challenge of sensitized animals. Such animals showed increased eosinophil and basophil numbers, which correlated with augmented local T_H2 cytokine production, systemic IgE levels, decreased alveolar macrophage and plasmacytoid dendritic cell numbers, and an altered phenotype of conventional dendritic cells. Also,

recolonization with the complex flora of SPF mice could completely reverse the exaggerated allergic phenotype of GF animals.¹²⁰

The role of the composition of transferred microflora is still controversially discussed. On the one hand, Chung et al⁴⁸ elegantly demonstrated that a full restoration of intestinal immune maturation depends on the colonization with a host-specific microbiota. Even though bacterial numbers and phylum abundance were similar to those seen in mouse microbiota, transfer of human or even rat-derived microbiota to GF mice was not sufficient to fully restore levels of T-cell subpopulations in their intestines and further characteristics of GF mice. Moreover, mice recolonized with human microbiota exhibited distinct epithelial gene expression patterns. This implies a potential role for segmented filamentous bacteria, which are specifically abundant in the mouse microbiome for full intestinal immune maturation. In contrast, Rodriguez et al¹²¹ observed a significant protection of GF mice from cow's milk allergy symptoms after transfer of infant human gut microbiota with a predominant presence of *Bifidobacterium* and *Bacteroides* species. However, these authors described still immature ileal T-cell responses in the recolonized compared with conventional mice, probably because of low translocation of the human-derived bacteria into Peyer patches of the recipients. *Vice versa*, mice with food allergy showed a distinct microbiota signature, which significantly differed from that of nonallergic animals. Interestingly, this disease-associated microbiota was able to promote allergic responses when transferred to GF mice. This points to a mutual interaction between the effective host immune status and microbiota composition. In this context disease-associated microbiota might play a pathogenic role, at least in food allergy.¹²²

Generally, several mechanisms are discussed to be involved in transduction of the allergy protective effects of intestinal microbial components. Recognition of commensal bacteria by TLRs and other *pattern recognition receptors (PRRs)* seems to be an indispensable prerequisite for immunodeviation toward an allergy-protective status.^{123,124} Mainly, TLR2 as the major receptor for gram-positive bacteria-derived components plays an essential role^{116,125}; however, other PRRs, such as TLR4, TLR9, or nucleotide-binding oligomerization domain-containing protein 1 (NOD1), might also contribute to these processes.^{123,126,127} Engagement of TLR2 does act (1) through control of epithelial barrier function,¹¹⁶ (2) through appropriate activation of dendritic and other antigen-presenting cells with subsequent induction of T_H1 responses,¹²⁵ and also (3) through direct activation of fork-head box protein 3-positive *regulatory T (Treg) cells*, which themselves express TLR2 at their cell surface.¹²⁸ Actually, specific probiotic bacterial strains were demonstrated to induce the proliferation and activation of Treg cells, leading to increased numbers of this tolerance-inducing T-cell subpopulation.^{129,130} Mainly, extrathymically induced Treg cells were shown to be involved in the regulation of T_H2-type pathologies at mucosal sites, such as the gut and lung.¹³¹ Again noteworthy, transfer of allergen-specific Treg cells might not only alleviate allergic immune mechanisms but also lead to a tolerance-associated micro-biota signature in otherwise allergy-prone mice.¹²²

In contrast, the potential role of T_H17 cells is controversially discussed. Zaph et al¹³² observed an increased frequency of T_H17 cells in GF mice. The number of these cells is limited in the presence of commensal bacteria by intestinal epithelial cell expression of

IL-25. In the presence of IL-25, the production of macrophage-derived **IL-23** is suppressed, which otherwise supports T_H17 development. Other authors report an increased number of steady-state intestinal T_H17 cells after colonization with commensal or probiotic bacteria.^{133–135} PRR-mediated release of IL-1 β ¹³⁴ or ATP (also directly released from commensal bacteria)¹³⁵ might drive T_H17 differentiation under these conditions. Additionally, also natural killer cell priming depends on the presence of instructive signals provided by commensal microbiota that lead to *epigenetic* changes in cytokine promoters necessary for the binding of transcription factors (eg, nuclear factor κ B and interferon regulatory factor 3) relevant for appropriate NK cell development.¹³⁶

Aside from PRR-mediated mechanisms, other ligand receptor systems might also be involved in the transduction of allergy-protective effects of commensal bacteria. Among them, short-chain fatty acids (SCFAs), which are produced by intestinal bacteria during the fermentation of dietary fibers, and their specific G protein–coupled receptors GPR41 and GPR43 came recently into focus. SCFAs were demonstrated to represent potent suppressors of inflammatory reactions, and thus mice lacking SCFAs or GPR43 showed exaggerated T_H2-driven allergic responses.¹³⁷ However, the mechanisms by which SCFAs interact with immune system components are still to be resolved.

Taken together, a variety of different animal models has meanwhile proved the concept that intestinal colonization is indispensably necessary for appropriate development of the adaptive immune system (Table II). Disturbances in early-life colonization processes or innate immune signaling pathways might therefore lead to pathophysiologic consequences, even outside the gut, such as the development of allergic immune reactions at other mucosal or dermal sites. Several mechanisms, including TLR-mediated signaling, in different cell types are involved in the transduction of signals from commensal bacteria to developing components of the immune system. Vice versa, composition of the intestinal microbiota might be influenced by inflammatory immune reactions. Further efforts are required to better understand these mutual interactions.

Environmental microbes and allergy

One of the best studied examples linking the hygiene hypothesis and the hypotheses on biodiversity and microbiota is the epidemiologic observation that living in a traditional farming environment results in the protection of particularly respiratory allergies.

Maternal exposure during pregnancy has a strong effect on innate and adaptive immune responses at birth. The high microbial load, which is present in the unique farming environment, is strongly associated with a consistent upregulation and increased production of IFN- γ and TNF- α at birth. In several studies no direct effects on T_H2 cytokine levels or on IL-10 or IL-12 production could be detected. Furthermore, an inverse relationship between neonatal IgE production and cord blood IFN- γ levels was noted.¹³⁸ These data further support the concept that the stimulation of T_H1 immunity in prenatal life is an important mechanism to prevent a T_H2 immune response.

Early-life exposures have a strong effect on the TLR expression, as indicated by increased TLR5 and TLR9 mRNA levels at birth. When microbial exposures continue through early

childhood, the effects are even stronger. The higher the number of different farm animal species the mother had contact with during pregnancy, the higher the levels of TLR2, TLR4, and CD14 at school age.¹³⁹ This effect has been related to a natural dose of endotoxin exposure,¹⁴⁰ as measured in mattress dust.

These exciting data from epidemiologic studies resulted in the development of a new concept regarding the effect of microbial exposures on early immune development. As an important step forward in this direction, it could be demonstrated that local (mucosal) administration of natural or synthetic TLR agonists triggers a cascade of immune responses which result in the protection of experimental asthma in several murine models. This has been shown for TLR2 (lipopeptides),¹⁴¹ TLR2/4 (peptidoglycan),¹⁴² TLR4 (LPSs),^{143,144} TLR3 and TLR7 (poly I:C and R848),¹⁴⁵ and TLR9 agonists (DNA from *Bordetella pertussis*).¹⁴⁶ In most of these studies the T_H2 protective effect could be related to the development of either a strong T_H1 immune response or increased IL-12 and IL-10 levels. Further studies then provide evidence that a similar asthma-protective effect could be achieved by intranasal application of intact bacteria, belonging to both the gram-negative and the gram-positive genera, including *Acinetobacter lwoffii*, *Lactobacillus lactis*, *Bacillus licheniformis*, and *Staphylococcus sciuri*.^{147–149} These proof-of-principal experiments performed in adult animals provide the foundation for the exploration of transmaternal asthma-protective effects. Although several asthma-protective bacterial strains have been identified, the modulation of the asthmatic phenotype showed strain-dependent qualitative and quantitative differences, with the highest degree of protection by *A lwoffii*.¹⁵⁰ Intranasal administration of this strain throughout pregnancy resulted in strong and long-lasting protection of the offspring with regard to airway inflammation, airway hyperresponsiveness, and (IgE) antibody production. Intranasal administration augments TLR expression patterns in the maternal lung, resulting in local and systemic mild inflammatory responses (IL-6, TNF- α , and IL-12p40). That maternal mucosal TLR signaling is responsible for the initiation and eventual transmission of the asthma-protective effect to the next generation was demonstrated by using quintuple-TLR knockout mice deficient in functional expression of TLR2, TLR3, TLR4, TLR7, and TLR9. The absence of functional TLR signaling through these receptors completely abolished the asthma-protective effect in the F1 generation, despite functional TLR signaling in the offspring. These data provide clear evidence that intact airway mucosal TLR expression and signaling are intimately involved and responsible for triggering asthma-protective immune responses operating in a transgenerational and transmaternal fashion.

Recently, a novel mechanism was discovered through which environmental microbes, such as *A lwoffii*, mediate asthma protection in the offspring. It was shown that exposure to these strains brings about marked epigenetic modifications of the IFN- γ genes, resulting in increased IFN- γ production.¹⁵¹

The effect of environmental microbes on the development of clinical phenotypes, such as asthma, provides a new dimension in the relationship between microbes, immune functions, and development of chronic inflammation. Although many experimental data, particularly those obtained in animal model systems, point to a possible cause-effect relationship, this causality needs to be addressed in appropriate clinical studies.¹⁵² It will be particularly

important to investigate whether the same mechanisms operate in the human immune system as identified in animal studies. If a collection of environmental microbes could be characterized and defined with a cause-effect relationship on immune development and maturation, this could be a starting point for preventive clinical studies.

CONCLUSION

The microbial communities on the surfaces of our mucosal tissues and the skin have coevolved with the evolution of the innate and adaptive immune system. We use these microbes to develop a functional immune response pattern; part of this is the development of mucosal tolerance. The perinatal and postnatal environment is of critical importance to achieve this goal. The first few years of life represent a vulnerable period during which several fundamental decisions regarding appropriate or inappropriate immune responses are made. The host-microbe interaction plays a decisive role in this regard. There exists ample evidence from epidemiologic, clinical, and experimental studies for this concept. However, the underlying mechanism, the cause-effect relationship, and particularly the identification of individual microbes and microbial compounds still need to be established and discovered (Table III). It is the expectation that further delineation of the complex relationship between the development of our immune system in life, the qualitative and quantitative patterns of microbial colonization, and the effect of other environmental factors, such as nutrition, smoking, and toxins, will lead to the development of novel preventive strategies that combat the dramatic increase in NCDs, including allergies and autoimmunities.

Acknowledgments

H.G. and H.R. are supported by the Deutsche Forschungsgemeinschaft (DFG; SFB/TR22), the German Lung Centre (DZL), and the LOEWE Centre "Universities Giessen and Marburg Lung Centre" (UGMLC). R.S.B. is supported by National Institutes of Health grants DK044319, DK051362, DK053056, and DK088199 and the Harvard Digestive Diseases Center (DK0034854).

GLOSSARY

CD1D	CD1d is an MHC class I-like molecule that presents glycolipid molecules to CD1d-restricted cells, such as natural killer T (NKT) cells. The classic ligand is α -Gal-Cer (α -galactosylceramide). CD1d tetramers are used in research to identify NKT cells
EPIGENETIC	Genetic changes affecting cellular phenotype (as defined by gene expression) through DNA methylation, chromatin remodeling, and histone. These changes are transmissible to progeny but are also reversible because the genetic code remains unchanged
GENOME-WIDE ASSOCIATION STUDY (GWAS)	GWASs use GeneChip technology and bioinformatics to analyze the human genome for single nucleotide polymorphisms in diseased and nondiseased states. Haplotypes (in blocks) that vary between diseased and

	nondiseased subjects are considered to be associated with the disease state
GERM-FREE MICE	Also known as gnotobiotic, these mice are born in germ-free conditions and then can be reconstituted with specific strains of bacteria to evaluate effects on mucosal immunity, immune system development, and diseases, such as colitis
IL-25	IL-25 is also known as IL-17E and is produced by mast cells and T _H 2 cells, its levels are increased after airway challenge, and it can cause airway eosinophilia
MYELOID DIFFERENTIATION PRIMARY RESPONSE GENE-88 (MYD88)	Most TLRs and IL-1 receptors use the MyD88 and IL-1 receptor–associated kinase 4 (IRAK-4) signaling pathways. Subjects with MyD88 or IRAK-4 deficiencies have impaired polysaccharide antibody responses, invasive bacterial infections, and impaired fever and C-reactive protein responses during infections
NATURAL KILLER T (NKT) CELLS	NKT cells are CD1d-restricted lymphocytes that function in immunity against tumors, bacteria, and viruses and that can suppress autoimmunity. The majority appear to be invariant NKT cells that can be CD4 ⁺ CD8 ⁻ or CD4 ⁻ CD8 ⁺ , although there are also noninvariant NKTs that have a broader T-cell repertoire and do not recognize α -Gal-Cer
PATTERN RECOGNITION RECEPTOR (PRR)	Pattern recognition receptors (PRRs) bind to pathogen-associated molecular patterns, danger-associated molecular patterns, and bacterial products, such as peptidoglycan, N-formylmethionine, flagellin, and LPS. PRRs include Toll-like receptors, Nod receptor, C-type lectin receptors, and mannose receptors
POLYMORPHISMS	Genetic variant in a single nucleotide that might be normally present in the population and associated with risk for certain complex polygenic diseases. “Functional” SNPs are those that clearly alter gene transcription/translation, such as splice site changes or alterations in transcription factor binding sites
PYROSEQUENCING	New second-generation technology for sequencing is available that allows rapid sequencing using the generation of light on chain termination. Techniques, such as pyrosequencing, can be applied to sequencing transcripts, a method known as “RNA Seq”
REAL-TIME QUANTITATIVE (RT-qPCR)	Quantitative RT-qPCR analyzes the amount of a specific mRNA, as normalized to a housekeeping gene. RT-qPCR can be done with TaqMan or SYBR technology, which uses fluorescence-tagged primers or a fluorescent intercalating

REGULATORY T (TREG) CELL

agent. Multiplexing RT-qPCR allows the concurrent assessment of multiple mRNAs in the same well by using different fluorescent color signals

Treg cells function to dampen the immune response and can express Forkhead box protein 3 (FoxP3) and/or TGF- β , CD25, and IL-10. Congenital absence of Foxp3 Treg cells causes immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, an immunodeficiency associated with poly-organ autoimmunity. There are a number of different Treg cells that can be divided into natural Treg cells and inducible Treg (iTreg) cells. Naturally occurring Treg cells come from the thymus, whereas iTreg cells (eg, IL-10-producing Treg cells or T_R1 cells or TGF- β 1-producing Treg cells or T_H3 cells) arise in the periphery, are CD80/86 independent, and are specific for tissue antigens

T_H17

IL-23, T_H17 cells are defined by production of IL-17A, IL-17F, IL-6, IL-21, IL-22, and TNF- α and are involved in autoimmunity. IL-17 production is increased by dendritic cell-derived IL-23. IL-23 then activates the transcription factor signal transducer and activator of transcription 3 and maintains the T_H17 phenotype of the CD4⁺ T cells

TOLL-LIKE RECEPTORS (TLRs)

Essential members of the innate immune system, TLRs are pattern recognition receptors that bind both endogenous and exogenous ligands. TLR4 binds LPS from gram-negative bacteria, heat shock protein 6, and respiratory syncytial virus protein F. TLR7 and TLR8 bind single-stranded RNA and are important for antiviral defense

Abbreviations used

CD	Crohn disease
GF	Germ free
GWAS	Genome-wide association study
IBD	Inflammatory bowel disease
NCD	Noncommunicable disease
OR	Odds ratio
PRR	Pattern recognition receptor
SCFA	Short-chain fatty acid
SPF	Specific pathogen free

TLR	Toll-like receptor
UC	Ulcerative colitis

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FIG 1.
Microbe-host interaction and immunoprogramming: contribution of the environmental microbiota and barrier microbiomes.

FIG 2.

Factors that influence IBD development. IBD is caused by dysfunction in the composition of and interactions between the commensal microbiota, the intestinal epithelium, and the immune system. Each of these factors is under the influence of genetic and environmental factors. *NKT*, Natural killer T cells; *T_{EFF}*, T effector cell; *T_{REG}*, T regulatory cell.

FIG 3.

Development of barrier microbe homeostasis. The development of a stable colonization pattern (intestinal colonization) takes time and is influenced by many variables, including conditions operating during the first few years in life. They include maternal factors, feeding, ethnic differences, lifestyle conditions, and more. *OTUs*, Operational taxonomic units.

FIG 4.

Effect of barrier microbes on organ-based inflammation. Fundamental differences exist between the normal homeostatic colonization pattern and the pattern identified in healthy children (*green*) and intestinal colonization in allergic children (*red*). However, the detailed qualitative and quantitative changes are still inconsistent and might differ for various allergic phenotypes. A common pattern, which is currently emerging, is reduced diversity in allergic subjects that precedes the onset of allergic diseases later on in life. However, a cause-effect relationship needs to be formally established. *OTUs*, Operational taxonomic units.

TABLE I

Intestinal dysbiosis and childhood allergies

• Reduced microbial diversity in allergic infants
• Strongest association for IgE sensitization, eczema, and food allergies
• Dysbiosis precedes development of clinical symptoms and is detectable already early in life
• Inconsistent results for respiratory allergies

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TABLE II**Key mechanistic results from animal models**

• Normal microbiota is required for full development of the immune system.
• Normal microflora is required for (oral) tolerance.
• Early exposure to antibiotics increases susceptibility to asthma and colitis.
• The microbiota can promote but also protect against development of intestinal inflammation.
• GF mice lack oral tolerance.
• GF mice can be more susceptible to colitis (oxazolone-induced colitis model).
• Deficient TLR signaling in the gut results in dysbiosis and has similar effects on immune homeostasis.
• GF mice have stronger T _H 2 responses, higher IL-4 production, and augmented allergic phenotypes.
• Colonization of GF mice with several defined species abrogates this T _H 2 bias.
• GF mice show reduced T _H 1 responses.
• Oral tolerance is restored in GF mice by <i>Bifidobacterium infantis</i> and other species.
• Restoration must occur in the neonatal period.
• The genetic background of the host has a strong influence on susceptibility to colitis.

TABLE III

Open questions

• Does a cause-effect relationship exist between dysbiosis and allergic phenotypes/intestinal inflammation?
• What causes dysbiosis? Candidates include host genetics, environmental factors, or both.
• Is dysbiosis the result of a loss of protective microbes or the acquisition of disease-promoting strains?
• How can dysbiosis be corrected?
• Does intestinal dysbiosis contribute to allergic manifestations outside the gut, and, if so, how?
• Does lung dysbiosis contribute to allergic manifestations outside the lung and perhaps to intestinal inflammation?
• How stable is dysbiosis over time?
• What are the reasons for heterogenic results between the studies?

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