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Microbiome and its Impact on Gastrointestinal Atopy

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Abstract

The prevalence of allergic conditions has continuously increased in the last few decades in Westernized countries. A dysbiotic gut microbiome may play an important role in the development of allergic diseases. Genetic, environmental and dietary factors may alter the commensal microbiota leading to inflammatory dysregulation of homeostasis. Murine and human studies have begun to elucidate the role of the microbiota in the pathogenesis of atopic diseases including asthma, atopic dermatitis and food allergies. However, the role of the microbiome in most eosinophilic gastrointestinal diseases (EGIDs) is not yet known. This review provides an overview of what is currently known about the development of tolerance from both molecular and clinical standpoints. We also look at the gut specific microbiome and its role in atopic conditions with the hope of applying this knowledge to the understanding, prevention and treatment of EGIDs, particularly EoE.

Keywords

asthma; atopic dermatitis; bacteria; eosinophilic esophagitis; eosinophil

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I. Introduction

Atopic gastrointestinal diseases are a diverse group of antigen-mediated disorders that encompass IgE-mediated disorders related to anaphylaxis as well as delayed hypersensitivity responses that cause eosinophilic infiltration of the gut. While there is some evidence linking early gut dysbiosis to gut atopy, very little is known about the role of the microbiome in atopic and eosinophilic disorders of the gut. It is the goal of this review to examine the role of the gut microbiome in atopic disorders and attempt to explain how gut commensals influence specific gastrointestinal atopy.

II. Commensal bacterial exposure drives tolerance

The increasing incidence and prevalence of atopic diseases is most often attributed to the hygiene hypothesis. It is thought that the highly sterilized western lifestyle does not provide adequate exposure to pathogens, leading the immune system to mistake innocuous food and environmental particles for pathogens. Recent studies have found that subjects raised in rural communities with exposure to animals are less likely to develop atopic disease than subjects exposed to sterilized urban environments (1). Germ-free and antibiotic treated mouse models have also been used to test this hypothesis, and these models have supported the idea that early life exposure to diverse commensals is essential for the development of immunologic tolerance.

In murine models of allergic airway disease, failure of commensal population development causes dysregulation of the IgE-basophil axis (2). IgE is the immunoglobulin specifically linked to type 1 hypersensitivity anaphylactic reactions and in addition is responsible for binding mast cells and basophils characteristic of other atopic conditions.. Eosinophils express a number of anti-microbial products, including granule cationic proteins and defensins, and they generate DNA-containing extracellular traps (3) (4) (5). Similarly, basophils have recently been reported to kill bacteria through formation of extracellular traps (BETs) containing mitochondrial DNA and granule proteins (6). These products may change the local microbiota in atopic diseases where there is significant infiltration by these granulocytes. Hill et al. found that depletion of murine microbial populations enhances serum IgE and basophils in both antibiotic treated mice as well as germ free mice (2). These mice displayed enhanced basophil-mediated allergic inflammation of the lungs in a house dust mite model of asthma. This link between a commensal population of bacteria and IgE-basophil-mediated inflammation was found to be B cell intrinsic and MYD88 dependent, as MYD88 depleted mice had similarly enhanced levels of IgE and basophils to antibiotic-treated and germ free mice when compared to control populations. The authors postulate that the commensal population stimulates B cell MYD88 inhibition of IgE, thus decreasing basophil progenitor formation in the bone marrow.

Commensal bacteria are also important for maintaining a balance of type 1 T helper (Th1) cells and type 2 T helper (Th2) cells. Th1 cells are the effector cells against bacterial invasion, whereas Th2 cells historically deter parasitic invasion. However, in the setting of altered tolerance the Th2 response leads to the allergic responses seen in atopic disease. A careful balance of these cell types is important for tolerance. Improper exposure to

commensals in infancy may be responsible for the enhanced Th2 response in atopic conditions. Oyama et al. found that treatment of mice with antibiotics in infancy resulted in an exaggerated IgE/IgG1 response with diminished IgG2 (7). Furthermore, the spleen and Peyer's patches of these mice had decreased lymphocytes compared to untreated mice. Similar antibiotic exposure in older mice, presumably after gut microbial colonization, did not cause any of these immunologic changes, and both antibiotic treated and untreated mice had similar IgE levels. These results were supported in studies with the opposite approach, wherein exposure of germ-free mice to ova-albumin (OVA) resulted in Th2 predominant inflammation (8). Reconstitution of the microbiota with *Bifidobacterium infantis* in germ-free mice resulted in tolerance to OVA, though only if exposure was in the neonatal period.

Correspondingly, an intact response to the commensal bacteria and its components is essential for the development of tolerance. The gut has evolved several systems to monitor the bacterial populations within the lumen, including signaling through toll-like receptors (TLRs). Previous studies have shown that defective TLR signaling has been implicated in development of food allergies; for example, mice with a mutation in TLR4 exposed to peanut extract undergo a systemic allergic response (9, 10). Interestingly, TLR4 wild-type mice neonatally exposed to antibiotics produce a similar response to peanut extract, suggesting that there is a TLR4-commensal response necessary for development of tolerance (11). Furthermore, exposure of the TLR4 mutant mice to the TLR9 agonist, CpG oligonucliotides (CPG-ODN), resulted in an attenuated response to the peanut extract. This CpG-ODN likely causes a shift toward the Th1 response (12), and acts to rescue the development of tolerance. Other mouse models of allergic disease have also shown that administration of CPG-ODN reduces airway inflammation and decreases IgG1 and IgE responses (12). Lastly, exposure to the commensal population's polysaccharide A induces TLR2-dependent activation of the T regulatory population (Tregs) (13). Induction of this population of Tregs is necessary to suppress the development of autoimmunity and successfully attenuates pro-inflammatory Th17 responses that can be seen in the context of asthma and atopy (14).

In summary, exposure to commensals is necessary for proper development of tolerance through a number of mechanisms. Sterility in the murine neonatal period can lead to a shift in the IgE-basophil axis, an imbalance in Th1/Th2 activation, as well as improper activation of Tregs. Human studies to evaluate these mechanisms are limited, but some have implicated polymorphisms of TLR2 as well as TLR4 co-receptor CD14 in atopic disease (15, 16) (Figure 1). These genetic variations may provide a link between commensal bacteria and genetic disposition to atopic disease. These bacterial ligands also represent potential therapeutic targets in atopic disease.

III. Early life environment contributes to development of atopy

The quantity and quality of the population of commensals in the human gut during the neonatal period is shaped by early life exposures. Even the mode of birth can cause alterations in the gut microbiota that lead to altered immunologic responses. Jakobsson et al. showed that infants born by Cesarean section have decreased diversity of the Bacteroides phylum throughout the first two years of life compared to those born vaginally (17).

Stokholm et al. showed that Cesarean section was significantly associated with colonization of the intestinal tract by *Citrobacter freundii, Clostridium species, Enterobacter cloacae, Enterococcus faecalis, Klebsiella oxytoca, Klebsiella pneumoniae,* and *Staphylococcus aureus* at 1week of life, whereas colonization by *Escherichia coli* was associated with vaginal birth (18). A recent pilot study by Dominguez-Bello et al. started to investigate the long-term health consequences of Cesarean section-delivered infants. They found that the microbiota of Cesarean-born infants could be partially restored via vaginal microbial transfer (19). Likewise, those born vaginally have enhanced circulating levels of Th1 cytokines compared to their Cesarean counterparts. The sterility of the surgical procedure and the lack of exposure to maternal vaginal microbiota have been blamed for this decreased colonization (20). While there is some variation in the results, there have been meta-analysis and population studies showing an increased risk of asthma and allergic rhinitis in infants born by Cesarean (21, 22), however very little is known about the relationship of delivery mode and gastrointestinal (GI) atopy.

While diet has been linked to gut microbial changes, dysbiosis and gut inflammation (23), most studies show few microbial differences between formula and human milk fed infants. There are some differences noted between the populations, with human milk fed infants having proportionally more *Staphylococcus, Lactobacillus* and fewer *Clostridium difficile, Bacteroides, Enterococci* and *Enterobactericeae* colonies (24). There may be some evidence that human milk is protective against early childhood wheezing, but there is no evidence that breastfeeding provides protection against food allergy (24–26). However, studies looking at breastfeeding are inherently limited due to inability to randomize and differences in duration of breastfeeding.

Antibiotic exposure is common in the neonatal period due to prematurity, maternal group B Streptococcus status, and sepsis protocols for febrile infants. It is difficult to control for this diverse population of patients who receive antibiotics early in life. There is a marginally significant increase in atopic disease in children treated with antibiotics prior to 3 months of life, but this may have been confounded by upper respiratory infections (27). In a more homogeneous population controlling for premature birth, Kummeling et al. found that antibiotic exposure in the first 6 months of life correlated with wheeze but not allergic sensitization or eczema (28). Further work must be done to understand the connections between neonatal antibiotic exposure and later development of atopic conditions.

While mode of delivery, formula feeding, and early antibiotic exposure are important factors in shaping the infant microbiome, there have been very few studies evaluating how these exposures shape the development of GI atopy. Jensen et al. performed a case-control study looking at the development of eosinophilic esophagitis (EoE) and found a 6-fold increase in EoE in children with antibiotic exposure in the first 6 months of life (29). While Cesarean delivery, preterm birth, and formula-only feeding trended toward increasing incidence of EoE, these measures were not significant. Moreover, dizygotic twins have a 10-fold higher EoE concordance rate compared with non-twin siblings, providing strong evidence for a profound impact of early-life exposures in addition to known genetic underpinnings for susceptibility to EoE (30). Sensitization to food antigen at 1 year of life was found to be associated with variations in the microbiome between 3 and 12 months of life. Specifically,

infants with food sensitization by skin prick testing had increased Enterobacteria and decreased Bacteroides compared to those with negative skin prick testing (31). However, larger prospective studies looking at gastrointestinal atopy will be needed to help verify these causal relationships.

IV. Atopy and the microbiome –other organ systems

a. Asthma

The micromilieu at the site of inflammation creates a specific environmental niche where certain bacteria can proliferate and the gastrointestinal microbiota, as the central microbial habitat, can influence asthma and allergy development at other body sites. Recent evidence suggests that immune dysfunction in allergic diseases such as asthma and atopy is related to the function and composition of the gut microbiota. Arrita et al. suggest that fecal microbiota dysbiosis in the first 100 days of life, particularly reductions of the genera *Faecalibacterium, Lachnospira, Veillonella* and *Rothia* (FLVR), are linked to the development of allergic diseases (32).

Early studies using culture-based approaches have demonstrated that detection of a high abundance of either *Escherichia coli* or *Clostridium difficile* in neonatal feces is associated with significantly higher risk of developing childhood allergic disease (33) (34). Additionally, the absence of certain genera such as *Bifidobacterium* in neonatal stool has also been associated with an increased risk of developing childhood atopy in the first 5 years of life (35). These studies demonstrate that altered microbiota development in the neonatal gut lays the foundation for allergic disease and asthma development in childhood. *Bifidobacterium adolescentis* has recently been shown to be lower in fecal samples from adult allergic subjects with long term asthma and may represent a novel therapeutic target for modulation of the gut microbiota in this group of subjects (36).

b. Atopic dermatitis

Atopic dermatitis (AD) is often associated with other allergic diseases including asthma, allergic rhinitis (37) and eosinophilic gastrointestinal diseases such as EoE (38, 39) (40). With recent increase in methicillin resistant *Staphylococcus aureus* strains, antibiotic therapy is not the first choice for AD treatment. Corticosteroids, anti-inflammatory reagents and diluted bleach baths are more commonly used. Just as fecal microbiota therapy from healthy donors has shown success in recent years in reverting gut microbiota dysbiosis in subjects with recurrent *Clostridium difficile* infection, and seems promising for some other gastrointestinal diseases (41), in the future microbial skin transplant therapy may be useful for treating AD.

The gut microbiota in AD has not yet been as deeply investigated as the skin microbiota, but it has been recently reported by Song et al. that the gut microbiota in 90 AD subjects was enriched in *Faecalibacterium prausnitzii,* a species previously described to be deficient in the gut of Crohn's disease subjects. Additionally, the gastrointestinal microbiota was enriched in genes encoding the use of nutrients that could be released from damaged gut epithelium, indicating a bloom of auxotrophic bacteria. Furthermore, anti-inflammatory bacterial

metabolites such as butyrate and propionate had decreased levels in fecal samples from AD subjects (42). Specifically, studies have shown that disease improvement in infants was correlated with an increased abundance of fecal butyrate-producing bacteria *Coprococcus eutactus* (including Clostridial families Lachnospiracea and Ruminococceae) (43). These results suggest that the dysbiotic gut microbiota in AD and dysregulation of the gut inflammation and epithelial barrier could lead to an aberrant Th2-type immune response to allergens in the skin.

b. Food allergies

The increasing use of antibiotics in humans (particularly in infancy) and in agriculture for growth-promoting properties of livestock, and the increasing consumption of a high-fat/low fiber diet, have had a major impact on the gut microbiota, and have been associated with an increased allergic response to food in industrialized countries in the last few decades (44) (45). Animal models suggest that the gut microbiota, particularly in early life, play a crucial role in susceptibility to food sensitization and food allergy. Murine models of neonatal exposure to antibiotics have shown reduced gut microbiota diversity, and are associated with aberrant immunity to respiratory and dietary antigens and enhanced food allergen sensitization (46).

Atarashi et al. have identified Clostridia species in both mice and humans as being potent inducers of colonic Tregs capable of suppressing food allergy (47, 48). Stefka et al. reported that Clostridia colonization increased colonic Tregs and IL-22 expression in the small and large intestine. This alteration led to enhanced epithelial barrier function and prevented uptake of food allergens (46). Interestingly, Noval Rivas et al. have shown that the intestinal microbiota regulates susceptibility to food allergy. They identified a unique microbial signature in genetically food-allergy susceptible mice that can transmit food allergy susceptibility when transferred to germ free mice (49). Additionally, that same group recently identified a Th2-like phenotype in Tregs of food allergic mice; these specific Tregs play a key role in controlling food allergy (50).

Co-administration of bacterial adjuvants with oral immunotherapy (OIT) has been suggested as a potential treatment for food allergy. In the first double-blind, placebo-controlled randomized trial with 62 children on the probiotic *Lactobacillus rhamnosus* and peanut OIT (probiotic and peanut oral immunotherapy [PPOIT]) in children with peanut allergy, Tang et al. found that sustained unresponsiveness was achieved in 82.1% receiving PPOIT and 3.6% receiving placebo. Of the subjects receiving PPOIT 89.7% were desensitized. Although these results are very promising, further work is required to confirm sustained unresponsiveness after a longer period of secondary peanut elimination, and to clarify the relative contributions of probiotics versus OIT. In addition, the active group had more reactions suggesting an imbalance in treatment, which would affect efficacy (51).

As previously demonstrated in murine models, human studies also link the use of antibiotics with food allergies; maternal use of antibiotics during pregnancy and in the first month of life was associated with increased risk of cow’s milk allergy in infants (52). Infants with cow's milk allergy have a fecal microbial community dominated by *Lachnospiraceae* and *Ruminococcaceae*. Dietary intervention with extensively hydrolyzed casein formula

supplemented with *Lactobacillus rhamnosus* GG was shown to expand butyrate-producing bacterial strains and accelerated tolerance acquisition in infants with cow's milk allergy (53).

The fecal microbiota of food allergic infants is characterized by increased relative abundance of *Clostridium I* and *Anaerobacter* and a decrease of *Bacteroides* and *Clostridium XVIII* (54) (Table 1). Chen et al. have recently shown that children with food sensitization in early life have an altered fecal microbiota compared to healthy controls, with lower microbiota diversity. Children with food sensitization had a significantly decreased number of Bacteroidetes and a significantly increased number of Firmicutes compared with healthy children. The most differentially abundant taxa in children with food sensitization was characterized by increased abundances of *Clostridium* IV and *Subdoligranulum* and decreased abundances of *Bacteroides* and *Veillonella* (55) (Table 1). Exploring novel Clostridia-based biotherapeutics or butyrate administration as adjunct therapy to promote tolerance to food allergens may be useful in the future.

V. Microbiome in EoE

a. Philadelphia Experience

Once thought sterile or composed of few cultivable microbes, the esophagus has been shown to have a composition of around 300 bacteria species. New culture independent techniques available today have allowed scientists to identify the microbial composition of the esophagus in children and adults. Investigators at Children's Hospital of Philadelphia (CHOP) sought to characterize the oral and esophageal microbiota in 33 children diagnosed with EoE as well as 35 controls. Oral samples were obtained through a swab method prior to endoscopy and esophageal samples from esophageal biopsies. The majority of subjects included in the study were on proton pump inhibitor therapy and on a food elimination or reintroduction diet. Subjects on antibiotics or presenting with any GI inflammatory process were excluded from the study.

Benitez et al. reported marked differences between the oral and esophageal microbiota with strains exclusively present in esophageal samples (56). However, some lineages were shared between the oral and esophageal samples, mostly members of the genus *Prevotella, Streptococcus* and *Neisseria*. Further analysis of the microbial composition of the esophagus revealed significant differences between EoE and control subjects. These differences were driven by EoE subjects with active esophageal inflammation when compared to controls, and only when taking into account relative abundance of species. An enrichment of the genus *Neisseria* and *Corynebacterium* was reported in esophageal samples of subjects with EoE and an enrichment of *Streptococcus* and *Atopobium* in the non-EoE control group.

Changes in the fecal microbiota have been largely associated with dietary interventions. Benitez et al. found that dietary changes affected the esophageal microbiota. Even though there were no significant differences in bacterial composition between active and control groups, an enrichment of the genus *Granulicatella* and *Campylobacter* was detected in subjects who reintroduced a highly allergenic food from the six-food elimination diet (Table 1).

This study in many ways is in agreement with previous reports in adults where the genus *Streptococcus* dominated the microbial composition of the healthy esophagus, while the genus *Neisseria* dominated the inflamed esophagus. Importantly, it would be of future interest to determine if specific bacterial lineages drive the inflammatory response or can be used to ameliorate the inflammation arising from the condition itself.

b. Denver and Chicago experience

Using the minimally invasive esophageal string test (EST), it was determined that the esophageal microbiota in 15 normal individuals was similar to the microbiota detected on esophageal biopsies, and that the esophageal bacterial genera were different from the bacterial contents of samples collected from the nasal and oral cavity (57).

More recently Harris et al. have shown that in 70 EST samples examined from children and adults, the bacterial load was increased with inflammation in the esophagus of subjects with EoE and gastroesophageal reflux disease (GERD) compared to controls (58). This increase in bacteria was independent of the degree of esophageal mucosal eosinophilia and treatment status. Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria were identified as the four predominant phyla in the esophagus of EoE, GERD and control subjects.

In about half of the subjects on proton-pump-inhibitor treatments in both Denver and Chicago, it was found that the phylum Proteobacteria was enriched in untreated (no steroids or diet) EoE subjects, similar to what has been observed in the Philadelphia study in active EoE subjects on PPI by Benitez et al. (56). The results from both of these studies demonstrate that active esophageal inflammation in EoE is associated with Proteobacteria enrichment on mucosal biopsies and EST samples (59).

Harris et al. showed that *Haemophilus* genus from the Proteobacteria phylum was significantly increased in the esophagus of untreated EoE subjects (Table 1). *Streptococcus,* the predominant genus in the esophagus, was decreased in PPI treated GERD subjects, and *Aggregatibacter* was enriched in the esophagus of PPI treated GERD subjects. Results from Benitez et al. and Harris et al. indicate that not only the degree of inflammation but also the treatment regimen has a strong impact on the esophageal microbiota in EoE. These results offer a baseline of knowledge of bacterial communities in the esophagus in EoE subjects, and provide important findings for the design of future studies, longitudinal analyses and the impact of specific treatments on the microbiota in EoE and other EGIDs. Further studies in different countries with larger numbers of subjects will help delineate the effect of alterations in the microbiota and treatment on esophageal inflammation, and potentially help to identify novel therapeutic targets for these diseases.

c. Pros and Cons and future needs

According to Hippocrates (460-377 BC), all diseases begin in the gut. The mucosal surface of the gastrointestinal tract and its microbiota act as an important organ for host defense. In the EoE microbiome studies performed in Philadelphia, Chicago and Denver it was observed that independent of the sites of collection, subjects with active EoE have an enrichment in Proteobacteria. However, each center has different treatment approaches, and we know that treatment has an impact on the esophageal microbiota. In the future it will be necessary to

examine the microbiome of EoE patients on dietary therapy and compare it to the steroid treated population. Further complicating the microbiome of the EoE population, a new population of patients with PPI responsive esophageal eosinophilia has been recently identified. These patients have esophageal eosinophilia and inflammation, but show clinical and histologic improvement in response to PPI therapy. Further investigation of the microbiota in these individuals may help determine which type of bacteria are associated with inflammation and which are associated with specific treatments (60).

Additionally, to determine the functional consequences of specific alterations in microbiome composition the field is in need of models development (animal, germ-free, *in vitro* and *ex vivo*) to better understand the impact of an altered microbiota or specific bacteria to demonstrate causality of the microbiota in allergic diseases and inflammation. There are multiple mouse models of EoE (61–68). Some of these models are transgenic combined with antigen exposure while others involve inducing an immune response with cutaneous or inhaled antigen exposure. The inflammation in these models varies in location (whole gut vs. esophagus alone), the type of granulocytes infiltrating, as well as position of inflammation within the esophagus (lamina propria vs. epithelia). Because of these differences, teasing the functional role of the microbiota in EoE will have additional obstacles to overcome before the data translates to the human population and will likely need to be performed in multiple murine and germ-free models.

Future multicenter studies will be necessary to determine if the gastrointestinal microbiome in subjects with atopy (asthma, AD, EGIDs) have some similar bacterial communities characteristic of allergic disease that may have the potential to be targeted and altered to reconstitute a physiologically “normal microbiota”.

VI. Conclusion

We are only at the beginning of untangling the complex network and relationships between the human gut microbiota, environmental factors and the genetics in health and atopic disease. However, the number of publications describing an altered microbiota in allergic disease has significantly increased in recent years. Further work including longitudinal studies and multicenter projects are still needed to better understand if the dysbiotic microbiota identified in various atopic diseases is a cause or consequence of the disease. Changes in lifestyle such as increase in Cesarean sections, early life formula feeding and increased use of antibiotics in infancy have all been linked to disturbed microbial homeostasis and appear to significantly influence childhood allergic disease susceptibility. However, the use of supplementation with probiotics to stabilize microbial homeostasis seems promising in some studies but conflicting in others, indicating that large multicenter trials, with well characterized populations and long term follow up will be necessary in the future to better understand the potential beneficial impact from probiotics, prebiotics and bacterial produced metabolites in the treatment of allergic disease.

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Abbreviations

AD	atopic dermatitis
BETs	basophil extracellular traps
EETs	eosinophil extracellular traps
EGIDs	eosinophilic gastrointestinal diseases
EoE	eosinophilic esophagitis
EST	esophageal string test
GERD	gastroesophageal reflux disease
IG	Immunoglobulin
GI	gastrointestinal
CPG-ODN	CpG oligonucliotides
OVA	ova-albumin
PPI	proton pump inhibitor
PPOIT	probiotic and peanut oral immunotherapy
TLR	toll-like receptor

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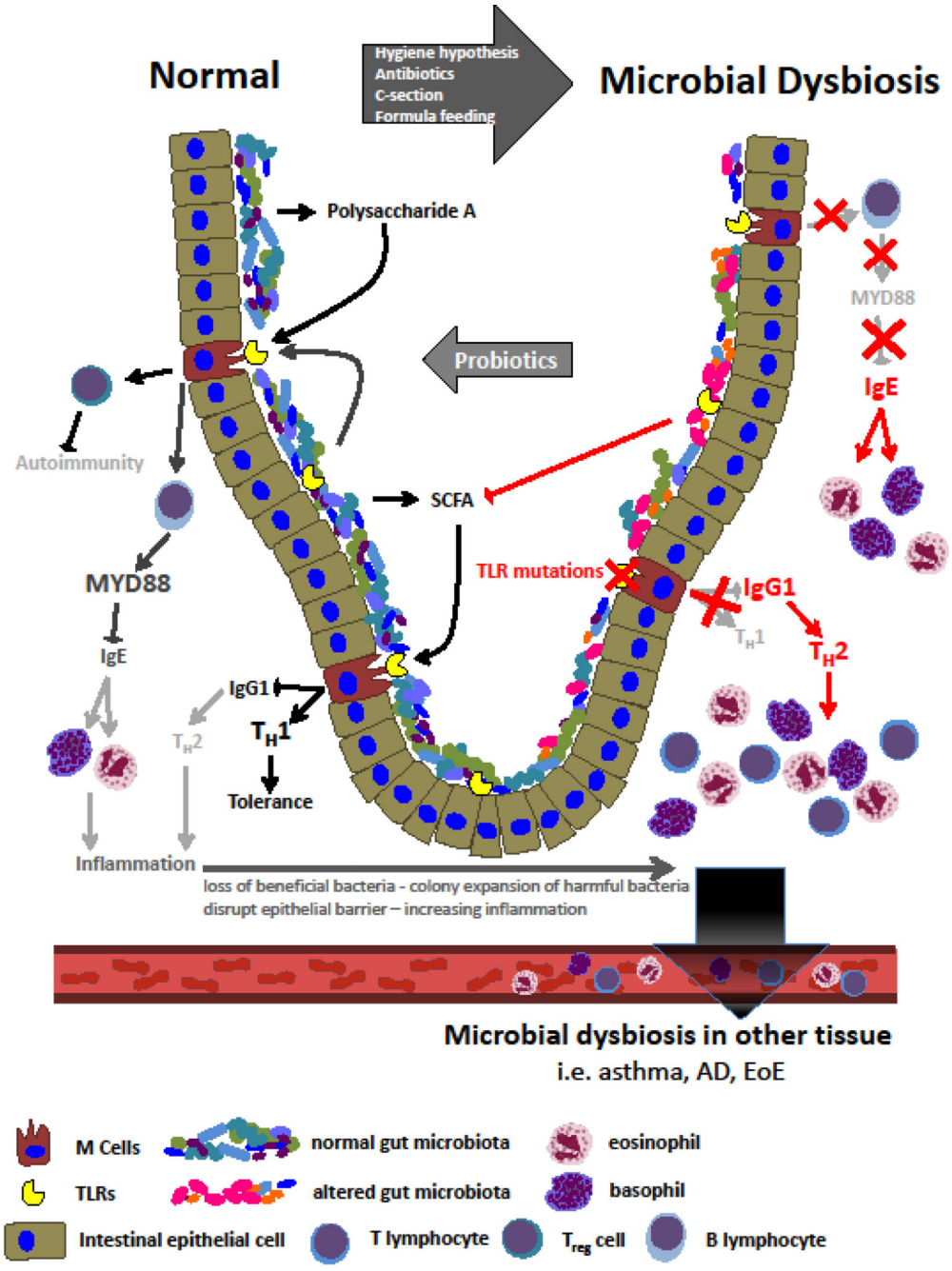

Figure 1. Commensal bacteria and their role in the development of tolerance

Commensal colonization decreases IgE-basophil axis and increases TLR stimulation thus promoting tolerance. Dysbiosis and ineffective TLR signaling leads to enhanced Th2 response and IgE-mediated disease.

Table 1

Microbiota associated with human gut atopy

Disease	Microbiota	Behavior of microbiota	Site	References and Authors
Cow Milk Allergy	*Lachnospiraceae* and *Ruminococcaceae*	increased relative abundance	fecal	(53) Berni Canani et al.
Food Allergies	*Clostridium I* and *Anaerobacter*	increased relative abundance	fecal	(54) Ling et al.
	Bacteroides and *Clostridium XVIII*	decreased relative abundance	fecal	(54) Ling et al.
	Sphingomonas, Sutterella, Bifidobacterium, Collinsella, Clostridium sensu stricto, Clostridium IV, Enterococcus, Lactobacillus, Roseburia, Faecalibacterium, Ruminococcus, Subdoligranulum, and *Akkermansia*	increases in the numbers	fecal	(55) Chen et al.
	Bacteroides, Parabacteroides, Prevotella, Alistipes, Streptococcus, and *Veillonella*	decreases in the numbers	fecal	(55) Chen et al.
EoE	*Prevotella, Streptococcus* and *Neisseria*	shared lineages	oral and esophageal	(56) Benitez et al.
	Neisseria and *Corynebacterium*	enrichment in active EoE	esophageal	(56) Benitez et al.
	Granulicatella and *Campylobacter*	enrichment with reintroduction of highly allergenic foods	esophageal	(56) Benitez et al.
	Proteobacteria (*Haemophilus*)	enriched in untreated EoE	esophageal	(58) Harris et al