



The impact of gut microbes in allergic diseases

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Purpose of review

The prevalence of allergic diseases continues to rise globally in developed countries. Since the initial proposal of the hygiene hypothesis, there has been increasing evidence to suggest that the intestinal microbiota, particularly during early infancy, plays a critical role in regulating immune responses associated with the development of atopy. This review evaluates the key epidemiologic and mechanistic data published to date.

Recent findings

Epidemiological data have provided the framework for animal studies investigating the importance of gut commensals in allergy development. These studies provide new insights about the microbial regulation of mucosal immune responses inside and outside the gut, and how these effects may drive allergic inflammation in susceptible individuals. Specific immune cells have been identified as mediators of these microbiota-regulated allergic responses.

Summary

In the last year, technological advances have provided us with a better understanding of the gut microbiome in healthy and allergic individuals. Recent studies have identified the associations between particular gut microbes and different disease phenotypes, as well as identified immune cells and their mediators involved in allergy development. This research has provided a number of host and microbe targets that may be used to develop novel therapies suitable for the treatment or prevention of allergic diseases.

Keywords

allergy, asthma, epigenetics, gut microbiota, perinatal immune development

INTRODUCTION

Coinciding with the major advances in medicine, sanitation, and other modern societal practices over the last 50 years, there has been a surge in the prevalence of allergic diseases including asthma, eczema, and food allergy in the developed world [1]. The hygiene hypothesis was initially proposed to explain this phenomenon, suggesting that microbial exposures during childhood are crucial for proper development of the immune system thereby avoiding allergy development [2]. Recent evidence points to the intestinal microbiota as playing a definitive role in influencing the immunological events that could lead to the development of atopic disorders [3]. It has been suggested that changes in diet, hygiene, and antibiotic use associated with urbanization have caused the disappearance of ancestral species of our indigenous microbiota. These cooperative niches are lost because the microbial communities in our guts are changing faster than our immune systems can adapt, promoting the rise of dysregulated

immunological disorders such as atopy, inflammatory bowel disease (IBD), and autoimmunity [4].

Human epidemiological data suggest that altered microbial exposures during childhood influence the outcome of allergic sensitization [5,6] and that the composition of the intestinal microbiota differs between allergic and nonallergic infants [7–9,10^a]. To date, there is no definitive subset of the microbiota that is associated with atopy, aside from limited evidence that *Clostridium difficile* may be overrepresented in some atopic infants [10^a]. Variability between cohorts is likely due in large

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Curr Opin Gastroenterol 2012, 28:563–569

DOI:10.1097/MOG.0b013e3283573017

KEY POINTS

- There is substantial evidence from human and animal studies to suggest that gut microbes play specific roles in the development of allergic diseases.
- The significance of perinatal programming in the development of atopic disorders has been the focus of several studies over the last year, highlighted by data that suggest that timing and pattern of microbial colonization in early life affects allergic sensitization.
- Several mechanistic studies demonstrate that gut commensals regulate specific immune cell populations (i.e. invariant natural killer T cells, basophils, and regulatory T cells) important for allergic responses, particularly in models of allergic asthma.
- Microbial exposure during the perinatal period has been linked to the epigenetic regulation of genes involved in allergic inflammation, hence altering the susceptibility to allergic diseases.

part to the differences in study design, sample preparation, and method of microbiota analysis. Interestingly, certain environmental factors seem to offer protection from allergic sensitization, including early life exposure to farm antigens [11[■]] and vaginal flora [12]. There is also evidence that antibiotic use in early infancy influences asthma susceptibility later in life [13,14[■]]; however, protopathic bias, reverse causation, and confounding by indication (i.e. respiratory infections) are factors that need to be considered when evaluating these datasets [15]. Although most studies have focused on the gut microbiome and allergy development, there is new evidence to suggest that the airway microbiome may also play a role in allergic asthma [16,17].

GUT MICROBES INFLUENCE ALLERGIC SENSITIZATION

In accordance with the data obtained from human studies, a similar role for the gut microbiota has been reported in animal models of allergy. Altering the microbiota by antibiotic or probiotic means was sufficient to change the outcome of disease in several allergy models [18]. In allergic airway disease, it has been shown that antibiotic treatment in combination with oral administration of *Candida albicans* enhanced the disease severity [19], while oral administration of *Mycobacterium vaccae* [20], *Helicobacter pylori* [21], and probiotics [22,23] ameliorated the disease symptoms in mice. Our studies show that antibiotics can have different effects on disease outcome when administered at clinically relevant doses. Vancomycin caused an

exacerbated allergic response, whereas streptomycin did not, suggesting that microbial composition, not simply bacterial numbers, can influence allergic sensitization [24[■]]. Monocolonization studies provide similar insights; colonizing germ-free mice with *Escherichia coli* or *Lactobacilli* failed to induce proper tolerogenic responses to food antigens compared to conventionalized controls, arguing for microbe-specific immune modulation rather than indiscriminant colonization effects [25]. In another study, depletion of specific *Clostridium* species promoted elevated IgE levels in the blood [26[■]], a trend also observed in mice whose gut flora was depleted by a cocktail of antibiotics [27[■]]. Adult mice treated with this antibiotic cocktail generated heightened allergic responses after intranasal house dust mite sensitization or papain [27[■]]. Thus, it is not surprising that germ-free mice exhibit dysregulated immune responses in models of asthma [28[■]] and food allergy [29[■]]. Additionally, mice deficient in Toll-like receptor (TLR) 4 or the short-chain fatty acid receptor GPR43 have exacerbated inflammation in models of asthma [30,31], suggesting that exposure to microbial products or metabolites rather than the bacteria themselves may be involved in modulating allergic responses.

Despite evidence in human and animal models supporting a role for intestinal bacteria in atopy, there have been far fewer successes in reducing allergy symptoms in humans using probiotics. Whether related to efficacy of the probiotic strains selected, time of administration, or variability in the individuals, the majority of these studies remain largely inconclusive (reviewed in [32[■]]). Very little is known about how individual bacterial species influence allergy development, so perhaps the choice of probiotic is the limiting factor in these studies.

SIGNIFICANCE OF PERINATAL PROGRAMMING

There is now substantial evidence in human and animal studies indicating that colonization of the gut early in life plays an important role in directing proper immune development. It has been hypothesized that there may be a 'critical window' during which this developmental program is initiated, and if this program is disrupted by an altered pattern of microbial colonization as a result of environmental factors such as mode of birth delivery, antibiotics, or diet, the host may be predisposed to a number of chronic diseases associated with aberrant mucosal immune responses [18].

At birth, the neonate begins its transition from a sterile fetal environment to an environment rich in

microorganisms. Initial colonization of the neonate is largely dependent on the mode of delivery [33]. Colonization of mucosal surfaces including the gut is essential for the maturation of both innate and adaptive immune responses, stimulating appropriate pattern recognition receptors and mediating specific immune cell interactions. There is evidence in germ-free as well as antibiotic-treated mice that absent or altered colonization disrupts normal T-helper-type 1/2 (Th1/Th2) balance by hindering Th1 cell maturation and promoting Th2 cell-polarized immune responses [34,35]. The enhanced susceptibility to allergy observed in these animals suggests that a similar microbiota-driven deviation in perinatal immune development could exist in humans.

The importance of normal microbial exposures during the perinatal period has been extensively studied in a number of animal models over the last year. Early life exposure to vancomycin was shown to enhance the severity of allergic asthma in mice, while the same antibiotic given during adulthood did not [24²²]. Similarly, Olszak *et al.* [36²²] showed microbial colonization of neonatal but not adult germ-free mice restores the inflammation associated with models of IBD and asthma to levels observed in conventional controls, a phenomenon they believe to be due to a reduction in the number of invariant natural killer T (iNKT) cells localized to the gut and lung mucosa. In another study, recolonizing germ-free mice at 5–6 weeks of age reduced the lung inflammation to levels observed in conventional controls [28²¹], emphasizing the need to further investigate the limitations of a defined perinatal program if one does exist.

CROSSTALK BETWEEN MUCOSAL SURFACES: MECHANISTIC INSIGHTS

As well as affecting intestinal mucosal immune development (reviewed in [37²²]), intestinal bacteria can influence the outcome of diseases affecting the tissues outside the gut. The strongest evidence exists for allergic asthma [3]. These studies support the existence of a 'gut–lung axis', a theory originally proposed to explain the effectiveness of probiotics on lung inflammation [32²¹]. It suggests that mucosal tissues function as a system-wide organ, where immune responses between mucosal sites can be integrated by mechanisms that remain largely undefined [38]. In the last year, several ground-breaking studies have explored the relationship between intestinal microbiota, the gut and lung mucosal immune system and allergic inflammation, and have provided some fascinating mechanistic insights.

Olszak *et al.* [36²²] used germ-free mice to show that the microbiota is required for the epigenetic regulation of immune mediators involved in the allergic response. Early microbial colonization reduced the methylation of the gene encoding the chemokine ligand 16 (CXCL16), a chemokine responsible for iNKT cell homing to mucosal tissues. Hypermethylation of CXCL16 enhances the iNKT cell populations at mucosal sites and could be responsible for the enhanced susceptibility in models of allergic inflammation (Fig. 1a).

In another recent study, Hill *et al.* [27²²] showed that mice treated with antibiotics have elevated IgE levels and higher numbers of circulating basophils in their blood compared with conventionally reared mice. They demonstrated that more severe allergic inflammation in antibiotic-treated mice was the result of basophil-mediated Th2 cell responses, and that increased IgE was the result of B-cell-intrinsic MyD88 signaling induced by bacteria-derived signals. On the basis of these data, they also show that increased IgE production as a result of dysregulated B cell responses may be linked to the upregulation of IL-3 receptor subunit CD123 on basophil precursors in the bone marrow, driving basophil hematopoiesis in animals lacking sufficient microbiota [27²²] (Fig. 1b). These data suggest that IgE may play a broader immunoregulatory role in allergic responses and that microbiota-derived signals can influence hematopoietic programs in the bone marrow as well as in the periphery to control allergic inflammation.

Another T-cell subset, the regulatory T cell (Treg), may be involved in mediating crosstalk at mucosal sites. Induction of immunological tolerance is essential for the control of allergic responses [39]. Human studies reveal that atopic children have reduced Treg expression profiles [40] and mothers with lower cord blood Treg populations have elevated IgE levels [41]. A study in mice by our group suggests that mechanisms of tolerance may be altered when microbe populations in the gut have shifted. An exacerbated allergic response in the lung after neonatal vancomycin treatment corresponded with a reduction in CD25⁺Foxp3⁺ regulatory T cells in the colon [24²²] (Fig. 1c). This observation is consistent with the data published by Josefowicz *et al.* [42²²], suggesting that extrathymically generated Tregs may be responsible for restraining aberrant allergic-type (Th2) inflammation in mucosal tissues. Mice deficient in inducible Treg cells spontaneously develop Th2-type pathologies at gut and lung mucosal interfaces.

Mucosal B cells residing in gut-associated lymphoid tissue can control Treg suppression of Th2 cell responses. B cells isolated from Peyer's patches of

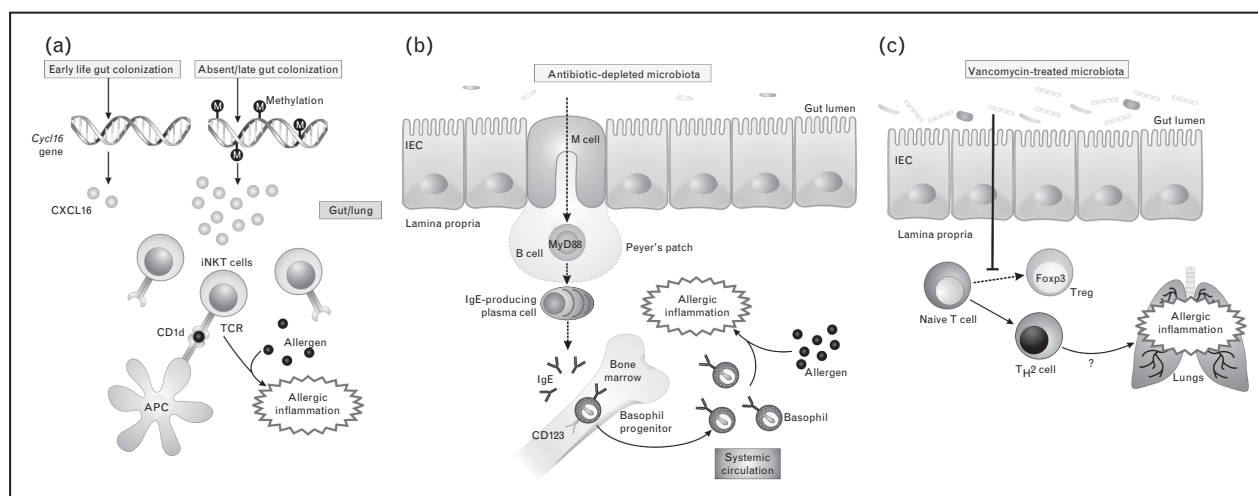


FIGURE 1. Mechanisms of allergy development linked to alterations in the intestinal microbiota. (a) Gut colonization alters the epigenetic regulation of CXC-chemokine ligand 16 (CXCL16) expression, affecting downstream populations of invariant natural killer T (iNKT) cells. Mice colonized with gut bacteria early in life have lower levels of CXCL16 compared to germ-free mice or mice colonized in adult life. CpG-rich regions of *Cxcl16* were hypermethylated in mice with late or absent colonization, which leads to increased CXCL16 protein levels, higher iNKT cell numbers in colon and lung tissue, and more severe inflammation in models of allergic asthma and colitis (panel based on Ref. [36[■]]). (b) Depletion of the microbiota with a cocktail of antibiotics results in elevated IgE levels in the blood that is linked to MyD88 signaling induced by bacterial signals in B cells residing in the intestinal lamina propria (based on Ref. [27[■]]). Circulating IgE leads to the upregulation of the IL-3 receptor CD123 on basophil precursors in the bone marrow, which is responsible for the increased number of circulating basophils in the blood and the enhanced allergic inflammation observed in murine models of asthma and skin allergy. (c) Shifting the composition of the intestinal microbiota with vancomycin (rather than reducing total numbers of bacteria) early in life is sufficient to induce exacerbated inflammation in a murine model of allergic asthma (based on Ref. [24[■]]). The enhanced T-helper-type 2 (Th2) cell responses in these antibiotic-treated mice have been associated with a reduction in Foxp3⁺ regulatory T (Treg) cells in the colon; however, the systemic defect remains to be characterized.

healthy mice were able to induce the production of Tregs, which, when adoptively transferred into irradiated mice, could alleviate the symptoms of allergic asthma [43[■]]. Perhaps in the presence of a healthy microbiota, mucosal B cells promote Treg induction in a MyD88-dependent manner (perhaps via TLRs); however, when the microbiota is altered or absent, signaling through MyD88 is inhibited, altering B cell-intrinsic programming, promoting increased IgE production, and production of allergic mediators like basophils in the blood and Th2 inflammatory responses at the sites of allergen exposure.

There is also evidence to suggest that innate immune mechanisms may be involved in allergy. Genetic polymorphisms and signaling defects in TLRs, predominantly TLR2 and TLR4, have been strongly associated with the development of atopy [44,45[■],46], while promoter variants of TLR2 have even been linked to neonatal Treg proliferation depending on maternal atopy status [47]. Innate and adaptive immune responses are likely linked in allergy development, as there is already data to suggest that symbiotic gut microbes signal through TLRs to induce tolerance in their hosts. Polysaccharide A from *Bacteroides fragilis* requires TLR2 signaling

on Foxp3⁺ Tregs to promote tolerance [48[■]], so perhaps other commensal microbes require similar innate immune receptors to influence systemic adaptive immune responses.

Now that we are starting to understand how microbial signals from the intestine are capable of regulating immune cell homeostasis in mucosal tissues, it will be necessary to characterize which immune cells or their mediators are able to 'bridge the gap' between mucosal interfaces. It is known that regulation of many immune cell populations by gut microbes is not restricted to the intestinal compartment (reviewed in [49]). Dendritic cells, which have also been implicated in allergic disorders [50], may also be involved in mediating crosstalk between mucosal tissues [51]; however, whether or not signals from intestinal bacteria regulate systemic populations of dendritic cells remains to be directly tested.

COLONIZATION OF THE GUT AND EPIGENETICS

Because of the complex cause of allergic diseases, many experts believe that gene–environment

interactions are responsible for triggering allergic sensitization. Epigenetic regulation is one mechanism through which signals from the environment could interact with genes involved in allergy development. The landmark study by Olszak *et al.* [36^{***}] was able to directly link the epigenetic modification of an important iNKT cell modulator, CXCL16, to early life microbial exposure in a model of allergic airways disease. They showed that the timing of gut colonization (early life versus adult life) is a key determinant of the methylation states of certain genes. There is also evidence that key immunological events like effector T-cell polarization (i.e. Th1/Th2 differentiation) are regulated by epigenetic mechanisms. Interestingly, these can be reversed to ameliorate the symptoms of experimental asthma in mice [52^{*}].

There has been substantial effort in recent years to understand how environmental exposures affect epigenetics in the context of allergic diseases in humans as well as in animal models (reviewed in [53]). For example, recent work by Brand *et al.* [54^{*}] demonstrated that exposure to a farm-derived environmental bacterium during pregnancy is sufficient to confer protection against asthma in mice, a phenomenon that has been linked to modifications in histone acetylation within the genes of the Th1/Th2 loci in CD4⁺ T cells. Although it is known that some environmental exposures are positively or negatively associated with the onset of atopy in infants (e.g. Caesarean delivery, use of antibiotics, perinatal stress, breastfeeding and probiotic use), it remains to be seen whether these factors elicit similar epigenetic modifications in the developing neonate.

CONCLUSION

There is increasing evidence from human clinical data and animal models that allergic diseases emerge as a result of interactions from genetic, environmental, and microbiota-driven factors during perinatal development. It is likely that varying contributions from these different risk factors cause the variability observed in allergic symptoms presented in the population: asthma in infants with atopic versus nonatopic mothers, children with eczema who do not complete an atopic march, children who develop food allergy *de novo*, or adult-onset atopy. These complexities may explain the difficulties experienced in human trials using helminth therapy or probiotics.

Despite the challenges presented by the complex nature of allergic diseases, it is encouraging that so much progress has been made in the last year to further our understanding of the gut microbiome

and its inter-relationship with host immune homeostasis. We are beginning to understand the importance of colonization in early life, and that altering microbial exposures during the perinatal period may promote dysregulated immune responses for a lifetime. For years, it has been hypothesized that mucosal tissues may function as a system-wide organ; however, we are only now beginning to understand the complex cellular and molecular mechanisms that mediate these interactions. This new knowledge has extended our understanding of allergy development and will likely pave the way for novel therapeutic or preventive approaches in the future.

Acknowledgements

The authors thank Marie-Claire Arrieta, Marta Wlodarska and Benjamin Willing for their critical review of the manuscript and for many thought-provoking discussions.

Conflicts of interest

The authors declare no conflicts of interest.

S.L.R. is funded by the Canadian Institutes of Health Research (CIHR) and work in our laboratory is supported by a CIHR Emerging Team Grant in partnership with Genome BC and the AllerGen NCE.

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This study describes how epigenetic modifications in TH1/TH2 cytokine genes during allergic sensitization and challenge affect differential secretion of cytokines by CD4⁺ T cells and ultimately the outcome of experimental asthma. These data suggest that environmental factors early in life could affect the epigenetic regulation of these genes and alter an individual's susceptibility to atopy.

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This study emphasizes the effect of microbes on the epigenetic regulation of gene-expression patterns. They show that bacterial exposure during pregnancy changes the DNA methylation patterns and expression of TH1/TH2 cytokine genes in the offspring, conferring protection against experimental asthma in mice.