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Title

The Role of Gut Microbiota in Atopic Asthma and Allergy, Implications in the Understanding of Disease Pathogenesis.

Running title

Gut Microbiota in Atopic Asthma and Allergy.

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Abbreviations

IgE, immunoglobulin E; Th, T helper; IL, interleukin; GIT, gastrointestinal tract; GF, germ-free; APCs, antigen-presenting cells; DCs, dendritic cells; CCL5, chemokine ligand 5; TGF-beta, transforming growth factor beta; TNF-alpha, tumor necrosis factor alpha; TLRs, Toll-like receptors; CXCR2, CXC chemokine receptor 2; Treg, regulatory T; SCFA, short-chain fatty acid; IgA, immunoglobulin A; iNKT, invariant natural killer T.

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Abstract

Asthma is a clinical syndrome characterized by chronic airway inflammation. There is mounting evidence on the role of microbiota in the development of asthma. This review focuses on the role of microbiota in maintaining the integrity of the epithelia and their role in regulating the immune response. The review compiles data from multiple studies on the role of microbiota in the innate immune response and the development and differentiation of CD4⁺ T cells, a major component of the adaptive arm of the immune response. As a result of dysbiosis, invariant natural killer T cells may induce T helper 2 cell differentiation and immunoglobulin E isotype switching through the release of Interleukin-4 and Interleukin-13. Furthermore, degradation of immunoglobulin A antibodies, increased circulating mast cells and basophils, and inflammation are among other mechanisms by which dysbiosis can induce or exacerbate asthma. After explaining the underlying mechanisms, the review derives conclusions from studies that investigate dysbiosis in infancy and the development of asthma later in life. The review also includes studies that investigate asthmatic mothers and the development of asthma in children and the role of dysbiosis in that regard. Finally, the review explains the statistical relationship between eczema and asthma through multiple studies that investigate the role of dysbiosis in both atopic states. This review provides insight into the role of dysbiosis in asthma, and an understanding that is required to establish clinical trials which aim to modulate the gut microbiota as a means of preventing and treating asthma.

Keywords

Asthma, Microbiota/Microbiome, Dysbiosis

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b. Conflict of interest statement

The authors declare no conflict of interest.

Main text

Introduction

Asthma is a clinical syndrome characterized by an immunological response of the airways which includes chronic inflammation, hyperreactivity, and airway obstruction in response to various stimuli.¹ A study estimated that 300 million people, or 4.5% of the world's population, suffer from asthma.² Allergic (extrinsic) asthma typically develops in childhood with the majority of affected children displaying at least one form of allergy, as opposed to adult-onset non-allergic (intrinsic) asthma.³

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It is well established that the microbiota plays a central role in modulating both the innate and adaptive arms of the immune response.⁴ Multiple factors may lead to an imbalanced gut microbiota (dysbiosis). This review focuses on the specific mechanisms by which dysbiosis can induce the inflammation that underlies asthma and derives conclusions from studies that investigate dysbiosis in infancy and the development of asthma later in life.

Papers for this review were gathered primarily via PubMed searches using the following keywords in various combinations: microbiota, microbiome, gut, intestinal, dysbiosis, inflammation, signaling, asthma, allergy and/or atopy. Papers were chosen from a 20-year timeframe between 1999 and 2019.

Role of Gut Microbiota in Modulating the Immune Response

The vast array of microorganisms that inhabit the human gastrointestinal tract (GIT) constitute the gut microbiota with an estimated number of 3.8×10^{13} cells. This number has been recently reported as the same order as the total number of human cells.⁵ This dynamic population of microorganisms maintains a mutually beneficial relationship with their host by performing several physiological functions for the host while benefiting from the nutrient-rich environment of the human gut.

Besides their role in metabolism, the gut microbiota are involved in maintaining the integrity of the gastrointestinal epithelia, protecting against pathogens, and regulating the host immune response. The gut microbiota play an essential role in this immune homeostasis. Microbiota modulate a large repertoire of signaling mechanisms that control the functioning of the immune system, including epigenetic remodeling which affects gene expression as explained in this section.

In the innate immune system, gut microbiota modify the function of antigen-presenting cells (APCs) of the GIT to be different than that of other APCs in the body. Dendritic cells (DCs) of Peyer's patches produce higher levels of interleukin (IL)-10, an anti-inflammatory cytokine, than DCs of the spleen.⁶ This may be mediated by beneficial bacteria which induce the production of IL-10 and prevent the secretion of proinflammatory cytokines through their secreted metabolites.⁷ Gut macrophages, under homeostatic conditions, reside in a state of anergy in which they do not produce cytokines including IL-1, IL-6, IL-12, chemokine ligand 5 (CCL5), transforming growth factor beta (TGF-beta), and tumor necrosis factor alpha (TNF-alpha) in response to microbe-associated molecular patterns binding to their Toll-like receptors (TLRs).⁸ The study revealed that multiple down-regulatory mechanisms are responsible for suppressing NF- κ B signaling pathway proteins and preventing the translocation of NF- κ B into the nucleus and induction of gene expression. Gut macrophages, however, maintain their microbicidal activities and host defense mechanisms despite not having an inflammatory response to the vast array of microorganisms of the gut.

Gut microbiota produce metabolites such as short-chain fatty acids (SCFAs) which may stimulate IgA production through class switching, resulting in the immune tolerance of the gut. Low levels of IgA at the intestinal surface barrier may contribute to allergies and asthma.⁴

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Gut microbiota play an important role in the homing of mast cells to the intestine, which controls the systemic immune response. A study found that gut microbiota are important for the proper intestinal epithelial expression of ligands for CXC chemokine receptor 2 (CXCR2), which is expressed on mast cells.⁹ The study compared the transcriptional levels of three ligands in germ-free (GF) mice to that of wild type mice. GF models of animals with sterile GITs demonstrate the role of gut microbiota in modulating a balance in the immune response.¹⁰ The three ligands investigated were keratinocyte-derived cytokine, lipopolysaccharide-induced CXC chemokine, and macrophage inflammatory protein 2. The transcription of all three ligands was significantly reduced in GF mice indicating a possible transcriptional effect of commensals on intestinal epithelial cells via TLR signaling pathway. Therefore, GF mice have fewer mast cells in the intestine and increased mast cells in the blood, which corresponds to an inflammatory state and is observed in diseases like asthma (Figure 1).⁹

Furthermore, gut microbiota affect the development and differentiation of CD4⁺ T cells. GF mice demonstrated a decreased number of CD4⁺ T cells in their lamina propria and a bias towards Th2 cells relative to Th1 cells.¹¹ In addition, gut microbiota affect the development and function of regulatory T (Treg) cells by expressing an outer membrane pili-like protein. This protein induces Treg cells which suppress the excessive activation of Th2 cells involved in atopic diseases including asthma.^{7,12} Figure 1 illustrates the role of gut microbiota in modulating the homeostatic adaptive immune response both systemically and in the gut.

In summary, gut microbiota downregulate the release of pro-inflammatory cytokines to modulate the immune response and maintain tissue homeostasis in noninflamed mucosa. The association between recurring inflammation of the intestinal mucosa and loss of the epithelial barrier has been established.¹³ Consequently, microbiota may indirectly affect the integrity of the gut epithelial barrier. Moreover, microbiota produce SCFAs which directly affect the epithelial integrity, as explained in the next section.

Role of Gut Microbiota in Modulating Epithelial Integrity

The gastrointestinal epithelial barrier plays an essential role in the regulation of the immune system and acts as a semipermeable barrier that allows for the absorption of nutrients and limits the transport of harmful antigens and microorganisms. Intestinal barrier defects result in an increase of the gut permeability, exposing the body to foreign substances. This may lead to abnormal immune responses triggered by the different inflammatory cytokines present in the blood circulation. This phenomenon has been associated with a broad range of diseases including allergies.¹⁴

Epithelial permeability is modulated by the microbiota through SCFA signaling. SCFAs are the products of nonstarch polysaccharide metabolism by the microbiota and are typically 2-4 carbons long.¹⁵ The cells of the colon are heavily reliant on SCFAs as energy sources - butyrate in particular supplies up to 70% of the energy requirements of colonocytes.^{4,16}

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The mucus layer coating the epithelium is an important physical barrier to microbial entry. The main component of intestinal mucus is glycoprotein mucin 2, which polymerizes to form a gel with a mesh-like structure that can trap microbes. Gut microbiota reside partly within the superficial mucus layer and partially feed off the glycans attached to the mucin core.¹⁷ Extraneous pathogens are trapped within the mucin meshes and are flushed out of the gut through regular bowel movements. The mucus layer may change in its thickness and hence become more permeable to pathogenic invasion. Mucin 2 production by goblet cells is upregulated in response to acetate and butyrate, the metabolic products of gut microbiota.¹⁸

The permeability of the epithelium is also dependent on tight junctions. Tight junctions include large transmembrane proteins which link cells in the extracellular matrix and are bound to cytoskeletal proteins in the cytoplasm. The tightness of the junction is modulated by the interaction of cytoskeletal actin with myosin, which in turn, is regulated by kinases such as myosin light chain kinase and Rho kinase.¹⁹ SCFA signaling from gut microbiota can alter the activity of myosin light chain kinase and Rho kinase which may induce the assembly of new tight junctions.²⁰ The most important signals for tight junction modulation are both inflammatory and anti-inflammatory cytokines including interferon-gamma, TNF-alpha, IL-1, IL-6 and IL-10.¹³ Through modified signalling, an imbalance or disruption in the gut microflora may affect the epithelial integrity of the GIT.

Gut Microbiota and Dysbiosis

A disruption in the balance of the gut microbiota impairs their ability to perform one or more of the aforementioned functions which can lead to both intestinal and extra-intestinal diseases such as asthma.²¹ A loss in the homeostatic balance is called dysbiosis and can be due to an imbalance in the bacterial composition or a change in its distribution through the GIT.²² Dysbiosis can come in a myriad of forms, yet can be grouped within one of three categories including: loss of beneficial microorganisms; excessive growth of a pathogenic microorganism; and loss of microbial diversity.²²

The three categories are not mutually exclusive and, in fact, usually occur together. The excessive use of antibiotics contributes to dysbiosis which should be concerning, particularly in regards to infants, as it has been shown that the use of antibiotics in infancy induces dysbiosis later in life.²³ In fact, antibiotic use in the first 3 years of life has been directly linked to an increased risk of developing asthma which is discussed later in the review.²⁴ Breast milk is also a key factor that influences the microbiome diversity in infancy.⁴

Impact of Dysbiosis on the Immune Response and on Epithelial Integrity and Link to Asthma

As described in the previous sections, the microbiota modulate the immune response and epithelial integrity which maintain tissue homeostasis in noninflamed mucosa by downregulating the release of pro-inflammatory cytokines and by producing SCFA. Therefore, dysbiosis results in loss of

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homeostasis and epithelial integrity which may lead to what is known as “leaky gut syndrome”. Leaky gut is a syndrome of intestinal permeability due to disruption of tight junctions which allows for the entry of antigens into the blood stream. This stimulates the immune response and is thought to be related to many chronic diseases including asthma. In fact, asthma can be managed, at least in part, by improving intestinal permeability via repairing the gastrointestinal mucosa to prevent immunological cascades.²⁵

It is important to note that the immune system functions to prevent dysbiosis. However, once established, dysbiotic gut microbiota affect local and systemic immune cells. This includes both innate and adaptive arms of the immune system (Figure 1).

The association between a dysbiotic microbial environment and immune-related diseases has been set for numerous diseases including inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, as well as, asthma, the focus of this discussion. The type of dysbiosis and the mechanisms of causation is different and in many cases, unclear. An example of such mechanisms is the production of inflammatory cytokines and the induction of costimulation of APCs in response to an infection. Infection acts as a danger signal and activates self-recognizing immune cells that respond to self-antigens.²⁶ A more recent study proposed a new mechanism that involves post-translational modification of proteins.²⁷ This study suggests that a dysbiotic microbial environment may contain enzymes that abnormally process luminal proteins which can result in the production of new epitopes and induce an immune response.

Furthermore, certain bacteria common in a dysbiotic community lead to uncoupling of bacterial eradication from the inflammation that they cause. In other words, they have mechanisms that allow them to be safe from the inflammation that they cause. For example, *Porphyromonas gingivalis* stimulates the degradation of the TLR2 signaling adaptor MYD88 whilst maintaining inflammation by means of cross talk of the TLR2 and the receptor for the complement protein C5a. This uncoupling provides bacteria with the ability to perpetuate inflammation whilst maintaining their presence in the gut.²⁸

Likewise, a dysbiotic environment within the gut can impact the function of the adaptive component of the immune system. An example of this relationship is the effect of gut microbiota on the degradation of IgA manifested through the levels of IgA in feces. Increased levels of IgE and decreased levels of IgA are associated with asthma. As discussed later, it was postulated that certain signals from a healthy microbiome limits isotype switching to IgE and increases levels of IgA.²⁹ On the other hand, certain pathogenic bacteria such as genus *Sutterella* is able to cause degradation of IgA and its stabilizing peptide. This genus is therefore capable of inducing intestinal inflammation and so is the fecal transplant from an infected individual.³⁰

All these are generic mechanisms tied to the gut microbiome that apply to asthma as well as other diseases. The following section provides examples of studies that found specific connections between dysbiosis and the pathogenesis of asthma.

The Link between the Pathogenesis of Asthma and Dysbiosis

With the emerging evidence supporting the role of gut microbiota in the immune system, dysbiosis is increasingly being associated with asthma. The allergic response in asthma is characterized by the Th2 cytokines IL-4, IL-5 and IL-13. IgE antibodies circulate in blood and bind to the high-affinity receptor FcεRI.³¹ It is important to note that there is an increased localization of mast cells in the airways of an asthmatic individual than in a control.³²

A study showed that antibiotic treatment, increased levels of lung inflammation, IgE titers, and the number of circulating basophils, are three important factors in the pathophysiology of asthma. It was hypothesized that commensal-driven signals limit isotype switching to IgE and direct it towards IgA and reduce levels of circulating basophils which in turn alleviates lung inflammation.^{4,29} A recent study revealed that antigens contributing to childhood airway diseases were associated with alterations in the composition of gut microbiota and elevated levels of fecal IgE.³³ Another study showed that early exposure to intestinal microbiota reduces the levels of invariant natural killer T (iNKT) cells which produce IL-4 and IL-13 that accumulate in the lungs of GF mice and promote isotype switching to IgE.³⁴ An increase in iNKT cells caused by microbial deficiency leads to increased susceptibility to asthma (Figures 1 and 2). It is postulated that the microbiota work partly through epigenetic modification of the gene that encodes for the chemokine ligand CXCL16 in the lungs to which iNKT cells migrate and bind.³⁵ A corroborating study demonstrated that early exposure to microbial organisms for GF mice reduces the accumulation of iNKT cells in the gut and lungs.³⁶

A clinical study that included 58 asthmatic and healthy subjects explored the link between the composition of gut microbiota and asthma. Results revealed a positive correlation between asthma and the relative abundance of *Bifidobacterium* and *Lachnospiraceae* family. Conversely, the relative abundance of *Bacteroides* and *Enterobacteriaceae* family decreased in asthmatic subjects.³⁷ A recent study reported the significant association between *Lactobacilli* and *E. coli* and asthma in 80 adult asthmatic patients.³⁸ Figure 2 summarizes some of the possible mechanisms by which gut dysbiosis may increase the risk of asthma.

Dysbiosis in Infancy and Asthma Later in Life

Multiple studies have shown that there is an increased likelihood for a patient to develop asthma if they have had gut dysbiosis early in life. A study showed that children with *Clostridium difficile* colonization at 1 month were more likely to develop asthma and eczema, but only if born to atopic parents. Interestingly, the prevalence of *C. difficile* colonization in this study was found to be

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positively correlated with hospital births and C-sections.³⁹ Furthermore, a study by the Canadian Healthy Infant Longitudinal Development on 3-month-old infants found that the prevalence of 4 genera of bacteria, *Faecalibacterium*, *Lachnospira*, *Veillonella* and *Rothia*, is negatively associated with the prevalence of asthma.⁴⁰ An expansion of this study proved the immunomodulatory function of these specific types of bacteria. A mouse model of experimental asthma showed a reduction in inflammation of airways after colonization of the mice GIT with stool supplemented with these bacterial taxa.⁴¹ A study on the association between bacterial gut colonization patterns during the first year of life and later risk of asthma among children revealed that gut microbiota were associated with asthma at age 5 in 690 children. The study also reported that the greatest significant changes in the population structure among children were found between ages 1 month and 1 year with dominating genera *Bacteroides*, *Bifidobacterium* and *Veillonella*. Furthermore, there was a significant difference in the structure of microbial populations at one year in nonasthmatic children compared to children who had asthma at age five, which was positively associated with higher abundance of *Veillonella* and lower abundance of *Roseburia*, *Alistipes*, *Faecalibacterium*, *Bifidobacterium*, *Ruminococcus*, *Lachnospiraceae incertae sedis* and *Dialister*. Interestingly, this association between developing asthma before age 5 and the structure of microbial population was only confined to the children who were born to asthmatic mothers.⁴² The association between asthmatic mothers and development of asthma in children has been explained through dysbiosis in a few studies which are discussed in the following section.

Dysbiosis and the Development of Asthma in Children Born to Asthmatic Mothers

To further expand on the linkage between gut microbiota and asthma, a study was conducted on the gut microbiota of infants born to asthmatic mothers. The experiment showed that the effect of being born to an asthmatic mother was gender specific where 3-4 month old male infants showed decreased abundance of *Lactobacillus* bacteria while female infants of the same age showed increased abundance of *Bacteriodaceae*.⁴³ The mechanism by which *Lactobacillus* acts to prevent the emergence of asthma and other allergic diseases is not exactly known. A study however, showed that continuous administration of 10^7 colony-forming units *Lactobacillus gasseri* for 4 weeks prevented *Dermatophagoides pteronyssinus* (house dust mites) induced inflammation of airway in mouse models of allergic asthma.⁴⁴ However, A randomized trial on humans showed that *Lactobacillus* supplementation for the first 6 months does not seem to cause a decreased rate of development of asthma.⁴⁵ In terms of increased abundance of *Bacteriodaceae*, a separate study showed that children that develop asthma show low diversity of gut microbiota with predominance of *Bacteriodaceae*.⁴⁶ This shows that an asthmatic mother can increase the chance of the child of developing asthma in a non-genetic mechanism via increasing abundance of *Bacteriodaceae* and decreasing that of *Lactobacillus*.

Dysbiosis Explaining the Link between the Seemingly Unrelated Conditions: Asthma and Eczema

Eczema is a reaction pattern of the skin to different diseases which is characterized by skin inflammation.⁴⁷ A study found that adult eczema was associated with higher prevalence of asthma. The same study also found that eczema is associated with asthma severity measured in terms of the number of attacks in the past year and disease persistency.⁴⁸ Another study showed that very early-onset eczema, i.e. onset at less than 6 months of age, was related to current asthma. Late-onset eczema, i.e. onset at 2-5 years of age, was associated with increased risk of asthma at 12 years of age.⁴⁹ This association between asthma and eczema can be explained, partially, through the gut microbiota and dysbiosis. In a study, the gut microbiota of participants with non-food allergies that include asthma and eczema had higher tendency to have 9 altered bacterial taxa.⁵⁰ Furthermore, it has been found that children who have IgE-associated eczema (atopic eczema) and subsequently developed asthma, had lower gut microbial diversity than those who did not.⁵¹

Conclusion

The root causes of asthma remain elusive, but its primary mechanisms - loss of epithelial integrity and a hyperreactive immune response - may well be explained, at least in part, by a dysbiotic microbial environment in the gut. Whether dysbiosis is the sole cause or a contributing factor among many, it is evident that it plays an important role in the pathogenesis, severity, and persistence of asthma. A deeper and a more thorough understanding of the role of microbiota in asthma is required before initiating clinical trials that target gut microbiota in the aim of preventing and treating the disease.

Author contributions

Ghizlane Bendriss, Noha A. Yousri and Dalia Zakaria conceived the original idea of the review. Mohamed Salameh, Zain Burney and Nada Mhaimeed searched for the data and compiled most of the articles that were used in the review under the supervision of Dalia Zakaria. Mohammad Salameh developed the idea and worked on setting the outline and structure of the review with the assistance of Zain Burney and Nada Mhaimeed. Mohammad Salameh and Zain Burney drafted the manuscript with a major contribution by Mohammad Salameh. Dalia Zakaria reviewed all the different phases of the manuscript and designed the diagrams. Nada Mhaimeed and Ibrahim Laswi also contributed to the preparation of the diagrams. Ibrahim Laswi compiled more articles, reviewed and edited the manuscript. All authors provided critical feedback and helped shape the review and manuscript.

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Figure legends

Figure 1. Gut microbiota plays an essential role in modulating the homeostatic adaptive immune response both systemically and in the gut. In a healthy gut, commensal bacteria produce SCFA which play an essential role in maintaining the epithelial integrity and mucosal homeostasis including the stimulation of IgA class switching.⁴ DCs produce higher levels of anti-inflammatory cytokines such as IL-10.^{6,7} Furthermore, gut macrophages, under homeostatic conditions, enter a state of anergy while maintaining their microbicidal activities and host defense mechanisms. Besides driving APCs into a state of anergy, intestinal epithelial cells express the chemokine CXCL1 to which mast cells expressing CXCR2 are attracted. This leads to the homing of mast cells to the intestine.⁹ Gut microbiota is important for the proper expression of these ligands through the TLR signaling pathway of intestinal epithelial cells. In addition to the innate immune system, gut microbiota also affects the development and the unbiased differentiation of CD4⁺ into Th1, Th2 or Th17 cells.¹¹ In addition, gut microbiota also affects the development and function of Treg cells which play a significant role in immune homeostasis.¹² Gut microbiota modulates the epithelial barrier through the production of certain metabolites that maintain epithelial integrity and induce tissue repair. Conversely, dysbiosis is characterized by a state of inflammation which plays a role in the disruption of tight junctions and reducing epithelial integrity. For example, colonization of the gut by *C. difficile* may induce a cascade of inflammatory responses which results in mucosal inflammation and the disruption of the epithelial barrier. This state of inflammatory process involves TLR5 binding and the activation of NFκB which is the key transcription factor controlling the expression of inflammatory genes.^{52, 53} A dysbiotic

environment within the gut may also result in the degradation of IgA and increased levels of IgE which is linked to the pathogenesis of asthma as well as the other allergies.^{4, 29}

Figure 2. Gut dysbiosis may increase the risk of asthma through a number of possible mechanisms. Dysbiosis may lead to upregulation of TLR4, IgA degradation, and increased costimulation which may result in inflammation. Furthermore, as a result of dysbiosis, the immune system may fail to terminate inflammation following clearance of the infection. Inflammation, along with the loss of epithelial integrity caused by it, lead to an increased risk of asthma. Downregulation of the chemokine CXCL1 reduces homing of mast cells to the intestine which consequently increases circulating mast cells. Production of IL-13 and IL-4 by circulating iNKT induces IgE isotype switching and Th2 cell differentiation which may increase the risk of asthma.



