HHS Public Access

Author manuscript

Curr Opin Pediatr. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

Curr Opin Pediatr. 2016 December ; 28(6): 764–771. doi:10.1097/MOP.0000000000000419.

The Microbiome in Asthma

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Abstract

Purpose of review—Asthma is a complex and heterogeneous disease with strong genetic and environmental components that manifests within a variety of clinical features and diverse patterns of immune responses. Asthma prevalence has dramatically increased over the last decade in Westernize societies, thereby suggesting a key function of environmental factors in disease promotion and development.

Recent Findings—"Early-life" microbial exposures and bacterial colonization are crucial for the maturation and the education of the immune system. The commensal flora is also critical in order to maintain immune homeostasis at the mucosal surfaces and may consequently play an important function in allergic disease development. Recent evidences demonstrate that asthma influences and is also impacted by the composition and function of the human intestinal and respiratory microbiome.

Summary—In this review, we will summarize the most recent findings on how asthma development is connected with respiratory and intestinal microbial dysbiosis. We will highlight and discuss the recent research that reveal the existence of a "gut-lung" microbial axis and its impact on asthma development. We will also analyze how "early-life" microbial exposure affects the immune response and their consequences on asthma development.

Keywords

Asthma; Microbiome; Mycobiome; Lower airways; Gut; Metabolites

Introduction

Asthma is a chronic inflammatory disease of the respiratory airways characterized by an inappropriate immune response resulting in reversible airflow obstruction, airway hyper-responsiveness (AHR), mucus overproduction, tissue eosinophilia, and intense airway wall remodeling [1,2]. It is a complex and heterogeneous disease which exists under different phenotypes, known as allergic and non-allergic or intrinsic asthma. Allergic asthma affects

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Conflicts of interest

There are no conflicts of interest.

mostly children and is triggered by aeroallergens, such as house dust mite (HDM), pollen, and fungal spores. Predominantly developing during the first years of life, allergic asthma involves the production of allergen-specific immunoglobulin type E (IgE) and the participation of an adaptive T helper cells Type 2 (Th2) immune response. On the contrary, non-allergic intrinsic asthma occurs later in life independently of aeroallergen sensitization, results from air pollution, chronic or recurrent bacterial and viral infections of the bronchi and sinuses, is more severe and predominant in women [3]. Compared with non-allergic patients, allergic asthmatic subjects respond better to a medical treatment combining β2-adrenergic receptor agonists and inhaled corticosteroids. However, 5 to 10% of bronchial asthmatic subjects are unresponsive to this treatment and considered as refractory or steroid resistant asthmatic [4,5].

Asthma and allergic diseases have become a major health issue worldwide with increased prevalence over the last 50 years. In the USA, subjects affected with asthma rose from 20.3 millions in 2001 to 25.7 million in 2010 [6]. Although genetic polymorphisms have been associated with asthma development [7], this steep increase in asthma prevalence over the last decades implicates the existence of environmental factors which promote disease on genetically predisposed hosts. Among them, early-life sensitization to aeroallergen [8] and microbial exposure [9] as well as infections with respiratory viruses [10] and changes in the host microbiome composition [11] have been associated with increased risk of asthma development.

In the present review we will highlight and discuss major findings demonstrating a connection between the gut and lung microbiome with asthma development and how host-microorganism interactions in early-life affect the immune response and their consequences on allergic diseases development.

Immunology of asthma

Asthma is a complex and heterogeneous disease initially thought to be mediated by eosinophils and Th2 immune cells as evidenced by elevated numbers of interleukin 4 (IL-4) and IL-5 producing $CD4^+$ T cells in bronchoalveolar lavage (BAL) fluid collected from allergic asthmatic patients [12]. Asthma heterogeneity was further characterized by gene expression analysis and the identification of two human endotypes, $Th2^{high}$ and $Th2^{low}$, which differ mainly by their Th2 cytokine expression pattern and their response to medical treatment [13] (Figure 1). The $Th2^{high}$ endotype is associated with increased activation of dendritic cells (DCs), $CD4^+$ Th2 T cells, innate lymphoid cells type 2 (ILC2) and B cells resulting in heightened allergen-specific IgE and Th2 cytokine production as well as elevated numbers of lung infiltrating and circulating eosinophils. On the other hand, the $Th2^{low}$ endotype display reduced levels of Th2 cells activation, Th2 cytokine production and eosinophil accumulation in the lungs (Figure 1). Strategies aiming to block the α chain of the IL-4 receptor and thereby disrupting both IL-4 and IL-13 signaling (Dupilumab) [14] or targeting directly IL-13 (Lebrikizumab) [15] improve asthma clinical aspects for the $Th2^{high}$ phenotype. However, those therapeutical approaches appear to be less successful for patients with a $Th2^{low}$ profile. Recent studies demonstrate that intrinsic non-allergic, non-IgE dependent, late-onset asthma triggered by environmental factors such as respiratory airway

infection, air pollution and/or smoking, involves a Th1/Th17 immune response and intense neutrophils recruitment in the lungs [16–18] (Figure 1). The secretion of IL-8, IL-17, IL-21, IL-22, and monocyte colony stimulatory factor by either $CD4^+$ Th17 T cells or ILC3s will promote the recruitment of neutrophils and airway inflammation [19,20]. This Th17-driven asthma phenotype is linked to a more severe pathology and a poor response to corticosteroid treatment [21–23] (Figure 1). Asthma endotype classification and disease heterogeneity are not fixed and exclusive. While each endotype is characterized by specific immune responses and pathological patterns, the possibility that those pathways concur and act synergistically in asthmatic patients cannot be excluded.

The microbiome

The advancement and the development of affordable next generation high-throughput sequencing techniques has expanded our knowledge on the composition of the human microbiome as well as its relation to disease [24]. Recently, it has been estimated that the human body is composed of $3x10^{13}$ eukaryotic cells and colonized by $4\ x10^{13}$ bacteria, roughly representing a 1:1 ratio [25]. The microbiome is constituted of bacteria, fungi, and viruses that colonize body surfaces exposed or opened to the outside environment such as the skin [26], the lung [27,28], the oral cavity [29] and the gastrointestinal tract [30]. Most of the current studies focus on the characterization of the bacterial microbiota and omit the presence of the virome and the mycobiome. Fungi are often considered as pathogenic, however many fungal species do not trigger disease and coexist with the bacterial microbiota. Compared to the bacterial microbiome, the characterization of the mycobiome composition is rendered more complicated by the lack of a complete fungal database; it is currently estimated that only 1% of fungal species are present in the NCBI GenBank database and among those sequences a large part are not classified correctly or of uncharacterized origin [31]. Currently, up to 75 different fungal genera have been described and are present at different body sites such as the oral cavity [32], intestine [33], skin [34] and lungs [35]. Commensal fungi also regulate immune responses and host physiology as fungal dysbiosis is associated with changes in the commensal bacteria composition that could potentially fuel further inflammation and disease [31,36,37].

Early microbial exposure influences the atopic status

The continuous rise in asthma incidence in industrialized societies cannot be attributed to genetic factors alone and implies that some environmental factors resulting from the modern lifestyle promotes asthma [38] (Figure 2). The “hygiene hypothesis” states that personal hygiene improvement, declining family size and decreased infection burden result in reduced early-life microbial exposure and promotion of atopic diseases [39]. The current dogma is that microbial colonization begins at birth from exposure to the mother’s vaginal and fecal microbial flora, although recent studies demonstrate that microbial exposition may start earlier, *in utero*, as indicated by the presence of bacterial DNA in the placenta and meconium of pre-term babies [40,41]. Maternal farming microbial exposure during pregnancy is correlated with elevated levels of regulatory T cells (T_{Reg}) and decreased Th2 cytokine secretion [42]. Those studies suggest the possibility that *in utero* bacterial exposure could initiate colonization at different fetal surfaces during pregnancy. Alternatively, it is the maternal exposure that directly influences the fetal immune development. The mode of

delivery is also known to strongly influence the infant gut bacterial colonization [43]. Infants born by Caesarian section (C-section) are at higher risks of developing atopic diseases [44,45] and are principally colonized by microbial communities similar to the mother's skin, such as *Staphylococcus* species [43,46]. Early colonization patterns of the neonatal gut, but not the airways, is also affected by the mode of delivery; C-section promotes the emergence of *Citrobacter*, *Klebsiella*, *Enterobacter*, and *Enterococcus* species whereas *E. coli* colonization was associated with natural delivery [47]. Right after birth, inhalation of environmental microorganisms will result in lung and airways colonization [48,49]. In mice neonates, the main bacterial phyla of the lung microbiome, Proteobacteria and Firmicutes, will stabilize during the first month of life and aging is correlated with an outgrowth of Bacteroidetes [49]. Similar bacterial clusters were detected in the gut and respiratory airways of young infants diagnosed with cystic fibrosis, thereby suggesting the possibility of dynamic crosstalk and interactions between the intestinal and the respiratory microbiota [48].

Breast- versus formula-feeding is another factor that influences the infant microbiome composition and development. Breast milk shapes the infant gut microbial communities and promotes intestinal homeostasis through diverse mechanisms; it is a source of bacterial communities transmitted to the infant [50], it provides maternal antibodies, such as secretory IgA, that further promotes gut homeostasis [51], and supplies nutrients that shape the microbial flora composition during the first year of life [46]. Consequently, compared to formula-fed infants, breast-fed infants harbor a more uniform commensal flora composition associated with higher abundance of *Bifidobacteria* and *Lactobacillus,* whereas formula-fed infants display higher abundances of *Bacteroides*, *Clostridium*, *Streptococcus, Enterobacteria, and Veillonella* species (reviewed in [52]). Compared to formula-fed infants, breast-feeding is also associated with decreased risks of developing asthma and this trend is even higher in infants with family history of asthma [53–55]. However, whether infant feeding mode can be correlated with increased risks to develop atopic diseases still remains uncertain and will require further studies in order to identify the specific microbial communities present in breast-versus formula-fed infants.

Early microbial exposure and bacterial colonization are important for the maturation and the generation of a normal immune system. Compared to specific pathogen-free (SPF) mice, Germ-free (GF) mice which are devoid of any microbiota harbor distinct physical differences such as increased size cecum, decreased gastrointestinal motility, abnormal morphology, and smaller Peyer's patches and mesenteric lymph nodes [56]. Absence of microbial flora in GF mice is linked to a Th2-skewed immune response and elevated levels of serum IgE [57,58]. Mono-colonization of GF mice with either *Bacteroides fragilis* or segmented filamentous bacteria will redirect and balance the T cell immune response towards stable Th1/Th2 or Th17 phenotypes respectively [59,60]. There is a critical "time window of opportunity" in early-life for an appropriate education of the immune system by the microbiota [56]. Therefore, early-life events able to alter the microbiota composition or disrupt microbiota-immune interactions can be deleterious and promote immune deviation towards atopy. In mice, interruption of microbial sensing by the immune system promotes the development of allergic inflammation and food allergy development has been associated with the emergence of a specific microbial signature [61–63]. In humans, the use of

antibiotics during the pre- or post-natal period is associated with increased development of atopic dermatitis and asthma [64,65]. Early-life intestinal microbial flora dysbiosis in children is correlated with increased asthma development and a reduction of four specific bacterial genera: *Faecalibacterium, Lachnospira, Veillonella* and *Rothia* [66]. Supplementation of these 4 bacterial species in GF mice reduced asthma incidence in their adult offspring by decreasing neutrophil recruitment in the lungs and reducing the Th1/Th17 immune response associated with severe human asthma [66]. Whether early-life microbial dysbiosis triggered by environmental factors elicit allergic disease or is a consequence of allergic disease development remains unknown and will require further investigations.

Asthma and the lung microbiome

Until recently, healthy lungs and respiratory airways were considered sterile. The use of 16S rDNA sequencing techniques demonstrated the presence of a lung microbiota in healthy subjects as well as its specific topographical distribution since bacterial biomass decreases from upper to lower respiratory airways [27]. The characterization of the lung microbiome is rendered more difficult due to its low microbial load compared to other organs such as the skin, the oral cavity and the gut, and the possible presence of bacterial contaminants from either the upper respiratory airways during sample collection by bronchoscopy or contaminants present in DNA extraction kit [67].

In mice, absence of lung microbial colonization in OVA-sensitized and intranasally challenged GF mice results in enhanced allergic airway inflammation with increased levels of Th2 cytokines and serum IgE as well as augmented lymphocytes and eosinophils infiltrations. Colonization of GF mice by SPF flora before allergen exposure protects from allergic airway inflammation development [68]. Furthermore, SPF mice intra-nasally treated with either *Staphylococcus sciuri* or *Escherichia coli* were protected from allergic airway development [69,70].

In humans, 16s rDNA sequencing of various type of specimens, including bronchial brushing [71,72], BAL [73], and induced sputum samples [74] allowed the detection in the lower airways of bacterial species belonging to the 5 major phyla (Proteobacteria, Firmicutes, Actinobacteria, Fusobacterium and Bacteroidetes). The asthmatic airway microbiota composition differs from healthy subjects and is characterized by a higher bacterial load and diversity as well as increased abundance of species belonging to the Proteobacteria phylum such as Comamonadaceae, Nitrosomonadaceae, Oxalobacteraceae, Pastereurellaceae and Pseudomonadaceae families [71,72,74]. On the other hand, members from the Bacteroidetes and Firmicutes phyla have been found more abundantly in the airways of healthy controls subjects [71,72,74]. The microbial airway composition also differs based on clinical features and the type of immune response triggered by the aeroallergens. For instance, the airways of corticosteroid-resistant and severe asthmatic patients are predominantly colonized by pathogenic organisms such as *M. catarrhalis* or members of the *Haemophilus* or *Streptococcus* genera [75]. Th17 immune-mediated asthma exhibit a predominance of Proteobacteria belonging to the Pasteurellaceae, Enterobacteriaceae, and Bacillaceae families [76]. This specific bacterial signature was only observed in Th17-driven inflammation and did not overlap with a Th2-driven airway

inflammation [76]. Studies linking the metabolic activities of those dysbiotic bacteria to asthma severity and treatment prognosis are further required. For example, steroid-resistance treatment could be related to the presence and expansion in the airways of bacterial species that are capable of degrading steroids.

Fungi are also present in the sputum of allergic and healthy subjects [77]. *Malassezia pachydermatis*; a fungus already associated with the development of atopic dermatitis, was present in the sputum of asthmatic patients and absent from healthy controls [77]. The last couple of years have seen huge improvement in high-throughput fungal rDNA sequencing techniques and refinement of fungal sequences databases [31]. Therefore, in order to gain a better understanding of the lung mycobiome function during asthma, further studies taking advantage of those advances are required to define the role of fungal colonization in asthma.

Asthma and the gut microbiome

The intestinal microbiota is composed of hundreds to thousands of bacterial species belonging mostly to the Firmicutes and Bacteroidetes phyla [24]. Its composition varies within the distinct section of the gastrointestinal tract and their physiological characteristics, resulting in different intestinal micro-habitats such as the gut lumen, colonic mucus and colonic crypts and their capacities to promote differently the growth of bacterial species (Reviewed in [78]). The bacterial composition of the intestinal microbiota is known to affect the development and the phenotype of immune responses [79]. Defined bacteria or their derived microbial products will trigger and modulate distinctively either a regulatory or an effector immune response. Commensal flora derived products, such as polysaccharide A of *Bacteroides fragilis* [80], several short-chain fatty acids (SCFAs) generated by fermentation of dietary fibers [81–83], or the colonization of GF mice by a defined mix of Clostridium strains [84,85], prevent intestinal pathology by promoting the differentiation of suppressor T_{Reg} cells.

On the other hand, the gut microbiota is a potent stimulus for the generation of pro-inflammatory and harmful autoimmune responses. Alterations in the intestinal flora composition are associated with disease development, however how intestinal dysbiosis affects the host systemic immune response remains uncertain. Diet is known to modulate the gut microbiota composition in humans and mice [48,86]. Increased asthma incidence in developed countries could be related to recent lifestyle changes characterized by reduced dietary fibers consumption and higher fat intake. Intestinal microbiota as well as their derived metabolites production can be influenced by those dietary components. Bacterial-derived metabolites are not specifically confined to the intestinal tract but can enter the blood circulation and affect immune cells and responses at distant sites. Trompette *et al.* demonstrated in a murine model of allergic airway inflammation that dietary fibers influence the intestinal microbial flora composition and increase the circulating levels of SCFAs [86]. Treatment with the SCFA propionate promotes the hematopoiesis of DCs and convert those cells into poor drivers of lung Th2 immune responses resulting in reduced airway inflammation [86].

The strong influence of diet on intestinal flora composition and bacterial metabolite production on atopic disease development has also been highlighted in human studies. In

young children, development of allergic disease is often preceded by alteration of the intestinal microbial flora composition [11,87]. The intestinal human microbiota coevolves with the diet as demonstrated by a study of the intestinal microbiota composition of 2 different children cohorts; one from Europe and the other one from Burkina-Faso. The fiber rich diet from Burkinabe children is associated with intestinal microbiome compositional changes and the emergence of bacterial species producing high levels of SCFAs and specialized in energy intake from polysaccharides [88]. On the other hand, the western diet with reduced fiber and increased animal protein, sugar and starch content consumed by European children is correlated to a complete lack or under representation of those SCFAs bacterial producers and consequently with a significant decrease in SCFAs production [88].

How the commensal fungi within the gastrointestinal tract influences the systemic immune response and affects the development of allergic airway inflammation is still under investigation. Recently, in a murine model of allergic airway inflammation Wheeler *et al.* demonstrated that commensal fungi dysbiosis, obtained by means of oral antifungal treatment, is associated with exacerbated allergic airway inflammation and the growth of *Aspergillus*, *Wallemia*, and *Epicoccum* species [37]. Oral gavage of these 3 fungi into HDM-sensitized mice was sufficient to recapitulate similar and exaggerated levels of allergic airway inflammation as the ones observed in mice treated with antifungal drugs [37]. Importantly, fungal dysbiosis was also associated with a complete restructuring of the commensal microbial communities, including a decrease in abundance of Bacteroides, Clostridium, and Desulfovibrio and an increase of Anaerostipes, Coprococcus, and Streptococcus in mice with allergic airway inflammation [37]. On the other hand, gut bacterial dysbiosis induced by antibiotics treatment also promotes allergic airway inflammation by boosting the overgrowth of intestinal Candida fungal species and the polarization of lung M2 macrophages [89]. These data demonstrate that commensal fungi and microbial communities are deeply connected and dependent on one another thereby suggesting that the impact of the mycobiome on atopic disease development in underestimated and should be further investigated.

Conclusion

Asthma is an heterogeneous and complex disease that can manifest within a variety of different clinical features and patterns of immune responses. A growing number of studies demonstrate that asthma influences and is also impacted by the composition of the intestinal and respiratory microbiota. As discussed above, evidences generated from human and murine investigations indicate that lung microbial as well as intestinal microbial and fungi communities diverge between healthy and allergic airway inflammation. Additionally, the composition of those communities is also impacted by a wide variety of environmental factors such as diet and microbial exposures in “early-life”. The specific mechanisms by which this “gut-lung” microbiota axis impact health and disease requires further investigation. However, modulation of systemic immune responses by the intestinal microbial flora can occur through the release in the circulation of bacterial-derived metabolites with immuno-modulatory properties. Deciphering further the mechanisms and mediators of this “gut-lung” axis could potentially lead to a new therapeutic strategies

aiming to modulate the composition of the respiratory and/or intestinal microbiome in order to promote resistance to allergic airway disease.

Acknowledgments

Financial support and sponsorship.

This work was supported by the following grants: R01AI117968-01A1 (to M.A.) and R21AI112826-02 (to T.R.C.).

Abbreviation

AHR	Airway hyper-responsiveness
HDM	House Dust Mite
IgE	Immunoglobulin type E
Th2	T helper cells Type 2
IL-	Interleukin
BAL	Bronchoalveolar lavage
ILC	Innate lymphoid cells
DCs	Dendritic cells
T_{Reg}	Regulatory T cells
SPF	Specific pathogen free
ICSs	Inhaled corticosteroids
SCFA	short-chain fatty acid

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Key points

- Asthma is a complex and heterogeneous disease with strong genetic and environmental components that manifests within a variety of clinical features and immune responses.
- Lung tissues are not sterile; fungi and bacterial species have been detected in various type of airway specimens collected from both healthy and asthmatic subjects.
- The lower airway and intestinal microbial composition is different between healthy and asthmatic subjects and early-life intestinal microbial flora dysbiosis is correlated with increased asthma incidence.
- Existence of a “gut-lung” axis that could potentially be used for therapeutics purposes.

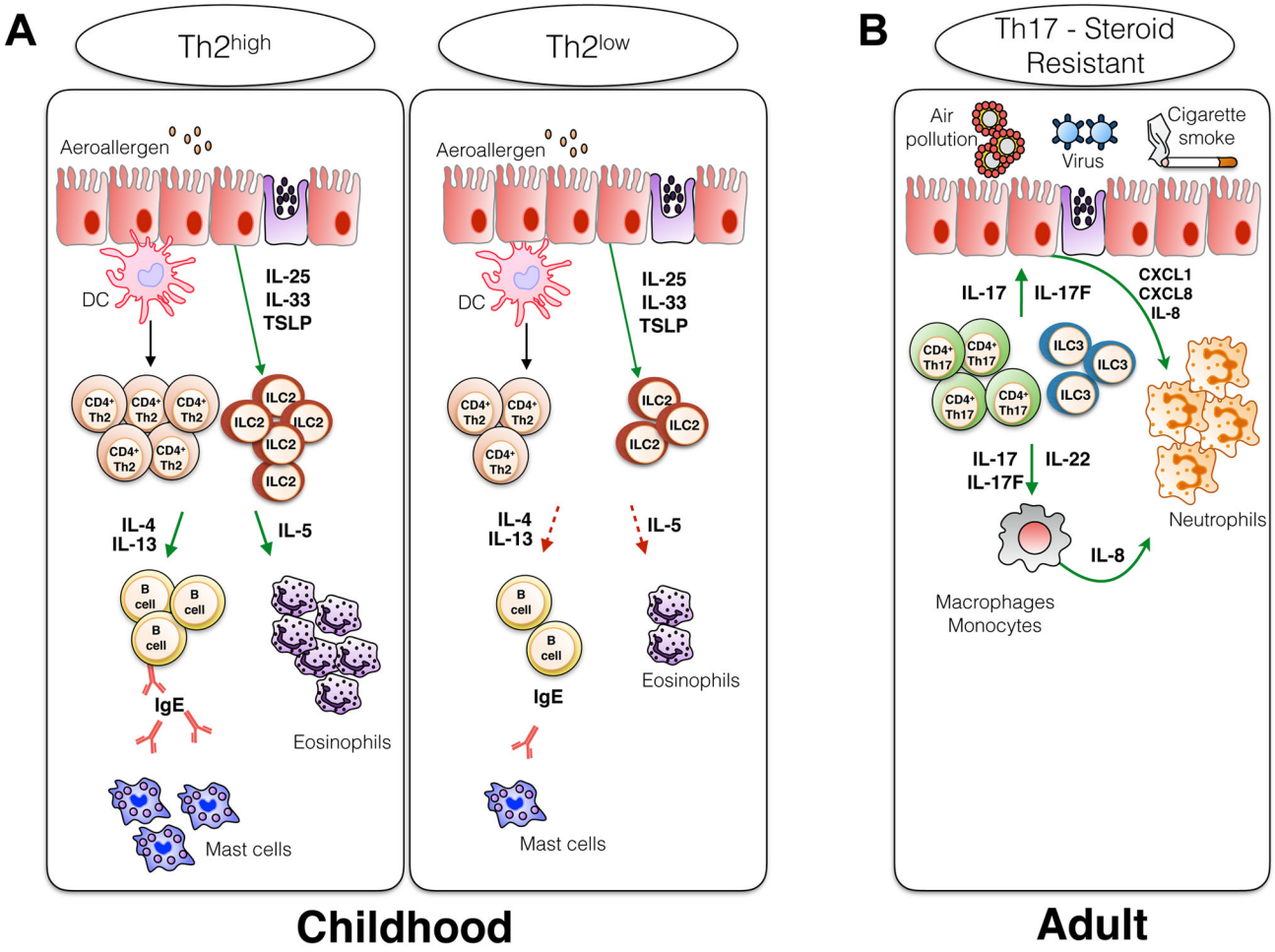

Figure 1. Asthma heterogeneity and immunological patterns involved in disease pathology

(A) The Th2^{high} endotype is characterized by increased activation of DCs, Th2 CD4^{+} T cells, ILC2s and B cells which result in heightened allergen-specific IgE and Th2 cytokine production as well as elevated numbers of lung infiltrating and circulating eosinophils. On the other hand, the Th2^{low} endotype display reduced activation of Th2 cells and Th2 cytokine production as well as decreased numbers of eosinophils. **(B)** Intrinsic non-allergic and steroid resistant asthma is IgE and Th2 independent, associated with a Th1/Th17 immune response and the participation of ILC3s as well as intense neutrophils recruitment in the lungs.

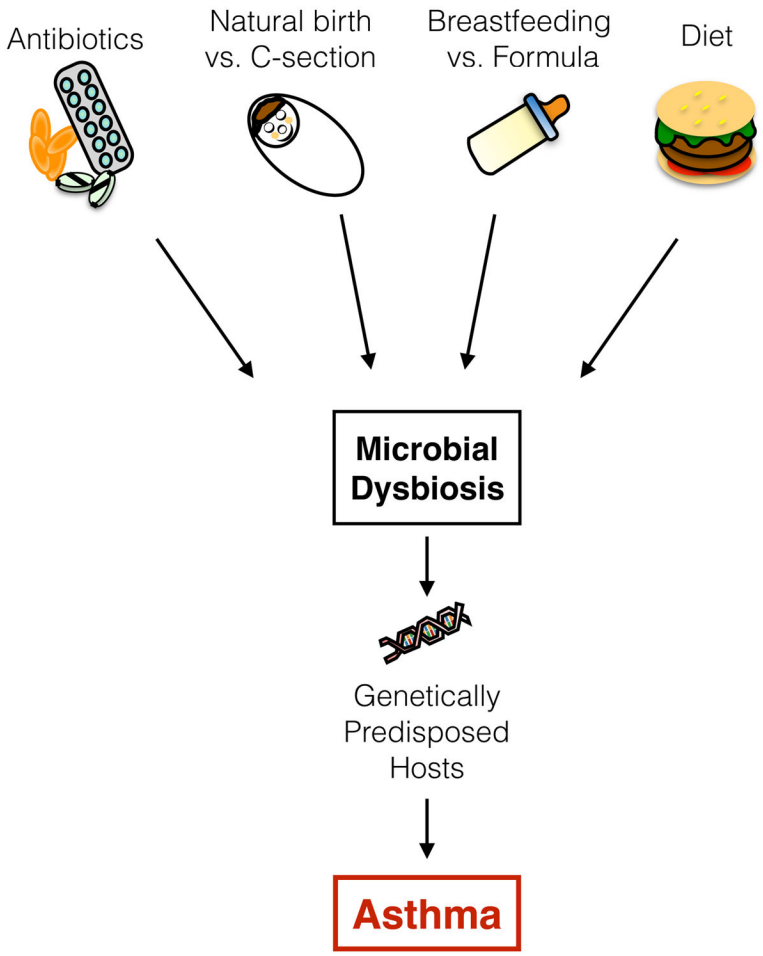

Figure 2. Environmental factors influencing asthma development

The continuous rise in asthma incidence in "westernized" societies cannot be only attributed to a genetic component alone; environmental factors resulting from a modern lifestyle are involved in this increased prevalence. The composition of the microbial flora is constantly fluctuating and strongly influenced by environmental factors. Microbial dysbiosis can be triggered by stress, the modern-lifestyle diet, antibiotic use, birth and feeding mode. By promoting the growth of pathogenic bacteria, microbial dysbiosis will also prevent early exposure to health-promoting bacteria. In genetically predisposed hosts, this alteration in microbial communities will promote the development of asthma.