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Review Article

The Role of Upper Airway Microbiome in the Development of Adult Asthma

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AMP, antimicrobial peptides; AR, allergic rhinitis; COPD, chronic obstructive pulmonary disease; CRS, chronic rhinosinusitis; CRSwNP,

ABSTRACT

Clinical and molecular phenotypes of asthma are complex. The main phenotypes of adult asthma are characterized by eosinophil and/or neutrophil cell dominant airway inflammation that represent distinct clinical features. Upper and lower airways constitute a unique system and their interaction shows functional complementarity. Although human upper airway contains various indigenous commensals and opportunistic pathogenic microbiome, imbalance of this interactions lead to pathogen overgrowth and increased inflammation and airway remodeling. Competition for epithelial cell attachment, different susceptibilities to host defense molecules and antimicrobial peptides, and the production of proinflammatory cytokine and pattern recognition receptors possibly determine the pattern of this inflammation. Exposure to environmental factors, including infection, air pollution, smoking is commonly associated with asthma comorbidity, severity, exacerbation and resistance to anti-microbial and steroid treatment, and these effects may also be modulated by host and microbial genetics. Administration of probiotic, antibiotic and corticosteroid treatment for asthma may modify the composition of resident microbiota and clinical features. This review summarizes the effect of some environmental factors on the upper respiratory microbiome, the interaction between host-microbiome, and potential impact of asthma treatment on the composition of the upper airway microbiome.

Keywords: Asthma; Eosinophils; Inflammation; Microbiome; Neutrophils; 16S rRNA

INTRODUCTION

Asthma is a common chronic airway disease that affects people of all ages. Over 300 million people worldwide are affected by asthma, and the prevalence is high in children and in the elderly (1,2). After 40-50 years of age, most new cases of asthma are nonallergic and related to obesity, more severe disease, and having a lower lung function (3,4). Asthma-related morbidity and mortality are higher in older patients (1). The pathophysiology of asthma is complex, and involves airway inflammation that contributes to respiratory symptoms, airway hyperresponsiveness, and airflow limitation leading to a variety of more permanent changes in the airway which is commonly known as airway remodeling (5).

chronic rhinosinusitis with nasal polyposis; DC, dendritic cell; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; GWAS, genome-wide association studies; hBD-2, human β-defensin 2; ICS, inhaled corticosteroid; ILC2, group 2 innate lymphoid cell; MUC5AC, mucin 5AC; NA, not applicable; NP, nasal polyposis; PM, particulate matter; PM10, particulate matter of less than 10 μm; PM2.5, particulate matter of less than 2.5 μm; PRR, pattern recognition receptor; SE-IgE, IgE against S. aureus superantigens.

Asthma is a polygenic, multifactorial disorder and its etiology is related with interactions between genetic susceptibility, host factors, and environmental exposures. Genome-wide association studies of asthma have identified potential risk alleles and loci in different ethnic groups, providing an understanding of its genetic basis and functional role involved in disease pathogenesis (6). Genotype-specific risk factors are critical modifiers of the disease development, and host factors include age, gender, obesity and diet, whereas environmental exposures include tobacco smoke, air pollution, pollens, infection, mold and damp, animal exposure, occupational exposures, antibiotics, aspirin and non-steroidal anti-inflammatory drugs (7,8). These factors have been associated with either risk for or protection against upper and lower airway disease. Imbalance of resident microbial communities in the airways is affected by these factors and could be important in defining states of health or disease. Microbial exposures can be considered as plausible mechanisms for many of these associations, and the upper airway might be the direct site of important host-environment interactions (9).

According to the united airway concept, upper and lower airways constitute a unique system and their interactions show functional complementarity (10). In healthy adults, microbiome biomass decreases from the upper to lower respiratory tract, and community composition in the lung is not largely indistinguishable from upper airway microbiota (11). Microbiome sampling in upper airway is non-invasive and easy to assess (12), and may predict similar changes occurring in the lower airways. Our team showed that *Staphylococcal* enterotoxin sensitization was associated with asthma, especially in the adult and elderly asthma with sputum eosinophilia and chronic rhinosinusitis (CRS), which suggests that microbiome in upper airway could play an important role in the pathogenesis of asthma (13-15).

The Human Microbiome Project reported bacterial distribution by body sites as a reference, and the majority of the bacteria reside in the gastrointestinal tract with 29%, and the distribution in the airway is 14% (16). The total number of bacteria across the whole body of the adult is approximately 3.8×10^{13} and this number is close to human cells in the body (17). Although members of the host microbiota are referred to as commensals, their interaction with a host can be contextual according to host genetic and other risk factors.

Most recent studies have been using advanced molecular techniques such as 16S rRNA gene sequencing (partial genomes), shotgun sequencing (complete genomes), metabolomics (non-protein small molecules), metaproteomics (protein) and metatranscriptomics (RNA) that are revolutionizing our understanding of the human microbiome (18). The pathobiological impact of the airway microbiome in asthma is only partially known. This review summarizes recent findings and emerging concepts on the role of environmental and genetic factors in the dysbiosis of upper airway microbiota, immune regulation of microbiome, and the effect of asthma therapies on the composition of nasal microbiome in adult asthma.

AIRWAY INFLAMMATION AND ASTHMA PHENOTYPES

Airway inflammation is a key component in the asthma pathogenesis and has been divided into two types based on dominant cells present in the airway: eosinophilic (T2) and neutrophilic (non-T2). T2 asthma can be further split into allergic eosinophilic asthma and non-allergic eosinophilic asthma, while non-T2 asthma can be divided in three inflammatory phenotypes based on sputum neutrophilia: neutrophilic, mixed granulocytic and paucigranulocytic (19).

Allergic T2 inflammation is mediated by antigen specific Th2 cells that produce IL-4, IL-5, IL-9, and IL-13 resulting IgE-mediated mast cell and basophil degranulation and eosinophil activation (20). Th2 derived cytokines play an important role in airway eosinophilia, airway hyperreactivity and mucus hypersecretion, and IgE mediated mast cell activation induce airway inflammation, mucus production, smooth muscle cell contraction and airway remodeling (21). Biological actions of current monoclonal antibody and corticosteroid therapies are both effective in these patients (19), while nonallergic eosinophilic asthma patients have persistent high numbers of eosinophils despite treatment of corticosteroids (22). Air pollutants, microbes and glycolipids induce the release of epithelium-derived cytokines such as IL-33, IL-25, or TSLP, stimulating group 2 innate lymphoid cells (ILC2s) to secrete high amounts of IL-5 and IL-13, leading to eosinophilic inflammation (20). Though the secretion of IL-4 by ILC2 is disparate, IL-4 is critical to promote Th2 activation (23,24). A recent study revealed that ILC2s are accumulated predominantly in lung tissue and led to a pronounced T2 inflammation in the lung after allergen challenge (25). Eosinophilic asthma that develops in adulthood has a greater probability of chronic rhinosinusitis with nasal polyposis (CRSwNP) and it is often nonallergic (26).

Nonallergic asthma is defined with negative skin prick or *in vitro*-specific IgE tests to common allergens (27). The mechanisms contributing nonallergic asthma is less understood, and suggested mechanisms are hypersensitivity reactions to unidentified allergens such as fungi, recurrent or persistent infections, autoimmune mechanism, neutrophil mediated immune response or the activation of the IL-17-dependent pathway (28). Non-T2 neutrophilic asthma biomarkers are YKL-40 (chitinase-like protein) and IL-17 that are correlated with airway neutrophilia, obesity and asthma severity by inducing relative steroid unresponsiveness (29,30). Paucigranulocytic asthma patients have lower levels of fractional exhaled nitric oxide (FeNO), eosinophil cationic protein (ECP) and IL-8 in induced sputum, and 14.8% of these patients had poor asthma control despite the absence of inflammatory cells in their sputum (31). As similar to neutrophilic asthma, inhaled corticosteroid (ICS) treatment could be less effective in these patients (32). Th2/Th17 (IL-4 and IL-17) predominant asthmatics show blood eosinophilia, airway obstruction and hyperresponsiveness, and relative corticosteroid insensitivity (33). Also, in mixed granulocytic asthma patients, sputum IL-8, ECP and FeNO levels are elevated (31). Macrolides could be effective for non-T2 asthma patients through decreasing airway concentrations of IL-8 and neutrophil numbers and improving quality-of-life scores (34).

Certain airway bacterial taxa have potential influence on immunity. For example, *Streptococcus pneumoniae* and *Haemophilus influenzae* increase IL-8 production through an NF-κB-dependent mechanism (35). *Staphylococcus aureus* manipulates the airway mucosal immunology by releasing IL-33 from respiratory epithelium and activates ILC2 and Th2 cells, mast cell degranulation, B-cell activation and IgE formation, and eosinophil stimulation leading to epithelial damage (36). Proteobacteria is associated with epithelial expression of Th17 inflammation-related genes and negatively correlated with bronchial eosinophil (37).

HOST-MICROBIOTA AND INTERRELATIONSHIPS BETWEEN MICROBIOTA

Commensals are a critical and active inducer of immune regulatory responses. The epithelial mucosa and the dendritic cells (DCs), which are in continuous contact with the airway
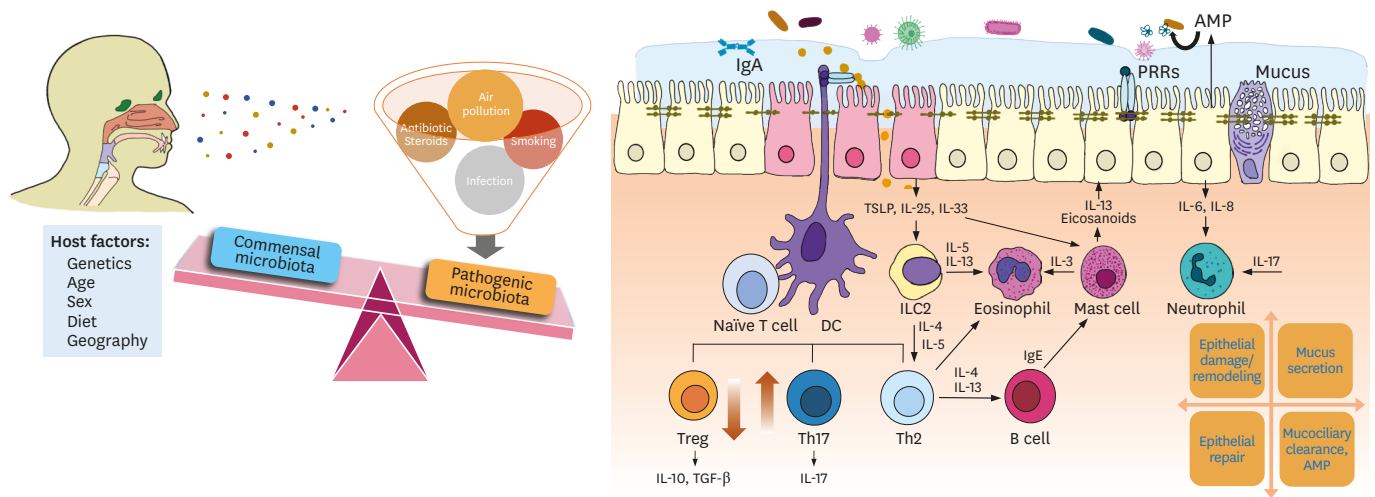
Figure 1. Upper-airway dysbiosis and potential immunopathology in the airway inflammation. Persistent exposure to pathogens and other risk factors promotes airway inflammation through induction of both innate immune cells and adaptive immune cells. Although some risk factors are prominently related to upper airway dysbiosis, host factors are also considerably overlap in the development of airway diseases. Mucins in interaction with IgA and AMP contribute to innate immunity. Upon microbial signals, DCs activate antigen-specific naïve T cells which further drive development in effector Th cells. Tregs can suppress various effector Th cell subsets releasing anti-inflammatory and immunoregulatory cytokines, yet are downregulated in chronic airway inflammation. Th2 derived cytokine IL-13 upregulates the expression of mucin genes that initiate mucus hypersecretion. Pathogen invasion also induces epithelium-derived IL-6 and IL-8 production via interaction with PRR leading to neutrophilic inflammation.

lumen, and antimicrobial peptides (AMP) produced by immune cells play an important role in the response to environmental agents (**Fig. 1**).

Respiratory mucin proteins contribute to mucociliary clearance and host defense in human nasal mucosa and are (mucin 5AC [MUC5AC] and MUC5B) produced at similar levels in the upper and lower airways (38). In patients with asthma, the abnormal expression of MUC5AC, MUC5B, and MUC2 are the principal gel-forming mucins secreted predominantly in upper airway diseases (39), and MUC5AC is upregulated in the epithelium of asthmatic airways (40). Excessive mucus formation in upper airway may trap more inhaled micro-organisms and this, in turn might result ciliary dysfunction and give bacteria opportunity to adhere and promote biofilm formation (41). Both gram-positive and gram-negative bacterial products upregulate mucin genes expression and mucin secretion in respiratory epithelial cells (42), and microbial binding to mucin is essential for nasal colonization (43). Airway inflammation with Th2 cells stimulates mucus production by IL-13 in asthma (44). IL-13 has been shown to increase goblet cell numbers in airways that asthma patients experience severe mucus obstruction and airway hyperresponsiveness.

Airway epithelium produces AMP that contribute to barrier function, including human *β*-defensin 2 (hBD-2). hBD-2 is expressed in nasal mucosa and the expression is induced by microbial components, proinflammatory cytokines and TLR signaling pathway (45). hBD-2 has chemotactic activity for T cells and immature DCs, and can activate mast cells leading to release histamine and prostaglandin D2 in allergic inflammation (46). The reduction of hBD-2 expression affects the abnormal microbial colonization and immune responses in CRSwNP (47).

Pattern recognition receptors (PRRs) are essential for host and microbial interactions in nasal sinus mucosa. Sinonasal epithelial cells express 10 TLRs, and the signaling through TLRs initiates immune responses in the respiratory epithelium (48). TLRs bind to and recognize damage-associated molecular patterns and pathogen-associated molecular

patterns such as peptidoglycan (TLR2 ligand), double-stranded RNA (TLR3 ligand) and LPS (TLR4 ligand) (49). Thus, TLR signaling induces distinct stimulation of cytokines, chemokines and costimulatory molecules based on the exposure (e.g., TLR2 and TLR4 potentially induce the release of IL-1β and IL-8, whereas TLR3 and TLR7/8 may induce the production of IL-4, IL-5, and IL-13) (50). Genetic variations in TLR-related pathway genes are important factors for asthma development, and our previous study demonstrated that *TLR2* and *CD14* impact the abundances of nasal microbiota and clinical phenotypes in adult asthma (51). Besides, bacterial pathogens express numerous virulence factors and seek to evade PRR-induced innate immune responses that assist the bacterium colonize the host (52).

Th17 cells are also important in host defense against pathogen, and IL-17 signaling has a significant role in maintaining the nasal normal microbiome composition by reducing colonization with potentially pathogenic bacteria (53). Inhibitory Tregs play opposing roles by suppressing effector T-cells to limit excessive immune responses during infections. Downregulated Treg-related cytokines and elevated Th2 cytokines exhibited in eosinophilic CRS, whereas upregulated Treg cells and increased Th1 and Th17 cytokines presented in non-eosinophilic rhinosinusitis (54). Therefore, microbiome dependent imbalance of Treg and Th cell subsets leads to different inflammatory patterns in airway diseases.

ILCs are preferentially located close to the epithelial barriers that are in direct contact with microbiota. ILC1s and ILC3s produce Th1 and Th17 cytokines, while ILC2s produce Th2 cytokines. *In vivo*, rhinovirus challenge induced IL-33 and T2 cytokines in bronchial and nasal fluid in asthma patients (55). In same study, culture of human T cells and ILC2s with supernatants of rhinovirus-infected epithelium led to IL-33-dependent Th2 cytokine production *in vitro* (55). ILC2s are elevated in the eosinophilic nasal polyp, and polyp ILC2 is reduced by systemic corticosteroid treatment (56).

Members of the microbiota interact with each other in mutualistic or antagonistic way. Particularly, the commensal genus *Dolosigranulum* spp. is responsible for the acidification of the local environment which promote the expansion of *Corynebacterium* spp. (57), while this effect inhibits the growth of acidophobic bacteria *S. aureus* (58). In addition, *S. aureus* forms polymicrobial biofilm with *Candida albicans* and enhance antimicrobial resistance (59) resulting persistent inflammatory changes in the upper airway. Also, common respiratory bacteria *H. influenzae* and *Pseudomonas aeruginosa* can promote the production of pro-inflammatory cytokines in response to subsequent infection with respiratory syncytial virus in airway epithelial cell (60).

NORMAL UPPER AIRWAY MICROBIOTA

The Human Microbiome Project Consortium evaluated microbiome composition sampling at 18 body sites from 242 healthy adults (61). The most abundant genera in the anterior nares were *Propionibacterium* (*Propionibacterium acnes*), *Corynebacterium* (*Corynebacterium accolens*) and *Staphylococcus*, and abundant pathogenic species were *P. acnes*, *S. aureus* and *S. epidermidis.* Similarly, Zhou et al. (62) determined prevalent genera in the nasal cavity of 236 healthy adults, and it included *Staphylococcus*, *Propionibacterium*, *Corynebacterium*, and *Moraxella*. When healthy adults sinus microbiota was assayed with CRS nasal samples, genera *Corynebacterium* and *Staphylococcus* were common in the majority of the nasal samples including healthy controls (63). These findings suggest that a healthy nasal cavity contains various opportunistic pathogenic bacteria within genera and potential to be as reservoir.

INFLUENTIAL FACTORS ON THE COMPOSITION OF UPPER AIRWAY MICROBIOME

Smoking

In asthma patients, tobacco smoking is associated with more severe asthma symptom, frequent exacerbation, and corticosteroid insensitivity (64). Persistent smoking exposure enhances mixed Th1/Th2 immune response that develops more severe asthma phenotype, and induces neutrophils and oxidative stress which cause resistance to corticosteroids (65). In general, smoking induces pro-inflammatory cytokines as well as IgE production, and alters immune cells such as alveolar macrophages, neutrophils, lymphocytes and NK cells which are the major cells in pathogen clearance and defense against pathogens (66).

Cigarettes are direct source of potentially pathogenic microorganisms that include fifteen different classes of bacteria and wide range of pathogenic organisms including *Acinetobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Klebsiella*, *Pseudomonas aeruginosa*, and *Serratia* (67). In healthy adults nasopharynx, smoking status is positively associated with *Corynebacterium* and *Staphylococcus* (68). In airway exposure study, the presence of nine bacterial genera in pharynx was correlated positively with smoking: *Actinomyces*, *Actinobacillus*, *Anaerococcus*, *Peptoniphilus*, *Staphylococcus*, *Streptococcus*, *Gemella*, *Psychrobacter*, and *Treponema* (69). In a univariate analysis and a machine learning approach, nasopharyngeal microbial communities were compared between smoking and non-smoking healthy adults (70). *Haemophilus* spp., *Campylobacter* spp. and *Abiotrophia* spp. were dominant in smokers, whereas only *Shigella* spp. was lower in nasopharyngeal communities of smokers compared with non-smokers. Newly detected genera with increased abundance in the nasopharynx of smokers included *Eggerthella*, *Erysipelotrichaceae I.S.*, *Dorea*, *Anaerovorax*, and *Eubacterium* spp. that constitute gram-positive anaerobic bacterial colonization in the nasopharynx (70). Recently, we reported that genera *Corynebacterium*, *Propionibacterium*, *Streptococcus*, *Haemophilus* and *Rothia* in the upper airway were correlated with the exposure to smoking and clinical phenotypes in adult asthma (51). In different studies, although smoking cessation improved asthma symptom and lung function in smoking asthma patients (64), it did not change the sputum bacterial community in 12-wk follow-up study (71). Thus, in addition to smoking and cessation duration, distinct bacterial colonization in smokers and potential influence of airway microenvironment on particular microbiota should be carefully considered in future research (72).

Air pollution

Air pollution is associated with the prevalence of adults asthma (with particulate matter [PM] of less than 10 μm [PM10] and nitrogen dioxide), decreased lung function, asthma severity and exacerbation (with ozone, sulfur dioxide, nitrogen dioxide) (73). PM contributes to airway inflammation through genetic variation, oxidative stress induction, airway epithelial barrier disruption, altered cell signaling pathways, production of T2 cytokines, and inflammatory cell recruitment (74).

Compositions of total airborne bacteria and pathogenic bacteria have been measured in different PM sizes and air quality levels (75). The dominant bacterial phyla in all the airborne PM samples were Proteobacteria, Cyanobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. The most common classes were Alphaproteobacteria, Actinobacteria, Betaproteobacteria, Oscillatoriophycideae and Clostridia. The most abundant genera were *Thiobacillus*, *Methylobacterium*, *Rubellimicrobium*, and *Paracoccus*. The relative abundance (RA) of pathogenic bacteria was different in PM10 samples from those of particulate

matter of less than 2.5 μm (PM2.5) and total suspended particulate. *Staphylococcus*, *Bacillus*, *Clostridium*, *Enterobacter*, and *Klebsiella* were the dominant pathogenic genera in all the airborne PM samples. The proportions of the total and pathogenic bacterial community were correlated with wind scale, relative humidity, carbon monoxide, sulfur dioxide and ozone concentrations (75).

Exposure to high levels of PM2.5/PM10 critically alters airway microbiota composition. Qin et al. (69) studied pharyngeal microbiota in 83 subjects after exposure to high concentrations of PM2.5/PM10. The RA of 38 phyla increased in the post-smog samples, such as Firmicutes, Fusobacteria, and Actinobacteria, and 11 newly detected phyla including Caldiserica, Chlamydiae, Parcubacteria, Atribacteria, and Microgenomates (69). A total of 559 genera were increased including *Leptotrichia*, *Corynebacterium*, *Veillonella*, *Dolosigranulum*, *unidentified-Chloroplast*, *Moraxella*, *Gemella*, *Actinomyces*, *Granulicatella*, and *Haemophilus*. Main recognized prevalent respiratory pathogens in the post-smog swabs were *Streptococcus*, *Haemophilus*, *Moraxella*, and *Staphylococcus*, while significantly reduced genus was *Neisseria* after exposure to smog for two days. At the species level, RA of *H. influenzae* and *Moraxella catarrhalis* were dominant in the smog exposed samples (69). In a study on the association among air pollutants, immune markers and adult-onset asthma, 15% of the effect of air pollution on the risk of adult-onset asthma was mediated through immune system (76). IL-1RA was suggested to be associated with adult-onset asthma and air pollutants in the study. IL-1RA has an anti-inflammatory effect and its functional role was identified in the inflammatory process of asthma (77) and fungal-associated allergic airway inflammation (78). Although air pollutants are well known to cause asthma exacerbations, there is lack of supporting evidence on how air pollution may influence on upper airway microbiome in adult asthma.

Host and microbial genetics

Host genetic factors can contribute to the susceptibility of asthma. Recent advances in sequencing technology bring enormous genetic variation data from a large number of individuals. Igartua et al. (79) performed genome-wide association studies (GWAS) to determine host-microbial interaction using 16S rRNA gene sequencing of nasal swabs in 144 adult Hutterite population and whole genome sequencing data of the same population. They identified 37 microbiome quantitative trait loci which was associated with the RA of 22 genera and highly contained genes involved in mucosal immunity pathways. The most significant association was between RA of *Dermacoccus* (phylum Actinobacteria) and an intergenic variant near *TINCR* (rs117042385), a long non-coding RNA that binds to peptidoglycan recognition protein 3 (*PGLYRP3*) mRNA. Another association was between the RA of an unclassified genus of family *Micrococcaceae* (phylum Actinobacteria) and a missense variant in *PGLYRP4* (rs3006458), a pattern receptor that binds to murein peptidoglycan of gram-positive bacteria. Currently no other research has done for host-microbiome association in adults' upper airway.

We previously observed higher composition of microbiome genes in non-asthmatics that are related with lysine degradation, N-glycan biosynthesis, caprolactam degradation, and peroxisome proliferator-activated receptor signaling pathway, and prevalent genes associated with pentose phosphate pathway, LPS biosynthesis, flagella assembly, and bacterial chemotaxis in young adult asthmatics (80), revealing possible role of microbiome in adult asthma.

	Increased	Decreased
Chronic rhinosinusitis	**Firmicutes** (*Lactobacillus, Turicibacter, Clostridium, Enterococcus, Romboutsia, Staphylococcus aureus*) **Proteobacteria** (*Escherichia-Shigella, Haemophilus influenzae*)	**Firmicutes** (*Dolosigranulum*) **Actinobacteria** (*Corynebacterium*)
Asthma	**Firmicutes** (*Streptococcus, Dialister invisus*) **Proteobacteria** (*Alkanindiges hongkongensis, Burkholderia*) **Actinobacteria** (*Gardnerella vaginalis*) **Bacteroidetes** (*Prevotella buccalis*)	**Bacteroidetes** (*Prevotella*) **Actinobacteria** (*Corynebacteriales*)
Asthma with chronic rhinosinusitis	**Firmicutes** (*Staphylococcus*) **Proteobacteria** (*Escherichia coli, Acinetobacter, Ralstonia*)	**Bacteroidetes** (*Prevotella*) **Proteobacteria** (*Campylobacter*) **Fusobacteria** (*Fusobacterium*)

Figure 2. A summary of upper airway dysbiosis in asthma and CRS.

MICROBIOTA COMPOSITION IN UPPER AIRWAY DISEASE

We summarized the upper airway dysbiosis in patients with CRS (with/without nasal polyposis [NP]) and asthma in **Fig. 2**. In a recent study, CRSwNP patients contained reduced *Corynebacterium* and *Dolosigranulum* in the nasal sample compared to healthy controls (81). Bacterial genera such as *Lactobacillus, Escherichia-Shigella, Turicibacter, Clostridium, Enterococcus,* and *Romboutsia* were positively correlated with the severity of CRSwNP, while no correlation observed with age, body mass index and symptom score (81). Another group compared bacterial communities in nasal tissues of CRSwNP with and without asthma and healthy controls. Interestingly, CRSwNP patients had significantly enriched *H. influenzae*, while healthy controls had higher *P. acnes*. *S. aureus*, common colonizer of the upper airways that aggravate allergy and induce IgE formation, was dominant in the CRSwNP without asthma group, and *Escherichia coli* was higher in CRSwNP with asthma group (82,83). *E. coli* was positively correlated with ECP and IL-5 in the nasal polyp tissues which promote T2 inflammation in CRSwNP patients.

UPPER AIRWAY MICROBIOTA IN ADULT ASTHMA

Microbiota composition in asthma phenotypes and upper airway varies between research articles due to population characteristics including age, sex, ethnicity, diet, geography, and study design, sampling site, technique, analysis method and disease status. As asthma is a heterogenous disease with different phenotypes and variable clinical manifestations, understanding airway microbiome diversity in disease progression and propagation is crucial and phenotype-specific alterations are summarized in **Table 1**.

Asthmatic patients with CRS had a lower abundance of *Prevotella, Fusobacterium,* and *Campylobacter* species, and more abundant of *Staphylococcus, Acinetobacter,* and *Ralstonia* species compared with non-asthmatic patients with CRS (84). Hilty et al. (85) identified nasal microbial communities in adults with asthma, chronic obstructive pulmonary disease (COPD) and healthy controls as part of their study. When nasal microbiota was compared with oropharynx and left upper lobe, nasal specimens were characterized by Actinobacteria and Firmicutes. However, the authors did not report the comparisons between the nasal

Table 1. Clinical relevance of upper airway microbiota in adult asthma

Cohort	Subjects	Collection site	Microbiota variation	Phenotype association	Ref.
CRSwNP	59 CRSwNP and 27 controls	Nasal swab	· CRSwNP: reduction of *Corynebacterium* and *Dolosigranulum*	*Lactobacillus*, *Escherichia-Shigella*, *Turicibacter*, *Clostridium*, *Enterococcus*, and *Romboutsia* are associated with severity of CRSwNP.	(81)

CRSwNP with and without asthma	21 CRSwNP patients without asthma, 20 CRSwNP patients with co-morbid asthma, 17 healthy subjects	Nasal swab	· Enrichment in each group:
			    CRSwNP: *H. influenzae*
			    Healthy group: *P. acnes*
			    CRSwNP without asthma: *S. aureus*
			    CRSwNP with asthma: *E. coli*
				CRSwNP is associated with high concentrations of IgE, SE-IgE, and IL5.	(82)

Asthma with and without CRS	56 patients with CRS and 26 control subjects	Sinus swabs	· Asthma patients with CRS: lower abundance of *Prevotella*, *Fusobacterium*, *Campylobacter* and higher *Staphylococcus*, *Acinetobacter*, *Ralstonia* compared with non-asthmatic patients with CRS	Higher relative abundances of Actinobacteria can be predictive of better surgical outcome of CRS.	(84)
Asthma and COPD	5 patients with COPD, 11 patients with asthma and 8 controls	Nose, oropharynx, left upper lobe	· Asthma and COPD: reduction of Bacteroidetes (*Prevotella* spp.). · Nasal samples were characterized by Actinobacteria and Firmicutes.	NA	(85)
Young adult and elderly asthma	60 patients with asthma and 20 controls	Nasopharyngeal swab	· Young adult asthma: high Proteobacteria · Young non-asthma: high uncultured *Corynebacteriales* · Elderly non-asthma: high *Moraxella*	· Young adult: *Prevotella*, *Neisseria*, *Fusobacterium* negatively correlated with FEV1. · Elderly: *Burkholderia* and *Psychrobacter* positively correlated with FEV1, while *Corynebacterium* negatively correlated with FEV1.	(80)
Asthma	72 exacerbated asthma, 31 non-exacerbated asthma and 21 healthy controls	Nasal swab	· Asthma: high Bacteroidetes and Proteobacteria	· *Prevotella buccalis*, *Gardnerella vaginalis* and *Alkanindiges hongkongensis* are associated with exacerbated asthma. · *Dialister invisus* is associated with non-exacerbated asthma.	(86)
Asthma	111 CRS: 46 asthma, 65 non-asthma	Nasal swab	· Asthmatic CRS: high *Streptococcus* genus	Asthmatics with emergency visit had high Proteobacteria phylum (*Burkholderia* spp.)	(87)

SE-IgE, IgE against *S. aureus* superantigens; NA, not applicable.

microbiota of asthmatics versus control groups. Previously we have shown a higher abundance of uncultured *Corynebacteriales* in non-asthmatic young adults, and a higher abundance of *Moraxella* in non-asthmatic elderly individuals (80). The comparison of upper airway microbiota between young adults and elderly also showed different composition including *Staphylococcus*, *Propionibacterium*, and *Moraxella*. The high abundances of *Prevotella*, *Neisseria*, and *Fusobacterium* were correlated with forced expiratory volume in 1 s (FEV1) in young adults, whereas *Burkholderia* and *Psychrobacter* and *Corynebacterium* revealed contrary relationship with FEV1 among elderly individuals.

In an association study of nasal microbiome in asthma exacerbation and healthy controls, taxa from Proteobacteria and Bacteroidetes were dominant in subjects with exacerbated and non-exacerbated asthma relative to healthy controls (86). More specifically, enrichment of *Prevotella buccalis*, *Alkanindiges hongkongensis*, and *Gardnerella vaginalis* were identified in patients with exacerbated asthma, while *Dialister invisus* was greater in patients with non-exacerbated asthma. According to Yang et al., asthmatic CRS patients had prevalent RA of the *Streptococcus* genus, and asthmatics with at least one emergency department visits were associated with higher RA of Proteobacteria phylum (*Burkholderia* spp.) (87).

Microbiome community in lower airways of adult asthmatic patients were reviewed to compare with upper airway microbiome. Similar to nasal microbiota, reduced prevalence

of sputum Bacteroidetes and Fusobacteria in patients with severe and non-severe asthma, and increased prevalence of Firmicutes were identified in severe asthma patients (88). In addition, higher abundance of *Lactobacillus* (Firmicutes), *Pseudomonas* (Proteobacteria) and *Rickettsia* (Proteobacteria) in asthmatic patients, and higher abundance of *Prevotella* (Bacteroidetes), *Streptococcus* (Firmicutes) and *Veillonella* (Firmicutes) were detected in healthy controls when measured in endobronchial brush samples (89).

These findings suggest that genera *Dolosigranulum* and *Prevotella* are more likely play protective effect against asthma, whereas enriched genus *Streptococcus*, and phyla Proteobacteria and Firmicutes in asthmatics are probably influencers of asthma development and disease severity.

THERAPEUTIC EFFECT ON THE COMPOSITION OF UPPER AIRWAY MICROBIOTA

Probiotics

Microbial colonization begins *in utero* and the composition is modified by consumption of probiotics during pregnancy, breastfeeding, mode of delivery, early respiratory infections (90), antibiotics, environmental factors and genetic/epigenetic regulation (9). Numerous studies reported the influential effect of the upper airway microbiome in the development of early onset rhinitis, wheeze and chronic inflammation in upper airway (91), and the symptoms were improved by probiotics intake (92). Probiotics are live microorganisms that provide health benefits to the host through promotion of epithelial barrier function, regulation of the mRNA levels of TLRs, and stimulate Tregs and reduce Th1 and Th2 cells development (93).

In a double-blind, randomized, placebo-controlled study, 49 young adults with perennial allergic rhinitis (AR) received either *Lactobacillus acidophilus* strain L-92 in fermented milk or without lactic acid bacteria (94). The result shows that oral administration of L-92 significantly improved nasal symptom-medication score, and decreased the scores of swelling and color of the nasal mucosa at 6 and 8 weeks of ingestion. However, there were no significant differences in serum specific IgE levels nor in Th1/Th2 ratio between groups (94). In different placebo-controlled study, co-administration of allergen specific immunotherapy with *Clostridium butyricum* significantly enhanced the therapeutic effect on AR (lowered 81.2% of the AR symptoms) (95). This combination effect decreased nasal symptom score, medication score, serum specific IgE, Th2 cytokines and skin prick test index, and elevated the frequency of regulatory B cell. When the immune modulatory effect of probiotic *Bifidobacterium lactis* NCC2818 was examined in individuals suffering from seasonal AR, concentrations of Th2 cytokines and total nasal symptom scores, and basophil cell activation were significantly lower in the probiotic group (96). Probiotic (*Lactobacillus gasseri* KS-13, *Bifidobacterium bifidum* G9-1, and *Bifidobacterium longum* MM-2) intake during spring allergy season improved rhinoconjunctivitis-specific quality of life in individuals with seasonal allergy (97). Although majority of the findings suggest the beneficial effect of probiotic treatment for CRS, nasal administration of honeybee lactic acid bacteria did not reduce symptom severity or inflammatory biomarkers and did not affect commensal bacteria of the nasal cavity (98). Due to the distinct study design, probiotic species and strains, administered dose and timing, the studies may represent inconsistent results which needs to be considered in future studies. Also, individual genetic variation, age, sex and resident microbiota should be taken into consideration when evaluating the effect of probiotics.

Antibiotics

Prolonged medical therapies such as corticosteroids, antihistamines, and antibiotics are the main therapy for inflammatory sinonasal disorders. Antibiotic perturbs the original microbiome composition as it damages bacterial cell wall, inhibits bacterial growth, and alters genes and protein fluxes (99). In addition to its anti-microbial effect, antibiotics also have an anti-inflammatory and immunomodulatory effects. Macrolide downregulates pro-inflammatory cytokine (IL-8) (100) and inhibits the activation of transcriptional factors (NF-κB, AP-1) (101) in human epithelial cells, which are important regulators in the inflammatory process.

The most common microbiota influenced by the antibiotic treatments are phyla Actinobacteria (*Bifidobacterium*-by 40 antibiotic treatments), Bacteroidetes (*Bacteroides*-30), Firmicutes (*Faecalibacterium*-30), and Proteobacteria (*Escherichia*-13) (102). Antibiotics for respiratory tract infection such as azithromycin and clarithromycin alone affect less than six genera each, whereas a combination of these macrolide antibiotics could alter the abundance of 15 genera (102).

The evidence supporting antibiotic efficacy in asthma symptoms and exacerbation are inconsistent. Although treatment with azithromycin in eosinophilic NP improved polyposis staging and quality-of-life (103), and decreased asthma exacerbation in persistent symptomatic asthma patients (104), these are not in the same line with other report (105). The previous study of sinus microbiota in CRS have shown that antibiotic use and asthma are associated with loss of microbial diversity and high *S. aureus* abundance (106). A combination of antibiotics and anti-inflammatory therapy significantly reduced sinus microbiota biodiversity in post-operative maxillary sinuses (107). Although the sinus bacterial composition after treatment was unique to each individual, the expansion was observed in *Pseudomonas*, *non-aureus Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Haemophilus*, and *Delftia*.

It is essential to choose pathogen-selective antibiotics in order to minimize disturbance to the microbiome, as inappropriate antibiotic treatments are able to shift the commensal microbiota to long-term alternative dysbiotic states, resulting in a variety of negative health consequences (108). As most common antibiotics reduce beneficial resident microbiota (*Faecalibacterium*, *Bifidobacterium*, *and Blautia*), it is crucial to consider combination or selective effect of antibiotics, administration type, concentration and resistance of antibiotics in clinical practice.

Corticosteroids

Inhaled corticosteroids are the most effective medication for asthma. In the meta-analysis study, the intranasal corticosteroid medication improved asthma-specific outcome measures in patients with both AR and asthma (109). The impact of intranasal corticosteroids in the management of CRS and asthma shows the alteration of nasal microbiota composition. In healthy adults with and without chronic non-infectious rhinitis, the administration of topical corticosteroid spray (mometasone furoate, 200 µg per day) for 1 month increased the RA of staphylococci (phylum Firmicutes), while suppressing *Moraxella* spp. (phylum Proteobacteria) and streptococci (phylum Firmicutes) in the nasal cavity (110). In asthma, nasal abundance of *P. buccalis*, *A. hongkongensis*, *G. vaginalis*, and *D. invisus* remained associated with asthma in multivariate models adjusted for steroids (86). In contrast, inhaled and oral corticosteroid treatments affected the alpha and beta diversity, and increased abundance of Proteobacteria and decreased abundance of Bacteroidetes and Fusobacteria in the lower airway samples of asthmatics patients (89). Effect of corticosteroids in lung microbiome concerning to

asthma has been summarized in another review and has shown strong impact on bacterial community which in turn contributes to corticosteroid responsiveness (111).

CONCLUDING REMARKS

Commensal microbiota plays an important role to maintain the host-microbe mutualism. Nasal commensals are primarily responsible for the health of respiratory tract. The microbial imbalance could have a causative role in the pathogenesis of upper and lower airway diseases including asthma. Environmental exposures, genetics and medications could shape the composition of upper airway microbiome through epithelial barrier disruption and the modulation of innate and adaptive immune response. Current review focused on most recent findings that assessed the composition of upper airway microbiota in adult asthma patients using 16S rRNA gene sequencing. However, we should count that genus can have a wide range of strains which are genomically distinct and may have either protective or disease-causing effects. Therefore, further investigation is warranted to perform network approaches to better understand the complex molecular mechanisms involved in these processes and develop effective therapies for asthma.

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