



Microbiome-focused asthma management strategies

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Asthma is a common, heterogeneous and serious disease with high prevalence globally. Poorly controlled, steroid-resistant asthma is particularly important as there are no effective therapies and it exerts substantial healthcare and societal burden. The role of microbiomes, particularly in chronic diseases has generated considerable interest in recent times. Existing evidence clearly demonstrates an association between asthma initiation and the microbiome, both respiratory and gastro-intestinal, although its' roles are poorly understood when assessing the asthma progression or heterogeneity (i.e. phenotypes/endotypes) across different geographical locations. Moreover, modulating microbiomes could be preventive and/or therapeutic in patients with asthma warrants urgent attention. Here, we review recent advances in assessing the role of microbiomes in asthma and present the challenges associated with the potential therapeutic utility of modifying microbiomes in management.

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Introduction

Globally, asthma is a major healthcare challenge that affects more than 330 million individuals. Notably, there have been significant increases in worldwide prevalence at an annual rate of 1.4% (4.2 million) in children and 2.1% (6.3 million) in adults from 2010 to 2015 [1]. Importantly, older patients are especially susceptible to asthma exacerbations and mortality risk increases with increasing age, and the elderly (>75 years) have the highest mortality (1.9% in-hospital mortality) [2,3]. Pathophysiologically, asthma is a complex and heterogenous inflammatory disease that includes multiple phenotypes (classified on the basis of clinical, demographic and pathological characteristics) that may be further (sub) categorised as endotypes (pathological and inflammatory characteristics, Figure 1) [4–7]. Despite the remarkable advances in defining the molecular and cellular mechanisms that underpin asthma pathogenesis in the recent decade [5–8], the role of microbiomes in pathogenesis is only just being unravelled [9,10]. This has led to a greater understanding the relationship between the host microbiome (both pulmonary and gastro-intestinal) and asthma pathogenesis, as well as asthma heterogeneity [9–11]. Here, we highlight the emerging concepts concerning the roles of microbiomes in asthma development and progression. We also discuss microbiome-directed therapies that may be beneficial in asthma.

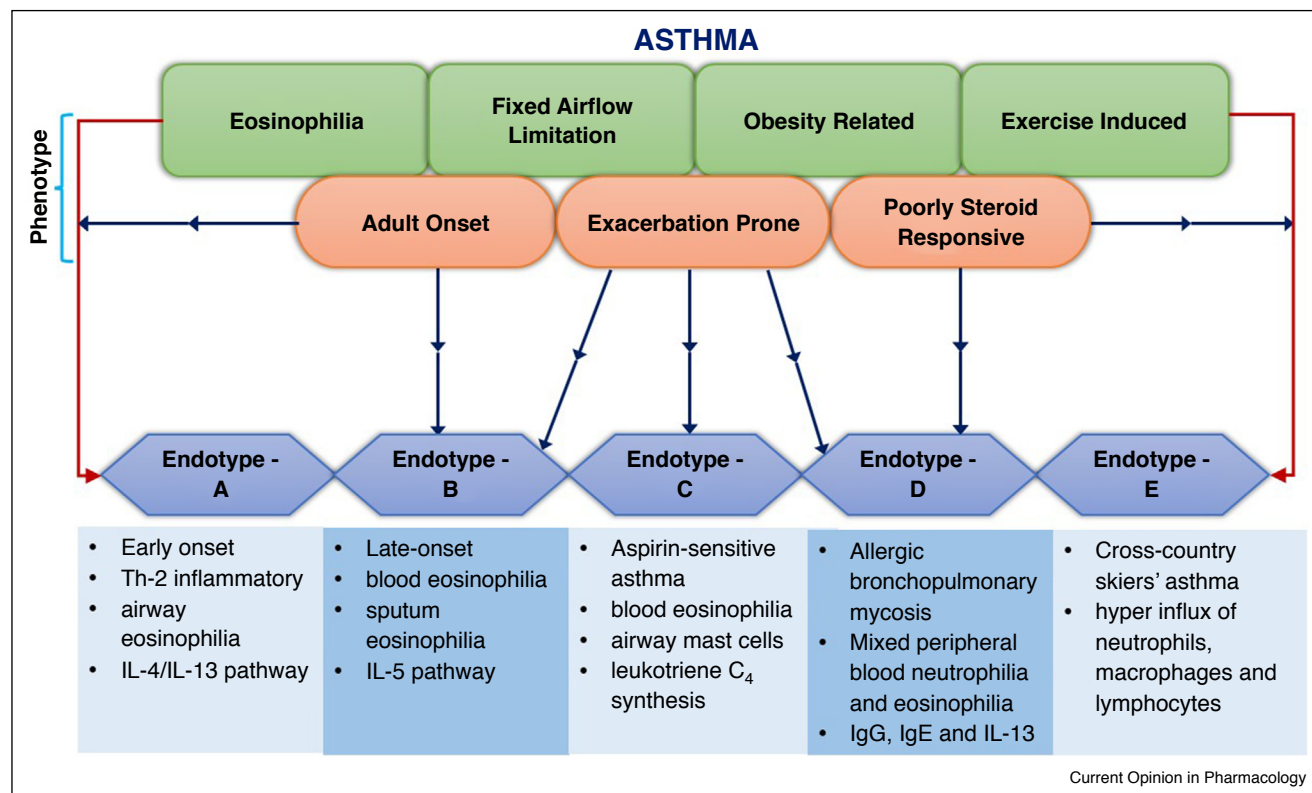
Data source and search strategy

We searched key terms on PubMed and Google scholar from Jan 1 2006, to Jan 2019. The search terms included asthma, microbiome, microbiota, gut, airway, respiratory, lung, viral, and fungal. Relevant articles provided insight into the current and/or developing therapeutics for treating asthma. Articles resulting from these searches and relevant references cited in those articles were reviewed. Only articles published in English were included.

Potential roles for the microbiome in asthma

The microbiome is defined as the sum of all microorganisms, bacteria, archaea, viruses, and fungi, along with their genomes and metabolites [10,12]. A rapidly evolving fields of microbiome investigation is metagenomics, a process that assesses the genomic DNA directly from the whole sample or niche [13]. Metagenomics provides an unbiased analysis of both the microbial community structure (i.e. species richness and distribution) and its'

Figure 1



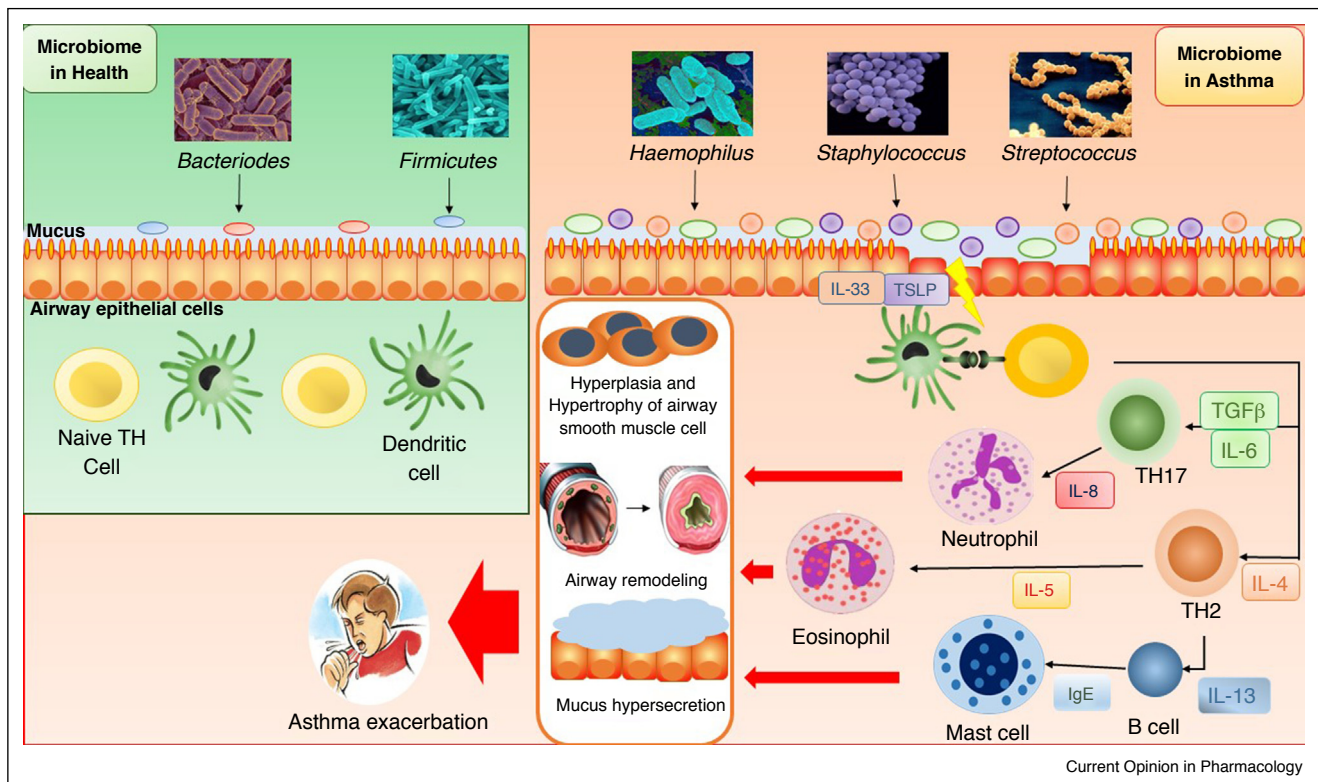
Major asthma phenotypes and endotypes based on demographic, clinical, and inflammatory profile of disease.

predictive functional (metabolic) potential [13]. Historically, the lungs were considered sterile; however, with remarkable advances in high-throughput sequencing and metagenomics, it is now known that micro-organisms (especially bacteria, but also fungi and viruses) in the respiratory tract (especially airways) may suppress or drive inflammation and asthma, in both children and adults (Figure 2) [9,10,12,14–17]. A number of factors determine the persistence of bacteria in the lower respiratory tract (i.e. the lower airways), including oxygen gradient, nutrient availability, temperature, pH gradient and surfactants [18]. The major bacterial phyla in the respiratory tract including in oral wash, oro-/nasopharyngeal swabs, bronchoalveolar lavage and lower airway protected brushings, of healthy individuals comprise *Bacteroides* and *Firmicutes*, which are primarily made up of bacterial genera *Faecalibacterium*, *Lachnospira*, *Veillonella* and *Rothia* [15]. In contrast, asthmatic individuals have increased pathogens in the airways (e.g. in nasopharyngeal aspirates), such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus* [19].

Dickson and Huffnagle, postulated that colonisation of the lungs is primarily dependent on the selective elimination of bacterial species by innate immune mechanisms

[18]. However, the altered immune system and pathophysiological characteristics of asthma may render several microbial clearance mechanisms ineffective, potentially allowing pathogenic bacteria to persist and colonise the respiratory tract [18]. Severe asthma patients have a higher prevalence of *Actinobacteria*, which correlates with disease outcomes, including total severity scores in Asthma Control Questionnaires, and sputum leucocyte and bronchial eosinophil levels [20]. The relative abundance of certain microbes, including members of *Comamonadaceae*, *Sphingomonadaceae*, and *Oxalobacteraceae* families, correlate with airway hyper-reactivity (AHR) in asthma [21]. Currently administered treatments, particularly corticosteroids, may also greatly affect the composition of lung microbiota in asthmatic individuals [21,22]. Segregating individuals with asthma on the basis of response to corticosteroid therapy, Durack *et al.*, reported the enrichment of bacterial families *Microbacteriaceae* and *Pasteurellaceae* at baseline in inhaled corticosteroid (ICS) non-responders, and *Streptococcaceae*, *Fusobacteriaceae* and *Sphingomonadaceae* in ICS responders, [22]. This suggests that the bronchial bacterial microbiome is associated with clinical and immunological features in steroid-naïve asthma that could be potentially targeted therapeutically [10]. An indirect association was also observed between

Figure 2



Role of lung microbiomes in health and asthma.

Core members of lung microbiota maintain immune homeostasis in health. Altered lung microbiota in asthma stimulate pro-inflammatory responses in already inflamed airway epithelia leading to the activation of Th1/Th2/Th17 pathways. The activated cellular pathways initiate a cascade of inflammatory events including the recruitment and activation of mast cells, eosinophils and neutrophils which further worsens the underlying chronic inflammation and promotes mucus hypersecretion, hyperplasia/hypertrophy of airway smooth muscle cells and airway remodelling. All these factors contribute to AHR and increasing the risk of asthma exacerbations.

pre-treatment (with clarithromycin) airway bacterial diversity and post-treatment asthma outcomes, that is, AHR. Greater bacterial diversity (determined by estimating the number of different bacteria present in a sample by 16S ribosomal RNA amplicon sequencing and analysis) before commencing clarithromycin treatment was associated with reduced AHR after antibiotic treatment [18]. Importantly, an increase or decrease in bacterial diversity due to treatment is often associated with asthma features [18]. Conversely, resident microbiota may alter the effects of administered drugs in asthma [23]. Durack *et al.* also found the predicted functions of bacterial communities of ICS non-responders were enriched in xenobiotic biodegradation pathways [22].

Recently, Segal *et al.*, reported that microaspiration of upper airway taxa (oral microbiome) into the lungs could result in subclinical inflammation, marked by increased expression of inflammatory cytokines and Th17 lymphocytes, as well as attenuated alveolar macrophage Toll-like receptor 4 responses in the lung [24]. These mechanisms may contribute to exaggerated host immune responses to

allergic triggers in asthma. We have shown experimentally that *Chlamydia*, *Haemophilus*, influenza and respiratory syncytial virus increase asthma severity in early life and adulthood [7,8,25–29], but that low pathogenic *S. pneumoniae* and its components suppress allergic airway disease in mice through the induction of regulatory T-cells (Tregs) [30–34].

The lung microbiome affects host responses in asthma through several key mechanisms [9,10,35]. Similar to microbiota-induced epithelial pathological features in the gastrointestinal tract [9], the lung microbiota may alter the permeability of the airway epithelium, increasing the risk of infection (bacterial or viral), which trigger asthma exacerbations [36,37]. Moreover, alterations in epithelial integrity may lead to invasion of epithelial cells by pathogens resulting in cell death [38]. Consequently, the compromised epithelial barrier is susceptible to the rapid uptake of other potential allergens (e.g. pollen, house dust mite, toxicants, air pollution), leading to increased exacerbation risk and detrimental immune responses [39].

Recently, the gut microbiome has also been implicated in initiating and/or modulating lung responses in various chronic inflammatory diseases, including asthma [9,12,40]. Notably, establishment of gut microbiota at an early age plays key roles in asthma development in later life [41–43]. Moreover, early life gut microbiome dysbiosis is linked to increased likelihood of allergen-induced airway inflammation in adulthood, in mice and humans [44,45]. Results from the Copenhagen Prospective Studies on Asthma in Childhood2010 (COP-SAC2010) birth cohort ($n = 690$) show that children born to mothers with asthma were at increased risk of asthma at five-years of age, which was associated with immature gut microbiomes (assessed by calculating β -diversity) at one-year of age [46]. Thus, the maturation of the gut microbial community during this window, that is, 1–5 years of age, may reduce the inherited asthma risk in pre-disposed children [46]. Notably, asthma in five year old children, particularly those born to mothers with asthma, was significantly negatively associated with relative abundance of several genera at age one, namely, *Faecalibacterium*, *Bifidobacterium*, *Roseburia*, *Alistipes*, *Lachnospiraceae incertae sedis*, *Ruminococcus* and *Dialister*, and there were positive correlations with one genera, *Veillonella* [46]. In addition, bacterial ligands and metabolites from the gut can enter the systemic circulation and reach distal organs, such as the lungs, that further increases the severity of respiratory inflammation. Experimentally, dextran sulfate sodium (DSS)-induced colitis in mice results in both systemic and pulmonary bacteraemia and subsequent IL-6-dependent neutrophilia [47], which may occur through increases in the platelet-activating factor receptor and the induction of inflammasomes [48]. Another class of major immunomodulatory molecules involved in the gut–lung axis are short-chain fatty acids (SCFAs), which are microbial metabolites of dietary fibre and have anti-inflammatory properties [9,12,49]. This further strengthens the concept of ‘common mucosal responses’ to external stimuli, forming ‘the gut–lung’ axis or ‘gut–lung’ cross-talk [9]. Further developments may lead to the generation of more robust technologies to quantify and characterise all types of microbiota (bacteriome, virome, mycobiome), as well as their effects on disease characteristics and therapeutic targeting/utilisation [10,12]. In particular, preparation of exhaustive referral libraries for fungi and viruses, as well as refining of the assessment of the functional effects of bacteria from respiratory samples is needed to fully elucidate the total and differential microbiome effects in asthma. Also, currently used metagenomic methodology could be further refined to better capture the low biomass present in pulmonary samples. Also, the addition of adequate negative controls will further reduce the mis-representation of microbial communities in respiratory samples [50]. This would ultimately inform the development of therapies targeting both the beneficial and deleterious effects of microbiota in asthma [9].

Microbiome-targeted therapeutics in asthma

As microbiome-focused research broadens, opportunities and novel treatment targets will be identified for both the prevention and treatment of asthma [10]. One possible early life intervention may involve strategies that promote a ‘healthy’ microbiome that may increase the tolerance of the immune system to allergies, including those linked to asthma [51]. A therapeutic strategy involving highly specific antimicrobials or vaccines targeted against pathogenic microbes in subgroups of asthmatics with neutrophilic inflammation may reduce disease-promoting colonisation and inflammation [28]. This was shown experimentally with the use of macrolides, which possesses both antimicrobial and anti-inflammatory properties, in mouse models of severe steroid-resistant asthma [27]. Moreover, in adults with asthma, long-term oral azithromycin (another macrolide) therapy (500 mg, thrice per week, for 48 weeks) significantly reduced exacerbations (including severe exacerbations), and improved asthma-related quality of life [52]. Notably, these beneficial effects were independent of asthma phenotype (eosinophilic versus non-eosinophilic), co-administration of maintenance therapy (inhaled corticosteroids), history of asthma exacerbations or symptoms of chronic cough and were irrespective of the isolation of bacterial pathogens (based on culturing). Azithromycin use also reduced sputum eosinophils, however, the number of neutrophils were not affected [52]. Another pilot study revealed that oral azithromycin therapy (250 mg/per day for 6-weeks) was associated with reduced airway bacterial richness, as well as airway dysbiosis leading to enrichment of *Anaerococcus* and reductions in classic respiratory pathogens (*Pseudomonas*, *Haemophilus* and *Staphylococcus*) [53]. Long term (six-months) azithromycin treatment affected the overall composition of the oropharyngeal microbiome in ~50% of patients, with increases in *Streptococcus salivarius* and marked reductions in *Leptotrichia wadei*, *Leptotrichia buccalis*/*Leptotrichia hofstadtii* and *Fusobacterium nucleatum* [54]. Although recent evidence suggests largely beneficial effects of azithromycin in limiting asthma exacerbations, it remains to be fully understood how macrolides affect the overall microbiome and disease progression.

Current research is focussing on developing effective vaccines against non-typeable *H. influenzae* (NTHi), a major respiratory bacterial pathogen in asthmatic individuals [55]. Two important vaccine targets are the outer membrane protein D and *Haemophilus* adhesion protein (Hap). However, laboratory and clinical trials are needed to define the efficacy of these vaccines in asthmatic individuals prone to NTHi colonisation [56]. Another area of high interest is the specific targeting of major pathogens by employing a combination of strategies, including broadening vaccine coverage in asthma patients against both bacterial (pneumococci) [57] and viral (influenza virus) [58], and/or promoting the growth of

commensal bacteria (*H. haemolyticus*) to inhibit pathogen colonisation (NTHi) [59].

Gut-lung axis-mediated therapeutics

Mechanistically, the inter-relationships between different mucosal tissues, particularly the lung and the GI-tract, remain inadequately defined. However, detailed knowledge of this phenomenon is pivotal to better understand disease aetiology and treatment. It may be possible to manipulate and enhance the immune system using commercially available products containing beneficial microbes (e.g. *Lactobacillus* spp., *Bifidobacterium* spp.), especially those producing SCFAs or prebiotics, which may impart significant health improvements in asthma patients [60,61]. In a meta-analysis of 20 cohorts involving >4000 participants, the administration of bacterial probiotics (single/multiple strains) in early life (prenatal or in the first year), significantly reduced total serum IgE levels and the risk of atopic sensitisation. However, the risk of developing childhood asthma was not affected [62]. In contrast, other studies did not find any protective effects of probiotic supplementation on the onset, diagnosis or outcomes of asthma in infants or children [63,64]. The disparities between these studies could be due to the heterogeneity of probiotic strains used, in terms of formulation and daily dosing, as well as the geographic locations where the original studies were conducted. Also, host-associated factors (genetic make-up, dietary habits, lifestyle) seems to play a crucial role. Furthermore, treatments were not selected with the aim to modify the underlying molecular mechanisms involved in asthma initiation and/or progression. Indeed, probiotics that release SCFAs that act through G-protein-coupled receptors to suppress inflammation may be more effective [9]. Optimising beneficial bacterial strains to improve immunotolerance or suppress pro-inflammatory responses of inflammatory cells may be an effective way forward.

Several reservations still exist when contemplating microbiome-focused strategies to be utilised therapeutically in asthma [65]. First, there is lack of studies that mechanistically demonstrate a definitive cause-and-effect relationship between the microbiome and asthma initiation and/or progression, and current studies, at best, only present a causal relationship between specific microbe(s) and asthma characteristics. Second, although extremely complicated, the interaction of host-associated factors, the microbiome and the environment need to be assessed to elucidate the cellular, molecular and inflammatory pathways that underpin asthma pathogenesis. This could be achieved by focused animal-based studies initially that could be then expanded in clinical setting. Finally, the mechanisms associated with individual members of microbiome need to be evaluated extensively to define modifiable microbial pathways that may significantly affect host inflammatory status with minimal risk of adverse effects [65].

Conclusions

In summary, the microbiome, both pulmonary and gastrointestinal, likely play a crucial role in modulating immune responses in asthma. Specific members of the microbiome are associated with asthma phenotypes/endotypes that further enhance the potential of therapeutically targeting the gut and/or lung microbiomes to improve asthma management. Improved understanding of mechanistic pathways would lead to microbiome-directed therapies that may alleviate the asthma burden for both patients and healthcare system.

Conflict of interest statement

Nothing declared.

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