Published in final edited form as:
Expert Rev Respir Med. 2011 June ; 5(3): 329–332. doi:10.1586/ers.11.33.

Bacteria and asthma—more than we thought

Daniel E. Dulek[1] and **R. Stokes Peebles Jr.**[2]

[1]Department of Pediatrics, Division of Pediatric Infectious Diseases, Monroe Carell Jr. Children's Hospital at Vanderbilt, Vanderbilt University Medical Center, Nashville, TN, USA, daniel.dulek@vanderbilt.edu [2]Department of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA

Abstract

In a recently performed case-control study, characteristics of the airway microbiota in suboptimally controlled adult asthmatics were compared to those of healthy, non-asthmatic adult subjects. Bacterial burden was significantly greater in asthmatic subjects. Further, increased airway microbiota variability and diversity were correlated with increased bronchial hyperresponsiveness. Though several limitations are present, this study provides an intriguing initial insight into the possible relationship between the airway microbiota and asthma pathogenesis.

Keywords

Microbiota; bronchial hyperresponsiveness; bacterial infection; asthma; microarray; macrolides

Introduction

Huang and colleagues recently published a case-control study examining the relationship between airway bacterial microbiota and clinical features of asthma [1]. The possibility that bacterial infections are responsible for asthma symptoms has been entertained for over 100 years [2]. In the first half of the 20th century, a widely held concept was that asthma exacerbations were related to 'bacterial allergy' in which a hypersensitivity reaction to bacteria was responsible for worsening asthma symptoms and could be improved with allergy shots to bacteria [3]. This was debunked in 1959 by the first randomized controlled trial of bacterial immunotherapy for the treatment of asthma [4]. The concept that bacteria were pathogenic in flares of asthma further fell out of favor after randomized controlled trials in the 1970s and 1980s showing that antibiotics did not improve pulmonary function or symptoms related to asthma exacerbations [5,6].

Subsequently, in the mid-1990s greater focus was placed on viral infections as precipitants of asthma as 80-85% of school aged children with asthma exacerbations were found to have viruses detected by PCR in respiratory secretions [7,8]. A reemergence of the role of bacteria occurred with the discovery that patients with unstable asthma despite medical therapy had *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* detectable by PCR in the

Corresponding author address: R. Stokes Peebles Jr, Department of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University Medical Center, T-1218 MCN, Nashville, TN 37232-2650, USA. stokes.peebles@vanderbilt.edu, Telephone: (615) 322-3412, Fax: (615) 343-7448.

Financial disclosure
The authors have no financial conflicts of interest to report.

lower airways [9]. Because of this finding, several clinical trials have addressed whether macrolide and macrolide-derived antibiotics might be useful therapeutically in patients with asthma. These studies vary in their approach and results with some demonstrating improvement and others not [10-14]. Further studies are needed to conclusively understand whether bacteria are important in asthma pathogenesis.

With this background Huang and colleagues evaluated whether there is a relationship between characteristics of the airway microbiota and clinical features of asthma. The ability to answer this question has been aided by the development of DNA sequencing and microarray technologies that allow for relatively rapid phylogenetic analysis of bacterial populations [15]. Recent findings using these technologies have correlated features of the human microbiome with specific disease states [15-18].

Summary of Methods and Results

Huang and colleagues found a significant relationship between characteristics of the airway microbiota and bronchial hyperresponsiveness (BHR), as defined by methacholine PC_{20} [1]. Methacholine PC_{20} is a measurement of BHR in which increasing concentrations of methacholine are administered by the inhaled route to determine the concentration of methacholine at which a subject's FEV_1 drops by 20%. Decreases in FEV_1 that occur at lower concentrations of methacholine indicate greater BHR. The authors performed a case-control study using patients participating in a larger multicenter trial of the effects of clarithromycin on asthma control [10]. Adult men and women (18-60 years of age) with suboptimal asthma control were targeted for inclusion. Suboptimal asthma control was defined as an Asthma Control Questionnaire (ACQ) score indicative of mild-persistent asthma, or worse, following 4 weeks of treatment with 88 ug of fluticasone propionate given twice daily through metered-dose inhaler. Exclusion criteria included a greater than 10 pack-year smoking history, unstable asthma, respiratory tract infection within the 6 weeks prior to enrollment, postbronchodilator forced expiratory volume 1 second (FEV_1) of less than 60% of predicted value, or other pulmonary disease. Patients were enrolled in the parent study through 10 Asthma Clinical Research Network clinical research centers.

Sixty-five case subjects participating in the parent study were included in the presently-reviewed pilot study. Flexible fiberoptic bronchoscopy was performed prior to treatment randomization within the parent study and three bronchial brushings were obtained using triple-lumen protected specimen brushes. These samples were shipped to the University of California, San Francisco where microbiome analyses were performed. Ten healthy, nonsmoking adult patients were included as controls and underwent bronchoscopy with airway sample collection as above following evaluation that indicated normal spirometry, negative methacholine challenge, and negative skin testing for local allergens.

Following collection, specimens were processed for DNA and samples were screened for the presence of 16S rRNA using universal primers. 16S rRNA PCR product was detected in 54 of the 65 case specimens and 8 of the 10 control specimens. These product-positive samples then underwent 8 additional PCR reactions using the same primers for the purpose of maximizing bacterial diversity captured. Amplicons for each sample were pooled, purified, and gel quantified. Amplicon concentration determined by gel quantification was verified with 16S rRNA quantitative PCR. Prior experience of the authors indicated that 250 ng of amplicon allowed for good respiratory microbiota characterization. Thirty-seven of the 54 case subject specimens and 3 of the 8 control subject specimens had at least 250 ng of amplicon DNA. The investigators compared microbiota diversity detected with PhyloChip using either 100 ng or 250 ng from 6 paired samples. As there was no significant difference in diversity between the amplicon amounts, 5 additional case specimens and 2 additional

control specimens were evaluated by PhyloChip using 100 ng of amplicon. Therefore, a total of 42 case samples and 5 control samples were analyzed for airway microbiota structure, diversity, and community composition in relation to clinical features of asthma. Following initial evaluation of microbial structure, three samples from asthmatic patients were identified as statistical outliers and excluded from further analysis of community structure and diversity.

Primary analyses were performed using PhyloChip microarray to detect bacterial taxa on the basis of 16S rRNA gene loci differences. Using these data the authors generated specific microbiota-related variables that included airway microbiota structure, diversity, and community composition as well as airway bacterial burden on the basis of gel-quantified 16S rRNA amplicon concentration (described above). Asthma-related variables evaluated included spirometry, sputum cell differentials, ACQ scores, total IgE, oral corticosteroid use, acute asthma exacerbation, as well as BHR. Validation of PhyloChip microarray results was performed using 16S rRNA clone library-sequencing in 6 asthmatic patients.

The 65 asthmatic case patients were well-matched to the 10 control subjects with no significant age or sex differences. Asthmatic patients had significantly lower mean FEV_1 as a % of predicted compared to controls (74.9% vs. 94.1%) and greater BHR indicated by lower methacholine PC_{20} (1.3 vs. >16 mg/mL). Thirty-five percent of asthmatic specimens did not possess enough DNA to be analyzed by PhyloChip. The investigators compared clinical characteristics of patients with sample analyzed by PhyloChip to those unable to be analyzed. There were no differences between these groups with regard to age, sex, FEV_1, sputum cell differentials, ACQ scores, allergen skin test positivity, total IgE, last oral corticosteroid use, or asthma exacerbation events during the study. The authors found that in samples able to be analyzed by PhyloChip there was no association between study center and variability of microbiota composition, indicating no effect of study center on subsequent findings. Bacterial burden was significantly greater in asthmatic samples than in control samples. However, no significant relationship was noted between bacterial burden and BHR. Therefore, further analysis was performed to assess characteristics of the airway microbiota in relation to features of asthma.

Multivariate analysis revealed no association between microbiota composition and spirometric measurements, sputum cell differentials, asthma exacerbation history, or systemic corticosteroid use in the prior 2 years. Notably, though, asthmatic and healthy samples had significant differences in microbiota structure. Further, variability in the microbiota composition correlated with both airway BHR and bacterial burden. In these measurements, patients with greater BHR had increased microbiota variability and patients with greater bacterial burden had increased microbiota variability.

Airway bacterial diversity, a measure of both the total number of taxa and the relative abundance of different taxa in a sample, was greater in asthmatic patients than in control patients. Furthermore, in a significant correlation, BHR increased along with increased airway bacterial diversity. The authors then evaluated the relative abundance of all taxa for relationship with BHR. Approximately 100 taxa demonstrated a significant linear relationship between increased abundance and increased BHR. As there was potential for some of these organisms to be detected from contaminating oral secretions, the investigators utilized an oral microbiome database as well as PubMed and showed that 87.5% of the associated taxa had not been previously noted in the oral cavity.

Finally, as mentioned previously, this study was performed within a larger trial of clarithromycin efficacy in asthmatic patients. Although post-treatment airway samples were not obtained, the investigators found a correlation between improvement in BHR after

clarithromycin and pre-clarithromycin airway bacterial diversity while those that did not have improvement in BHR had less bacterial diversity in the airway.

Discussion

The findings of this study by Huang and colleagues suggest a possible relationship between characteristics of the airway microbiota and clinical features of asthma. While significant relationships are identified, this paper raises more questions than it answers. As such the primary strength of the study is its hypothesis-generating capacity. One of the more interesting possibilities raised by the study is that specific microbiota taxa correlated in the study with BHR are involved in some manner with asthma pathogenesis. As the authors point out, several of the bacterial families identified in the microbiota of this study have members with features that could potentially impact asthma pathogenesis. For instance, members of one bacterial family influence nitric oxide synthesis by possessing active nitric oxide synthase and members of another possess steroid metabolism pathways, both areas of great interest in the asthma field. The discovery of these bacteria in the airways of asthmatic subjects will hopefully lead to future studies which more clearly define the role of these organisms and pathways in asthma pathogenesis. Further important questions raised from these associations include which bacteria or combinations of bacteria are related to inflammation and therefore may be harmful; which bacteria may be beneficial and actually protect against symptoms; which bacteria have a role in altering pulmonary function and which do not; elimination of which bacteria improves or worsens disease control; and, do relationships exist between specific bacteria and efficacy of currently established therapies for asthma?

Other specific issues are still unclear after the completion of this study. For instance, how does clarithromycin affect BHR in subjects with a high degree of microbiota diversity? Although association was noted between pretreatment diversity and post-treatment improvement in BHR, it is possible that clarithromycin did not alter either the microbiota burden or diversity through antibacterial mechanisms. Rather, the effects on the relationship between pre-antibiotic microbiota diversity and BHR may still be related to the medication's anti-inflammatory properties. Importantly, another issue centers around the inhaled steroids administered for four weeks prior to sampling of the airway by bronchoscopy. Although standardized across the asthma group, control patients did not receive inhaled corticosteroids which could conceivably have contributed to some of the observed between-group microbiota differences noted. It is possible that the microbiota burden and variability may have been different in the asthmatic subjects prior to and after inhaled corticosteroids. There may have been no relationship between the microbiota variability and diversity with BHR prior to inhaled corticosteroids. The relationship between microbiota variability and diversity with BHR may be a function solely of inhaled corticosteroids. Ideally, if such a study were to be repeated, the microbiome should be sampled before corticosteroid therapy, after the corticosteroid run-in period, and then again after macrolide treatment to fully understand the relationship between the microbiome, inhaled corticosteroids, antibiotic therapy and BHR.

Finally, lack of statistical power may have limited the ability to detect associations of airway microbiota with patient characteristics such as FEV_1, FVC, sputum cell differential, or asthma exacerbation history. Furthermore, it is important to note that 35% of the 65 asthmatic samples did not have sufficient PCR product for analysis by PhyloChip. The existence of these high and low amplicon concentration subgroups within asthmatic patients suggests that there may be heterogeneity within populations of asthmatic patients with regard to response to or clearance of airway microbiota.

It is important to note that the present study shares a similar finding with the one prior evaluation of airway microbiota in asthmatic patients. In this study 11 patients with asthma and 5 patients with COPD were compared to 8 control subjects [16]. Microbiota analysis was performed using 16S rRNA clone library-sequencing. This group noted that members of the phylum Proteobacteria were detected with significantly greater frequency in airways of asthmatic and COPD subjects than in healthy controls. A similar association was noted in the presently reviewed study.

Expert Commentary and Five-year view

In describing a relationship between airway microbiota characteristics and features of asthma, this study provides a foundation for further investigation in this exciting new area of research. Evaluation of airway microbiota should be incorporated into future studies of the role of antibiotics in treatment of both acute and chronic asthma. Further characterization of the airway microbiota in asthmatic patients and identification of specific bacterial groups that associate with poor asthma control may lead to greater appreciation of cellular and molecular pathways critical for the development and persistence of asthma.

Acknowledgments

Grant Support:

This work was supported by the National Institutes of Health (R01 HL 090664, R01 AI 070672, R01 AI059108, GM015431, R21 HL106446, UL1 RR024975) and the Department of Veteran Affairs (1I01BX000624).

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1••. Huang YJ, Nelson CE, Brodie EL, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol. 2011; 127(2):372–381. e371–373. [PubMed: 21194740] A pilot case-control study in which several airway microbiota characteristics are correlated to bronchial hyperresponsiveness in subjects with suboptimally controlled asthma pre-treated with inhaled corticosteroids for four weeks.

2. Osler, W. The principles and practice of medicine: designed for the use of practitioners and students of medicine. D. Appleton and Company; New York: 1892.

3. Chobot R, Uvitsky IH, Dundy H. The relationship of the etiologic factors in asthma in infants and children. J Allergy. 1951; 22(2):106–110. [PubMed: 14823822]

4. Helander E. Bacterial vaccines in the treatment of bronchial asthma. Acta Allergologica. 1959; 13:47–66.

5•. Graham VA, Milton AF, Knowles GK, Davies RJ. Routine antibiotics in hospital management of acute asthma. Lancet. 1982; 1(8269):418–420. [PubMed: 6121090] Randomized, double-blind trial of use of amoxicillin versus placeba in adult patients admitted to a hospital with acute exacerbation of asthma.

6•. Shapiro GG, Eggleston PA, Pierson WE, Ray CG, Bierman CW. Double-blind study of the effectiveness of a broad spectrum antibiotic in status asthmaticus. Pediatrics. 1974; 53(6):867–872. [PubMed: 4598933] Randomized, double-blind trial of hetacillin versus placebo in pediatric patients with status asthmaticus.

7. Dulek DE, Peebles RS Jr. Viruses and asthma. Biochim Biophys Acta. 2011

8••. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. BMJ. 1995; 310(6989):1225–1229. [PubMed:

7767192] Longitudinal community-based study using PCR-based respiratory viral detection in the setting of acute asthma exacerbation.

9••. Martin RJ, Kraft M, Chu HW, Berns EA, Cassell GH. A link between chronic asthma and chronic infection. J Allergy Clin Immunol. 2001; 107(4):595–601. [PubMed: 11295645] Case-control study of adult patients with chronic stable asthma compared to healthy controls in which the presence of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were assessed by PCR of bronchoscopy samples.

10. Sutherland ER, King TS, Icitovic N, et al. A trial of clarithromycin for the treatment of suboptimally controlled asthma. J Allergy Clin Immunol. 2010; 126(4):747–753. [PubMed: 20920764]

11. Johnston SL, Blasi F, Black PN, Martin RJ, Farrell DJ, Nieman RB. The effect of telithromycin in acute exacerbations of asthma. N Engl J Med. 2006; 354(15):1589–1600. [PubMed: 16611950]

12. Black PN, Blasi F, Jenkins CR, et al. Trial of roxithromycin in subjects with asthma and serological evidence of infection with Chlamydia pneumoniae. Am J Respir Crit Care Med. 2001; 164(4):536–541. [PubMed: 11520711]

13. Kraft M, Cassell GH, Pak J, Martin RJ. Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. Chest. 2002; 121(6):1782–1788. [PubMed: 12065339]

14. Strunk RC, Bacharier LB, Phillips BR, et al. Azithromycin or montelukast as inhaled corticosteroid-sparing agents in moderate-to-severe childhood asthma study. J Allergy Clin Immunol. 2008; 122(6):1138–1144. e1134. [PubMed: 18951618]

15. Flanagan JL, Brodie EL, Weng L, et al. Loss of bacterial diversity during antibiotic treatment of intubated patients colonized with Pseudomonas aeruginosa. J Clin Microbiol. 2007; 45(6):1954–1962. [PubMed: 17409203]

16. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. PLoS One. 2010; 5(1):e8578. [PubMed: 20052417]

17. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457(7228):480–484. [PubMed: 19043404]

18. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008; 455(7216):1109–1113. [PubMed: 18806780]

Key issues

- Differences in the bacterial microbiome between healthy and diseased states are increasingly appreciated.
- Asthmatic patients had significantly increased bacterial burden compared to healthy controls.
- Airway microbiota variability correlated significantly with bacterial burden and both airway microbiota variability and diversity correlated significantly with BHR.
- The presence of several bacterial taxa is associated with greater BHR.
- A correlation existed between improvement in BHR after clarithromycin and pre-clarithromycin airway bacterial diversity, while there was no improvement in BHR following clarithromycin in those subjects with less microbiota diversity prior to antibiotics.
- Future study will need to clarify the roles of airway microbiota in asthma pathogenesis.