Chronic Obstructive Pulmonary Diseases:
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Journal Club

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Abbreviations: chronic obstructive pulmonary disease, **COPD**; Human Microbiome Project, **HMP**; Phylogenetic Investigation of Communities of Reconstruction of Unobserved States, **PICRUSt**; upper bronchial tract, **UBT**

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COPD and the Lung Microbiome

With next-generation sequencing techniques that enable investigators to perform large-scale, parallel analysis of microbes, we have learned a great deal about the lung microbiome and alterations in diseased states such as chronic obstructive pulmonary disease (COPD), asthma and cystic fibrosis. It is ironic that the largest study of the human microbiome, the National Institutes of Health Human Microbiome Project (HMP) in 2007, did not include the lung because there was an erroneous assumption that the respiratory tract was sterile. Current research (see the 6 abstracts below) makes it clear there is indeed a significant lung microbiome composed of bacteria, viruses and fungal species and that there is quite a complex relationship that involves not only shifts in the microbiota present in health and disease but also shifts depending on the severity of disease and during times of a respiratory exacerbation. Further shifts in the lung microbiome appear to occur in response to treatments individuals receive for maintenance and for acute exacerbations. The ongoing study of the lung microbiome will improve our understanding of the etiology and pathogenesis of COPD (and other lung diseases) and the roles of current therapies such as inhaled and systemic corticosteroids and antibiotics. This line of investigation will also forge research directions with regard to future treatment strategies.

Abstract 1
COPD and the microbiome

Mammen MJ, Sethi S. *Respirology*. 2016 Jan 27. doi: http://dx.doi.org/10.1111/resp.12732. [Epub ahead of print]

Traditional culture techniques confirm that bacteria have an important role in chronic obstructive pulmonary disease (COPD). In individuals with COPD, acquisition of novel bacterial strains is associated with onset of acute exacerbation of COPD, which leads to further lung dysfunction and enormous health care costs. Recent study of the human microbiome, the total composite of the bacteria on the human body, posited the microbiome as the last human organ studied, as the microbiome performs a multitude of metabolic functions absent in the human genome. The largest project to study the human microbiome was the National Institutes of Health (NIH) Human Microbiome Project (HMP) started in 2007 to understand the 'normal' microbiome. However, due to the presumption that the healthy human lung was sterile, the respiratory tract was not included in that study. The advent of next-generation sequencing technologies has allowed the investigation of the human respiratory microbiome, which revealed that the healthy lung does have a robust microbiome. Subsequent studies in individuals with COPD revealed that the microbiome composition fluctuates with severity of COPD, composition of the individual aero-digestive tract microbiomes, age, during an acute exacerbation of COPD and with the use of steroids and/or antibiotics. Understanding the impact of the microbiome on COPD progression and risk of exacerbation will lead to directed therapies for prevention of COPD progression and exacerbation.

Comments

The authors provide an excellent up-to-date review with regard to our current understanding of the respiratory tract microbiome and the pathogenesis of COPD and COPD exacerbations. As they point out, the human microbiome refers to the constellation of microbes that live symbiotically in and on the human body. Indeed, it is likely that this relationship has evolved over time and that these microbes perform metabolic functions that human cells do not possess the capacity to perform. They make note that the National Institute of Health Human Microbiome Project (HMP), initiated in 2007, did not study the lung because of the presumption that the lung was sterile. The authors highlight the limitations of culture techniques and describe next-generation sequencing technologies that have allowed us to identify conserved 16S ribosome DNA sequences that are found only in bacteria and are well conserved within phyla and genera. Only 50% of bacteria sampled from human sites identified with these techniques have been recovered by traditional culture. They provide an excellent glossary of terms that are the common lexicon for experts in this field but foreign to most clinicians (such as operational taxonomic unit, α diversity and β diversity, etc.). This review is an excellent starting point to become familiar with the terminology and current concepts of the relationship between the respiratory tract and the microbiome both in health and in COPD and some of the directions for future research.

Abstract 2
The sputum microbiome in chronic obstructive pulmonary disease exacerbations

Huang YJ, Boushey HA. *Ann Am Thorac Soc.* 2015;12 (Suppl 2):S176-180. doi: http//dx.doi.org/10.1513/AnnalsATS.201506-319AW.

Acute exacerbations of chronic obstructive pulmonary disease (COPD) are thought to be associated with--and perhaps to mediate--accelerated loss of lung function in COPD. Although the application of culture-independent methods for detection of bacteria have shown COPD to be associated with marked differences in the burden, diversity, and composition of the bronchial bacterial microbiome, few studies have examined the changes associated with community-acquired exacerbations of the disease. In a longitudinal cohort study of COPD, the availability of sputum samples from subjects obtained at the onset of an exacerbation and during periods of clinical stability before and after the event enabled us to recently address this gap in knowledge, using culture-independent, 16S rRNA-based analysis methods combined with in silico inference of metagenomic functions. We observed sputum bacterial composition to be generally stable over the pre-exacerbation period of clinical stability, but to change at the time of exacerbation, with specific enrichment in not only typical COPD-associated bacterial species (e.g., Haemophilus influenzae) but also other phylogenetically-related species with pathogenic potential. Concurrently, we observed depleted abundance of other bacteria whose predicted metagenomes suggest functional capacities to produce a variety of anti-inflammatory compounds. Most strikingly, we found that resolution of these exacerbation-related changes in sputum microbiota composition differed significantly, depending on the exacerbation treatments prescribed. Treatment with corticosteroids resulted in microbiome enrichment for a number of bacterial communities, mostly members of the Proteobacteria phylum, whereas prolonged suppression of microbiota was seen in those treated with antibiotics alone. Taken together, our findings suggest that exacerbations of COPD are associated with heterogeneous changes in the bronchial microbiome, with increases in the abundance of species related to typical COPD pathogens and decreases in microbiota members that contribute to compositional and functional homeostasis. The findings further suggest that exacerbation treatments may have very different impacts on the bronchial microbiome's rate of return toward baseline composition.

Comments

This study examined 12 individuals who were part of a study cohort participating in a longitudinal study requiring serial sputum sampling. The individuals were all Global initiative for chronic Obstructive Lung Disease[1] Stage II and were able to provide spontaneous samples not requiring sputum induction. They were able to obtain a sputum sample at the time of exacerbation and at least 2 samples before and after the exacerbation. They studied 4 individuals for each treatment category: steroids alone, antibiotics alone and combined

steroids and antibiotics. The exacerbation sample was collected before treatment was instituted. As has been commonly reported there was a notable increase in the Proteobacteria phylum, particularly H influenza but also Gammaproteobacteria such as Enterobacteriacea, Pseudomonadaceae and Moraxellaceae. Reductions were noted in the Firmicutes and Actinobacteria phyla. Interestingly, the metagenomic analysis showed that functional pathways encoded by these depleted communities included their capacity to produce anti-inflammatory compounds (e.g., betalain, flavonoid, macrolide, and indole alkalid biosynthesis). There was a sharp decrease in Protobacteria noted in the *antibiotic only* treated group versus an increase in members from this phylum noted for individuals treated with oral corticosteroids. Also, the *antibiotic only* treated group was the only group to show further decreases in Proteobacteria in second post-exacerbation sputum samples, whereas the *corticosteroid only* and the *combined group* demonstrated either stabilization or a trend back to baseline values, respectively. These findings certainly give pause when considering the risk/benefit ratio with regard to the use of inhaled corticosteroids particularly in the group that appears to be more prone to repeated lower respiratory tract infections. The depletion of species typically associated with production of anti-inflammatory compounds may be as problematic as the increases in pathogenic species that produce pro inflammatory effects.

Abstract 3
Lung microbiome dynamics in COPD exacerbations

Wang Z, Bafadhel M, Haldar K, et al. *Eur Respir J.* 2016;47(4):1082-1092. doi: http://dx.doi.org/10.1183/13993003.01406-2015.

Increasing evidence suggests that the lung microbiome plays an important role in chronic obstructive pulmonary disease (COPD) severity. However, the dynamics of the lung microbiome during COPD exacerbations and its potential role in disease aetiology remain poorly understood. We completed a longitudinal 16S ribosomal RNA survey of the lung microbiome on 476 sputum samples collected from 87 subjects with COPD at four visits defined as stable state, exacerbation, 2 weeks post-therapy and 6 weeks recovery. Our analysis revealed a dynamic lung microbiota where changes appeared to be associated with exacerbation events and indicative of specific exacerbation phenotypes. Antibiotic and steroid treatments appear to have differential effects on the lung microbiome. We depict a microbial interaction network for the lung microbiome and suggest that perturbation of a few bacterial operational taxonomic units, in particular Haemophilus spp., could greatly impact the overall microbial community structure. Furthermore, several serum and sputum biomarkers, in particular sputum interleukin-8, appear to be highly correlated with the structure and diversity of the microbiome. Our study furthers the understanding of lung microbiome dynamics in COPD patients and highlights its potential as a biomarker, and possibly a target, for future respiratory therapeutics.

Comments

Wang and Colleagues provide data on 467 sputum samples that were longitudinally collected from 87 patients (largest cohort to date) during stable state (8 weeks exacerbation free), exacerbation (Anthonisen criteria[2]), 2 weeks post therapy and 6 weeks post therapy. All exacerbations were treated with corticosteroids and/or antibiotics. The investigators characterized exacerbations according to phenotypes: bacterial (n=35), eosinophilic (n=19), viral (n=15), bacterial/eosinophilic (n=3) and bacterial/viral combination (n=12) or pauci-inflammatory (n=27). Sputum and serum mediator data were collected in a 54 patient subset. There were 65 men and 22 women in the study with an average age of 68 and on average a 50 pack year smoking history. The majority were GOLD stage II (n=35) and III (n=32) with 19 stage IV and only 1 stage I individual. There were 21 individuals treated with antibiotics, 8 with steroids and 65 with both. Both sputum eosinophils ($p<0.01$) and neutrophils increased at the time of exacerbation ($p<0.001$). Serum neutrophils significantly increased ($p<0.001$) but eosinophils did not. The greatest differences were between the bacterial and eosinophilic exacerbation groups where the bacterial exacerbation group had a greater decrease in alpha diversity (diversity of organisms within a sample) at the phyla level, with decreases in Firmicutes but an increase in Protobacteria. At the genera level, the bacterial phenotype group had a significant decrease in streptococcus and an increase in Haemophilus compared to the eosinophilic group. The eosinophilic group also demonstrated a notable decrease in the Protobacteria: Firmicutes ratio. Also of note is that

treatment with oral corticosteroids lead to a decrease in α diversity and a decrease of Streptococcus and an increase of Haemophilus and Moraxella. Correlations were found with several clinical variables/biomarkers. Chemokine ligand8/interleukin-8 (CXCL8/IL8) in particular was significantly associated with the lung microbiome diversity and community structure and could represent a possible biomarker to assess the overall lung population.

While this study does represent the largest cohort examined to date, there is still a need for larger studies with more ethnically diverse populations and/or different biogeographical backgrounds. The study also does not contain healthy or non-exacerbator control individuals. While there was a mix of smokers (37), ex-smokers (48) and non-smokers (2), no comparisons were examined between these groups. Given the known inflammatory response related to cigarette smoke it would be helpful to know if active smoking had distinct effects on the microbiome. The authors also point out that the focus of this study was on the bacterial component of the lung microbiome but that it is increasingly appreciated that viruses and fungi also contribute to the lung microbiota pathogenesis of COPD exacerbations. Ideally, future studies will indeed look at the bacterial, viral and fungal microbiomes, integrating host response factors including transcriptome and metabolome profiles to further our understanding of the interactions between host and microbiome.

Abstract 4
Functional metagenomics of the bronchial microbiome in COPD

Millares L, Pérez-Brocal V, Ferrari R, et al. *PLoS One*. 2015;10(12):e0144448.
doi: http://doi.dx.org/10.1371/journal.pone.0144448.

The course of chronic obstructive pulmonary disease (COPD) is frequently aggravated by exacerbations, and changes in the composition and activity of the microbiome may be implicated in their appearance. The aim of this study was to analyze the composition and the gene content of the microbial community in bronchial secretions of COPD patients in both stability and exacerbation. Taxonomic data were obtained by 16S rRNA gene amplification and pyrosequencing, and metabolic information through shotgun metagenomics, using the Metagenomics RAST server (MG-RAST), and the PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) program, which predict metagenomes from 16S data. Eight severe COPD patients provided good quality sputum samples, and no significant differences in the relative abundance of any phyla and genera were found between stability and exacerbation. Bacterial biodiversity (Chao1 and Shannon indexes) did not show statistical differences and beta-diversity analysis (Bray-Curtis dissimilarity index) showed a similar microbial composition in the two clinical situations. Four functional categories showed statistically significant differences with MG-RAST at KEGG level 2: in exacerbation, Cell growth and Death and Transport and Catabolism decreased in abundance [1.6 (0.2-2.3) versus 3.6 (3.3-6.9), p=0.012; and 1.8 (0-3.3) versus 3.6 (1.8-5.1), p=0.025 respectively], while Cancer and Carbohydrate Metabolism increased [0.8 (0-1.5) versus 0 (0-0.5), p=0.043; and 7 (6.4-9) versus 5.9 (6.3-6.1), p=0.012 respectively]. In conclusion, the bronchial microbiome as a whole is not significantly modified when exacerbation symptoms appear in severe COPD patients, but its functional metabolic capabilities show significant changes in several pathways

Comments

This is a very small study, 8 individuals with severe COPD, but the findings are quite instructive. Shotgun metagenomics refers to production of millions of fragments of short DNA reads that, after processing, may be mapped to databases of orthologous (common origin) gene groups using *gene encyclopedias* such as the Kyoto Encyclopedia of Genes and Genomes that are databases that can be used to find matches to genes or proteins with previously described functions. Phylogenetic Investigation of Communities of Reconstruction of Unobserved States (PICRUSt) uses evolutionary modeling to predict metagenomes from 16S data and a reference genome database. The latter has the advantage that it does not require the relatively large quantities of bacterial DNA needed for shotgun metagenomics to detect microbial function and its variability. The limitation of PICRUSt is that it cannot distinguish differences at the strain level and cannot detect genes not included in the genomic database used. This study had only 8 individuals , all male, all on inhaled corticosteroid treatment and all with severely impaired lung function (mean post bronchodilator

forced expiratory volume in 1 second 37% predicted). Differences in the microbiome composition have been reported comparing severe and moderately severe COPD patients and hence, the findings are not generalizable. Further, metagenomic sequencing is expensive and computationally intensive further limiting its use on large populations. Despite these limitations, the study demonstrates that exacerbations may not be driven by significant changes in composition of the microbiome but more by metabolic functional differences as noted by the increase in *cancer and carbohydrate metabolism* functions and decrease in *cell growth and death* and *transport and catabolism* categories. While in this study it is not possible to distinguish whether the changes observed in metabolic function contribute to the development of symptoms or are simply a consequence of acute episodes, it is clear that answering this question may have significant implications for understanding the pathogenesis of exacerbations and offer directions for possible future therapeutic interventions.

Abstract 5
Metagenomic sequencing of the chronic obstructive pulmonary disease upper bronchial tract microbiome reveals functional changes associated with disease severity

Cameron SJ, Lewis KE, Huws SA, et al. *PLoS One*. 2016;11(2):e0149095. doi: http://dx.doi.org/10.1371/journal.pone.0149095.

Chronic obstructive pulmonary disease (COPD) is a major source of mortality and morbidity worldwide. The microbiome associated with this disease may be an important component of the disease, though studies to date have been based on sequencing of the 16S rRNA gene, and have revealed unequivocal results. Here, we employed metagenomic sequencing of the upper bronchial tract (UBT) microbiome to allow for greater elucidation of its taxonomic composition, and revealing functional changes associated with the disease. The bacterial metagenomes within sputum samples from eight COPD patients and ten 'healthy' smokers (controls) were sequenced, and suggested significant changes in the abundance of bacterial species, particularly within the Streptococcus genus. The functional capacity of the COPD UBT microbiome indicated an increased capacity for bacterial growth, which could be an important feature in bacterial-associated acute exacerbations. Regression analyses correlated COPD severity (forced expiratory volume in 1 second % of predicted) with differences in the abundance of Streptococcus pneumoniae and functional classifications related to a reduced capacity for bacterial sialic acid metabolism. This study suggests that the COPD UBT microbiome could be used in patient risk stratification and in identifying novel monitoring and treatment methods, but study of a longitudinal cohort will be required to unequivocally relate these features of the microbiome with COPD severity.

Comments

Despite the small numbers in this study, it is included because it compared COPD patients and healthy smokers and shows a difference in the abundance of bacterial species between the 2 groups with an increased functional capacity (sialic acid metabolism) for bacterial growth in the COPD UBT microbiome.

Abstract 6
Microbiota promotes chronic pulmonary inflammation by enhancing il-17a and autoantibodies

Yadava K, Pattaroni C, Sichelstiel AK, et al. *Am J Respir Crit Care Med*. 2015. Dec 2. [Epub ahead of print]

Rationale:
Changes in the pulmonary microbiota are associated with progressive respiratory diseases including chronic obstructive pulmonary disease. Whether there is a causal relationship between these changes and disease progression remains unknown.
Objective:
To investigate the link between an altered microbiota and disease, we utilized a model of chronic lung inflammation in specific pathogen free (SPF) mice and mice depleted of microbiota by antibiotic treatment or devoid of a microbiota (axenic).
Methods:
Mice were challenged with LPS/elastase intranasally over 4 weeks, resulting in a chronically inflamed and damaged lung. The ensuing cellular infiltration, histological damage and decline in lung function were quantified.
Measurements and Main Results:
Similar to human disease, the composition of the

pulmonary microbiota was altered in disease animals. We found that the microbiota richness and diversity were decreased in LPS/elastase-treated mice, with an increased representation of the genera Pseudomonas, Lactobacillus and a reduction in Prevotella. Moreover, the microbiota was implicated in disease development as mice depleted of microbiota exhibited an improvement in lung function, reduction in airway inflammation, decrease in lymphoid neogenesis and auto-reactive antibody responses. The absence of microbial cues also markedly decreased the production of IL-17A, whilst intranasal transfer of fluid enriched with the pulmonary microbiota isolated from diseased mice enhanced IL-17A production in the lungs of antibiotic treated or axenic recipients. Finally, in mice harboring a microbiota, neutralizing IL-17A dampened inflammation and restored lung function.

Conclusions:
Collectively, our data indicate that host-microbial cross-talk promotes inflammation and could underlie the chronicity of inflammatory lung diseases.

Comments

These investigators have performed some elegant bench studies to elucidate the potential relationship of the microbiome in the pathogenesis of COPD exacerbations and the role of IL-17. The investigators note differences in the microbiota in mice challenged with LPS/elastase that were not dissimilar to the changes noted in human COPD individuals. The fact that mice depleted of microbiota had improved lung function supports a possible causal relationship. The results will further help us in understanding the potential role and limitations of anti-IL17 therapeutic agents not only for COPD, but likely for asthma as well.

References

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