AMBIENTE Y SALUD RESPIRATORIA

Dra. Laura B. Moreno.
Servicio de Neumonología.
Hospital de Niños Santísima Trinidad de Córdoba
SAP Filial Córdoba, 2015

Ambiente interno. Microbioma pulmonar

Dra. Laura B. Moreno.
Servicio de Neumonología.
Hospital de Niños Santísima Trinidad de Córdoba
SAP Filial Córdoba, 2015

Objetivos de la presentación:

- Recuperar conceptos básicos y actuales sobre microambiente y **"microbioma pulmonar**" y sus posibilidades preventivas y terapéuticas en enfermedad respiratoria pediátrica.

- **Perspectiva ECOLOGICA del proceso SALUD-ENFERMEDAD respiratoria**

E. K. Costello et al.

The Application of Ecological Theory Toward an Understanding of the Human Microbiome. *Science Translational Medicine Review 2012*

Posibles agentes patógenos para el sistema respiratorio.

- **Infecciosos**:
  - virus, bacterias, parásitos, hongos.
- **Partículas inorgánicas**:
  - sílice, asbestos, carbón, hierro, talco, etc.
- **Gases tóxicos o irritantes**:
  - humo de tabaco, SO_2, NO_2, O_3, CO, oxigeno en altas concentraciones, etc.
- **Partículas orgánicas**:
  - pólenes, esporas de hongos, enzimas.
- **Sustancias químicas**:
  - humo de tabaco, polución
- **Cuerpos extraños:**
  - alimentos, piezas dentales, etc.
- **Secreciones**:
  - secreción bucofaríngea, gástrica.
- **Drogas**:
  - aerosoles, gotas nasales
- **Radiaciones:**
  - radioterapia, radiaciones

Mecanismos de defensa pulmonar

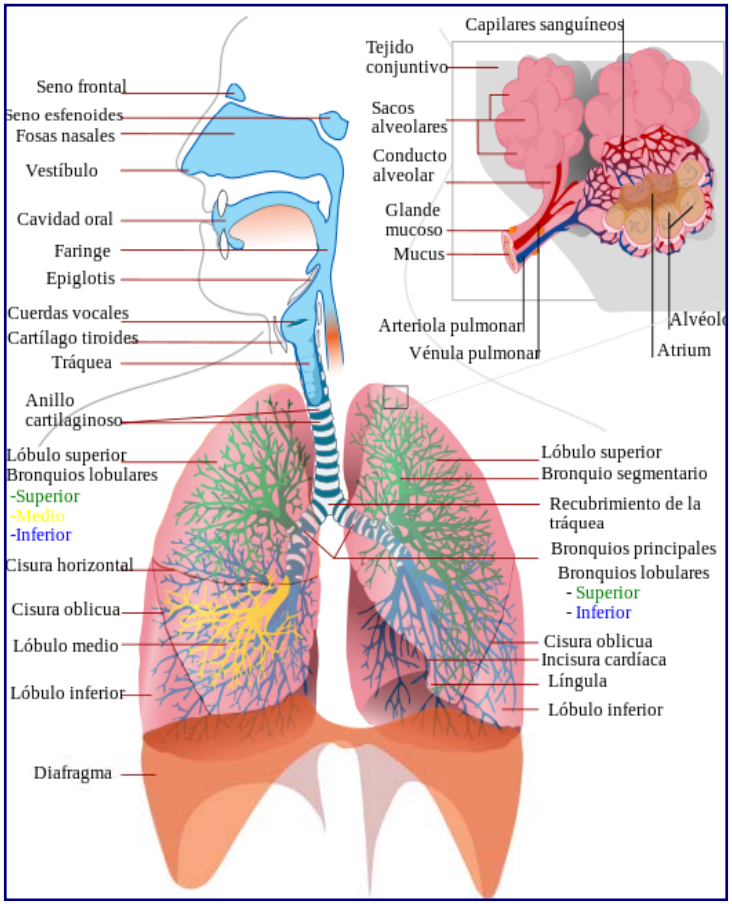
- Complejas estructuras anatómicas y funcionales del sistema RESPIRATORIO-INMUNOLÓGICO

- Objetivos
  - Mantener libre de agentes patógenos la zona inferior a las cuerdas vocales
  - Limitar el ingreso de elementos no deseados al pulmón (intercambio gaseoso).

$\Rightarrow$"Microambiente saludable"

Mecanismos intrínsecos/constitutivos

- Filtración aerodinámica de partículas
- Acondicionamiento del aire inhalado
- Reflejos de la vía aérea
  - Reflejo laríngeo de cierre
  - Estornudo
  - Broncoconstricción
  - Tos
- Transporte mucociliar e hidratación del moco.
  - Motilidad ciliar/composición del moco
  - Drenaje anatómico



Sistema inmunitario

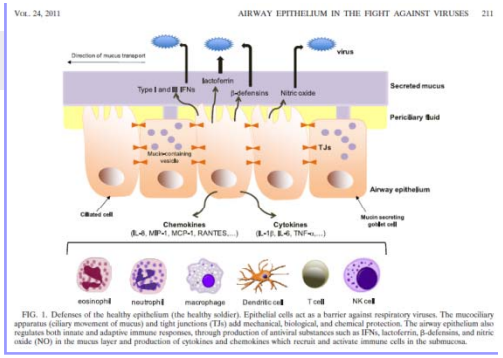


FIG. 1. Defenses of the healthy epithelium (the healthy soldier). Epithelial cells act as a barrier against respiratory viruses. The mucociliary apparatus (ciliary movement of mucus) and tight junctions (TJs) add mechanical, biological, and chemical protection. The airway epithelium also regulates both innate and adaptive immune responses, through production of antiviral substances such as IFNs, lactoferrin, β-defensins, and nitric oxide (NO) in the mucus layer and production of cytokines and chemokines which recruit and activate immune cells in the submucosa.

- Reconocimiento de células/partículas extrañas
- Eliminación de las mismas
- Reparación de los tejidos injuriados.
- Establecer adaptación al medioambiente:

Inmunidad innata ⇔ microambiente pulmonar

= MODULACIÓN INMUNOLÓGICA
(tolerancia, alergia o autoinmunidad)

L. V. Hooper et al. Interactions Between the Microbiota and the Immune System .
Science Translational Medicine Review 2012

Receptores celulares “tool-like receptor”(RLTs)

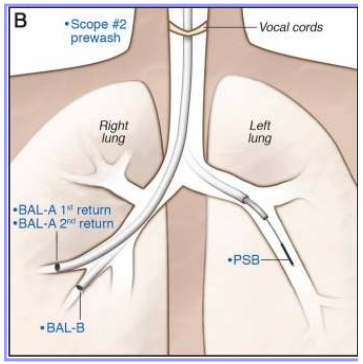
- Receptores proteicos que desempeñan un rol clave en la iniciación de la respuesta inmune innata celular.

- Se han descripto alrededor de 10 RLTs
- Tienen un dominio extracelular que reconoce algunos componentes microbianos
- La región intracelular contiene un receptor para IL-1 que desencadenan citocinas y quimocinas.

- Se expresan en macrófagos, neutrófilos y células epiteliales de la vía aérea.
- También en células dendríticas, linfocitos B y T, participando en la respuesta inmune adaptativa.

Microbioma Pulmonar

Microbioma…



- Todos los compartimientos humanos en contacto con el medio externo estarían <u>colonizados por diferentes poblaciones de microbios (bacterias-virus-hongos)</u>
- Relación de “mutualismo”-simbiosis

⇒ Rol de los microbios en SALUD - ENFERMEDAD humana

Microbioma pulmonar…

- Pruebas de detección de subunidades ribosomales 16S del ARNr y pirosecuenciación.
  ⇒los pulmones normales **no son estériles**!
  (múltiples microorganismos colonizantes -microbiota).

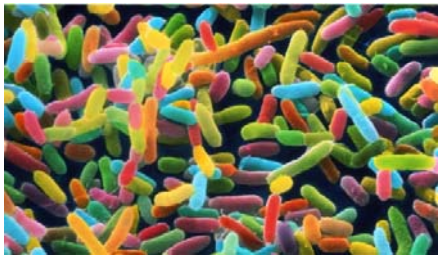
- Familias bacterianas asientan en las vías aéreas inferiores desde los primeros momentos de la vida
- Muestran diferencias entre los sujetos sanos y con patología respiratoria.

Microbioma: algunos conceptos (1)

- **Microbio:** forma de vida microscópica (bacterias, hongos, protozoarios, virus)
- **Microbiota:** microbios específicos en una "población" determinada
- **Microbioma**: la microbiota en un "hábitat" determinado (lugar-tiempo) que interactúa "funcionalmente y metabólicamente"

MICROBIOMA sinónimo de MICROBIOTA
(diferentes conceptos ecológicos)

The microbiome of the lung



JAMES M. BECK, VINCENT B. YOUNG, and GARY B. HUFFNAGLE

ANN ARBOR, MICH

Investigation of the lung microbiome is a relatively new field. Although the lungs were classically believed to be sterile, recently published investigations have identified microbial communities in the lungs of healthy humans. At the present time, there are significant methodologic and technical hurdles that must be addressed in ongoing investigations, including distinguishing the microbiota of the upper and lower respiratory tracts. However, characterization of the lung microbiome is likely to provide important pathogenic insights into cystic fibrosis, respiratory disease of the newborn, chronic obstructive pulmonary disease, and asthma. In addition to characterization of the lung microbiome, the microbiota of the gastrointestinal tract have profound influence on the development and maintenance of lung immunity and inflammation. Further study of gastrointestinal–respiratory interactions is likely to yield important insights into the pathogenesis of pulmonary diseases, including asthma. As this field advances over the next several years, we anticipate that studies using larger cohorts, multicenter designs, and longitudinal sampling will add to our knowledge and understanding of the lung microbiome. (Translational Research 2012;160:258–266)

Table 1

Examples of taxonomic classification for some of the major bacterial groups identified as members of the human microbiome, including the respiratory tract. Given the diversity of human microbiota, the table is not intended to be comprehensive. Listed bacterial phyla, classes, orders, families and genera include organisms that have been identified in lung microbiome studies.

Phylum	ACTINOBACTERIA	BACTEROIDETES	FIRMICUTES	PROTEOBACTERIA
Classes	Actinobacteria	Bacteroidia Cytophagia Flavobacteria Sphingobacteria	Bacilli Clostridia Erysipelotrichia	Alphaproteobacteria Betaproteobacteria Deltaproteobacteria Epsilonproteobacteria Gammaproteobacteria
Orders	Acidimicrobiales Actinomycetales Bifidobacteriales Rubrobacterales	Bacteroidales Flavobacteriales Sphingobacteriales	Bacillales Clostridiales Lactobacillales	Burkholderiales Campylobacterales Enterobacteriales Moraxellaceae Neisseriales Pasteurellales Pseudomonadales Sphingomonadales
Families	Actinomycetaceae Bifidobacteriaceae Corynebacteriaceae Mycobacteriaceae Nocardiaceae Streptomycetaceae	Bacteroidaceae Flavobacteriaceae Porphyromonadaceae Prevotellaceae Sphingobacteriaceae	Bacillaceae Clostridiaceae Enterococcaceae Lachnospiraceae Lactobacillaceae Ruminococcaeae Streptoccocaceae Veillonellaceae	Bartonellaceae Burkholderiaceae Enterobacteriaceae Helicobacteraceae Neisseriaceae Pasteurellaceae Pseudomonadaceae Sphingomonadaceae
Genera	Actinomyces Bifidobacterium Corynebacterium Mycobacterium Nocardia Streptomyces	Bacteroides Capnocytophaga Porphyromonas Prevotella Sphingobacterium	Clostridium Faecalibacterium Lachnospira Ruminococcus Streptococcus Veillonella	Acinetobacter Escherichia Enterobacter Haemophilus Moraxella Pseudomonas Sphingomonas

Microbioma: algunos conceptos (2)

- Unidades Taxonómicas Organizacionales (OTUs) permiten describir comunidades (<97% similares)
  - **Riqueza** (N° de OTUs o biomasa)
  - **Abundancia** (relativa proporción de diferentes OTUs)
  - **Dominancia** (emergencia de una OTU)
  - **Índice de diversidad/complejidad.**

Microbioma: algunos conceptos (3)

- Inmunomodulación. El sistema inmune se desarrolla a partir de su interacción con el medio ambiente (flora colonizante digestiva, respiratoria, etc).
  ⇒Tolerancia/alergia (patogenia del asma)

- Variabilidad-Diversidad: aumento o disminución de algunas poblaciones bacterianas (OTU/ Unidades Taxonómicas Operativas)
  ⇒Enfermedades crónicas obstructivas (EPOC)
  ⇒Exacerbaciones de Fibrosis Quística
  ⇒Exacerbaciones de asma

Microbioma.
algunas evidencias…

Topographical Continuity of Bacterial Populations in the Healthy Human Respiratory Tract

2011

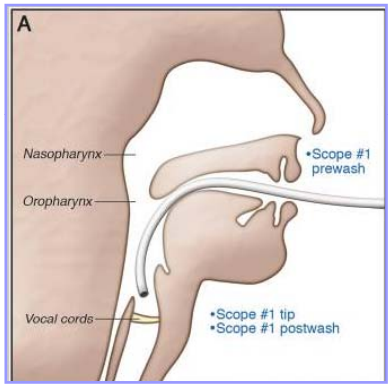
Emily S. Charlson[1,2], Kyle Bittinge[1], Andrew R. Haas[3], Ayannah S. Fitzgerald[3], Jan Frank[3], Anjana Yadav[2], Frederic D. Bushm[1]

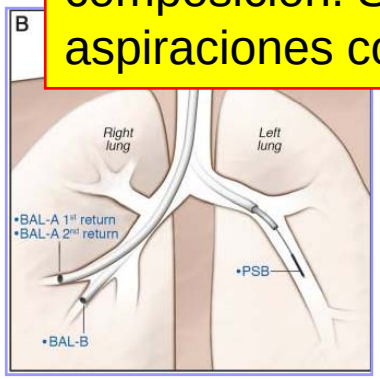
[1]Department of Microbiology; [2]Pulmonary ... of Pennsylvania School of Medicine, Philad...

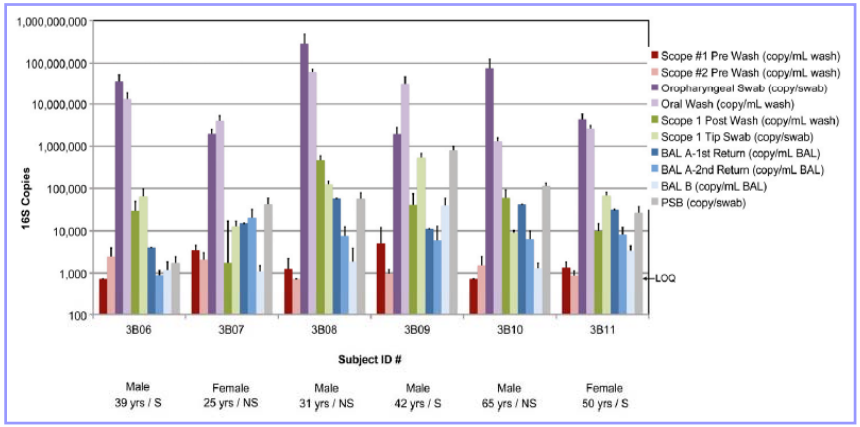
Am J Respir Crit Care Med Vol 184. pp 957–963, ...

•Existe un Microbioma pulmonar? O las bacterias identificadas son consecuencia del arrastre mecánico con el broncoscopio?

•Existen bacterias en los pulmones de individuos sanos en menor cantidad que en tracto respiratorio alto y de similar composición. Se sugiere que se originaría a partir de micro-aspiraciones continuas.







connections to the external environment, and it is increasingly clear that they play important roles in health, as well as disease when disrupted. The lung is classically thought to be sterile. New molecular techniques can comprehensively describe an entire population of bacteria without relying on the ability to culture specific organisms, and have suggested that the lung may harbor its own unique microbiome.

What This Study Adds to the Field

Bacteria are present in the lungs of healthy people at low levels compared to the upper respiratory tract, and are indistinguishable in community composition from upper airway microbiota. This finding suggests that they originate from the upper respiratory tract, most likely by microaspiration. There is no distinct lung-specific microbiome in healthy people, although very low levels of rare lung-specific bacteria may be present as well.

Nasopharyngeal Microbiota in Healthy Children and Pneumonia Patients

2014

Olga Sakwinska,[a] Viktoria Bastic Schmid,[a] Bernard Berger,[a] Anne Bruttin,[a] Kristina Keitel,[b] Mélissa Lepage,[a] Deborah Moine,[a]* Catherine Ngom Bru,[a] Harald Brüssow,[a] Alain Gervaix[b]

Nestlé Research Center, Lausanne, Switzerland[a]; Department of Pediatrics, University Hospitals of Geneva and Faculty of Medicine, Geneva, Switzerland[b]

Our study is the first to compare the nasopharyngeal microbiota of pediatric pneumonia patients and control children by 454 pyrosequencing. A distinct microbiota was associated with different pneumonia etiologies. Viral pneumonia was associated with a high abundance of the operational taxonomic unit (OTU) corresponding to *Moraxella lacunata*. Patients with nonviral pneumonia showed high abundances of OTUs of three typical bacterial pathogens, *Streptococcus pneumoniae* complex, *Haemophilus influenzae* complex, and *Moraxella catarrhalis*. Patients classified as having no definitive etiology harbored microbiota particularly enriched in the *H. influenzae* complex. We did not observe a commensal taxon specifically associated with health. The microbiota of the healthy nasopharynx was more diverse and contained a wider range of less abundant taxa.

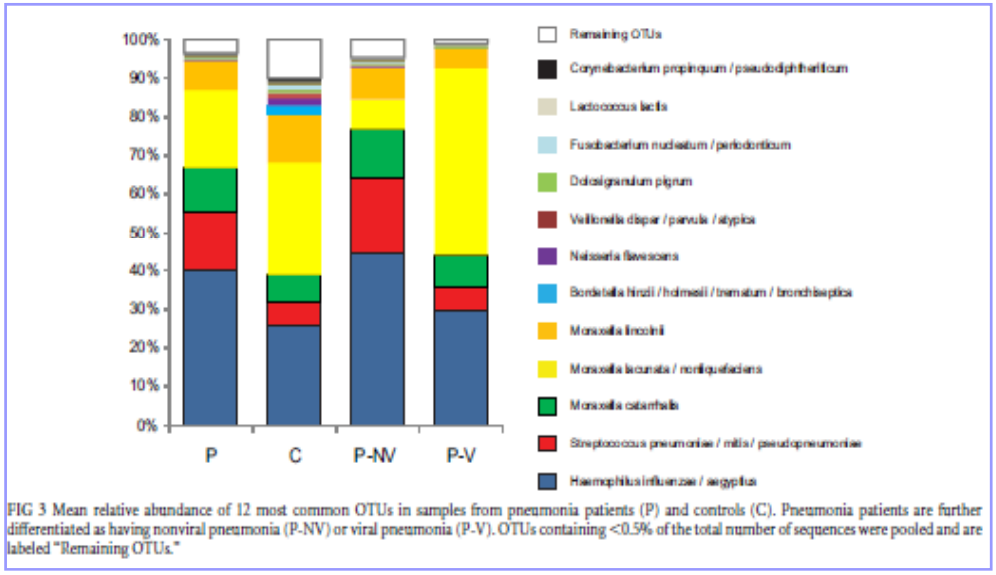


FIG 3 Mean relative abundance of 12 most common OTUs in samples from pneumonia patients (P) and controls (C). Pneumonia patients are further differentiated as having nonviral pneumonia (P-NV) or viral pneumonia (P-V). OTUs containing <0.5% of the total number of sequences were pooled and are labeled "Remaining OTUs."

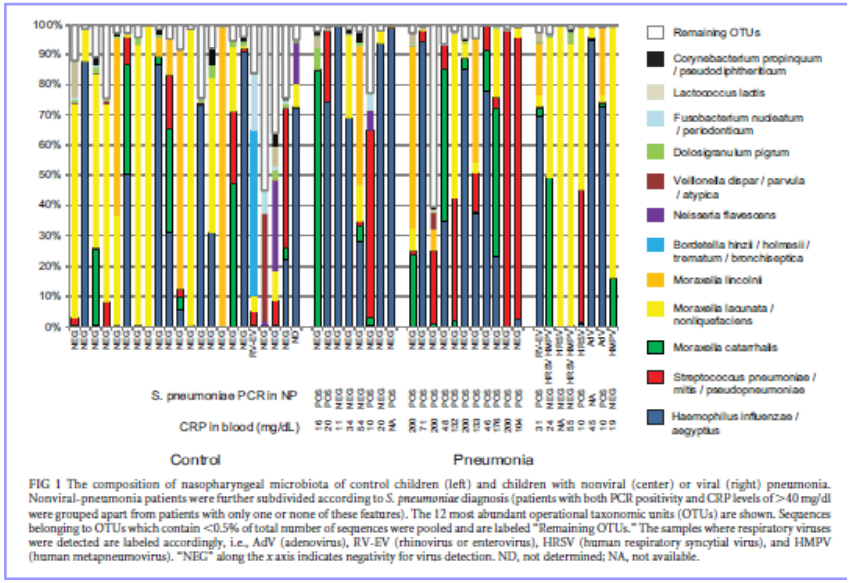


FIG 1 The composition of nasopharyngeal microbiota of control children (left) and children with nonviral (center) or viral (right) pneumonia. Nonviral-pneumonia patients were further subdivided according to *S. pneumoniae* diagnosis (patients with both PCR positivity and CRP levels of >40 mg/dl were grouped apart from patients with only one or none of these features). The 12 most abundant operational taxonomic units (OTUs) are shown. Sequences belonging to OTUs which contain <0.5% of total number of sequences were pooled and are labeled "Remaining OTUs." The samples where respiratory viruses were detected are labeled accordingly, i.e., AdV (adenovirus), RV-EV (rhinovirus or enterovirus), HRSV (human respiratory syncytial virus), and HMPV (human metapneumovirus). "NEG" along the *x* axis indicates negativity for virus detection. ND, not determined; NA, not available.

J. Clin. Microbiol. 2014, 52(5):1590. DOI: 10.1128/JCM.03280-13.
Published Ahead of Print 5 March 2014.

Nasopharyngeal Microbiota in Healthy Children and Pneumonia Patients

Olga Sakwinska,[a] Viktoria Bastic Schmid,[a] Bernard Berger,[a] Anne Bruttin,[a] Kristina Keitel,[b] Mélissa Lepage,[a] Deborah Moine,[a]* Catherine Ngom Bru,[a] Harald Brüssow,[a] Alain Gervaix[b]

Nestlé Research Center, Lausanne, Switzerland[a]; Department of Pediatrics, University Hospitals of Geneva and Faculty of Medicine, Geneva, Switzerland[b]

Our study is the first to compare t
pyrosequencing. A distinct microl
a high abundance of the operation
monia showed high abundances o
influenzae complex, and *Moraxell*
larly enriched in the *H. influenzae*
crobiota of the healthy nasophary

- Las diferentes etiologías en neumonía parecen estar asociadas a patrones específicos de "disbiosis" en la microbiota.
- La microbiota de los controles sanos fue mas "diversa" que los casos de neumonía.
- Podríamos especular que la disminución en la diversidad es causada por el sobre-crecimiento de patógenos, los que predispondría a la invasión de la infección . Sn embargo, es necesario confirmar esta hipótesis con estudios longitudinales.
- **Conclusiones**: Si bien no se encontraron bacterias especificas comensales asociadas a la salud, la nasofaringe sana fue colonizada con una flora con amplio rango de diversidad .

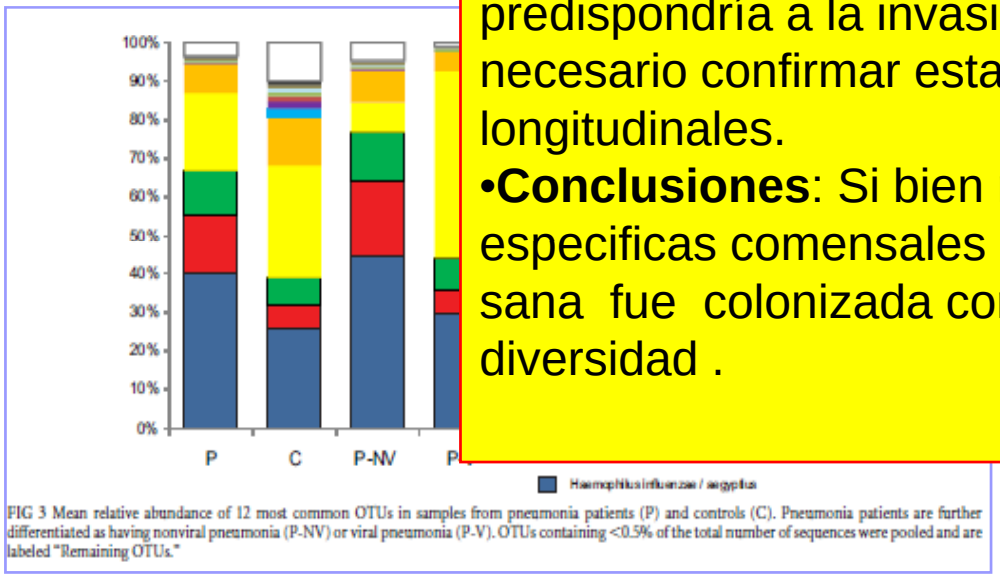


FIG 3 Mean relative abundance of 12 most common OTUs in samples from pneumonia patients (P) and controls (C). Pneumonia patients are further differentiated as having nonviral pneumonia (P-NV) or viral pneumonia (P-V). OTUs containing <0.5% of the total number of sequences were pooled and are labeled "Remaining OTUs."

J. Clin. Microbiol. 2014, 52(5):1590. DOI: 10.1128/JCM.03280-13.
Published Ahead of Print 5 March 2014.

OPEN ACCESS Freely available online

PLoS one

Analysis of the Lung Microbiome in the "Healthy" Smoker and in COPD

John R. Erb-Downward[1], Deborah L. Thompson[1], Meilan K. Han[1], Christine M. Freeman[1,2], Lisa McCloskey[1,2], Lindsay A. Schmidt[1], Vincent B. Young[1], Galen B. Toews[1,2], Jeffrey L. Curtis[1,2], Baskaran Sundaram[1], Fernando J. Martinez[1☯], Gary B. Huffnagle[1*☯]

1 University of Michigan, Ann Arbor, Michigan, United States of America, **2** Veterans Affairs Health System, Ann Arbor, Michigan, United States of America

Abstract

Although culture-independent techniques have shown that the lungs are not sterile, little is known about the lung microbiome in chronic obstructive pulmonary disease (COPD). We used pyrosequencing of 16S amplicons to analyze the lung microbiome in two ways: first, using bronchoalveolar lavage (BAL) to sample the distal bronchi and air-spaces; and second, by examining multiple discrete tissue sites in the lungs of six subjects removed at the time of transplantation. We performed BAL on three never-smokers (NS) with normal spirometry, seven smokers with normal spirometry ("heathy smokers", HS), and four subjects with COPD (CS). Bacterial 16 s sequences were found in all subjects, without significant quantitative differences between groups. Both taxonomy-based and taxonomy-independent approaches disclosed heterogeneity in the bacterial communities between HS subjects that was similar to that seen in healthy NS and two mild COPD patients. The moderate and severe COPD patients had very limited community diversity, which was also noted in 28% of the healthy subjects. Both approaches revealed extensive membership overlap between the bacterial communities of the three study groups. No genera were common within a group but unique across groups. Our data suggests the existence of a core pulmonary bacterial microbiome that includes *Pseudomonas, Streptococcus, Prevotella, Fusobacterium, Haemophilus, Veillonella, and Porphyromonas*. Most strikingly, there were significant micro-anatomic differences in bacterial communities within the same lung of subjects with advanced COPD. These studies are further demonstration of the pulmonary microbiome and highlight global and micro-anatomic changes in these bacterial communities in severe COPD patients.

Citation: Erb-Downward JR, Thompson DL, Han MK, Freeman CM, McCloskey L, et al. (2011) Analysis of the Lung Microbiome in the "Healthy" Smoker and in COPD. PLoS ONE 6(2): e16384. doi:10.1371/journal.pone.0016384

Editor: Stefan Bereswill, Charité-University Medicine Berlin, Germany

Received October 7, 2010; **Accepted** December 14, 2010; **Published** February 22, 2011

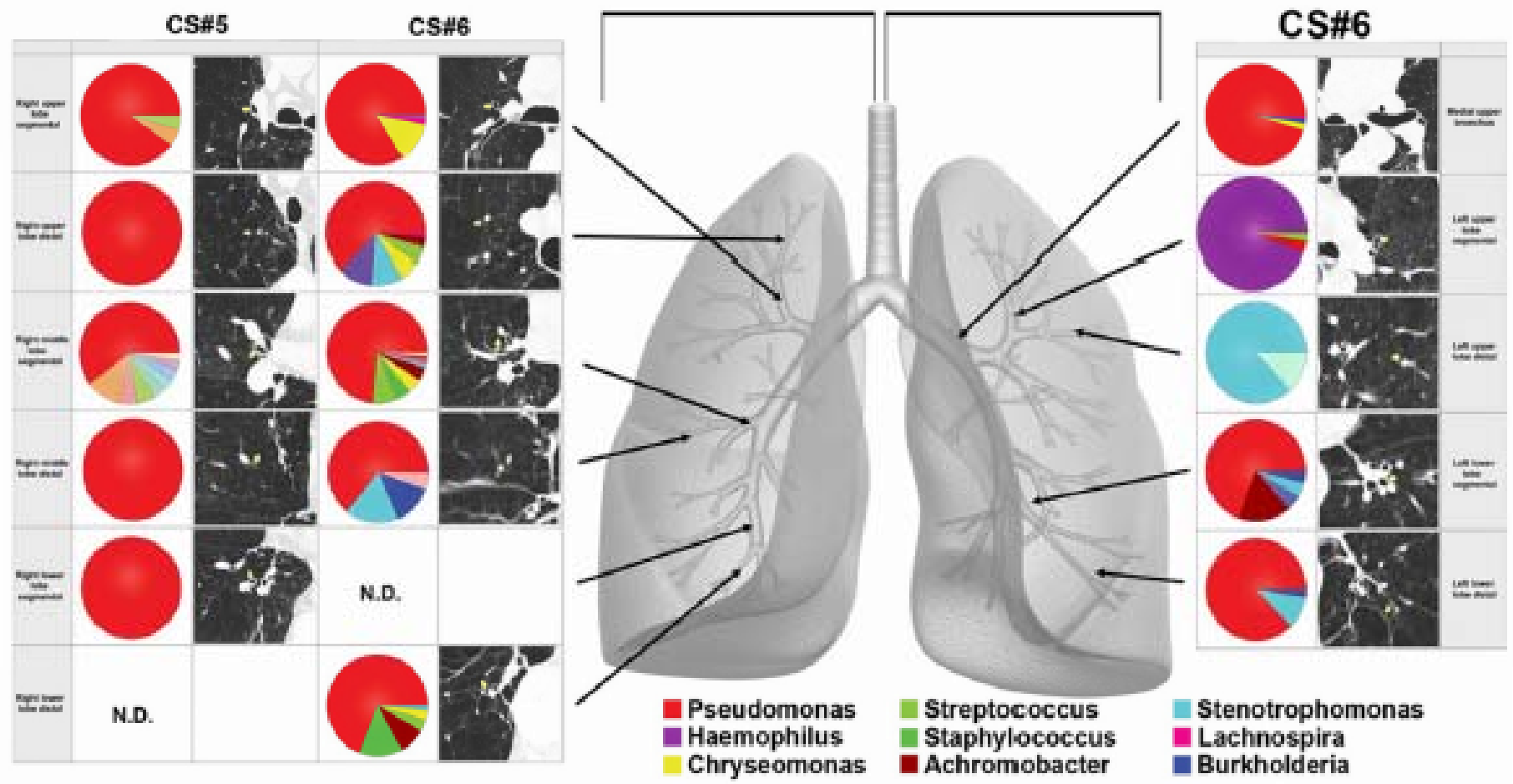


Figure 3.

Regional heterogeneity is noted in lung microbiota. Multiple samples were harvested from the regions of lung indicated by the arrows on the grey lung schematic in two chronic obstructive pulmonary disease (COPD) explants. Pie diagrams depict the genus level classification of 16S sequences, and the CT images demonstrate the absence of bronchiectasis in the airways adjacent to where samples were obtained. The key for the nine most abundant organisms is provided below the lung schematic. Significant heterogeneity is

2013

Lung Microbiota and Bacterial Abundance in Patients with Bronchiectasis when Clinically Stable and during Exacerbation

Michael M. Tunney[1,2*], Gisli G. Einarsson[1,2*], Lan Wei[1,2], Maire Drain[3], Erich R. Klem[4], Chris Cardwell[5], Madeleine Ennis[3], Richard C. Boucher[4], Matthew C. Wolfgang[4,6], and J. Stuart Elborn[1,3*]

[1]Cystic Fibrosis and Airways Microbiology Research Group, [2]School of Pharmacy, and [3]Centre for Infection and Immunity, School of Medicine, Dentistry, and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom; [4]Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; [5]Centre for Public Health, School of Medicine, Dentistry, and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom; and [6]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

of agreement was largely due to bacteria being detected by pyrosequencing but not by culture.

Conclusions: A complex microbiota is present in the lungs of patients with bronchiectasis and remains stable through treatment of exacerbations, suggesting that changes in microbiota composition do not account for exacerbations.

¿Qué rol juega la microbiota en la patogenesis de la exacerbación y qué impacto tiene cuando recibe tratamiento con ATB?

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Molecular techniques have emerged that can provide a comprehensive description of an entire population of bacteria at a body site. Despite the importance of the microbial–host relationship in the pathogenesis and progression of infectious diseases, these approaches have not been applied to define the lung microbiota in patients with bronchiectasis.

What This Study Adds to the Field

Diverse polymicrobial communities are present in the lungs of patients with bronchiectasis when clinically stable and during an exacerbation. A surprising degree of stability was observed in both microbial load and community composition before and after antibiotic treatment of patients with acute pulmonary exacerbations. This finding suggests that changes in lung microbiota composition do not account for pulmonary exacerbations in patients with bronchiectasis.

Am J Respir Crit Care Med Vol 187, Iss. 10, pp 1118–1126, May 15, 2013

Originally Published in Press as DOI: 10.1164/rccm.201210-1937OC on January 24, 2013
Internet address: www.atsjournals.org

La diversidad de bacterias se mantiene relativamente estable durante la exacerbación.
Conclusiones: una compleja microbiota está presente en los pulmonaes de pacientes con bronquiectasias y permanece estable durante el tratamiento de las exacerbaciones con ATB. sugiriendo que los cambios en la composición de la microbiota no estarían involucrados en las exacerbaciones.

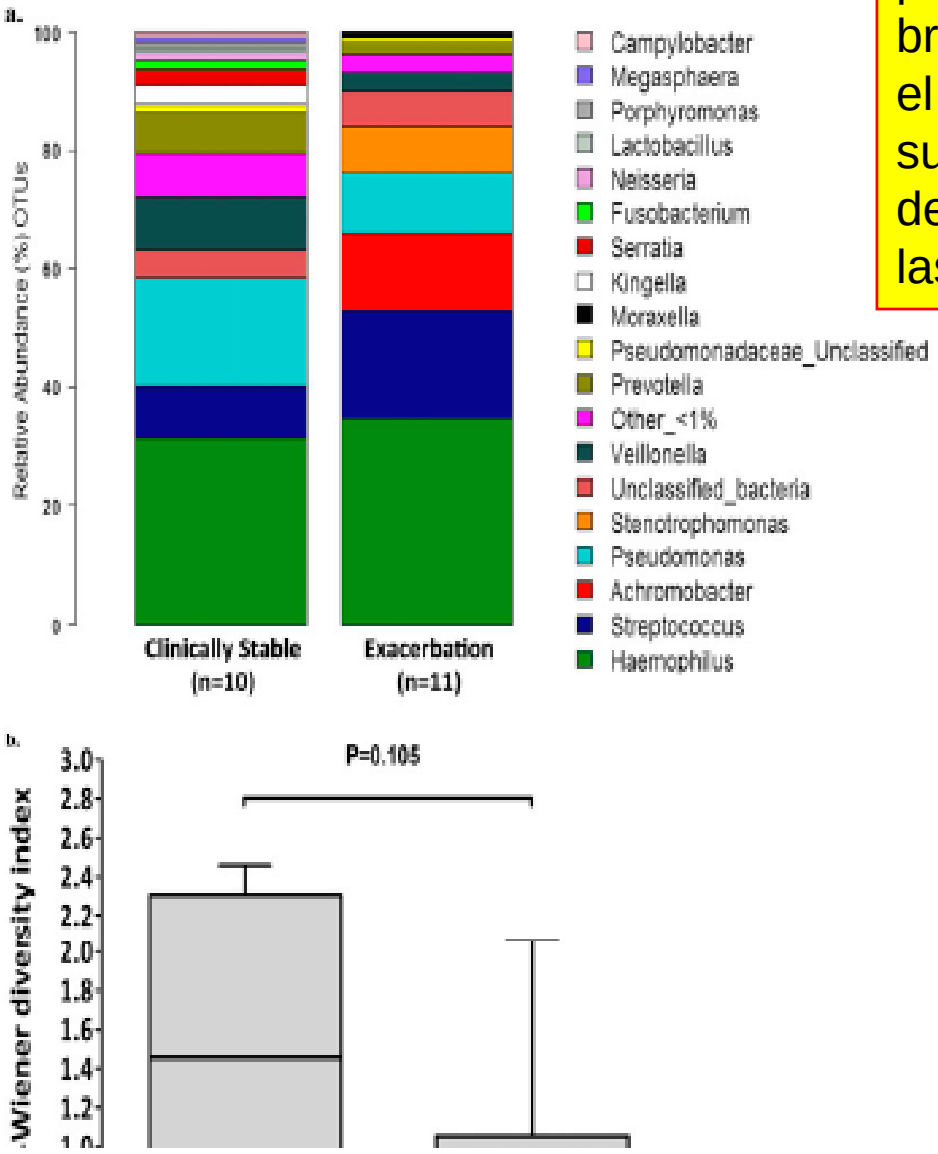


Figure 3. (*a*) Comparison of the percent abundance of the major identified phyla in pooled samples collected from patients when clinically stable (cross-sectional study, n = 10) and at the start of treatment for an exacerbation (longitudinal study, n = 11). Similar patterns of phyla distribution were observed in both groups. OTUs = operational taxonomic units. (*b*) Box plot comparison of microbial diversity (Shannon-Wiener diversity index) in samples from patients when clinically stable (cross-sectional study, n = 10) and at the start of treatment for an exacerbation (longitudinal study, n = 11), where higher values correspond to higher diversity. The top and bottom boundaries of each *box* indicate 75th and 25th quartile values, respectively, with the *black line* inside each *box* representing the median (50th quartile). The ends of the *whiskers* indicate the range. No significant difference ($P = 0.105$, Mann-Whitney test) in microbial community diversity is apparent between the two groups.

TRANSATLANTIC AIRWAY CONFERENCE

Significance of the Microbiome in Chronic Obstructive Pulmonary Disease

Fernando J. Martinez[1], John R. Erb-Downward[1], and Gary B. Huffnagle[1]

[1]Division of Pulmonary and Critical Care, University of Michigan Health System, Ann Arbor, Michigan

Abstract

Chronic obstructive pulmonary disease (COPD) is one of few chronic disorders with rising mortality and morbidity. It is a highly prevalent disorder, characterized by highly heterogeneous clinical symptoms, health status, and disease progression. COPD is also characterized by an inflammatory/immune response that persists despite smoking cessation and varies by the patient population, method of assessment, and timing of measurement. Bacterial colonization or infection is ubiquitous in patients with COPD and, until recently, has been predominantly assessed using culture-based methodologies. This colonization has been believed to be biologically relevant. It has been estimated that more than 70% of the bacterial species on body surfaces cannot be cultured by standard techniques. As such, advanced culture-independent techniques have been developed that target bacterial genes, such as the 16S ribosomal RNA gene, that function as molecular chronometers. Application of these techniques in patients with COPD has suggested microbial diversity that varies by age, disease severity, and medication use. All of these data provide unique and rapidly evolving insight into the potential role of the respiratory microbiome in disease genesis and expression.

Keywords: chronic obstructive pulmonary disease; microbiome; microbiota

OPEN ACCESS Freely available online

PLOS | ONE

Inflammation and Airway Microbiota during Cystic Fibrosis Pulmonary Exacerbations

Edith T. Zemanick[1]*, J. Kirk Harris[1], Brandie D. Wagner[2], Charles E. Robertson[3], Scott D. Sagel[1], Mark J. Stevens[1], Frank J. Accurso[1], Theresa A. Laguna[4]

1 Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado, United States of America, 2 Department of Biostatistics and Informatics, Colorado School of Public Health, University of Colorado School of Medicine, Aurora, Colorado, United States of America, 3 Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado, United States of America, 4 Department of Pediatrics, University of Minnesota Medical School and the University of Minnesota Amplatz Children's Hospital, Minneapolis, Minnesota, United States of America

Abstract

Background: Pulmonary exacerbations (PEx), frequently associated with airway infection and inflammation, are the leading cause of morbidity in cystic fibrosis (CF). Molecular microbiologic approaches detect complex microbiota from CF airway samples taken during PEx. The relationship between airway microbiota, inflammation, and lung function during CF PEx is

Objetivo: determinar las relaciones entre la microbiota de las vías respiratorias, la inflamación, y la función pulmonar en sujetos con FQ tratados por exacerbación pulmonar

(CRP) were measured.

Results: Thirty-seven sputum samples were collected from 21 CF subjects. At early treatment, lower diversity was associated with high relative abundance (RA) of *Pseudomonas* ($r = -0.67$, $p < 0.001$), decreased $FEV_{1\%}$ predicted ($r = 0.49$, $p = 0.03$) and increased CRP ($r = -0.58$, $p = 0.01$). In contrast to *Pseudomonas*, obligate and facultative anaerobic genera were associated with less inflammation and higher FEV_1. With treatment, *Pseudomonas* RA and *P. aeruginosa* by qPCR decreased while anaerobic genera showed marked variability in response. Change in RA of *Prevotella* was associated with more variability in FEV_1 response to treatment than *Pseudomonas* or *Staphylococcus*.

Conclusions: Anaerobes identified from sputum by sequencing are associated with less inflammation and higher lung function compared to *Pseudomonas* at early exacerbation. CF PEx treatment results in variable changes of anaerobic genera suggesting the need for larger studies particularly of patients without traditional CF pathogens.

Citation: Zemanick ET, Harris JK, Wagner BD, Robertson CE, Sagel SD, et al. (2013) Inflammation and Airway Microbiota during Cystic Fibrosis Pulmonary Exacerbations. PLoS ONE 8(4): e62917. doi:10.1371/journal.pone.0062917

Conclusiones,:

Al inicio del tratamiento de la Ex FQ, Pseudomonas se asociaron con disminución del FEV1 y mayores niveles de inflamación, no así los gérmenes anaeróbios.

Por otra parte, la diversidad microbiana se asoció con una mayor presencia de Pseudomonas, menor función pulmonar y un aumento de la inflamación.

Dado que el tratamiento Ex FQ da lugar a cambios en la variable de género anaeróbico se sugiere la necesidad de realizar estudios más grandes sobre todo en pacientes sin colonización con los tradicionales patógenos en FQ.

Table 1. Classification of the most prevalent bacterial genera detected from CF sputum samples.

Aerobic to facultative anaerobic genera	Obligate anaerobic genera
Atopobium	*Actinomyces*
Capnocytophaga	*Campylobacter*
Granulicatella	*Fusobacterium*
*Haemophilus**	*Leptotrichia*
Lactobacillus	*Porphyromonas*
*Pseudomonas**	*Prevotella*
Rothia	*Veillonella*
*Staphylococcus**	
*Stenotrophomonas**	
Streptococcus	

*Indicates genus that includes bacterial species typically detected by standard CF microbiologic culture.
doi:10.1371/journal.pone.0062917.t001

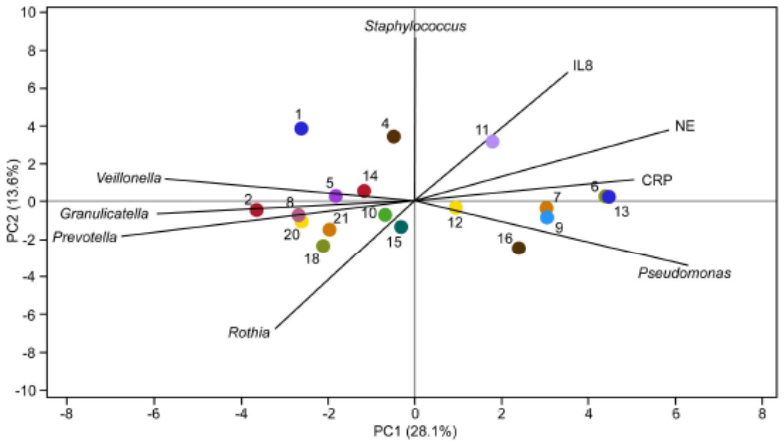


Figure 2. PCA plot of bacterial genera relative abundance and inflammatory markers at Early Treatment of PEx. Colored dots represent individual subjects with subject identification number (SID) (n = 21). The length of the vectors represents the PCA loadings of the variables on the first two principal components, which explain 42% of the variability. *Staphylococcus* and *Pseudomonas* are both positively correlated with NE and CRP, while *Veillonella*, *Granulicatella*, *Prevotella* and *Rothia* are negatively correlated.. The first principal component (PC) was negatively correlated with $FEV_{1\%}$ predicted indicating that sputum samples with higher inflammation and higher RA of *Pseudomonas* had lower FEV_1 and those with lower

Citation: Zemanick ET, Harris JK, Wagner BD, Robertson CE, Sagel SD, et al. (2013) Inflammation and Airway Microbiota during Cystic Fibrosis Pulmonary Exacerbations. PLoS ONE 8(4): e62917. doi:10.1371/journal.pone.0062917

OPEN ACCESS Freely available online

PLOS | ONE

The Adult Cystic Fibrosis Airway Microbiota Is Stable over Time and Infection Type, and Highly Resilient to Antibiotic Treatment of Exacerbations

Anthony A. Fodor[1*9], Erich R. Klem[2], Deirdre F. Gilpin[3,4], J. Stuart Elborn[3,5], Richard C. Boucher[2], Michael M. Tunney[3,4*9], Matthew C. Wolfgang[2,6*9]

1 Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, Charlotte, North Carolina, United States of America, **2** Cystic Fibrosis/Pulmonary Research and Treatment Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America, **3** CF and Airway Microbiology Research Group, Queen's University Belfast, Belfast, United Kingdom, **4** School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom, **5** Center for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, United Kingdom, **6** Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America

Abstract

Cystic fibrosis (CF) is characterized by defective mucociliary clearance and chronic airway infection by a complex microbiota. Infection, persistent inflammation and periodic episodes of acute pulmonary exacerbation contribute to an irreversible decline in CF lung function. While the factors leading to acute exacerbations are poorly understood, antibiotic treatment can temporarily resolve pulmonary symptoms and partially restore lung function. Previous studies indicated that exacerbations may be associated with changes in microbial densities and the acquisition of new microbial species. Given the complexity of the CF microbiota, we applied massively parallel pyrosequencing to identify changes in airway microbial community structure in 23 adult CF patients during acute pulmonary exacerbation, after antibiotic treatment and during periods of stable disease. Over 350,000 sequences were generated, representing nearly 170 distinct microbial taxa. Approximately 60% of sequences obtained were from the recognized CF pathogens *Pseudomonas* and *Burkholderia*, which were detected in largely non-overlapping patient subsets. In contrast, other taxa including *Prevotella*, *Streptococcus*, *Rothia* and *Veillonella* were abundant in nearly all patient samples. Although antibiotic treatment was associated with a small decrease in species richness, there was minimal change in overall microbial community structure. Furthermore, microbial community composition was highly similar in patients during an exacerbation and when clinically stable, suggesting that exacerbations may represent intrapulmonary spread of infection rather than a change in microbial community composition. Mouthwash samples, obtained from a subset of patients, showed a nearly identical distribution of taxa as expectorated sputum, indicating that aspiration may contribute to colonization of the lower airways. Finally, we observed a strong correlation between low species richness and poor lung function. Taken together, these results indicate that the adult CF lung microbiome is largely stable through periods of exacerbation and antibiotic treatment and that short-term compositional changes in the airway microbiota do not account for CF pulmonary exacerbations.

Citation: Fodor AA, Klem ER, Gilpin DF, Elborn JS, Boucher RC, et al. (2012) The Adult Cystic Fibrosis Airway Microbiota Is Stable over Time and Infection Type, and Highly Resilient to Antibiotic Treatment of Exacerbations. PLoS ONE 7(9): e45001. doi:10.1371/journal.pone.0045001

Editor: Suzanne Fleiszig, Univeristy of California Berkeley, United States of America

Received May 11, 2012; **Accepted** August 11, 2012; **Published** September 26, 2012

Microbioma.
algunas evidencias…

Asma bronquial

FOCUS ON ASTHMA REVIEW

nature medicine

Innate and adaptive immune responses in asthma

Stephen T Holgate

The recognition that asthma is primarily an inflammatory disorder of the airways associated with T helper type 2 (T_H2) cell-dependent promotion of IgE production and recruitment of mast cells and eosinophils has provided the rationale for disease control using inhaled corticosteroids and other anti-inflammatory drugs. As more has been discovered about the cytokine, chemokine and inflammatory pathways that are associated with T_H2-driven adaptive immunity, attempts have been made to selectively inhibit these in the hope of discovering new therapeutics as predicted from animal models of allergic inflammation. The limited success of this approach, together with the recognition that asthma is more than allergic inflammation, has drawn attention to the innate immune response in this disease. Recent advances in our understanding of the sentinel role played by innate immunity provides new targets for disease prevention and treatment. These include pathways of innate stimulation by environmental or endogenous pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) to influence the activation and trafficking of DCs, innate sources of cytokines, and the identification of new T cell subsets and lymphoid cells.

What is asthma?

Asthma is a disorder of the conducting airways leading to variable airflow obstruction in association with airway hyperresponsiveness (AHR). Airway inflammation is central to disease pathophysiology and relates to airway dysfunction partly through the release of potent

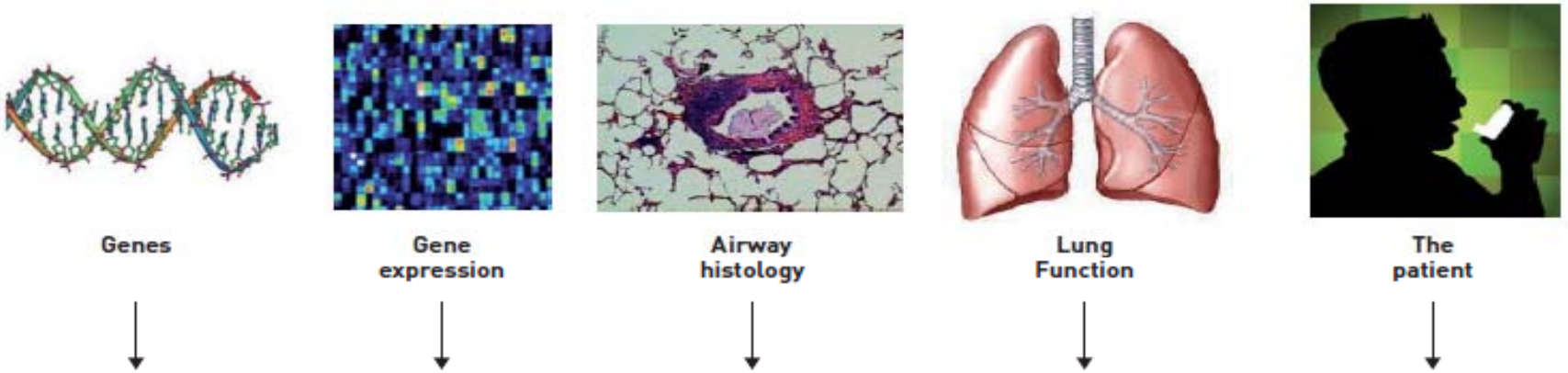
Activation of T_H2 CD4$^+$ cells occurs through phosphorylation of the *trans*-acting T cell–specific transcription factor GATA-3 (ref. 2). In other affected individuals, alternative immunological pathways drive the inflammatory response. These will be discussed later in this Review.

Stephen T Holgate

The recognition that asthma is primarily an inflammatory disorder of the airways associated with T helper type 2 (T_H2) cell-dependent promotion of IgE production and recruitment of mast cells and eosinophils has provided the rationale

El asma es más que inflamación alérgica. La respuesta inmune innata en interacción con el microambiente juega un rol clave en la patogenia de esta enfermedad.

International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma



URE 1 Integration of factors, beginning with genetics, which may contribute to the ultimate phenotype of the severe asthma patient.

Severe Asthma Research Program (SARP), using predominantly clinical characteristics, identified five

Algunas evidencias…(1)

- Relación inversa entre abundancia de ácaros, bacterias gram positivas (endotoxinas, etc) y atopia y asma. GABRIEL study). Comunidad Amish-granjeros en Suiza Vs comunidades urbanas.
- Madre embarazada expuesta/no expuesta…↑Cel T reg- ↑citocinas pro inflamatorias-INF-γ-FNTα en sangre de cordón del feto. Impronta en imbalance Th1/Th2.

Algunas evidencias…(3)

- Chlamydias-Mycoplasma- colonización o infección (bronquitis) utilización prolongada de ATB y mejoría clínica (fenotipo de asma?)
- Colonización temprana de bacterias (< 1mes) se asoció a mayor prevalencia de asma (Cohorte de Copenague/Dinamarca)

Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bonnelykke K, et al. Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med 2007;357:1487-95.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Childhood Asthma after Bacterial Colonization of the Airway in Neonates

Hans Bisgaard, M.D., D.M.Sc., Mette Northman Hermansen, M.D., Frederik Buchvald, M.D., Ph.D., Lotte Loland, M.D., Ph.D., Liselotte Brydensholt Halkjaer, M.D., Ph.D., Klaus Bønnelykke, M.D., Martin Brasholt, M.D., Andreas Heltberg, M.D., Nadja Hawwa Vissing, M.D.,

ABSTRACT

BACKGROUND

Pathological features of the airway in young children with severe recurrent wheeze suggest an association between bacterial colonization and the initiating events of early asthma. We conducted a study to investigate a possible association between bacterial colonization of the hypopharynx in asymptomatic neonates and later development of recurrent wheeze, asthma, and allergy during the first 5 years of life.

METHODS

The subjects were children from the Copenhagen Prospective Study on Asthma in Childhood birth cohort who were born to mothers with asthma. Aspirates from the hypopharyngeal region of asymptomatic 1-month-old infants were cultured for *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. Wheeze v
of life. Blood eos
of age. Lung fun

RESULTS

Hypopharyngea
Twenty-one perc
H. influenzae, or
of these organisms, but not colonization with *S. aureus*, was significantly associated with persistent wheeze (hazard ratio, 2.40; 95% confidence interval [CI], 1.45 to 3.99), acute severe exacerbation of wheeze (hazard ratio, 2.99; 95% CI, 1.66 to 5.39), and hospitalization for wheeze (hazard ratio, 3.85; 95% CI, 1.90 to 7.79). Blood eosino-

CONCLUSIONS

Neonates colonized in the hypopharyngeal region with *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis*, or with a combination of these organisms, are at increased risk for recurrent wheeze and asthma early in life.

Table 2. Colonization in Relation to Asthma Diagnosis, Lung Function, and Allergy.

Outcome	Colonized	Not Colonized	Odds Ratio (95% CI)	Percent Estimated Difference (95% CI)*
Asthma at 5 yr (no.)				
Yes	17	20	4.57 (2.18 to 9.57)	
No	35	188		
Specific IgE at 4 yr (no.)				
>0.35 kU/liter	15	49	1.28 (0.65 to 2.54)	
≤0.35 kU/liter	36	151		
Mean postbronchodilator specific airway resistance at 5 yr (kPa·sec·liter^{-1})†	0.94	1.00		−7 (−13 to 1)
Reversibility of specific airway resistance after β_2-agonist inhalation at 5 yr (%)‡	−23	−18		5 (0 to 10)
Blood eosinophil count at 4 yr (×10^{-9}/liter)§	0.42	0.29		31 (6 to 62)
Total IgE at 4 yr (kU/liter)¶	90	60		47 (1 to 115)

* Relative differences are given, except for reversibility of specific airway resistance after β_2-agonist inhalation, for which absolute differences are given. Estimated relative differences were calculated as the ratio of geometric means for the colonized and not colonized groups.
† Of 213 infants, 39 were colonized and 174 were not colonized.
‡ Of 213 infants, 39 were colonized and 174 were not colonized.
§ Of 239 infants, 47 were colonized and 192 were not colonized.

From the Danish Pediatric Asthma Center, Copenhagen University Hospital, Gentofte, Copenhagen. Address reprint requests to Dr. Bisgaard at the Danish Pediatric Asthma Center, Copenhagen University Hospital, Hellerup, Niels Andersens Vej 65, DK-2900 Gentofte, Copenhagen, Denmark, or at bisgaard@copsac.dk.

N Engl J Med 2007;357:1487-95.

Algunas hipótesis y teorías….

- Habría relación entre el ambiente, el microbioma gastrointestinal y el desarrollo de función inmune en la infancia.
  - Estudios epidemiológicos: relación inversa entre prevalencia de asma alérgica y exposición ambiental a microbios (hipótesis de la higiene)
  - Las bacterias intestinales ingresarían por ingesta oral
  - Alteraciones en la función respiratoria inmune estaría relacionada con la actividad inmuno-rregulatoria del microbioma intestinal

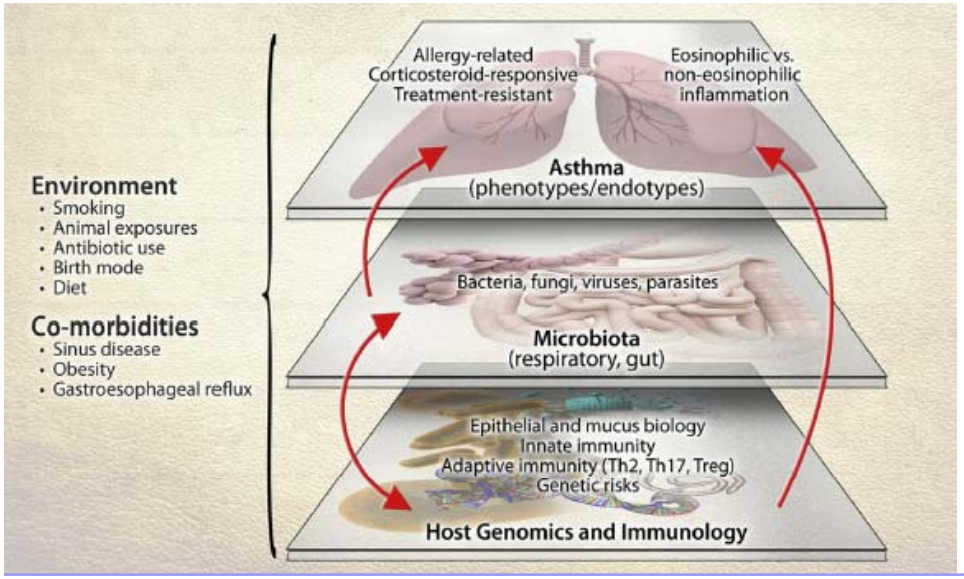
⇒"Respuesta común de mucosa"

Thorax. 2012 May ; 67(5): 456–463. doi:10.1136/thoraxjnl-2011-201183.

Current perspectives (J Allergy Clin Immunol 2015;135:25-30.)

The microbiome in asthma

Yvonne J. Huang, MD,[a] and Homer A. Boushey, MD[b]



The application of recently developed sensitive, specific, culture-independent tools for identification of microbes is transforming concepts of microbial ecology, including concepts of the relationships between the vast complex populations of microbes associated with ourselves and with states of health and disease. Although most work initially focused on the community of microbes (microbiome) in the gastrointestinal tract and its relationship to gastrointestinal disease, interest has expanded to include study of the relationships of the airway microbiome to asthma and its phenotypes and to the relationships between the gastrointestinal microbiome, development of immune function, and predisposition to allergic sensitization and asthma. Here we provide our perspective on the findings of studies of differences in the airway microbiome between asthmatic patients and healthy subjects and of studies of relationships between environmental

FIG 1. Interface of microbiota interactions with other factors that collectively influence susceptibility to asthma or its manifestations. Components of the depicted system (ie, host genetics and immunology, microbiota, environmental exposures, and asthma) are themselves heterogeneous entities, presenting challenges to more precisely dissect the role or roles of the microbiome in asthma.

Ann Am Thorac Soc Vol 11, Supplement 3, pp S139–S145, Apr 2014

NHLBI WORKSHOP

Asthma: NHLBI Workshop on the Primary Prevention of Chronic Lung Diseases

Daniel J. Jackson[1], Tina V. Hartert[2], Fernando D. Martinez[3], Scott T. Weiss[4], and John V. Fahy[5]

[1]Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin; [2]Center for Asthma Research, Vanderbilt University School of Medicine, Nashville, Tennessee; [3]Arizona Respiratory Center and BIO5 Institute, University of Arizona, Tucson, Arizona; [4]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School and Partners Center for Personalized Genetic Medicine, Partners Health Care, Boston, Massachusetts; and [5]Division of Pulmonary and Critical Care Medicine, Department of Medicine and the Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California

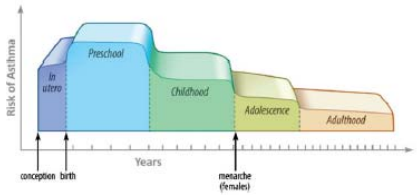


Figure 1. The risk for developing asthma is age dependent, with the majority of incident asthma beginning in the preschool years. *Box sizes* represent estimates of the relative importance of each developmental period in childhood.

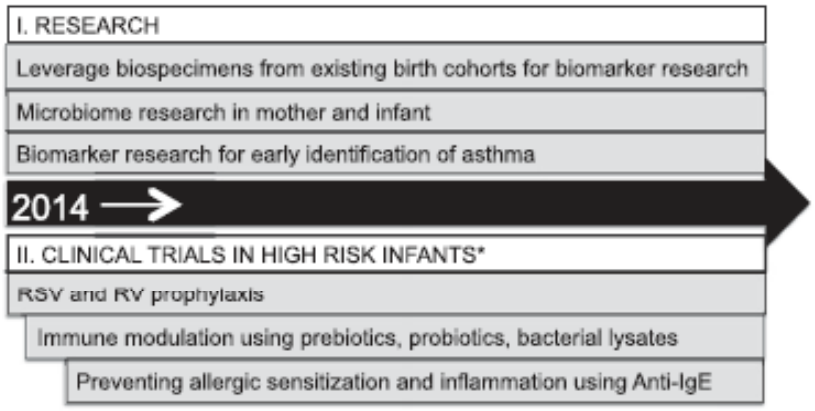


Figure 2. An integrated portfolio across the translational research spectrum is needed moving forward toward primary prevention of asthma. The figure illustrates how basic research efforts must continue alongside specific clinical intervention trials. RSV = respiratory syncytial virus; RV = human rhinovirus. *Covariates such as vitamin D should be assessed in asthma prevention trials; additional vitamin D trials may be necessary depending on the results of ongoing trials.

J Allergy Clin Immunol 2015;136:952-61.)

Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials

Carlos A. Cuello-Garcia, MD,[a,b] Jan L. Brożek, MD, PhD,[a,c] Alessandro Fiocchi, MD,[d] Ruby Pawankar, MD,[e] Juan José Yepes-Nuñez, MD, MSc(Epi),[a,f] Luigi Terracciano, MD,[g] Shreyas Gandhi, BHSc,[c,h] Arnav Agarwal, BHSc,[c,h] Yuan Zhang, MD,[a] and Holger J. Schünemann, MD, MSc, PhD[a,c] *Hamilton and Toronto, Ontario, Canada, Monterrey, Mexico, Vatican City, Tokyo, Japan, Medellin, Colombia, and Milan, Italy*

Conclusion: Probiotics used by pregnant women or breastfeeding mothers and/or given to infants reduced the risk of eczema in infants; however, the certainty in the evidence is low.

No effect was observed for the prevention of other allergic conditions.

Conclusiones

- Los pulmones no son estériles. Numerosas familias bacterianas asientan en las vías aéreas inferiores (microbioma) desde los primeros momentos de la vida mostrando diferencias entre sujetos sanos y pacientes con enfermedad respiratoria.
- La diversidad de las poblaciones microbianas estaría relacionadas con procesos inflamatorios y exacerbación de enfrermedades respiratorias crómicas.
- Quedan por establecer las complejas relaciones del microbioma pulmonar con el sistema inmune, su relación con la génesis de patología crónicas así como las posibilidades de intervención en la prevención de enfermedades alérgicas y asma bronquial.

Muchas gracias!

- lauramoreno@arnet.com.ar