



The Lung Microbiome

*challenging old paradigms about microbes
and the host respiratory tract*

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Vol. 72, No. 9

Role of Antibiotics and Fungal Microbiota in Driving Pulmonary Allergic Responses

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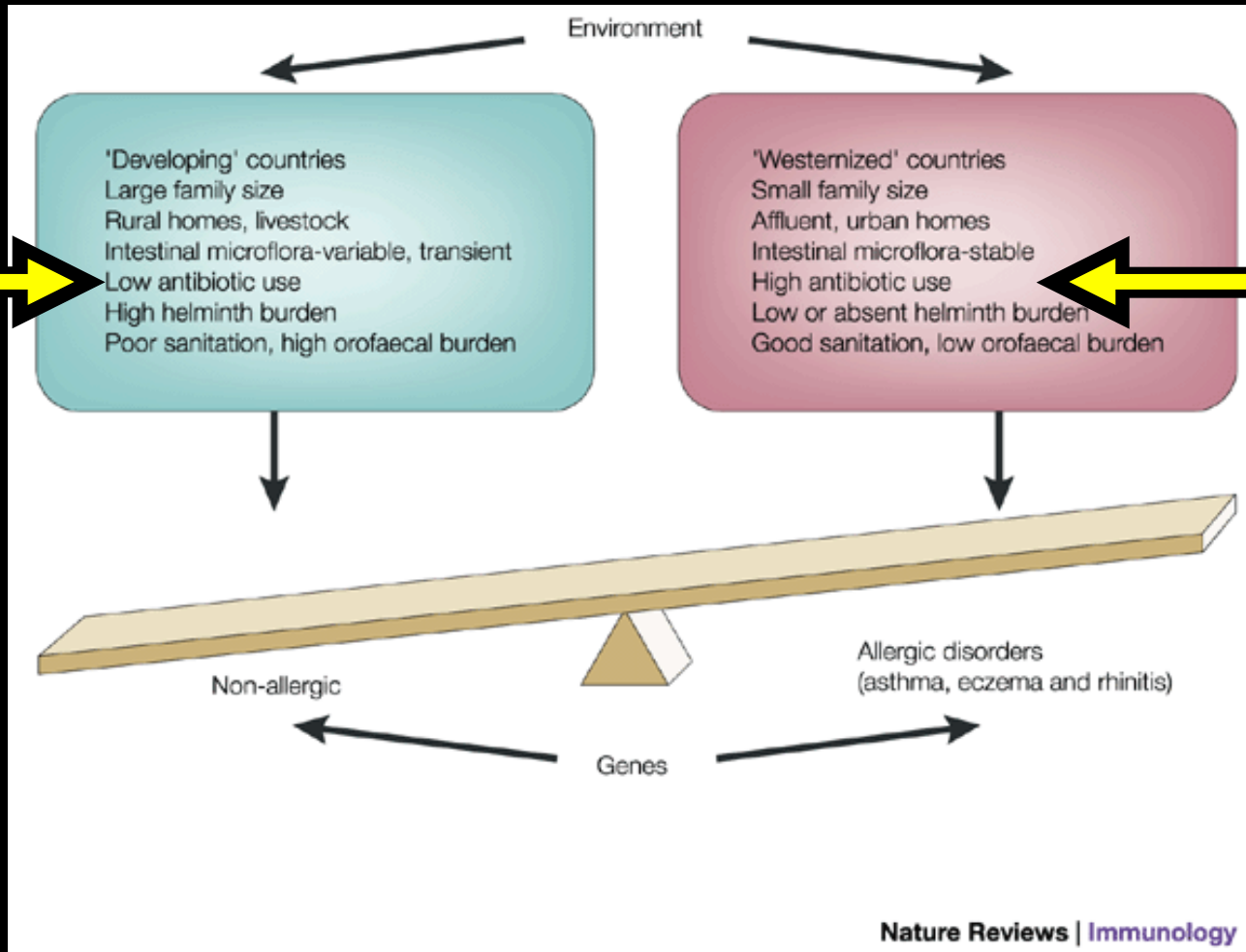
INFECTION AND IMMUNITY, Jan. 2005, p. 30–38
0019-9567/05/\$08.00+0 doi:10.1128/IAI.73.1.30–38.2005
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Vol. 73, No. 1

Development of Allergic Airway Disease in Mice following Antibiotic Therapy and Fungal Microbiota Increase: Role of Host Genetics, Antigen, and Interleukin-13

Mairi C. Noverr,¹ Nicole R. Falkowski,¹ Rod A. McDonald,¹ Andrew N. McKenzie,² and Gary B. Huffnagle^{1,3*}

The Hygiene Hypothesis

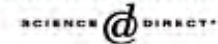




Review

TRENDS in Microbiology Vol.12 No.12 December 2004

Full text provided by www.sciencedirect.com



Does the microbiota regulate immune responses outside the gut?

Mairi C. Noverr¹ and Gary B. Huffnagle^{1,2}

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Subsequent Studies on the Effect of Antibiotics or Germ-Free State on Allergic Airway Response in Mice



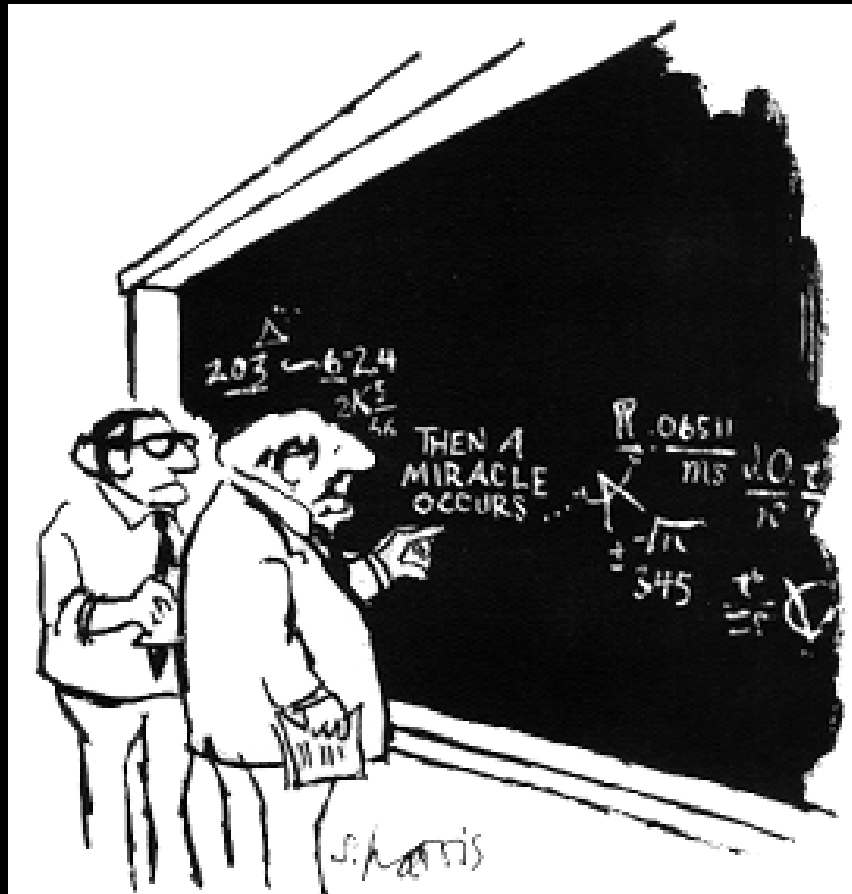
Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. Karimi K, Inman MD, Bienenstock J, Forsythe P. Am J Respir Crit Care Med. 2009 Feb 1;179(3):186-93.

Bacterial strain-specific induction of Foxp3+ T regulatory cells is protective in murine allergy models. Lyons A, O'Mahony D, O'Brien F, MacSharry J, Sheil B, Ceddia M, Russell WM, Forsythe P, Bienenstock J, Kiely B, Shanahan F, O'Mahony L. Clin Exp Allergy. 2010 May;40(5):811-9.

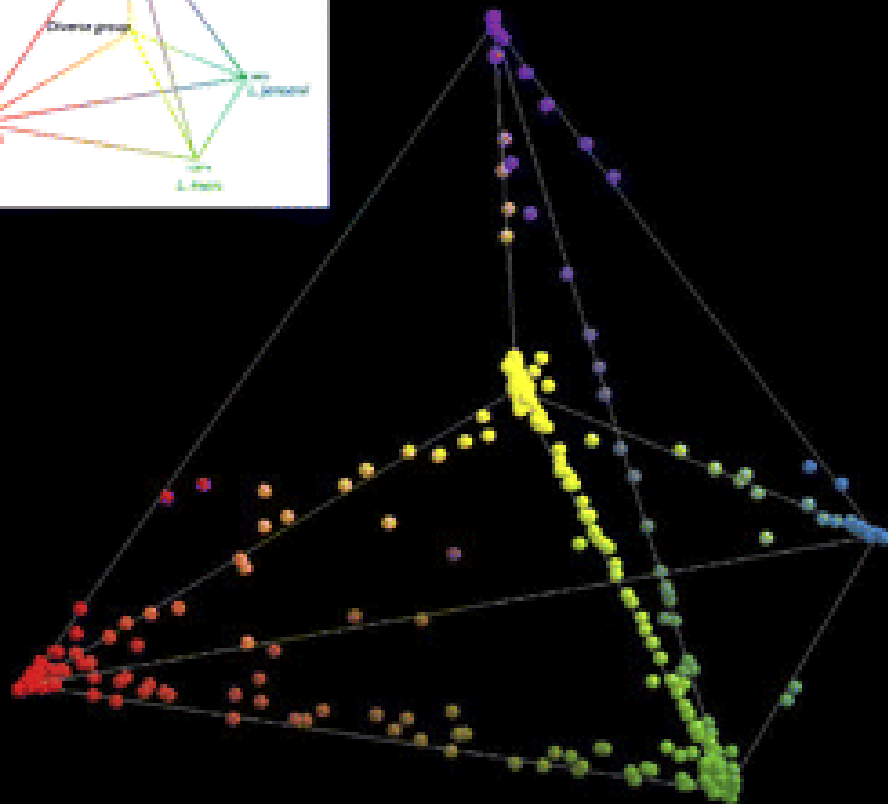
Dysregulation of allergic airway inflammation in the absence of microbial colonization. Herbst T, Sichelstiel A, Schär C, Yadava K, Bürki K, Cahenzli J, McCoy K, Marsland BJ, Harris. Am J Respir Crit Care Med. 2011 Jul 15;184(2):198-205.

Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, Gill N, Blanchet MR, Mohn WW, McNagny KM, Finlay BB. EMBO Rep. 2012 May 1;13(5):440-7.

How does the GI microbiome modulate inflammation at distal sites?



"I think you should be more explicit here in step two."



GAP

- Understanding HOW changes in the bacterial microbiota of the GI tract can affect distal mucosal sites

GAP

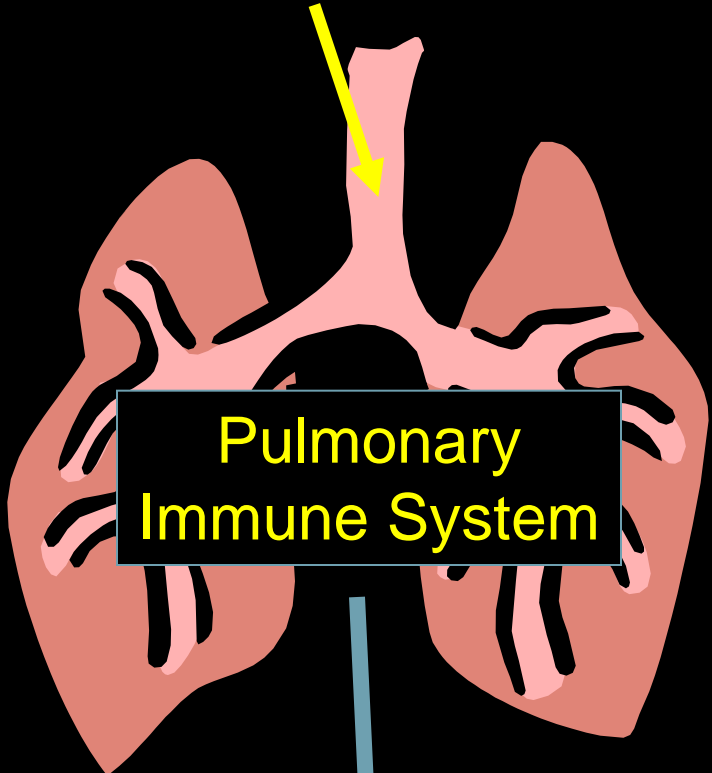
- Understanding HOW changes in the bacterial microbiota of the GI tract can affect distal mucosal sites

CHALLENGE

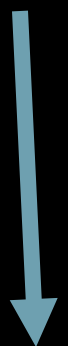
- Trying to get studies of complex in vivo systems reviewed positively by study sections dominated by reductionist biologists

Gut-Lung Axis of Immunoregulation

Antigen



**Pulmonary
Immune System**

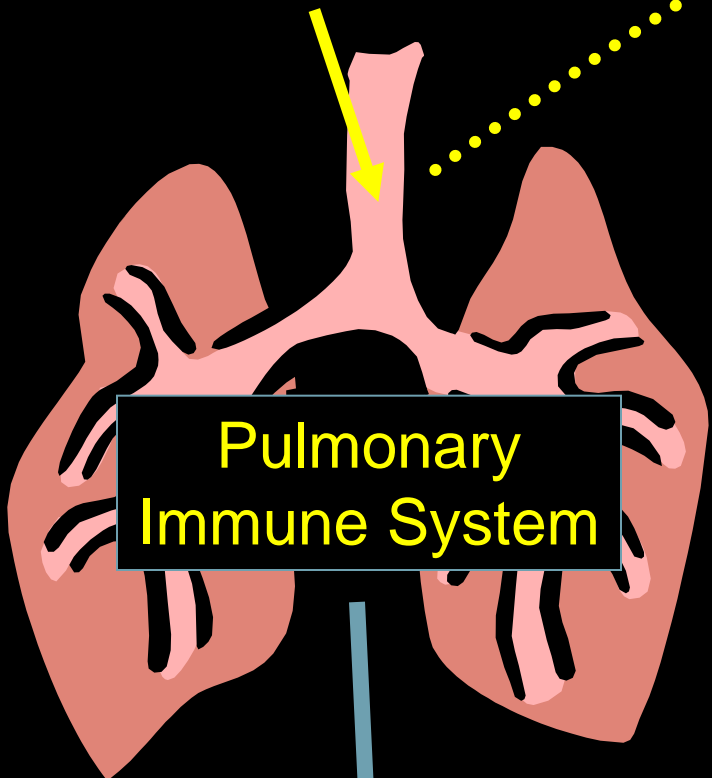


Outcome of Pulmonary Challenge

Gut-Lung Axis of Immunoregulation

Antigen

Antigen



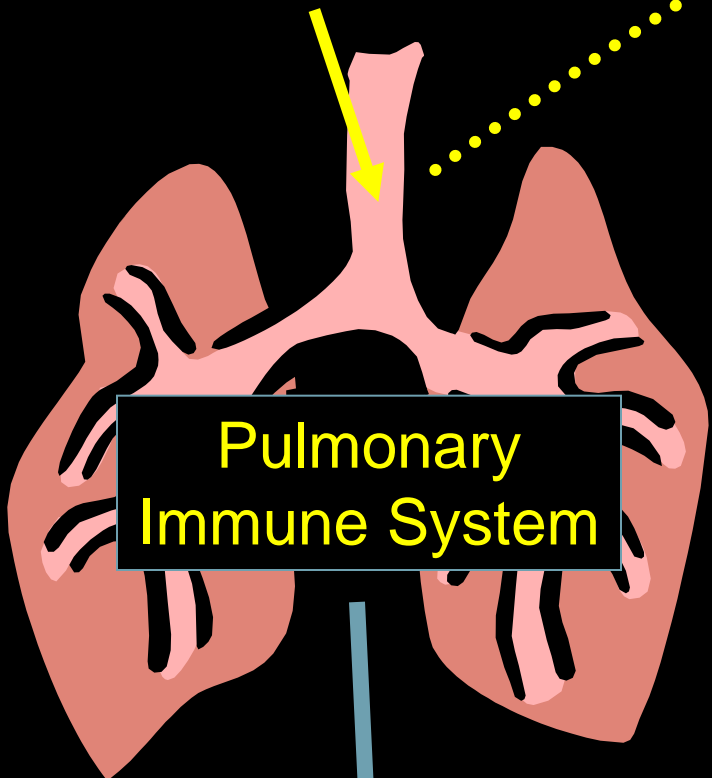
Pulmonary Immune System

Outcome of Pulmonary Challenge

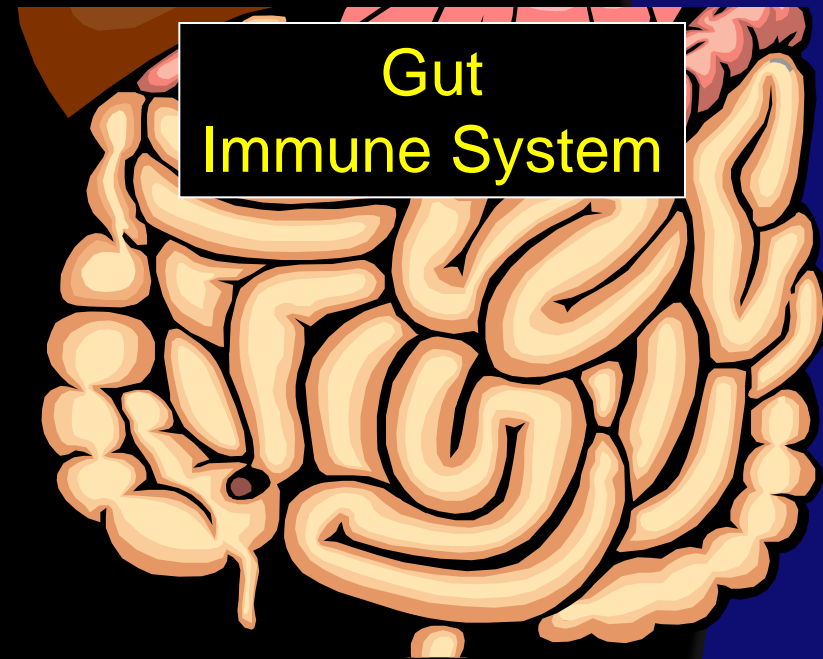
Gut-Lung Axis of Immunoregulation

Antigen

Antigen



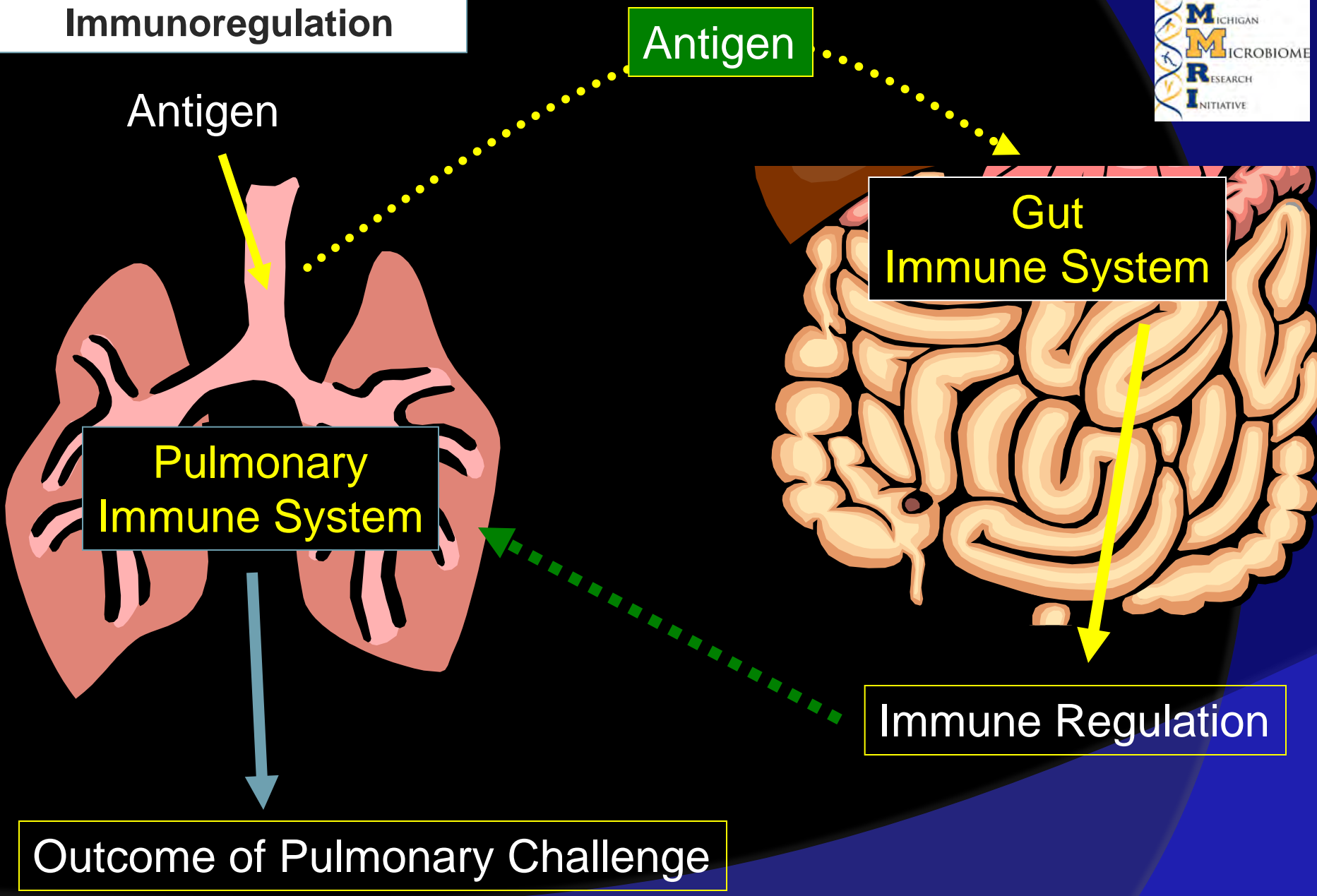
Gut
Immune System



Outcome of Pulmonary Challenge



Gut-Lung Axis of Immunoregulation



Antigen

Antigen

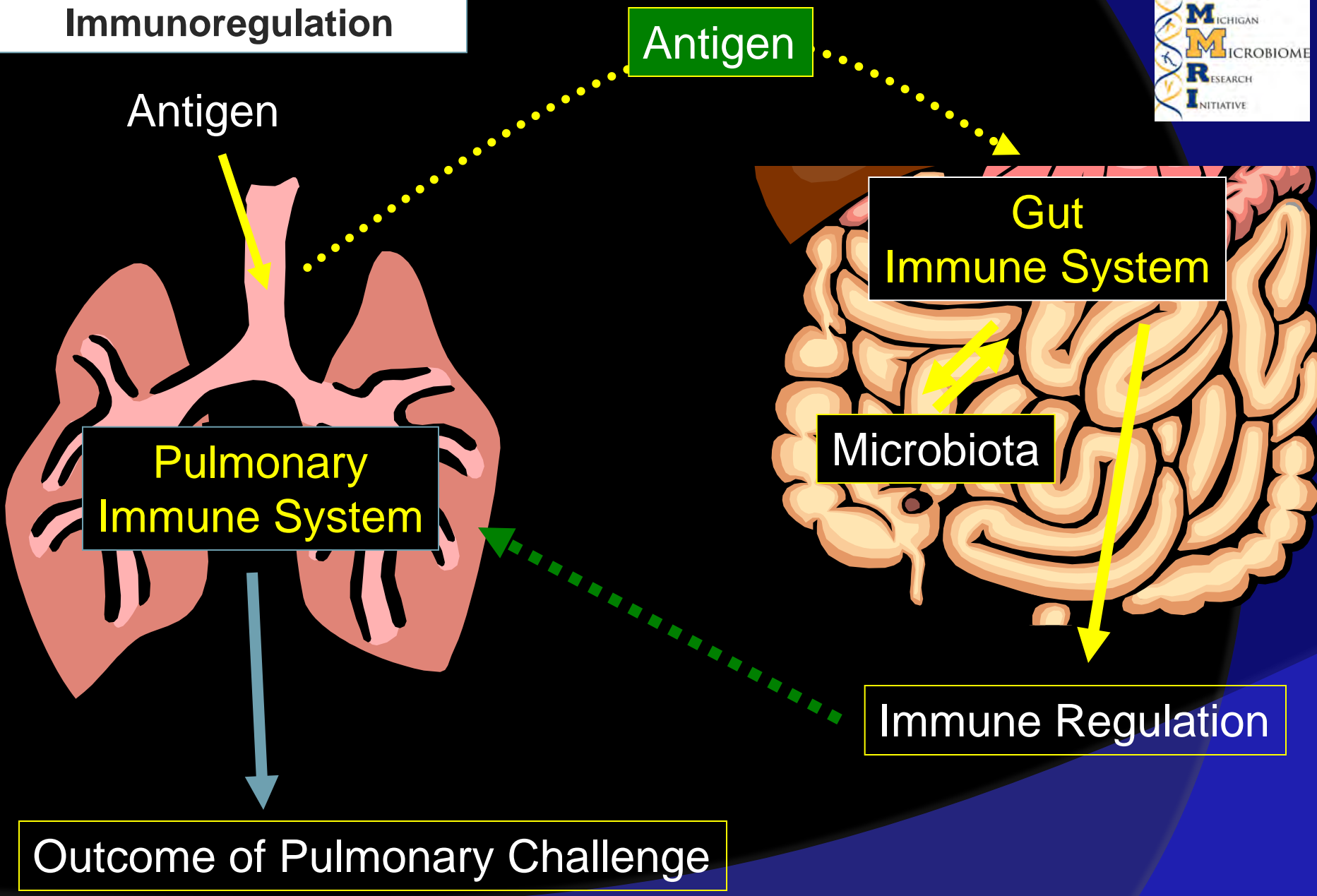
Gut Immune System

Pulmonary Immune System

Immune Regulation

Outcome of Pulmonary Challenge

Gut-Lung Axis of Immunoregulation



Antigen

Antigen

Gut Immune System

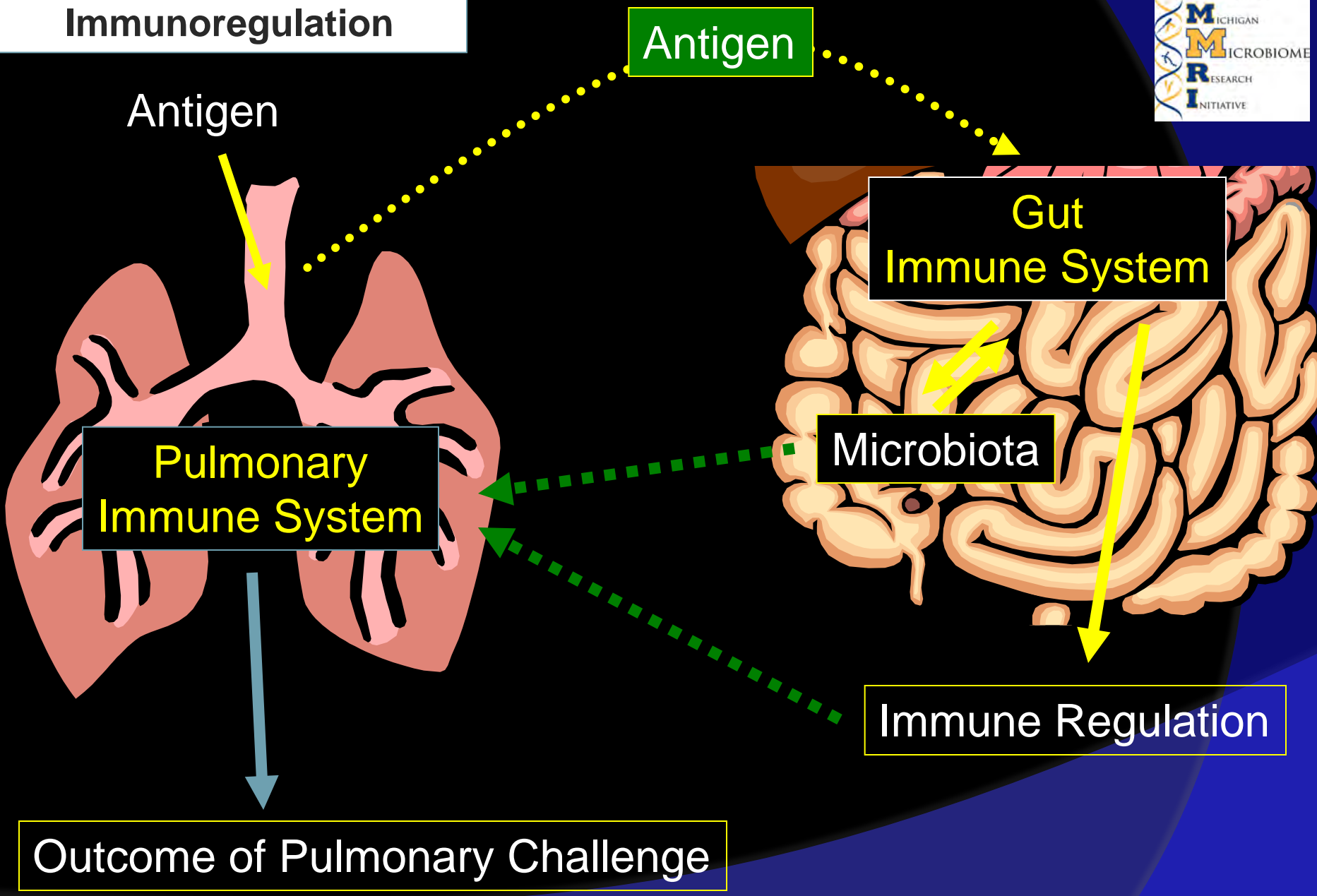
Microbiota

Immune Regulation

Pulmonary Immune System

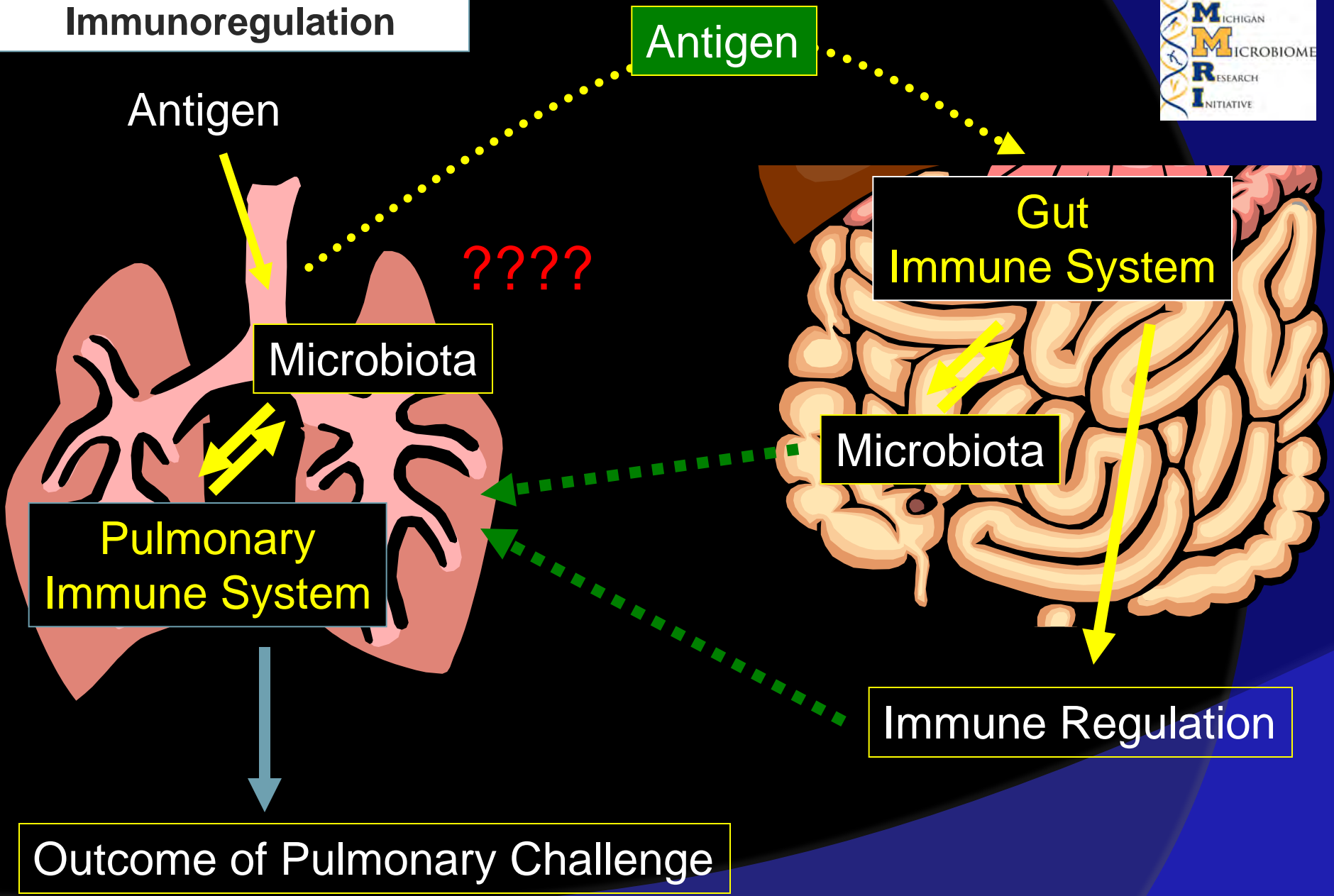
Outcome of Pulmonary Challenge

Gut-Lung Axis of Immunoregulation



Outcome of Pulmonary Challenge

Gut-Lung Axis of Immunoregulation



Outcome of Pulmonary Challenge



effectiveness with which antibiotics abort these infections and prevent the development of full-blown lobar consolidation.

These anatomic but still classic categorizations are often difficult to apply in the individual case because patterns



Figure 16-18

Lobar pneumonia—gray hepatization, gross photograph. The lower lobe is uniformly consolidated.

overlap. The lobular involvement may become confluent, producing virtually solid lobar consolidation; in common, limited involvement to a subsegmental consolidation. Although the same organism may produce lobar pneumonia in one patient, whereas in the more vulnerable individual a more extensive lobular involvement develops. Most infections, from the clinical standpoint, are identification of the causative agent and determination of the extent of disease.

Pathogenesis. Each day, the respiratory airways must swallow are exposed to more than 10,000 liters of air containing hazardous dusts, chemicals, and microorganisms. The fates of inhaled particles depend on their sizes. Thus, particles larger than 10 μm are deposited largely in the turbulent airflow of the nose and upper airways; particles of 1 to 10 μm lodge in the trachea and bronchi by impaction; and smaller particles, about the size of most bacteria, 1 to 5 μm , are deposited in the terminal airways and alveoli.

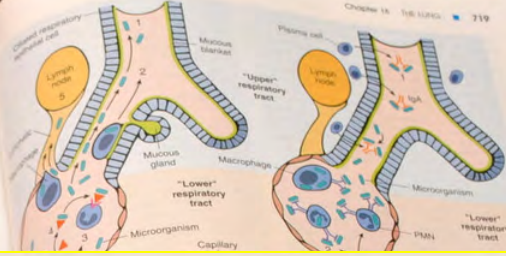
ted posteriorly to the nasopharynx, where they are swallowed.

Tracheobronchial clearance. This is accomplished by mucociliary action. The beating motion of cilia moves a film of mucus continuously from the lung toward the oropharynx; particles deposited on this film are eventually either swallowed or expectorated.

Alveolar clearance. Bacteria or solid particles deposited in the alveoli are phagocytosed by alveolar macrophages. A particle is either digested or carried to the proliferated bronchioles. From here, the macrophage is propelled to the oropharynx and then swallowed. Alternatively, the particle-laden macrophage may move through the interstitial space and either reenter the bronchioles or enter lymphatic capillaries. If the particle load is heavy and macrophage transport to the surface and alveolar pathways is overwhelmed, some particles may eventually reach the regional lymph nodes and, via the bloodstream, be carried elsewhere in the body.

Pneumonia can result whenever these defense mechanisms are impaired or whenever the resistance of the host in general is lowered. Factors that affect resistance in general include chronic diseases, immunologic deficiency, treatment with immunosuppressive agents, leukopenia, and unusually virulent infections. The clearing mechanisms can be interfered with by many factors, such as the following:

- Loss or suppression of the cough reflex, as a result of coma, anesthesia, neuromuscular disorders, drugs, or chest pain (this may lead to aspiration of gastric contents)



of the mucociliary apparatus, by either impairment of ciliary function or destruction of ciliated epithelium to the nasopharynx, where they are swallowed.

Other factors that may impair the mucociliary apparatus, by either impairment of ciliary function or destruction of ciliated epithelium to the nasopharynx, where they are swallowed. Factors that affect resistance in general include chronic diseases, immunologic deficiency, treatment with immunosuppressive agents, leukopenia, and unusually virulent infections. The clearing mechanisms can be interfered with by many factors, such as the following:

- Loss or suppression of the cough reflex, as a result of coma, anesthesia, neuromuscular disorders, drugs, or chest pain (this may lead to aspiration of gastric contents)

from one focus to other foci can occur, and secondary seeding of the lungs may be difficult to distinguish from primary pneumonias. Finally, many patients with chronic (nosocomial infection). Bacteria common to the hospital environment may have acquired resistance to antibiotics; such as intubations and injections, are common; and bacteria may contaminate equipment used in respiratory care units.

Etiology. For bronchopneumonia, the common agents are staphylococci, streptococci, pneumococci, Haemophilus influenzae, Pseudomonas aeruginosa, and the coliform bacteria, although virtually any lung pathogen may also produce this pattern. In the case of lobar pneumonia, 90 to 95% are caused by pneumococci (Streptococcus pneumoniae). Most common are types 1, 3, 7, and 2. Type 3 causes a particularly virulent form of lobar pneumonia.

The National Institutes of Health Human Microbiome Project



Disordered Microbial Communities in Asthmatic Airways

Markus Hilty¹, Conor Burke², Helder Pedro^{3,4}, Paul Cardenas¹, Andy Bush¹, Cara Bossley¹, Jane Davies¹, Aaron Ervine², Len Poulter², Lior Pachter⁴, Miriam F. Moffatt¹, William O. C. Cookson^{1*}

1 National Heart and Lung Institute, Imperial College London, London, England, **2** Department of Respiratory Medicine, Connolly Hospital, Dublin, Ireland, **3** Instituto Gulbenkian de Ciência, Instituto de Tecnologia Química e Biológica, Oeiras, Portugal, **4** Department of Mathematics, University of California, Berkeley, California, United States of America



Respiratory Diseases in Which the Lung Microbiome Has Been Reported to be Altered



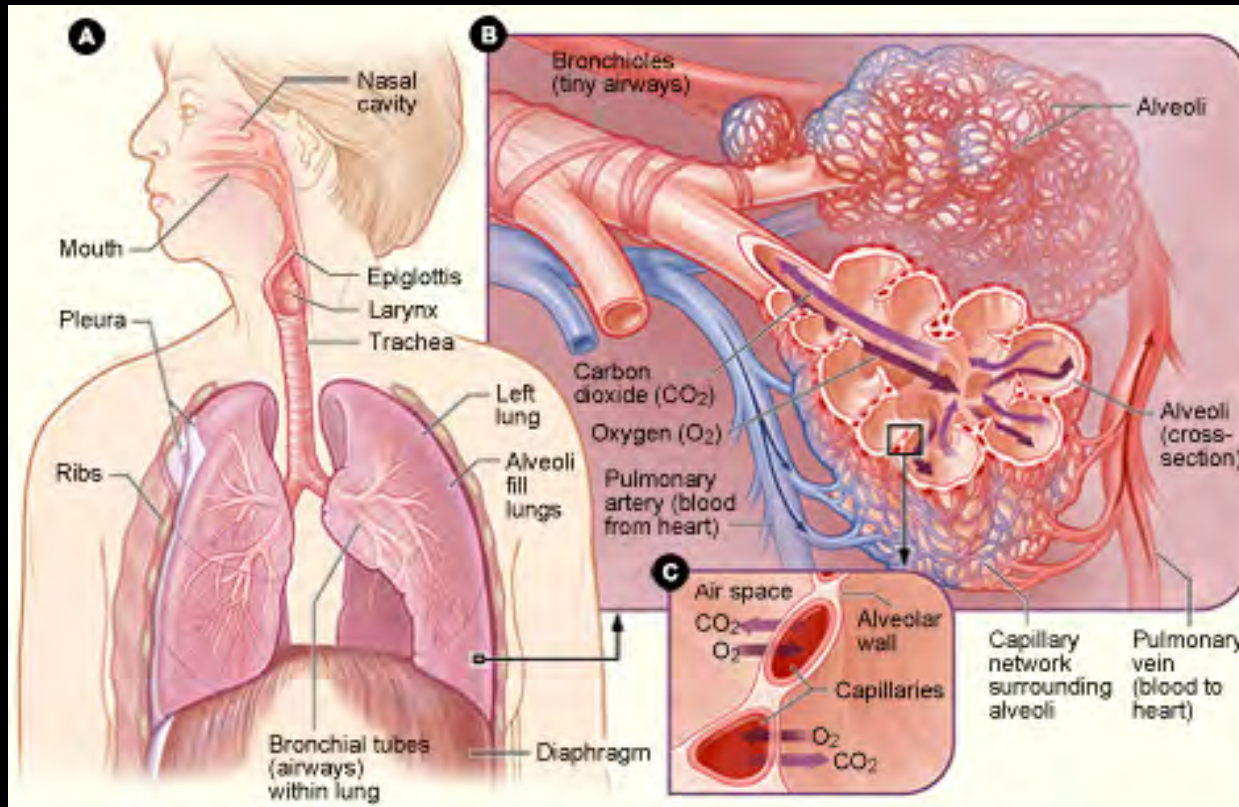
Cystic Fibrosis

Asthma

Chronic Obstructive Pulmonary Disease

**Bronchiolitis Obliterans Syndrome
following Lung Transplantation**

Airway Anatomy



-Anatomically contiguous surface

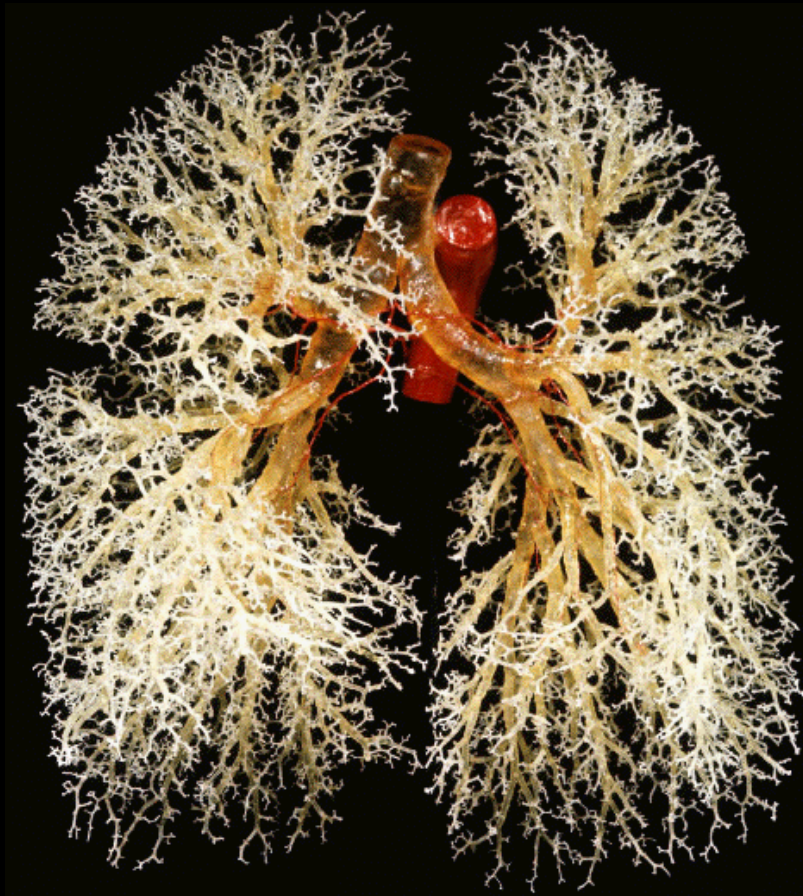
-Nasopharyngeal cavity has a significant resident microbiota

-A single “dam” between the upper and lower airways

-Cilia

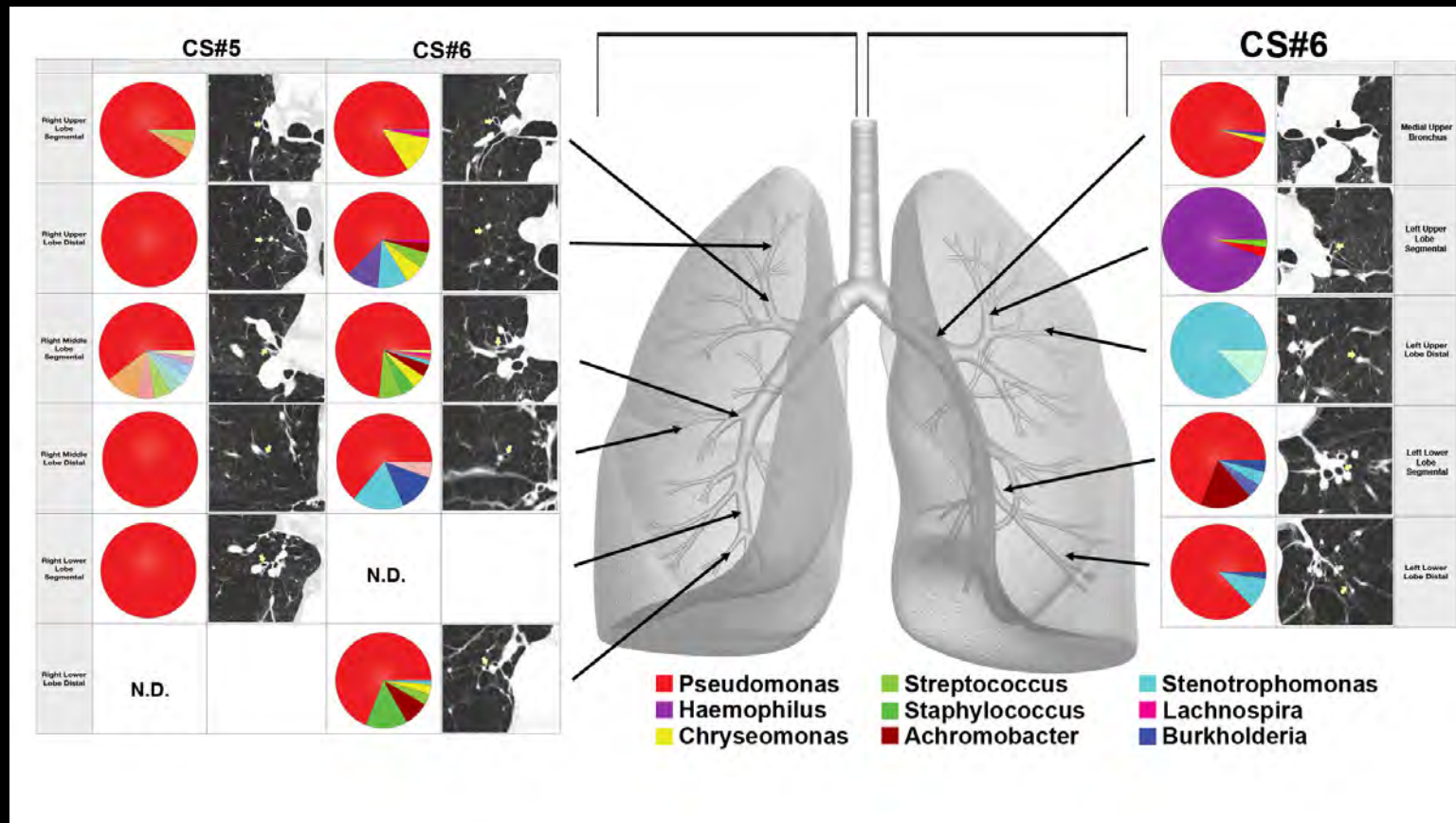
- Turns and branches

Airway Anatomy



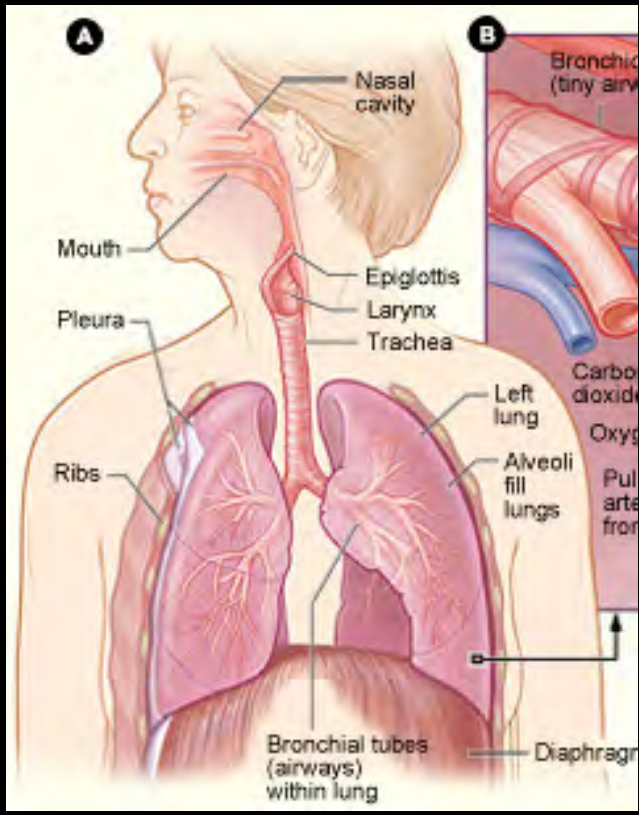
<http://capturedlightning.com/frames/Lung-Branching.gif>

Anatomical Heterogeneity of the Lung Microbiome in Advanced COPD



Sources of Microbes in the Lungs

- Nasopharyngeal aspiration
- Inhalation
- Reflux & aspiration
- (Bloodstream)



Can healthy lungs be considered sterile? If so, what do we mean by “sterile”?

Lack of microbial exposure?

Lack of microbial metabolism?

Lack of microbial replication?

Lack of microbial colonization?

What do we call the collection of persistent low
grade microbial immigrants in the lungs?

How to sample the airways

- Sputum (spontaneous and/or induced)
- Bronchoalveolar lavage
- Protected endobronchial brush
- Biopsy
- Sterile tissue sample

GAP

Determining the degree of bacterial transience vs. persistence vs. colonization in the lower airways

CHALLENGES

Type of sampling (invasive)

Infrequent (longitudinal = 2 to 3 samplings)

Potential for contamination of samples from nasal or oral microbiota

Using Analysis of the Oral Microbiome to Remove the Noise from Lung Microbiome Samples

Restricting Sampling Depth & Single-sided Outlier Test

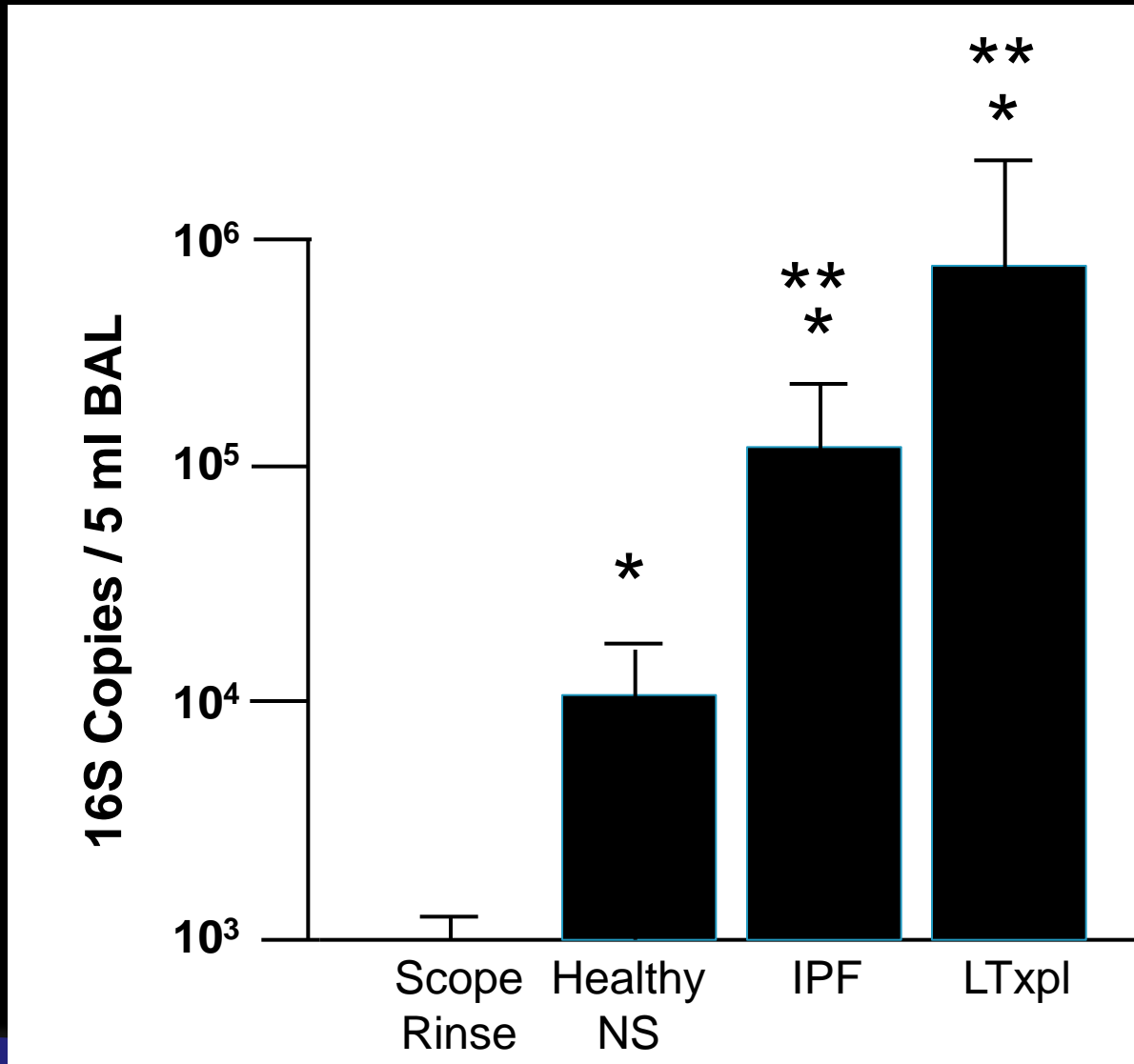
Charlson ES et al. Assessing bacterial populations in the lung by replicate analysis of samples from the upper and lower respiratory tracts. PLoS One. 2012;7(9):e42786.

Neutral Community Model

Sloan WT et al. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environ Microbiol. 2006 Apr;8(4):732-40

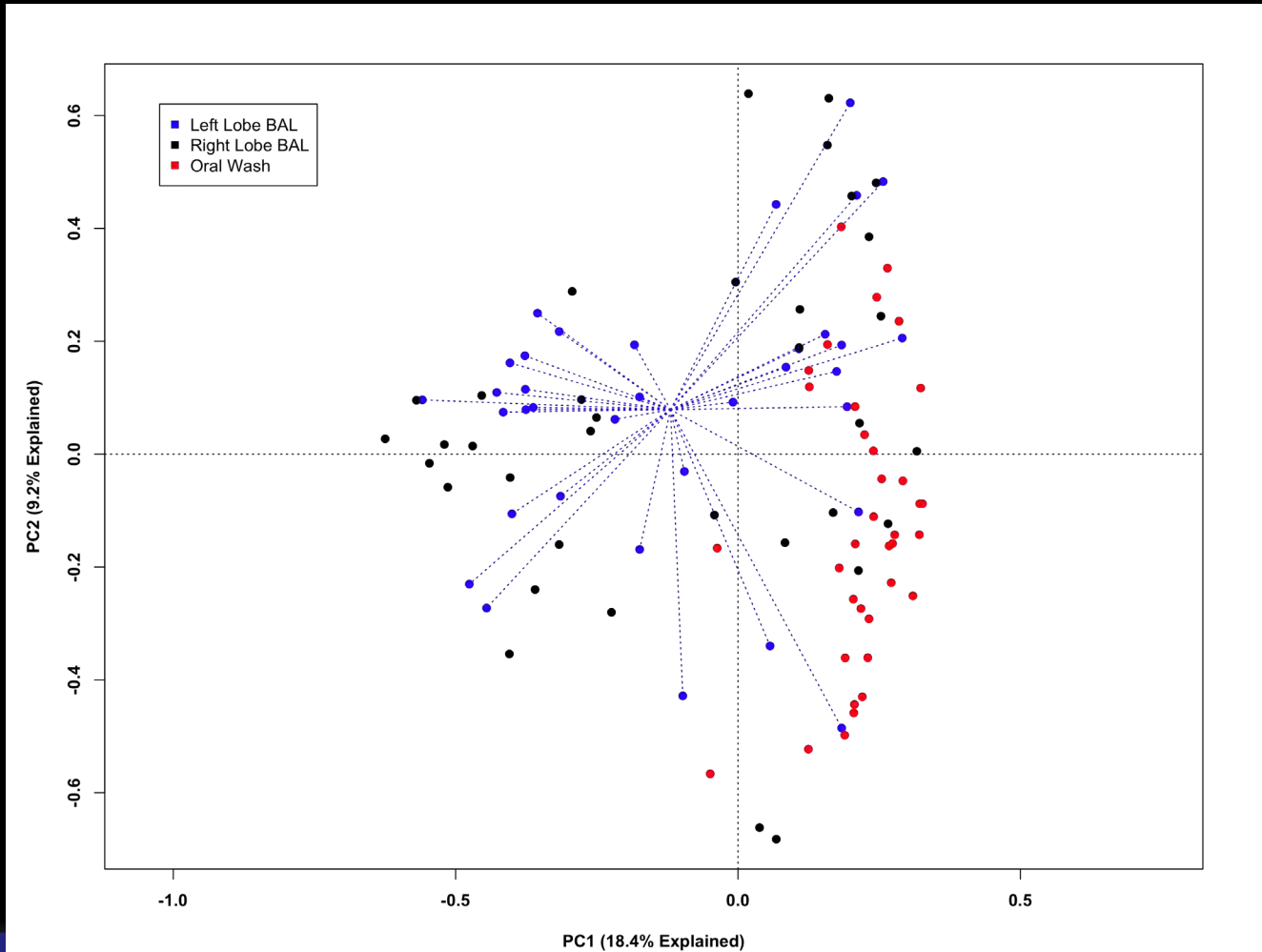
Morris A et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. Lung HIV Microbiome Project. Am J Respir Crit Care Med. 2013 May 15;187(10):1067-75.

Bacterial 16S Copy Numbers in the BAL fluid

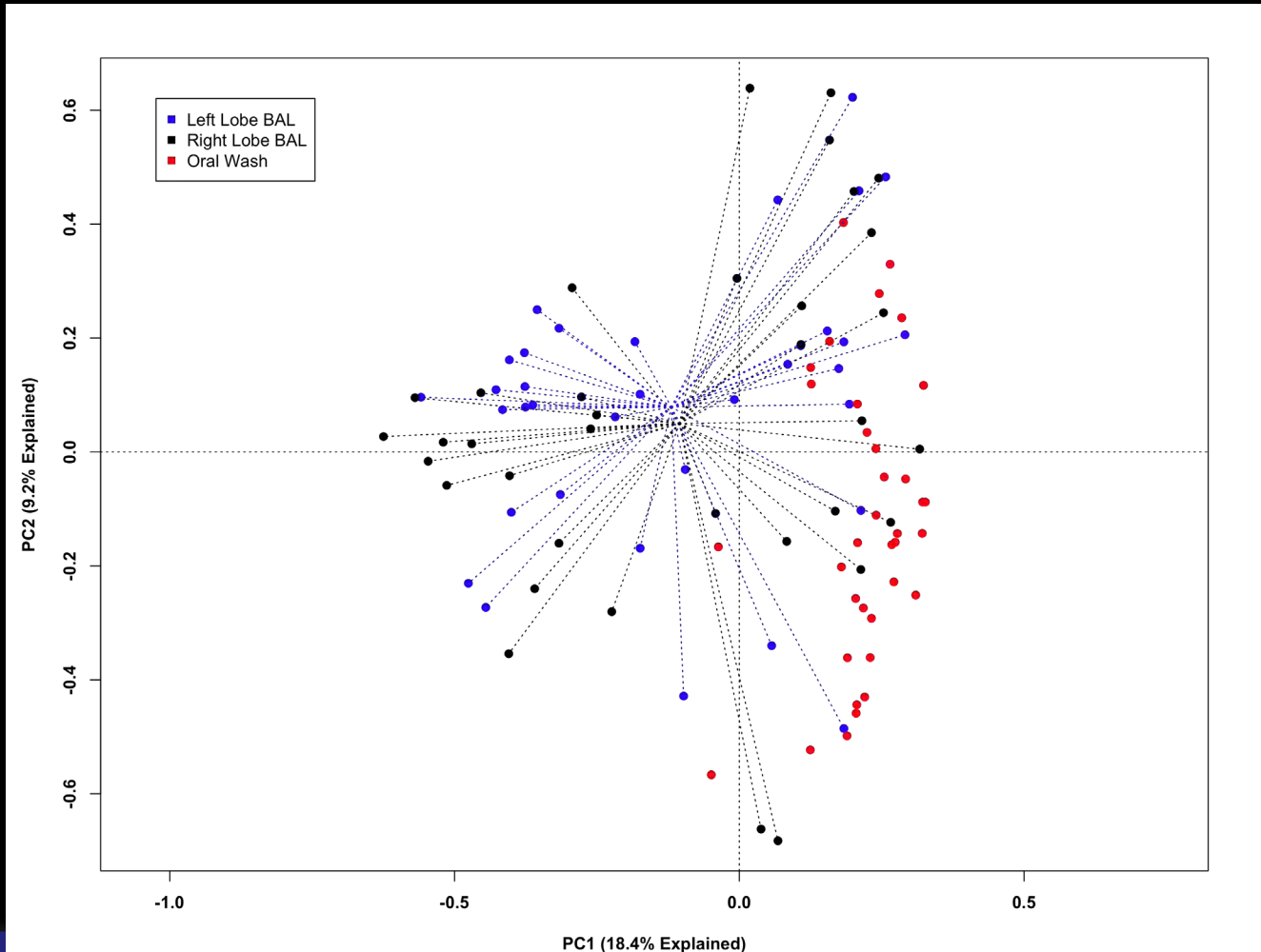


Thanks!
(TaqMan protocol)
Ric Bushman, Penn

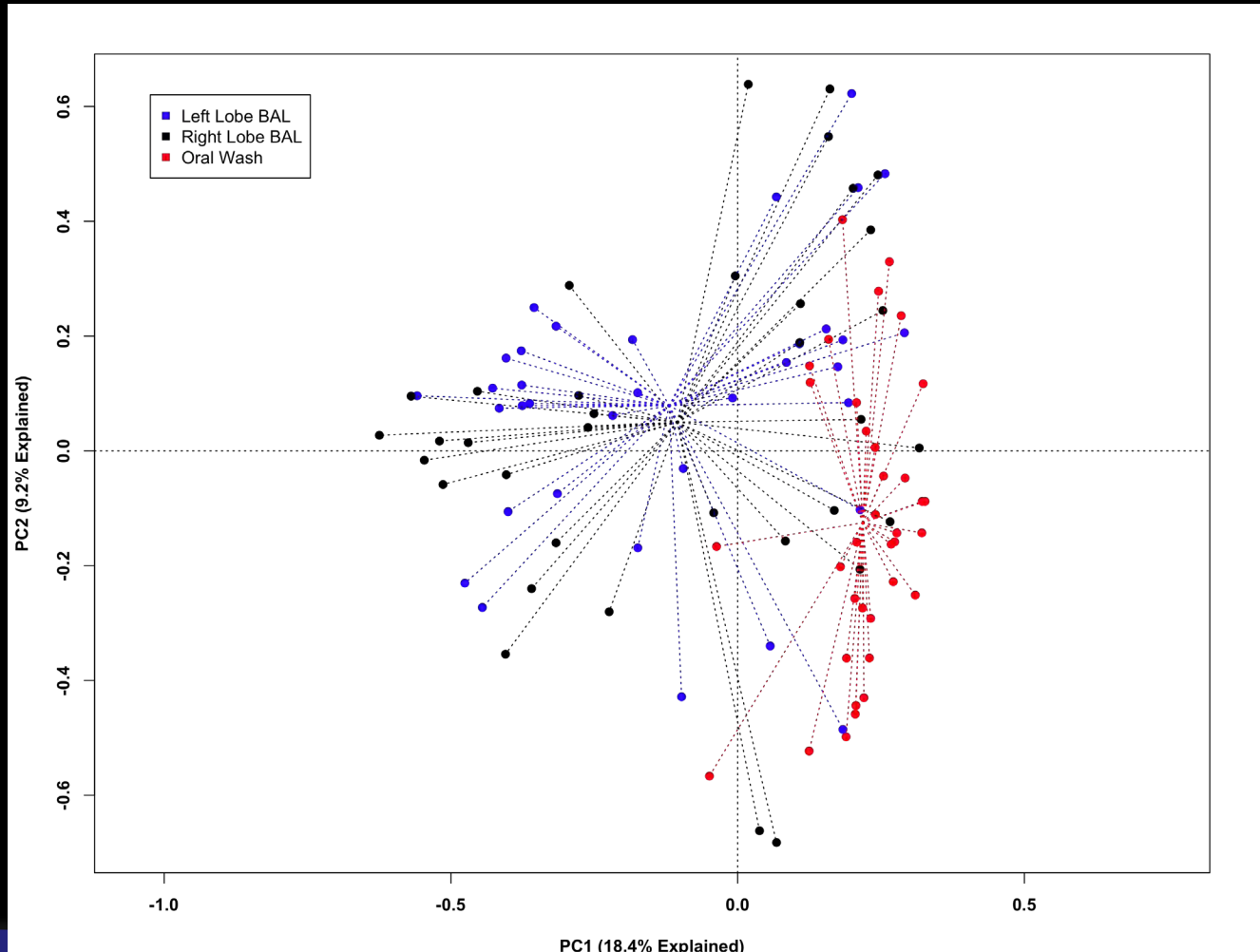
Comparison of BAL vs. Oral Rinse Bacterial Communities



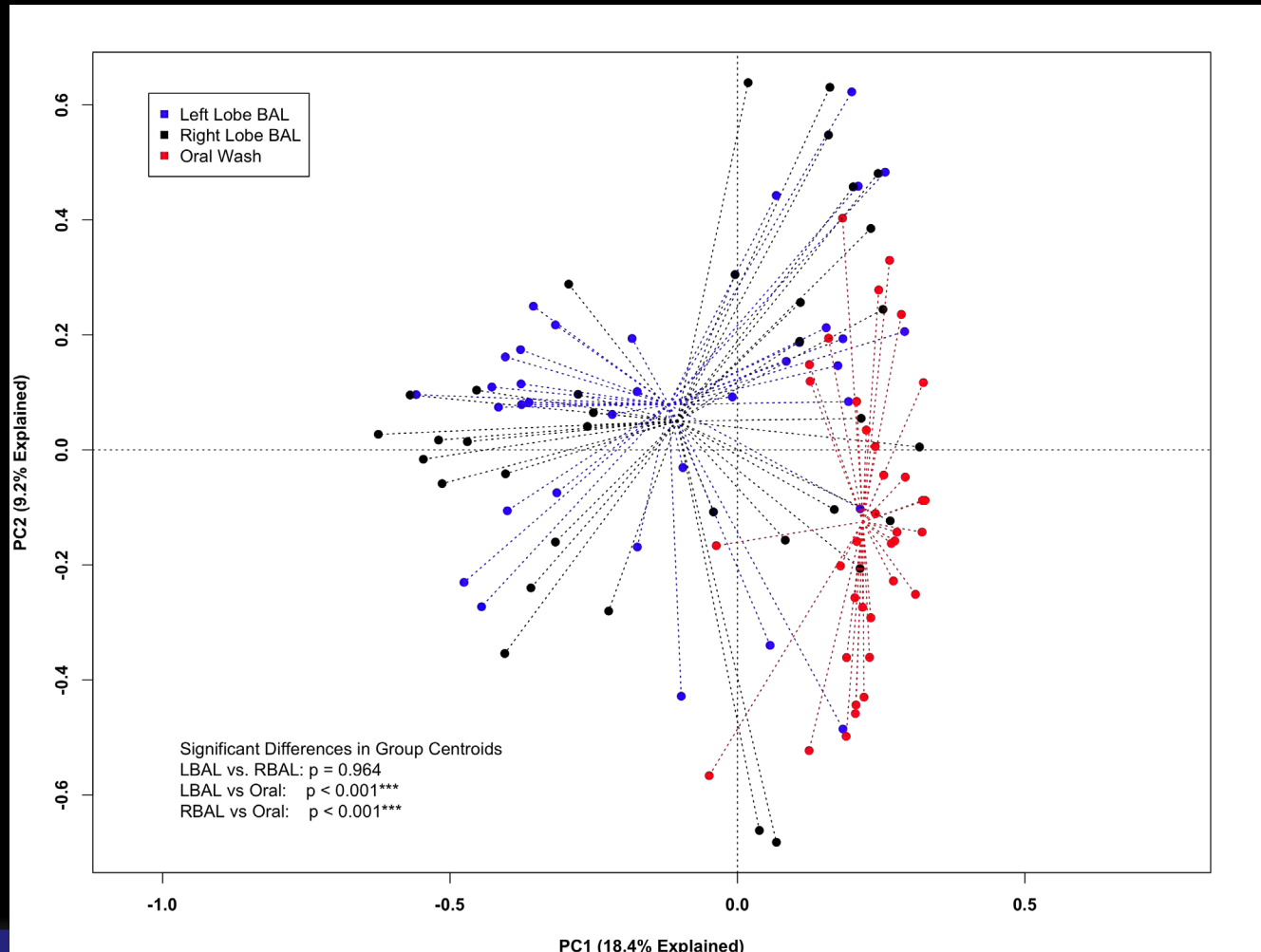
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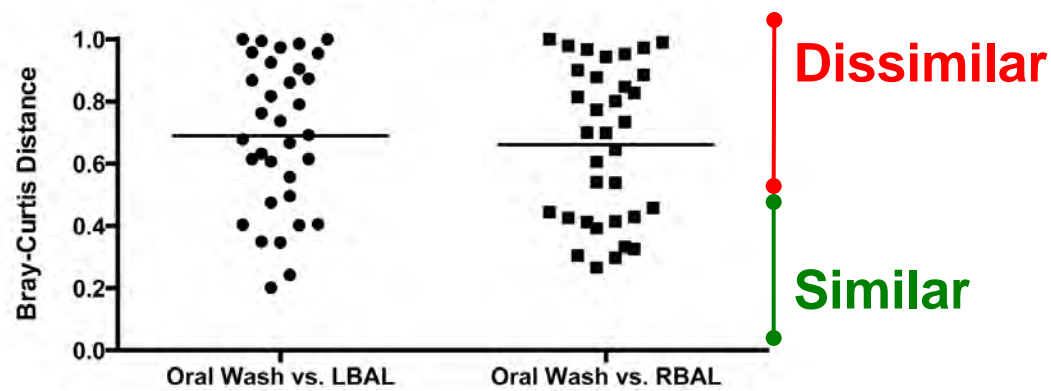
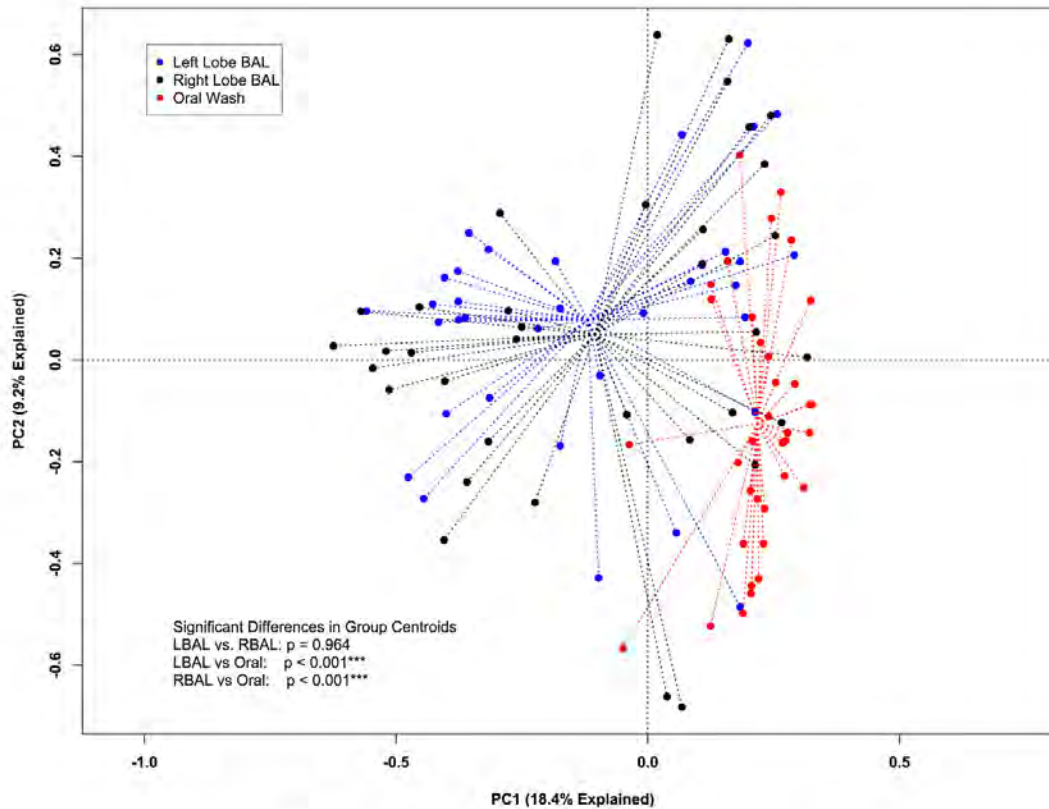


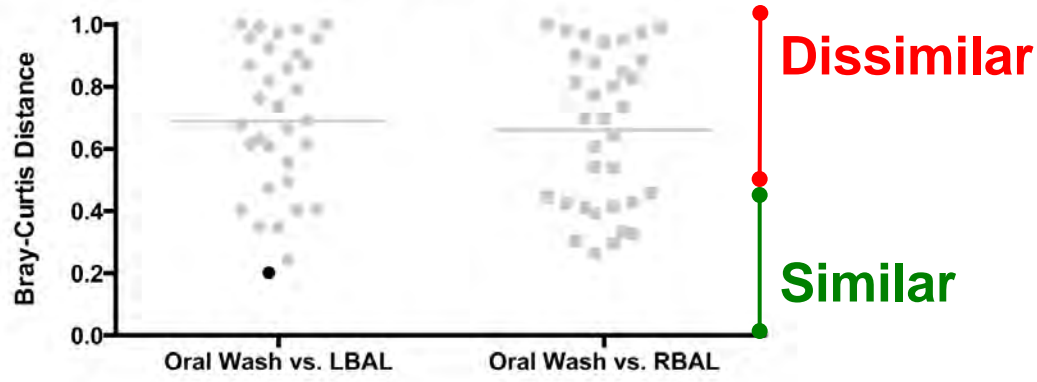
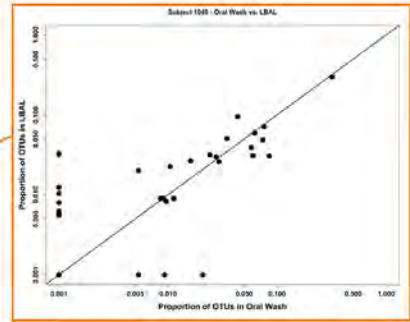
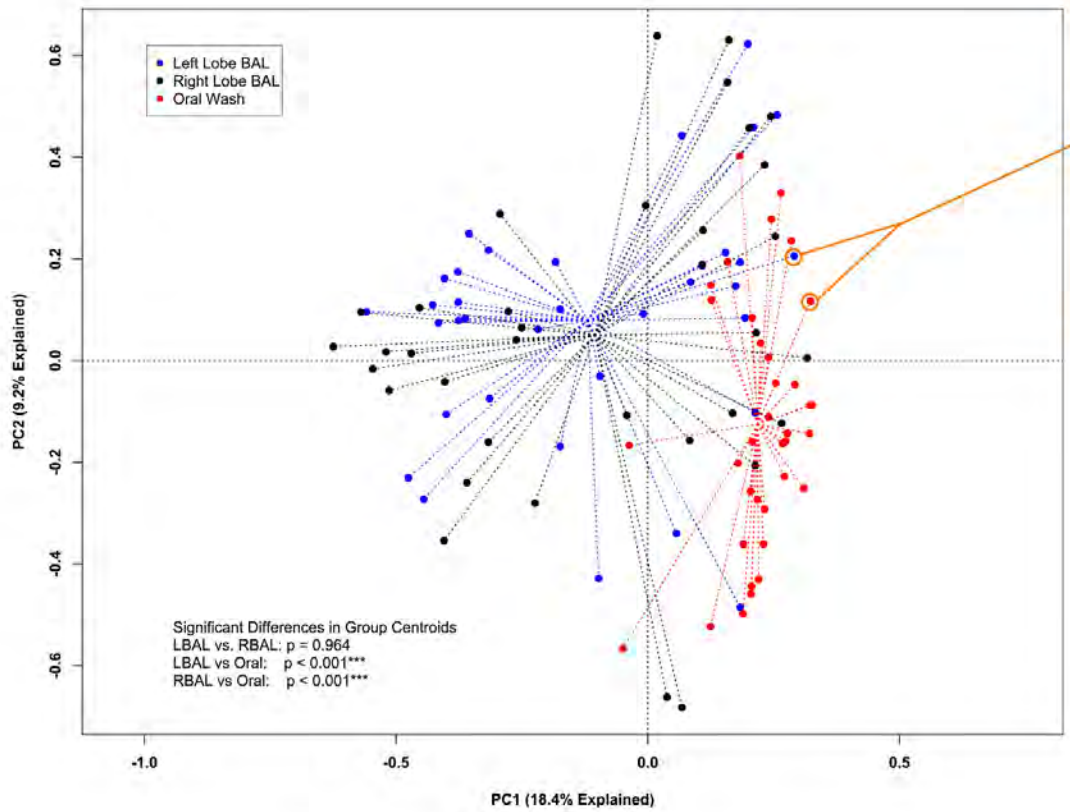
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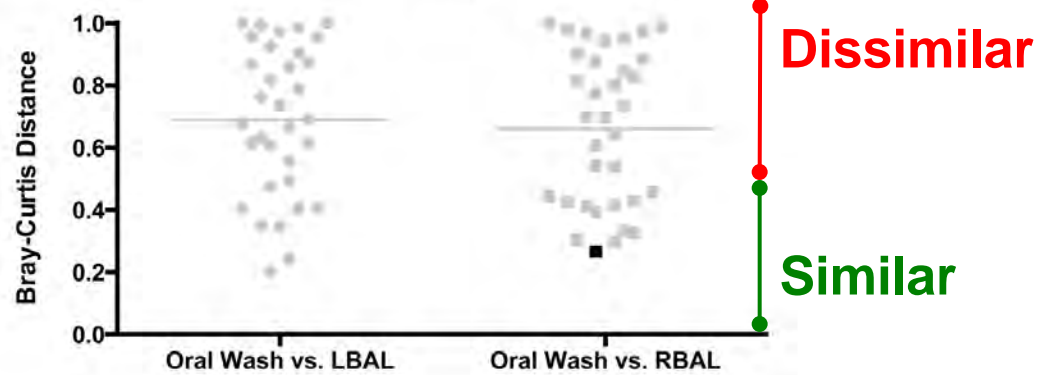
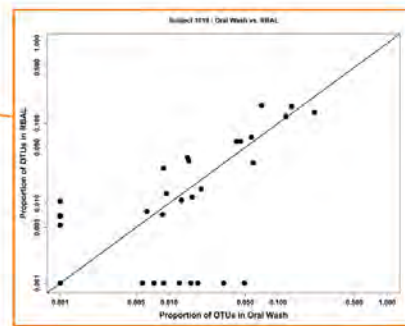
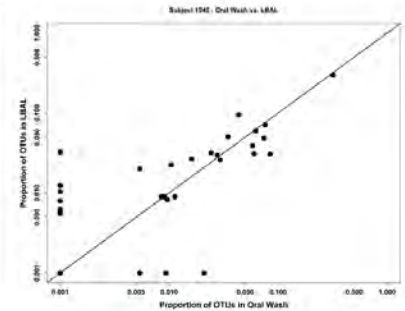
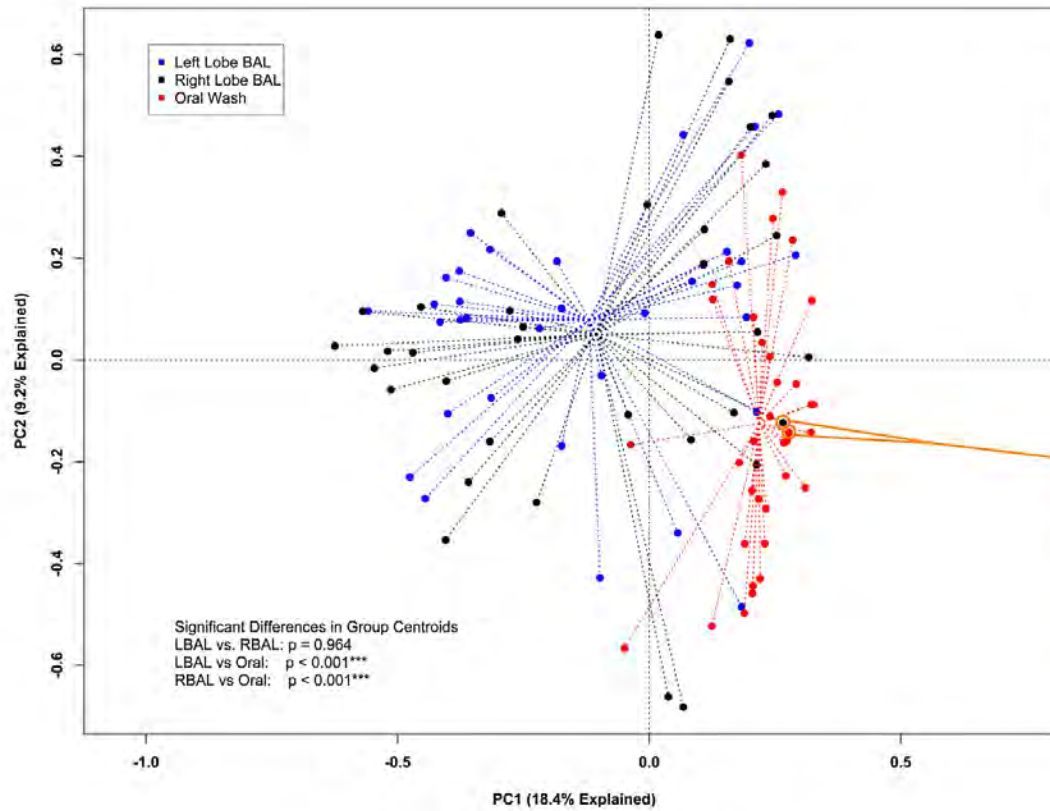


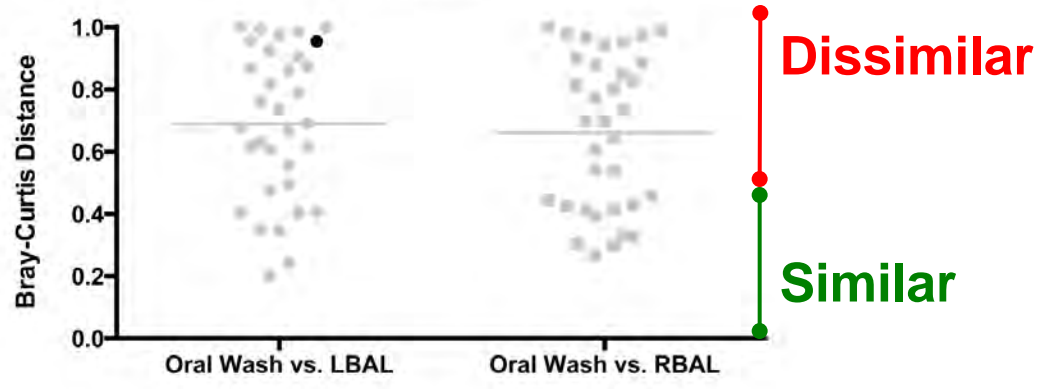
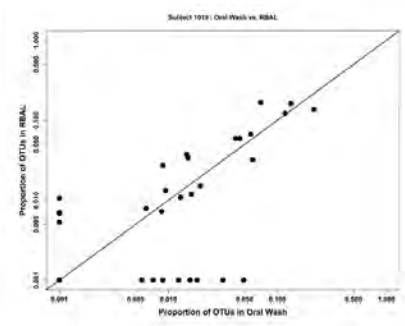
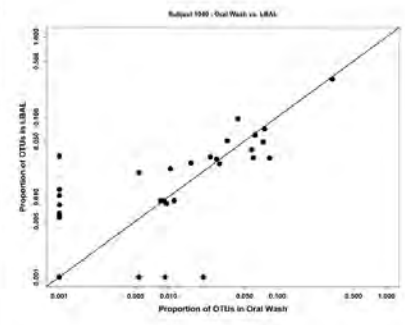
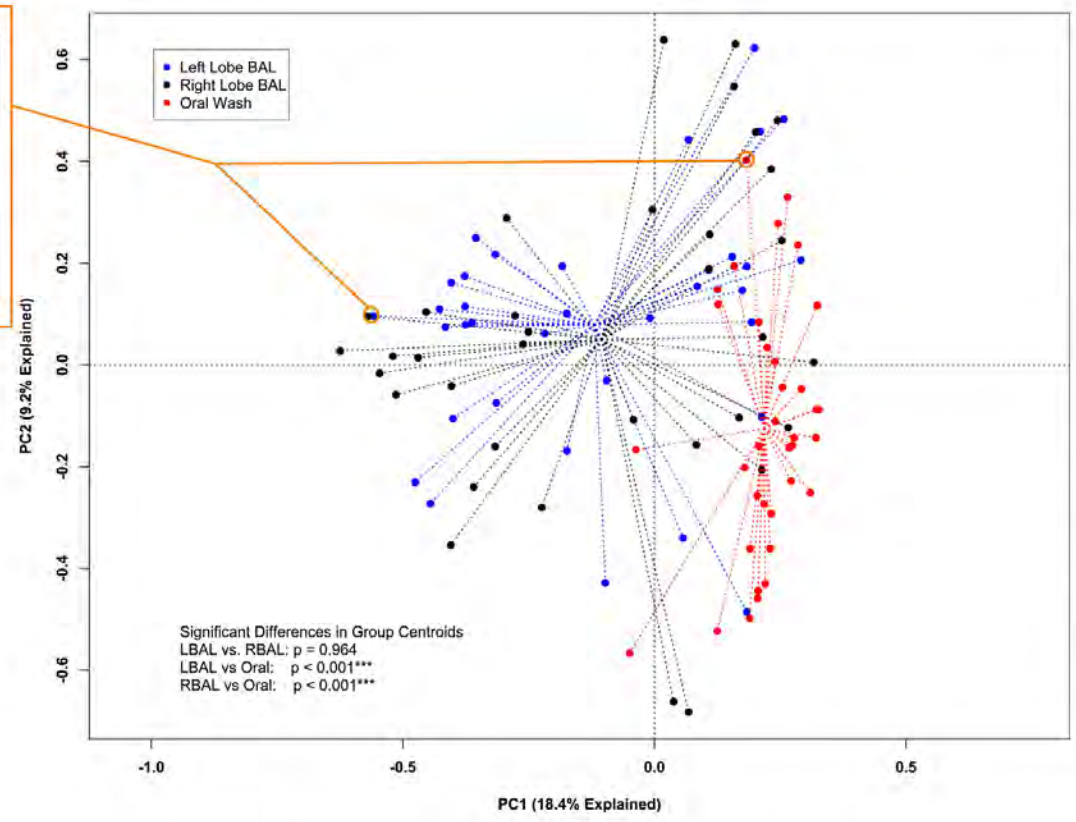
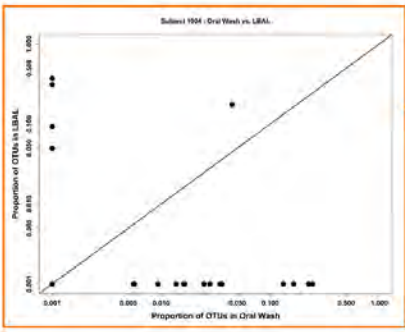
Comparison of BAL vs. Oral Rinse Bacterial Communities

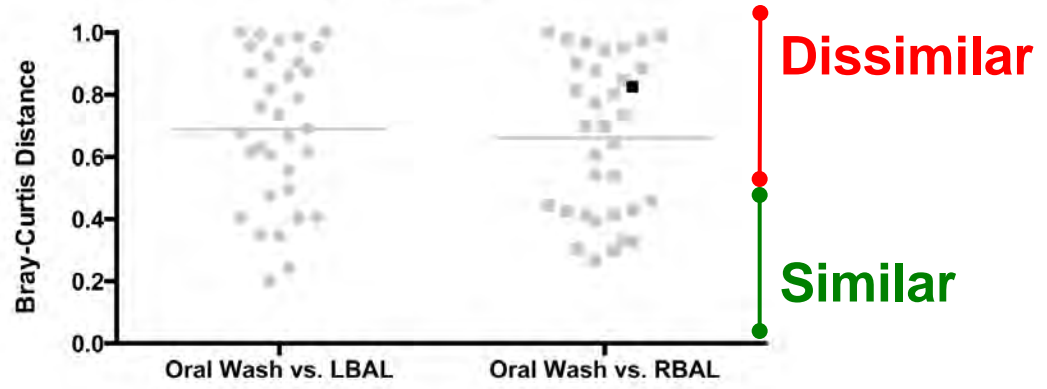
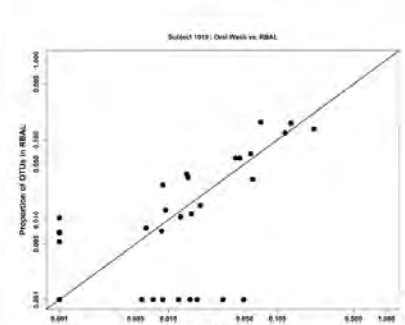
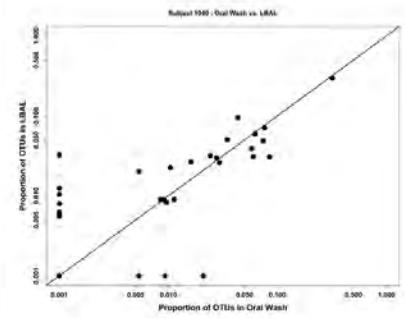
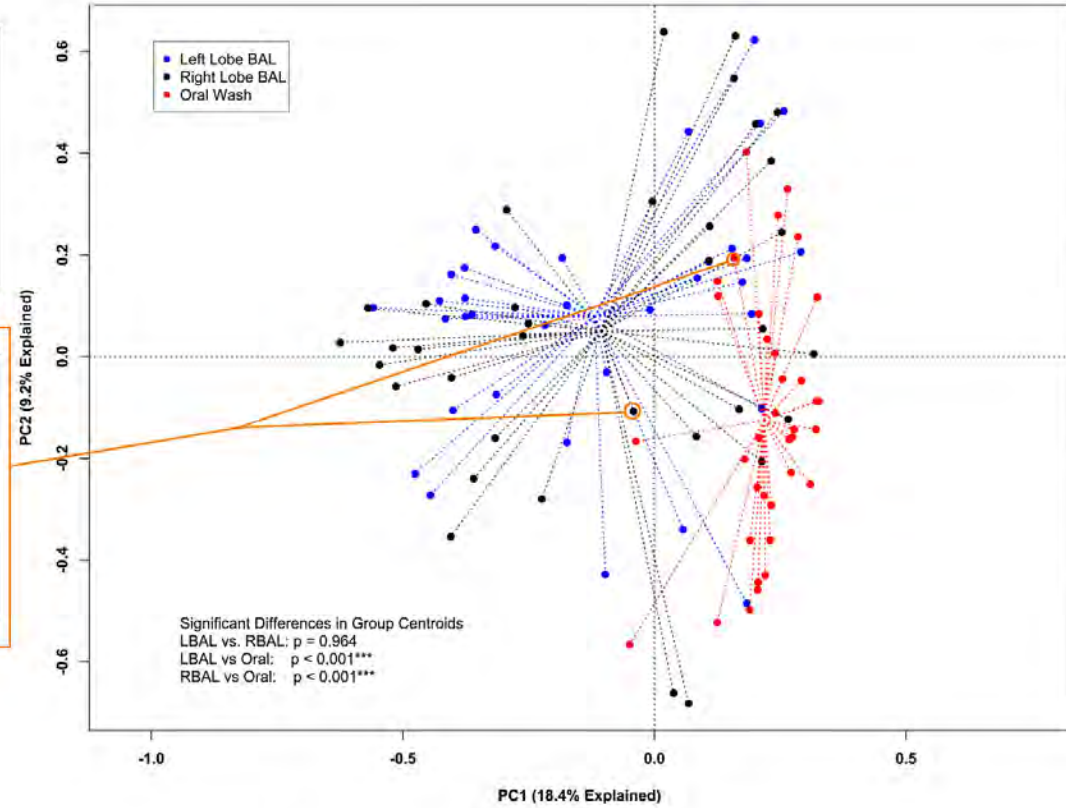
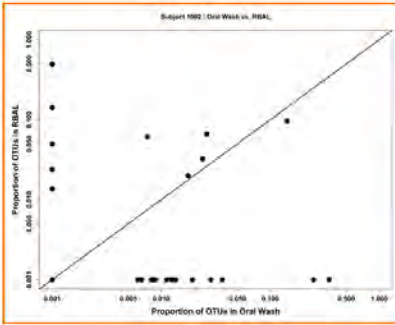
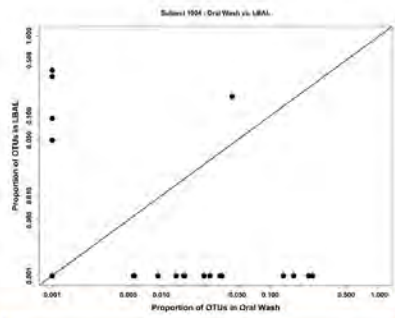










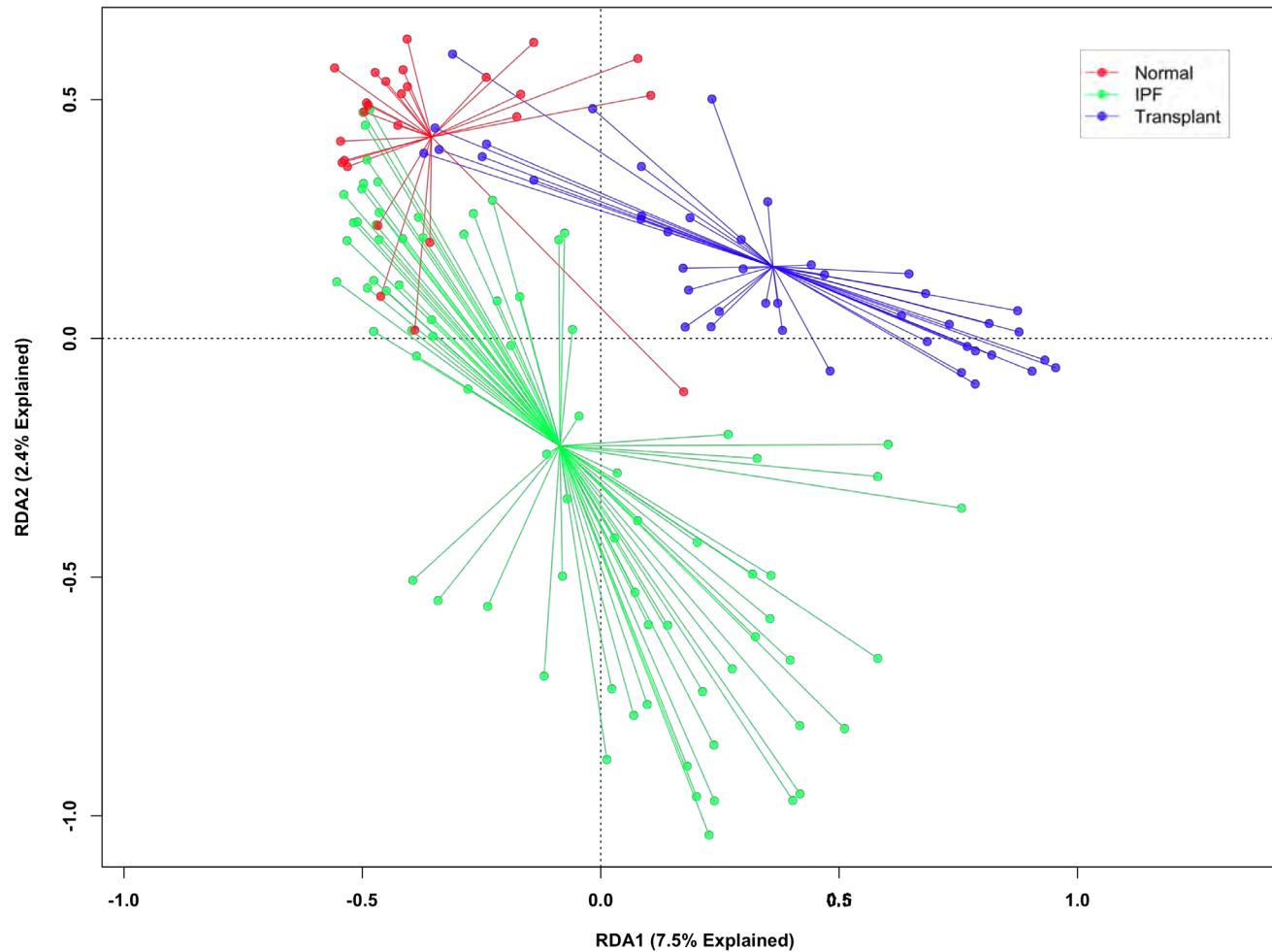


When healthy, the microbial load in the lungs is low and BAL samples contain a predominance of bacterial taxa also found in the mouth.

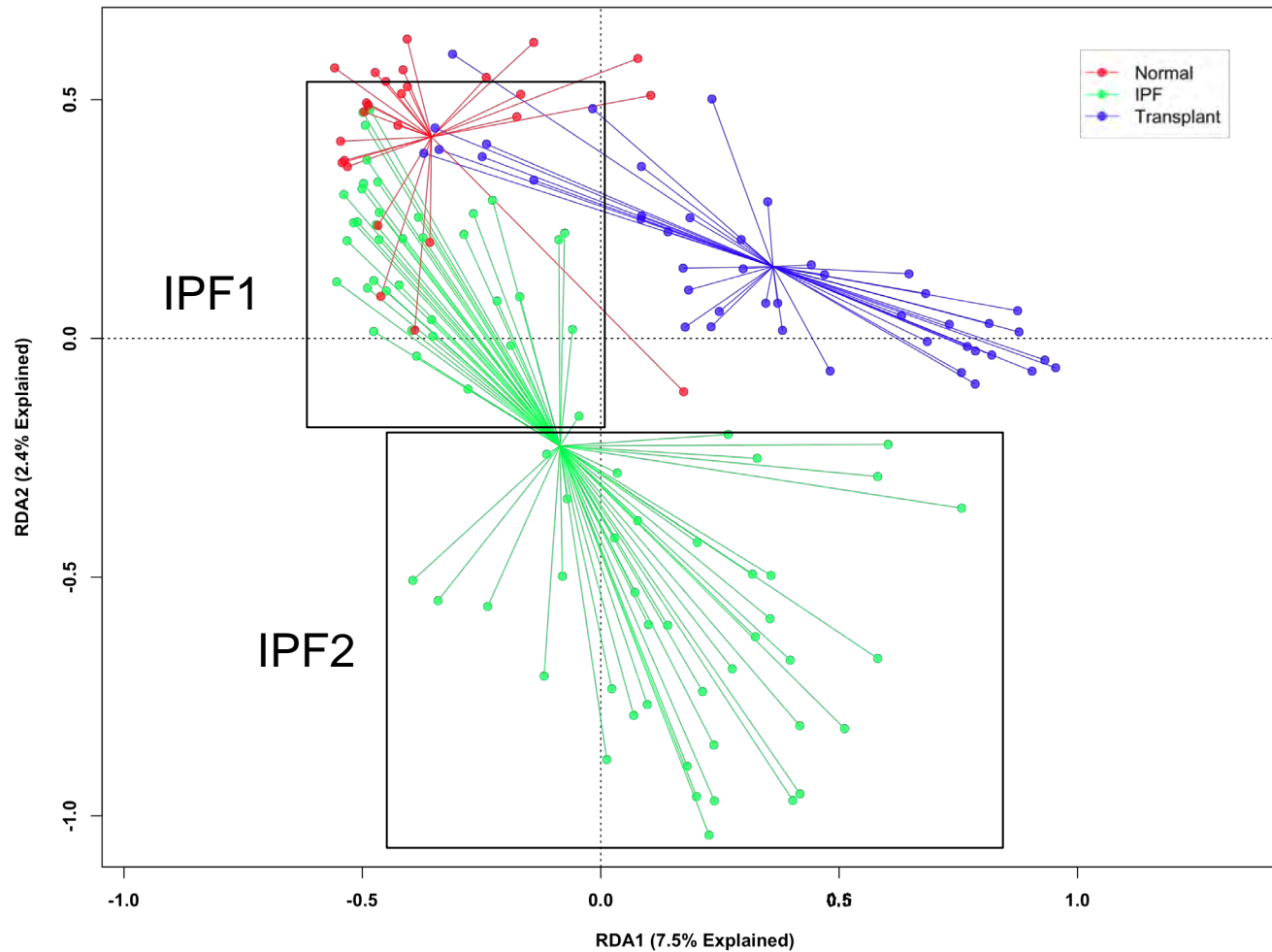
Bacterial diversity in the lungs is very low.

In some individuals, there are some differences suggesting that selective pressures exist in the lungs for elimination, persistence, colonization and growth.

Comparison of the BAL Microbiome in Healthy Non-Smoker, IPF & Lung Transplant Subjects



Comparison of the BAL Microbiome in Healthy Non-Smoker, IPF & Lung Transplant Subjects

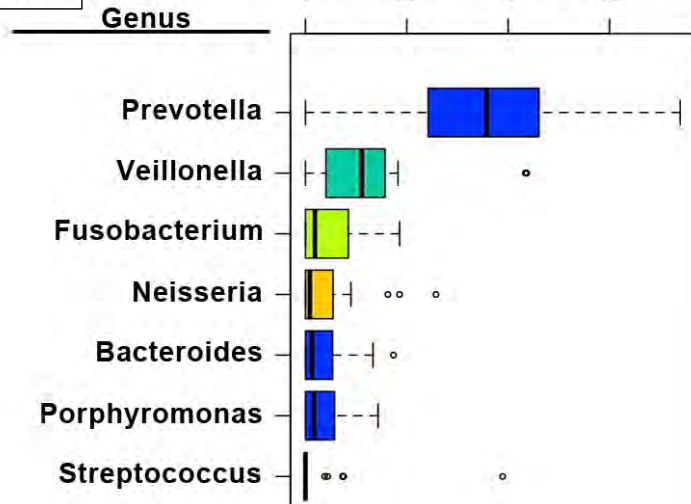


Genus-level OTU Cluster Analysis of the BAL Microbiome in Healthy NS and IPF1 Subjects

- Actinobacteria
- Bacteroidetes
- Firmicutes
- Fusobacteria
- Proteobacteria
- Spirochaetes

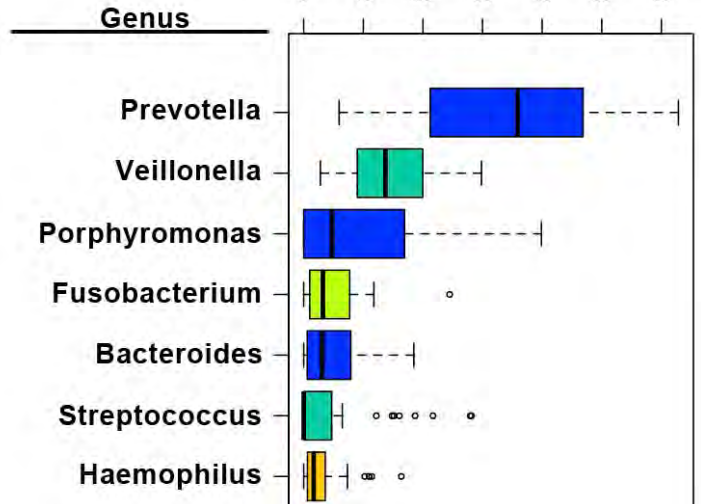
Most Abundant Phylotypes in Healthy BAL

Percent Relative Abundance

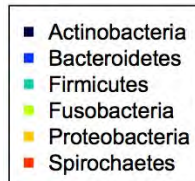


Most Abundant Phylotypes in "Normal" IPF BAL

Percent Relative Abundance

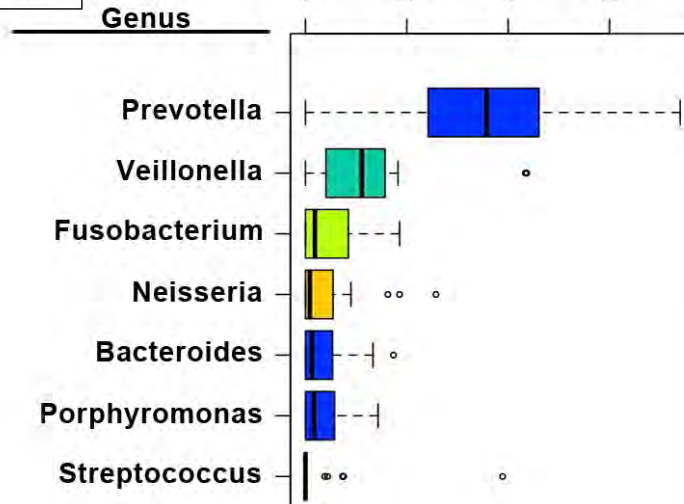


Genus-level OTU Cluster Analysis of the BAL Microbiome in Healthy NS and IPF1 Subjects



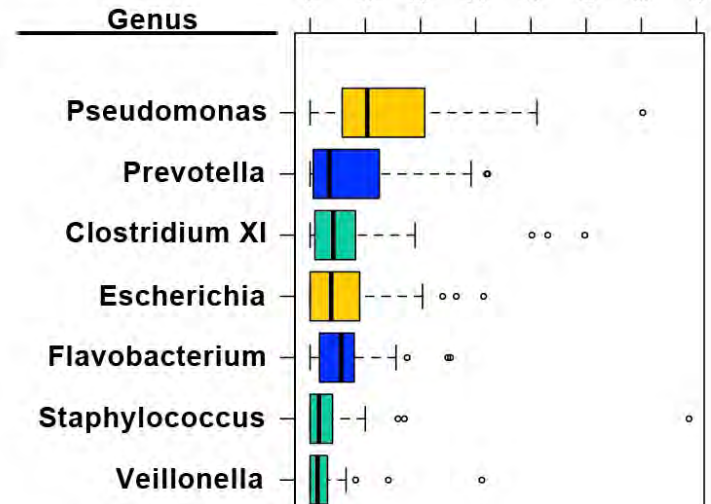
Most Abundant Phylotypes in Healthy BAL

Percent Relative Abundance

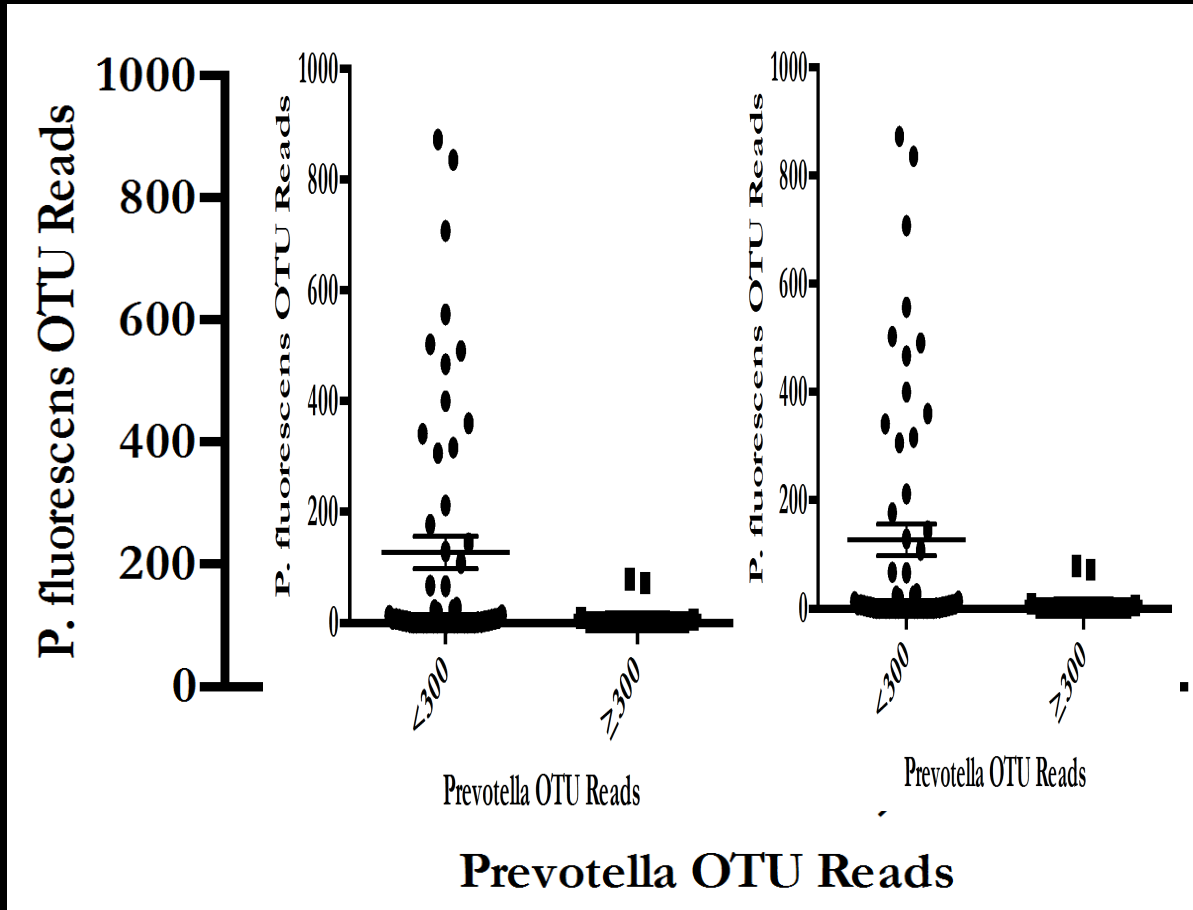


Most Abundant Phylotypes in "Non-Normal" IPF BAL

Percent Relative Abundance



The Lung Microbiome Following Lung Transplantation



When diseased, the microbial load in the lungs increases and BAL samples now often contain numerous bacterial taxa not found in the mouth, indicating selective pressures in the lungs for persistence, colonization and growth.

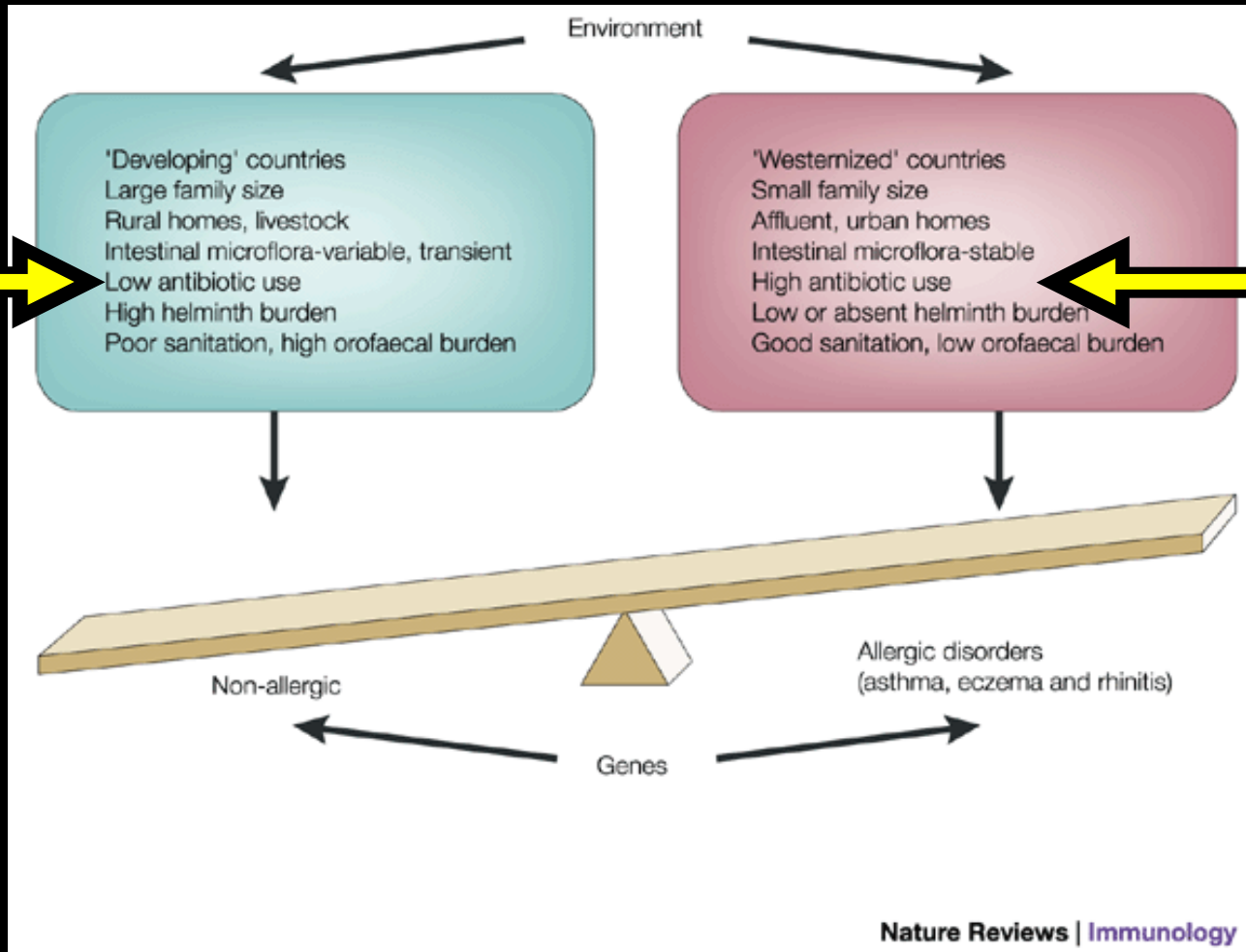
CHALLENGE

- Studies only involving human subjects will NEVER demonstrate causality, no matter how large the cohort. In vivo (animal) studies, model organisms, and in vitro experiments are needed to delineate mechanisms.

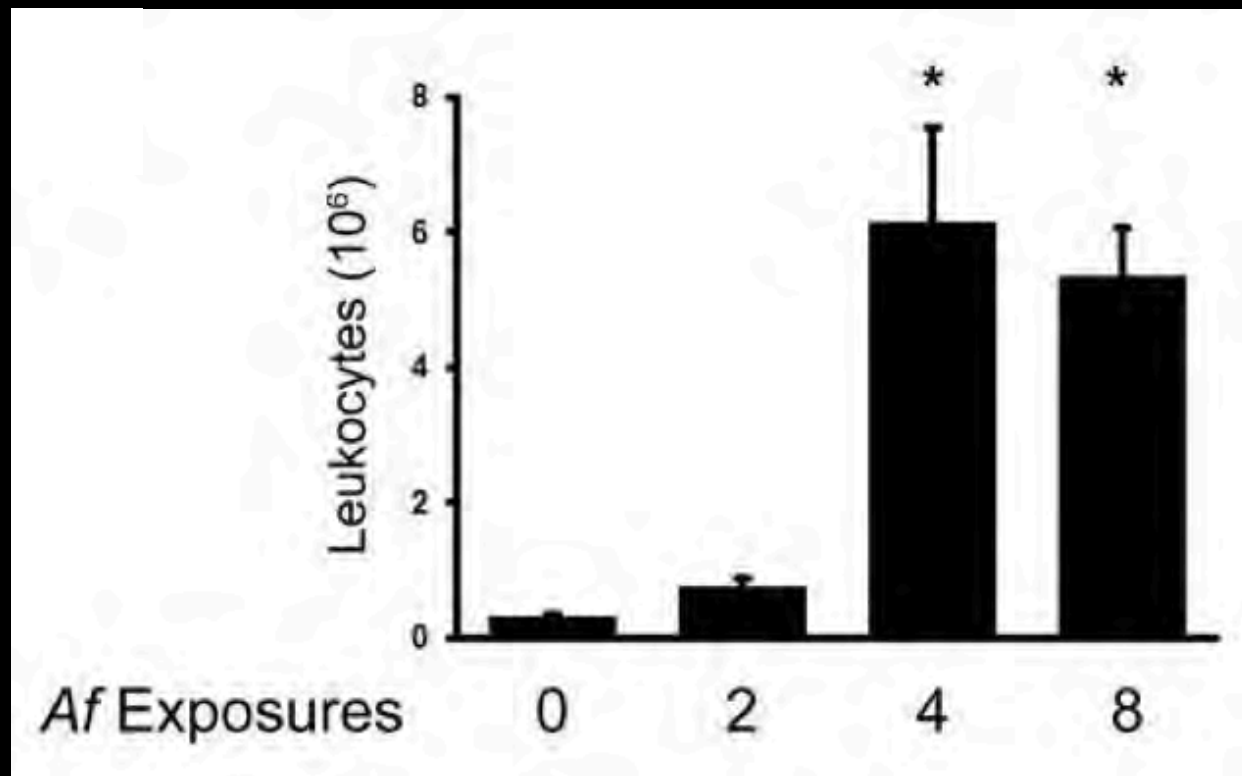
NEED

- Support for animal models in the study of the human microbiome

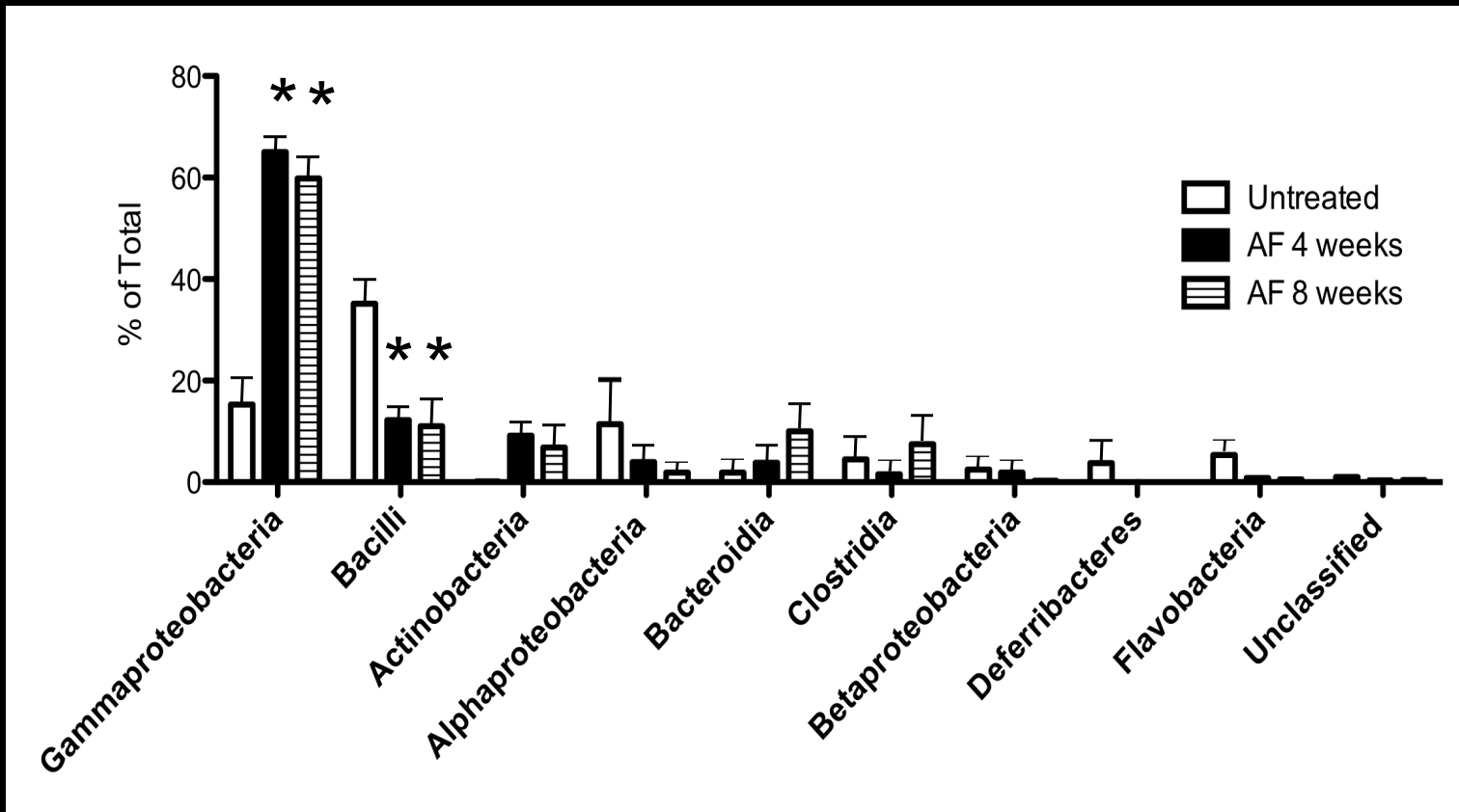
The Hygiene Hypothesis



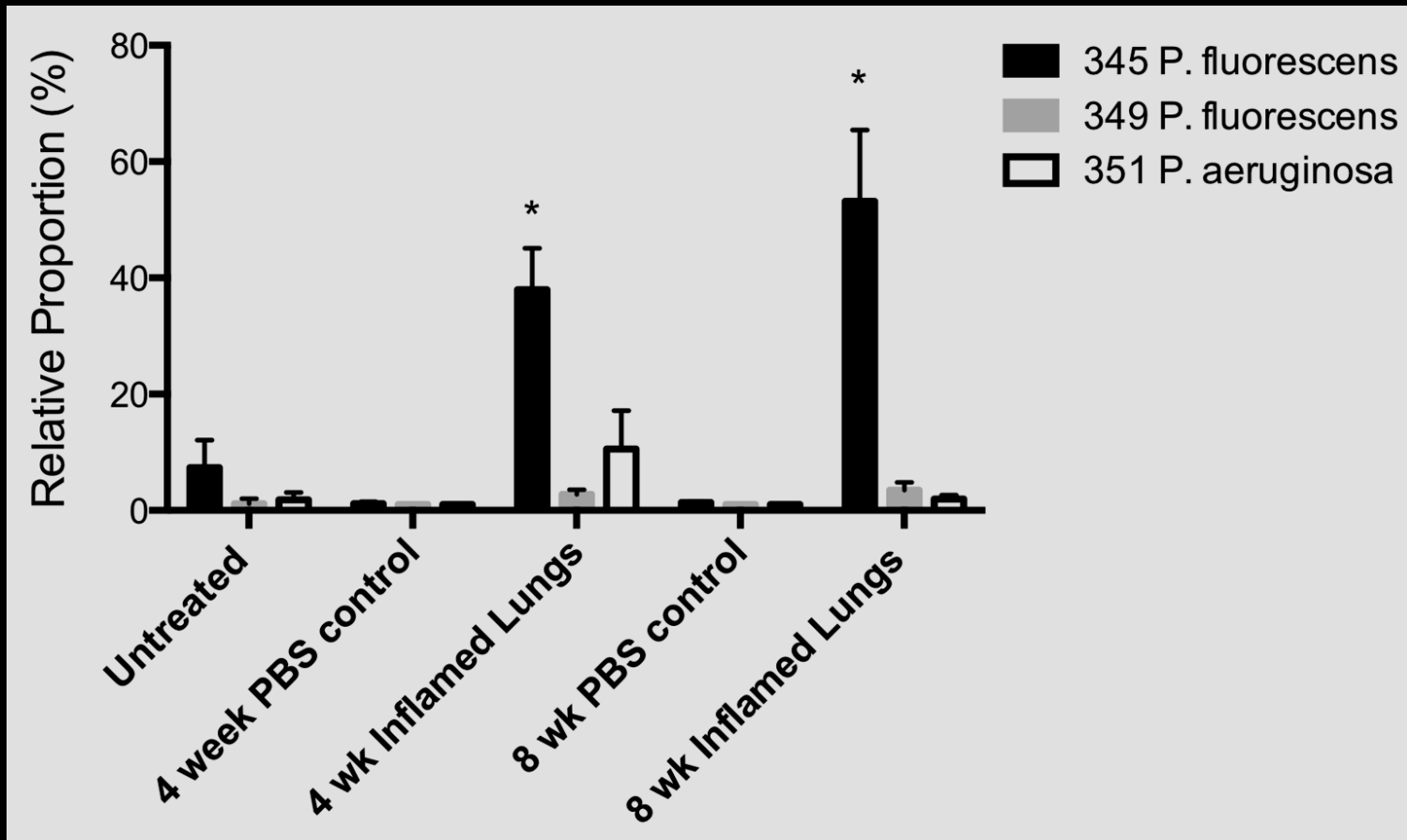
The Development of Pulmonary Inflammation Following Repeated *Aspergillus fumigatus* Conidia Exposure



Effect of Chronic Pulmonary Inflammation on Changing the Lung Microbiota

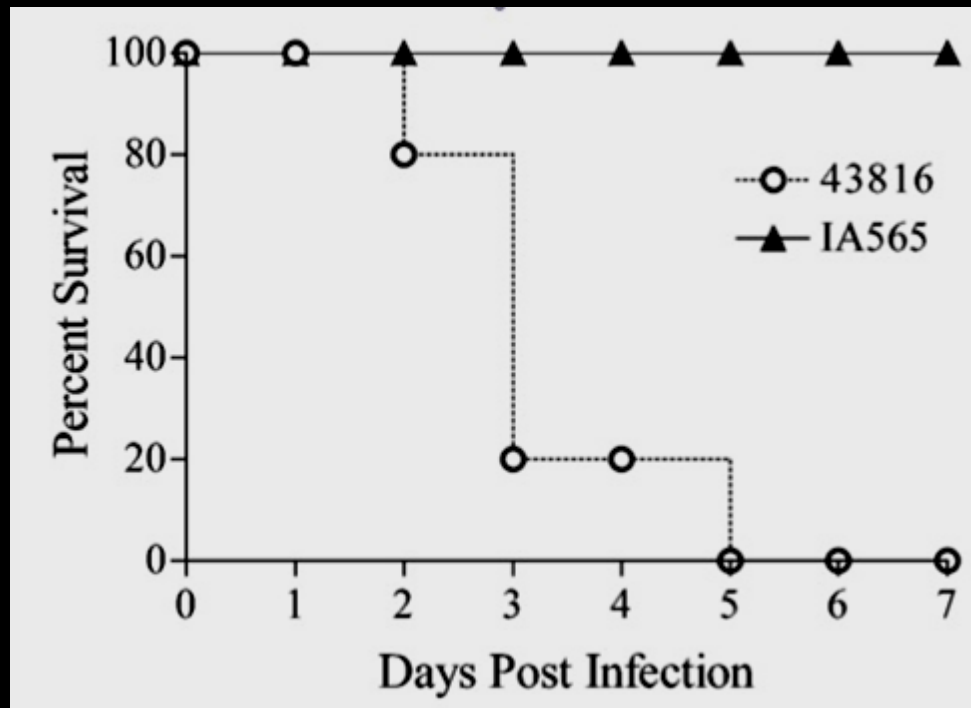


Effect of Chronic Pulmonary Inflammation on Changing the Lung Microbiota



Klebsiella pneumoniae as an etiologic agent of pneumonia

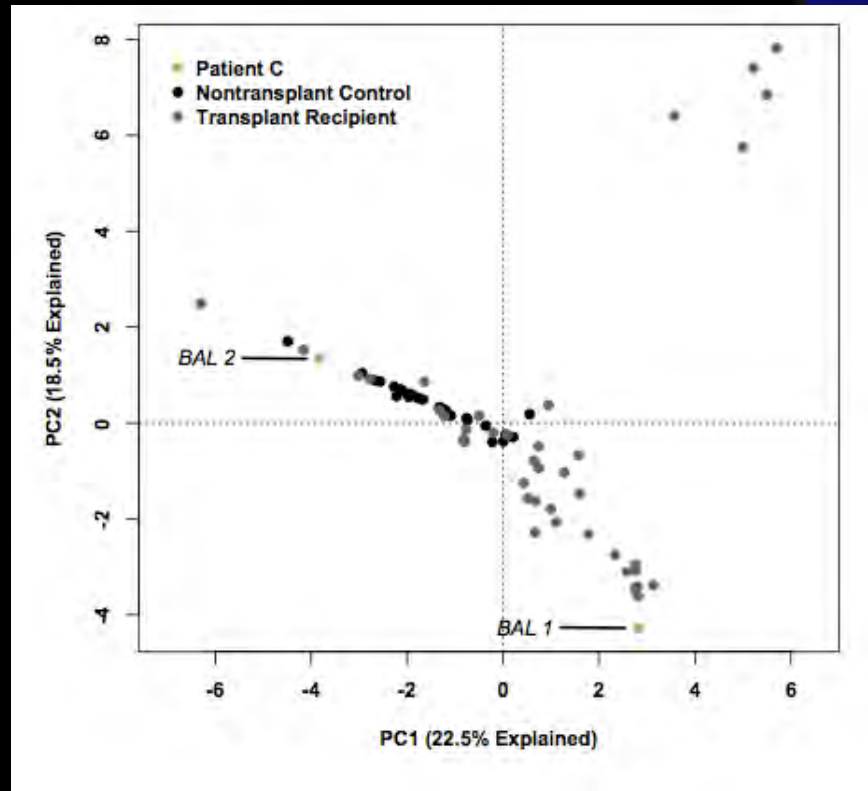
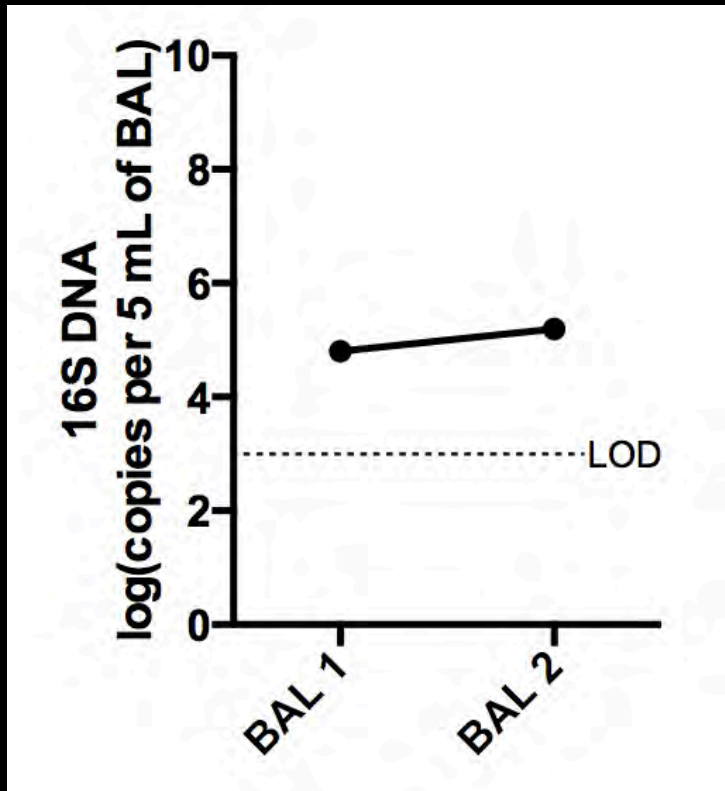
Two isolates of *K. pneumoniae* grown from two patients with presumed gram negative pneumonia (43816 and IA565)



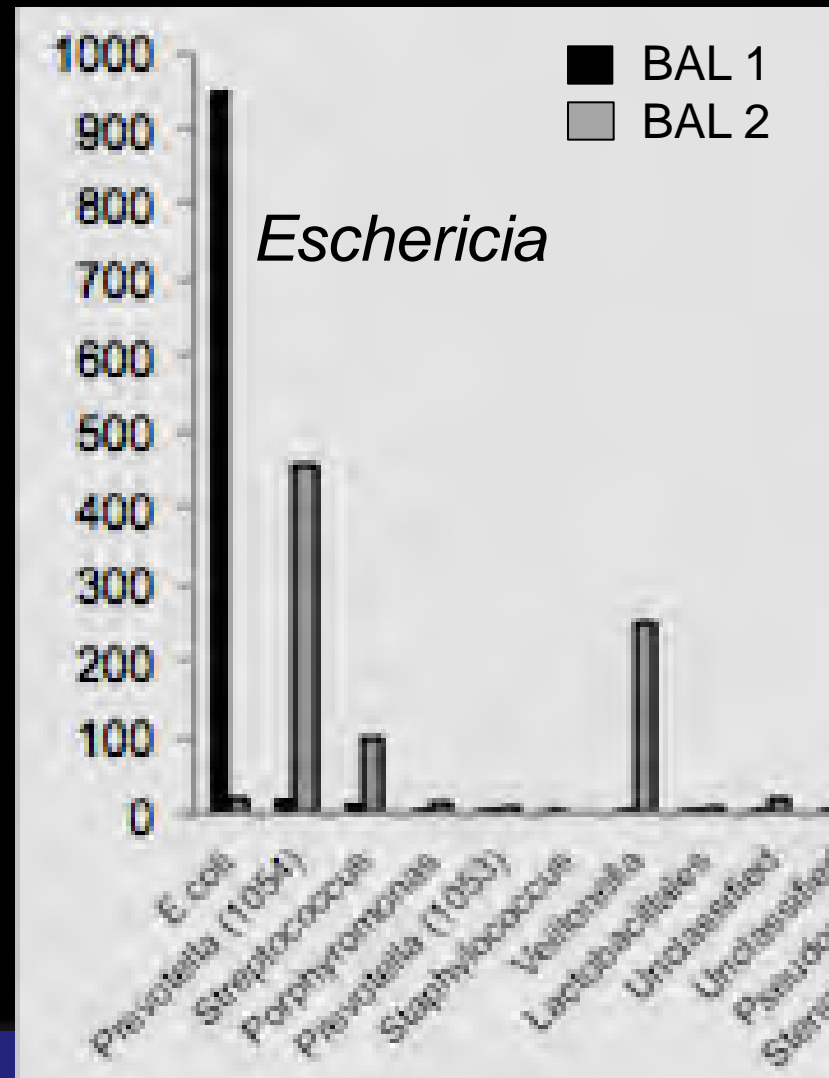
Lau HY et al. Microb Pathog. 2007;42(4):148-55.

Lau HY et al. Microbes Infect. 2008;10(12-13):1283-90.

Pyrosequencing of a culture-negative pneumonia



Pyrosequencing of a culture-negative pneumonia



GAPS

- understanding the implications of culturable and non-culturable states of bacteria in the lungs
- visualization of microbes in the lungs

NEEDS

- new cultivation strategies

OVERALL CHALLENGES

- more consistent and supportive peer review of microbiome/lung proposals
- accepting that sampling of the lower respiratory tract in humans will be imperfect and utilization of animal models to close the gap

The Research Group



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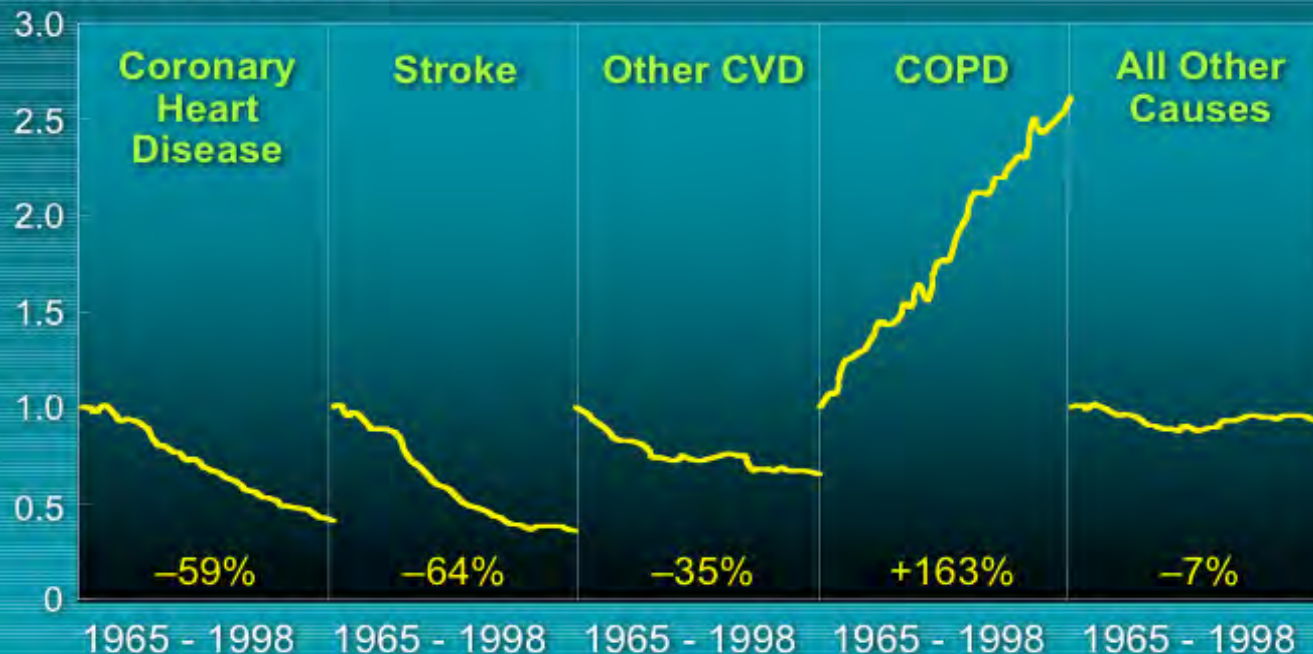
The future of internal medicine...how will you think about disease?





Percent Change in Age-Adjusted Death Rates, U.S., 1965-1998

Proportion of 1965 Rate



Source: NHLBI/NIH/DHHS