The Microbiome in Respiratory Diseases

James R. Brown
Computational Biology, Target Sciences
06/30/16
Outline

1. Microbiome and the pharma imperative

2. Antibiotics and the human microbiome

3. Targeting the host-pathogen interactome

4. The lung microbiome in respiratory diseases
The Pressures on Pharma Industry Continue...

- Largest cause of failure in clinical trials is lack of efficacy
- Choice of target is critical
We Are Not an Island

**Intrinsic**
- Genetics: Inherited
- Genetics: Mutations
- Gene Regulation: Space and Time
- Epigenetics
- Proteomics

**Extrinsic**
- Pathogens – Infections
- Drugs: Antibiotics
- Diet
- Medical History
- Environment: Allergens
- Environment: Behaviors

**Microbiome**
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Stop the killing of beneficial bacteria

Concerns about antibiotics focus on bacterial resistance — but permanent changes to our protective flora could have more serious consequences, says Martin Blaser.

- Average child in developed countries takes 10-20 courses of antibiotics before age 18 yr
GSK1322322 Inhibits Bacterial Peptide Deformylase

- Novel antibacterial target present in all bacterial organisms
  - Highly conserved active site
- Clinically unexploited
  - Inhibitors should be active against respiratory bacteria resistant to current antibiotics

**PDF Isozyme**

<table>
<thead>
<tr>
<th>PDF Isozyme</th>
<th>$K_i^*$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>0.064 ± 0.009</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>0.038 ± 0.007</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>0.011 ± 0.0004</td>
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</tbody>
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**Peptide Deformylase (PDF)**
Removes N-formyl group from newly-synthesized peptides.
Phase I GSK’322 Clinical Trial & Microbiome

- Phase I, randomized, double-blind, placebo-controlled, dose escalation study (PDF113376) initiated to determine safety, tolerability, and PK profile of GSK’322
- Administered IV as single and repeat dose infusions in 62 healthy subjects
- Three treatment regimens: 1) placebo; 2) IV-only and; 3) oral-IV BID
- Stool samples were collected with consent at pre-dosing and end-of-study for Illumina DNA sequencing of microbiome 16S rRNA V4 region (Total $n = 119$ samples)

Arat et al. 2015. *Antimicrobial Agents and Chemotherapy* 59:1182
Microbiota Overall Diversity & Drug Effects

- **Beta-diversity** is a relative index of microbial community diversity.

- **Oral/IV** dosing regimes notably changed the baseline microbiome while **IV only drug dosing and placebo** induced minimal change.

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Arat et al. 2015. *Antimicrobial Agents and Chemotherapy* 59:1182
Phylogeny of PDF proteins in pathogens and microbiota

- Firmicutes, Bacteriodetes decreased overall
- Proteobacteria, Bifidobacterium increased
- Enterobacter MICs > 8 and increased in gut
- Prevotella had low MICs and gut Prevotella decreased
- Many species were not significantly changed
Inferred Metagenomic Changes


- **Oral IV pre vs Oral IV end** – changes related to antibiotic stress
  - Increase in membrane transport – i.e. Efflux pumps
  - Increase in xenobiotic biogradation and metabolism
  - Decrease in overall metabolism and cell growth
  - No significant changes in ESBLs, QNRs, etc.

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Arat et al. 2015. *Antimicrobial Agents and Chemotherapy* 59:1182
Antibiotics, Microbiome and Human Health

- Global healthcare urgency for new classes of antibiotics

- Also growing evidence that long term exposure to antibiotics has negative effects on human health

- GSK study suggests antibiotic properties are important
  - Delivery regime – Oral vs IV-or
  - Specific versus broad bacterial targets

- Recommend microbiome studies in future antibiotic trials to understand risk vs benefits

"We are in danger of returning to a pre-antibiotic era"
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Recent studies have generated large scale human/host genomic datasets:

- siRNA
- mRNA
- Proteomics
- Yeast-2-Hybrid
- miRNA

Integrated pathway and network analyses attempt to define the host-pathogen interactome.
Drugging the Host-Pathogen Interactome

**Pros:**
- Pre-empt evolution of drug-resistant pathogens
- Broader collection of available targets and compounds
- Multi- or pan-pathogen therapies

**Cons:**
- Potential toxicity through modulation of human target
- New complex biology – discovery phase of host-pathogen interactions
- Unknown effects on healthy microbiome
Host Response to Respiratory Infection

- Analysis of human gene expression studies across seven common respiratory tract viruses
  - Respiratory syncytial virus (RSV)
  - Metapneumonia virus
  - Influenza A virus
  - Coronavirus (SARS)
  - Rhinovirus
  - Coxsackievirus
  - Cytomegalovirus

- 67 pathways in common among all seven viruses

- Suggest five new therapeutic indications for existing small molecules or biologicals
  - F3 – Recombinant Coagulation factor VIIa
  - IL1B – Antagonists such as Canakinumab
  - TNF – Antagonists such as Pranlukast
  - CASP1 – Antagonists to reduce inflammatory damage
  - MMP9 – Antagonists to modulate NLRP3 inflammasome
Novel Pathways in Infectious Disease

- Parkin-Ubiquitin Proteasomal System involved in the progression of Parkinson disease

- Pathway enriched across 5 viruses based on human mRNA microarray analysis


Smith et al. 2012 *PLoS One* e33174
Host-Respiratory Bacteria Interactome

- Meta-analysis of human gene expression data after *Pseudomonas aeruginosa* or *Streptococcus pneumoniae* infection
- SUMO conjugation pathway identified which also is essential for viral replication

Human-Microbe Disease Interactions

- Respiratory viral and bacterial pathogens activate common host pathways

- *Mycobacterium tuberculosis* (TB) interactome analysis in progress

- Further experimental validation of targets is on-going

![Gene and Pathway Venn Diagram](image-url)
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Pathogens, Host Response, Microbiome in COPD

Respiratory Infections
- Respiratory Synovial Virus
- Metapneumovirus
- Influenza virus
- Coronavirus
- Rhinovirus
- Haemophilus influenzae
- Moraxella catarrhalis
- Pseudomonas aeruginosa
- Streptococcus pneumoniae

Pathogen Driven COPD

Chronic obstructive pulmonary disease (COPD) is inflammation associated lung damage

78% of COPD exacerbations associated with viral/bacterial infections

Papi et al. 2006 Am J Respir Crit Care Med
The Lung Microbiome In Respiratory Disease

- Lung is not sterile

- Lung microbial community is associated with various respiratory diseases

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Erb-Downward et al. 2011

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Asthma

COPD

Cystic Fibrosis

Bronchiectasis

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Analysis of the Lung Microbiome in the “Healthy” Smoker and in COPD

Analysis of the Airway Microbiota of Healthy Individuals and Patients with Chronic Obstructive Pulmonary Disease by T-RFLP and Clone Sequencing

Decade-long bacterial community dynamics in cystic fibrosis airways

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

Lung Microbiota and Bacterial Abundance in Patients with Bronchiectasis when Clinically Stable and during Exacerbation
Lung Microbiome Observational Studies in COPD

1) COPD-BEAT Cohort (Wang et al. 2016. European Resp. J. 47:1082)
   - n = 87 COPD subjects
   - Single site study: University of Leicester
   - Samples: stable, exacerbation, treatment, recovery

2) COPD-MAP (in prep)
   - n = 285 COPD subjects
   - 3x sites: Imperial, Leicester, Manchester
   - Samples: stable + exacerbation

3) AERIS (GSK Vaccines) (in prep)
   - n = 127 COPD subjects
   - Single site study: University of Southampton
   - Samples: monthly visits (stable) + exacerbation
GSK/U Leicester COPD Microbiome Study

- 87 patients
- 139 visit series
- 476 sputum samples

Stable, Exacerbation, Post-Therapy and Recovery (collected from previously published patient cohort 2008 – 2010)

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Bafadhel et al. 2011 Am J Respir Crit Care Med 184: 662

Lung Microbiome Compared to Other Body Sites

- Beta diversity plot of microbiomes from the lung (this study) and other body sites (HMP)
- Lung microbiome is distinct
- Some overlap between oral and lung microbiomes

Exacerbation samples showed:

- (a) Overall reduced diversity during exacerbations
- (b) Increased ratio of Proteobacteria : Firmicutes driven by increased *Moraxella* sp and decreased *Streptococcus* sp.
- (c) 36 of 87 patients showed an increase in *Moraxella* sp.

Different Exacerbation Phenotypes

**Acute Exacerbations of Chronic Obstructive Pulmonary Disease**

Identification of Biologic Clusters and Their Biomarkers

Mona Bafadhel¹,², Susan McKenna¹, Sarah Terry¹, Vijay Mistry¹,², Carlene Reid¹, Pranabashis Haldar², Margaret McCormick³, Koirobi Haldar², Tatiana Kebadze⁴, Annelyse Duvoix⁵, Kerstin Lindblad⁶, Hemu Patel⁷, Paul Rugman⁵, Paul Dodson³, Martin Jenkins³, Michael Saunders³, Paul Newbold¹, Ruth H. Green¹, Per Venge⁶, David A. Lomas³, Michael R. Barer⁵,⁷, Sebastian L. Johnston⁴, Ian D. Pavord¹, and Christopher E. Brightling¹,²

¹Institute for Lung Health, and ²Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom; ³AstraZeneca R&D Charnwood, Loughborough, Leicestershire, United Kingdom; ⁴Department of Respiratory Medicine, National Heart and Lung Institute, Centre for Respiratory Infections, Imperial College London, United Kingdom; ⁵Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom; ⁶Department of Medical Sciences, Clinical Chemistry, University of Uppsala, Uppsala, Sweden; and ⁷Department of Clinical Microbiology, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom

Bafadhel et al. 2011 Am J Respir Crit Care Med 184: 662

- **Bacterial (B):** positive bacterial pathogen (HI, MC, SP, SA, PA) on routine culture, or total aerobic CFU >= 10^7 cells (micro_culture1)
- **Viral (V):** positive sputum viral PCR
- **Eosinophil (E):** eosinophil percent >= 3% nonsquamous cells
- **Pauciinflammatory (Pauci):** others, limited changes in the inflammatory profile
Exacerbation Microbiome / Phenotypes Profiles

* ANOVA FDR Corrected P < 0.05

Beta diversity plot of bacteria and eosinophil exacerbations

Antibiotic vs Steroid Treatment Effects

- Steroids and antibiotics had different effects on microbiome diversity and composition

**Haemophilus influenzae as a Keystone Species?**

- All 476 samples
- 119 OTUs with $\geq 10\%$ prevalence
- Top 200 significant correlations
- OTUs colored in the class level of taxonomy
- Red edges = negative correlation
- Green edges = positive correlation

Sputum IL8 Most Associated with Microbiome

- All 476 samples
- 119 OTUs with >=10% prevalence
- 67 clinical variables
- Top 100 significant correlations for OTU-OTU, OTU-clinical, and clinical-clinical
- OTUs colored in the class level of taxonomy
  - Red edges = negative correlation
  - Green edges = positive correlation

Emerging Model of the Microbiome in COPD

- Further confirmation on-going with larger patient cohorts

Lung Microbiome – Public Interest

New Science – New Therapeutic Paradigms

Disease
- Infectious disease: i.e. RSV, HIV, HCV, bacteria
- Chronic disease: i.e. Obesity, Diabetes, COPD, Asthma, etc.

Therapeutic
- Microbial Targets: Infectious agent, Microbiome
- Human Targets: Host interactome, Human pathways

Current strategies
Future strategies
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  - Sebastian L. Johnston (Imperial College)
  - Mohammadali Yavari Ramsheh
  - Michael R. Barer
  - Christopher E. Brightling

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The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.