The contribution of microbiota in gut and lung physiology

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More than one A co-evolution process

Individual organism

- 10 à 100 more microbes than our own cells;
- 100 fold more bacterial genes than human genes;
- 37% of similarity between microbes/archaea and human genomes: co-evolution.

McFall-Ngai et al., PNAS 2013
✓ Nasal and skin microbial ecosystems share similarities

✓ Gastrointestinal and oral communities are specific

Grice et al, Annu Rev Genomics Hum Genet 2012
HMP Consortium et al., Nature 2012
Microbial ecosystems in our body

Abondance relative

Skin

Vaginal

Intestinal

Buccal

Bacteroidetes

Firmicutes

Actinobacteria

Proteobacteria

Grice et al, Annu Rev Genomics Hum Genet 2012

HMP Consortium et al., Nature 2012
The intestinal microbiota and gut physiology
The digestive role of the intestine

**Function**
- Barrier
- Absorption: 80%
- Secretion: 20%

**Composition**
- Absorptive cells
- Goblet cells
- Paneth cells
- Tuft cells
- Stem cells
- Entero-endocrine cells

**Life cycle**
- M cells
- LGR5+ stem cell

The immune role of the intestine

**Procaryotic component of the gut**

**Function**
- Protection
- Digestion
- Synthesis of metabolites
- Immuno-modulation

**Composition**
- **Firmicutes** 60 à 80 %
- **Actinobacteria**
- **Bacteroidetes** 20 à 40 %
- **Proteobacteria**

**Life cycle**
- Age
- Diet
- Diseases

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The gut: is an hybrid organ

Co-evolution
Tolerance
Cooperation
Competition
The intestinal microbiota and gut physiology

Who are they?
What are they doing?
Most abundant groups

- **Bacteroides-Prevotella group**: 8.5–28%
- **Clostridium leptum group**: 21.1–25.2%
- **Clostridium cocoides-Eubacterium rectale group**: 22.7–28.0%

**Gram-negatives 10–30%**

**Enterobacteriaceae**: 0.1–0.2%

**Akkermansia group**: 1.3%

**High GC Gram-positives**: 8–17%

- **Bifidobacterium**: 4.4–4.8%
- **Atopobium group**: 3.1–11.9%
- **Eubacterium cylindroides group**: 1.1–1.4%
- **Eubacterium-Eubacterium rectale group**: <0.1–1.3%

**Low GC Gram-positives 46–58%**

- **Lactobacillus-Enterococcus group**: <0.1–1.8%

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1. Oro-anal gradient of bacteria

- **Duodenum**, $10^4$ UFC/g
  - *Streptococcus, Lactobacillus, Clostridium, Prevotella*.

- **Ileum distal**, $10^7-10^8$ UFC/g
  - *Lactobacillus, Clostridium, Ruminococcus, Eubacterium, Bacteroides, Bifidobacterium, Enterobacteriaceae*.

- **Jejunum**, $10^5-10^7$ UFC/g
  - *Streptococcus, Lactobacillus, Clostridium, Prevotella*.

- **Colon**, $10^{11}-10^{12}$ UFC/g
  - *Lactobacillus, Clostridium, Ruminococcus, Eubacterium, Bacteroides, Bifidobacterium, Enterobacteriaceae*.

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*Sartor et al., IBD 2007  
Costello, Lauber et al., Science 2009  
Coudeyras and Forestier, Rev Microbiol 2010  
Marchesi, Env. Microbiol 2011*
2. Luminal *versus* mucosa-attached microbes

*Friedrichsen and Stappenbeck, The ISME Journal 2011*
*Pedron, Mulet et al., mBio 2012*
*Lee, Donaldson et al., Nature 2013*
The proximity of microbes to the epithelium changes with diet.

Earle et al., 2015, Cell Host & Microbe 18, 1–11
The microbiota is fluctuating with our environment.
Selection forces that shape our intestinal microbiota

The intestinal microbiota is dynamic and alive.
Functions of the intestinal microbiota

100 000000000000 microbes in our digestive tract

What are they doing?

Protective role and Digestion

Structural and « educative » roles
Barrier effect of the intestinal microbiota

Protection

Pathogens

Health → Proliferation → Translocation → Disease

Barrier effect of the intestinal microbiota

Competition for niche and nutrients
High production of anti-microbial peptides
Metabolic activity

Competition between health-related microbes and pathogens
Energetic supply by the intestinal microbiota

- Fermentation of polysaccharides by microbes
  - Production
    - Acetate
    - Propionate
    - Butyrate
  - Absorption by epithelium
    - Butyrate is the energy source used by epithelial cells
      - anti-inflammatory effect
      - protection by mucus synthesis
      - Differentiation

Macfarlane et al, J Clin Gastroenterol, 2011
The intestinal microbiota: a main actor of digestion

**Homeostasis**

- Metabolism of amino acids
  - 1 - 20% lysine et threonine

- Metabolism of polysaccharides
  - Production of SCFA
    - 0.5 - 0.6 mol/day
    - 140-180 Kcal/day (European)

- Synthesis of vitamins
  - vit. K(3µg/day) ; B12 (15-20 ng/day)

- Metabolism of cholesterol

- Energy homeostasis

- Metabolism of micro-constituants
  - Glucosinolates, polyphenols
In germ free rodents: deficiency in T cells (CD4 and CD8)

Immuno-maturation by the intestinal microbiota

McPherson et al, 2004
Immuno-modulation by the intestinal microbiota

Hollister E et al; Gastroenterology, Volume 146, Issue 6, 2014, 1449–1458
**F. prausnitzii** : anti-inflammation

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**In Crohn’s disease:**

- **Fecal flora**
  - *Bacteroides*
  - *Peptostreptococci*
  - *Coprococci*
  - *Clostridium*
  - *Escherichia coli*

- **Swidsinski et al., 2002; Manichanh et al., 2006; Seksik et al., 2006; Sokol et al., 2009**

- **Bifidobacterium**
  - *Lactobacillus*
  - *Faecalibacterium prausnitzii*

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**F. prausnitzii** is considered as a sensor and an actor of intestinal health: New probiotic?

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The microbiota shapes the intestinal epithelium

Intestinal structure

Absorption
Secretion
Protection
Renewal

Epithelium education

Friedrichsen and Stappenbeck, The ISME Journal 2011
Pedron, Mulet et al., mBio 2012
Lee, Donaldson et al., Nature 2013
Morphogenic effect of microbiota

Epithelium education

Cherbuy et al, Am. J. Physiol, 2010
## The microbiota and intestinal epithelium

### Dynamic cross-talk

<table>
<thead>
<tr>
<th>Structure</th>
<th>Proliferation</th>
<th>Secretion (like mucus)</th>
<th>Function (absorption)</th>
</tr>
</thead>
</table>

- **Dominant Commensals**
- **Subdominant**

- Simplified model with mini-microbiota

*Tomas, Faseb J, 2013*
*Wrzosek, BMC Biol, 2013*
*Tomas, Isme J, 2015*
*Deschemin, Faseb J, 2015*
Germ free

Commensals: B. thetaiotaomicron

Primo-colonizing bacteria: E. coli

Gnotobiotic

Bacteria involved in Mucus secretion, composition, remodeling

Bacteria involved in epithelium renewal

The co-evolution and co-operation between microbiota and gut

- Digestion
- Protection
- Education

Better understand functional cross-talk
Nutritional innovation and new medicine
Integrated physiology

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Integration of the lung microbiota in our project

Intestinal strains
Human complex microbiota
Probiotic strains

Immunity and epithelium

Health impact?

Lung Microbiota

Immunity and Epithelium
The lung contains its own microbiota

- 2000 bacterial genome / cm² lung
- Different from other mucosa
- Several respiratory diseases are associated to lung dysbiosis
ALRIGHT project: **Applied Lung bacteria for Health**

- Lung microbiota
- Immunity / epithelium / sensitivity to asthma
Asthma is a chronic non-curable lung disease.

- Allergic extrinsic asthma
- Long-term disease that has no cure (risk of death from asthma is 1 in 10,000 persons with asthma)

Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells
DOI: 10.1126/science.aac6623
Inflammatory mechanism of Asthma

Th2 response is exacerbated in asthma
1. Influence of lung microbiota on lung physiology?
2. Viable bacteria in the lung at steady state or during asthma?
3. What are they doing?
4. Do the lung strains modulate a pathology like asthma?
1. Influence of lung microbiota on lung physiology?

- Adults C57BL/6 Germ free vs Adults C57BL/6 SPF
1. Influence of lung microbiota on lung physiology?

**A**

HES (x20)

GF

SPF

**B**

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<thead>
<tr>
<th>Gene</th>
<th>GF</th>
<th>SPF</th>
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<tbody>
<tr>
<td>TSLP</td>
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<td>IL-10</td>
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<td>IFNγ</td>
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<tr>
<td>Muc5AC</td>
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</tbody>
</table>

At steady-state, different expression of innate molecules
1. Influence of lung microbiota on lung physiology?

Lung DC, T and B cells: in the same proportion in GF and SPF mice
1. Influence of lung microbiota on lung pathology?

6-7 days old

Neonates C57BL/6

- **d0**: HDM (1µg) or PBS i.n
- **d7**: HDM (10µg) or PBS i.n, 5 consecutive days

vs

- Germ free
- SPF
1. Influence of lung microbiota on lung pathology?

HDM-induced asthma is not reduced / exacerbated in GF neonates
1. Influence of lung microbiota on lung pathology?

Lung histology

GF HDM  SPF HDM

HDM-induced asthma is not reduced / exacerbated in GF neonates
ALRIGHT conclusion 1

1. Influence of lung microbiota on lung patho-physiology?

At steady state: differences in GF vs SPF (innate immunity)
After HDM challenge: sensitivity of GF and SPF are equivalent
GF are armed to face environmental challenges
1. Influence of lung microbiota on lung physiology?

2. Recover viable bacteria from the lung?

Germ free vs SPF

Culture method
2. Viable bacteria in the lung at steady state?

Steady state

- BAL bacteria (cfu/g)
- Lung bacteria (cfu/g)

20 strains have been isolated from lung microbiota
2. Viable bacteria in the lung at steady state?

Cultivatable bacteria in the lung as early as 3 days ($10^4$ CFU/g)

20 strains were isolated
Lung bacteria have different immuno-modulating properties –

Strain 3 = pro-Th1 and Strain 4 = pro-Th2

After i.n, bacteria can be found in the nasal cavity, in BAL and Lung
1. Influence of lung microbiota on lung physiology?
2. Viable bacteria in the lung at steady state or during asthma?
3. What are they doing?
4. Do the strains 3 and 4 modulate the pathology?

**Protocol**

6-7 days old

- **d-2**
  - HDM (1µg) or PBS i.n

- **d0**
  - HDM (1µg) or PBS i.n

- **d7**
  - HDM (10µg) or PBS i.n (5 consecutive days)

- **d11**
  - 10^6 ufc strain 3 or strain 4 or PBS

- **d14**
  - 10µL i.n every two days

- **d16**
4. Do the strains 3 and 4 modulate asthma features?

End-points: Growth delay / eosinophils in BAL / infiltration of epithelium / mucus production / Ig E / Il5 / IgG1 production ...

Health parameters

- PBS: +++
- HDM: --
- Strain 3: +++
- Strain 3 + HDM: ++
- Strain 4: +/-
- Strain 4 + HDM: ----

HDM treatment induced asthma
Strain 4 exacerbated asthma
Strain 3 protected against asthma
Modification of microbiota ecology by i.n of bacteria changes asthma features

**Sterile environment**
- Mucosal and immune immaturity
- If aeroallergen: asthma

**Equilibrium state Bacteria / Host**
- Mucosal and immune maturation
- If aeroallergen: asthma

**Unbalanced Bacteria / Host**
- Inflammation
- If aeroallergen: severe asthma

Remot et al, in preparation
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Thanks a lot

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