Bacteriotherapy in IBD

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Micalis

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Saint-Antoine Hospital

AVENIR team U1057
Disclosures

- Consulting for Danone, Astellas, Enterome
Outline

- The gut microbiota
- Why targeting the gut microbiota in IBD?
- Therapeutic interventions targeting the microbiota
- Microbiota as a biomarker
Outline

• The gut microbiota

• Why targeting the gut microbiota in IBD?

• Therapeutic interventions targeting the microbiota

• Microbiota as a biomarker
The human intestinal microbiota

- faecal microbiota: $>10^{11}$ $\mu$org/g
- hundreds of species ...
- adapted and functionally stable ...
- nutrition, physiology, immunity & protection
- genetic repertoire: $\sim 150 \times$ human genome
> 1250 species identified belonging to 3 major phyla

- **Actinobacteria**
- **Bacteroidetes**
- **Firmicutes**

Wilson *et al* 1996  
Suau *et al* 1999  
Bonnet *et al* 2002  
Hold *et al* 2002  
Hayashi *et al* 2002  
Hayashi *et al* 2003  
Wang *et al* 2003  
Mangin *et al* 2004  
Manichanh *et al* 2006  
Eckburg *et al*. 2005
High Inter-individual variability at the bacterial level, but ………………………………………

High inter-individual variability at the bacterial level, but some species are present in everyone.

Core microbiome

**Faecalibacterium prausnitzii SL3**

*Roseburia intestinalis M50* 1

*Bacteroides vulgatus ATCC 848*

*Bacteroides sp. 9_1_42FAA*

*Ruminococcus sp SR1* 5

*Coprococcus comes SL7* 1

*Bacteroides sp. 2_1_7*

*Bacteriodes xylanisolvens XB1A*

*Ruminococcus torques L2-14*

*Bacteroides sp. 2_2_4*

*Bacteroides sp. D4*

*Bacteroides dorei*

*Ruminococcus obeum A2-162*

*Ruminococcus lactaris*

*Bacteroides capillosus*

*Bacteroides finegoldii*

*Clostridium sp M62* 1

*Clostridium nexile*

High Inter-individual variability at the bacterial level, but stability at the functional level.
• The gut microbiota

• Why targeting the gut microbiota in IBD?

• Therapeutic interventions targeting the microbiota

• Microbiota as a biomarker
Why targeting the gut microbiota in IBD?

- Role of fecal stream in post operative recurrence of CD
- Animal model of colitis depend on gut microbiota
- Spontaneous colitis in some genetically modified mice can be transmitted to WT mice via the gut microbiota (TRUC mice, NLRP6 KO...)
- Polymorphisms of innate immunity genes involved in bacterial sensing: associated with IBD (GWAS)
Genes in IBD

Crohn’s Disease  
140 risk loci

Ulcerative colitis  
133 risk loci

Identified in GWAS

30

110

23

Biological processes involved in IBD loci

Epithelial Barrier  
Innate Immunity  
Autophagy  
Adaptive immunity

Genes in IBD

Crohn’s Disease
140 risk loci

Ulcerative colitis
133 risk loci

Identified in GWAS

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Biological processes involved in IBD loci

- Epithelial Barrier
  - Microbiota
    - Innate Immunity
    - Autophagy
    - Adaptive immunity

...and the Microbiota is abnormal in IBD patients

Form of Principal Components Analysis

Composition

Morgan, Tickle, Sokol et al. Genome Biology 2012
...and the Microbiota is abnormal in IBD patients

Composition

- Bacteroidetes
- Actinobacteria
- Firmicutes
- Proteobacteria
...and the Microbiota is abnormal in IBD patients

Composition

- Bacteroidetes
- Actinobacteria
- Proteobacteria
- Firmicutes

Functions

- Secretion system
- Cyst/Meth Metab.
- Butanoate Metab.
- Lys biosynth
- Pentose Ph pathway
...and the Microbiota is abnormal in IBD patients

Changes in microbial function in IBD: more consistent than changes in composition

Morgan, Tickle, Sokol et al. Genome Biology 2012
Outline

- The gut microbiota
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- Microbiota as a biomarker
Therapeutic interventions targeting the microbiota

Normal microbiota

Adapted from Lozupone et al. Nature 2012
Therapeutic interventions targeting the microbiota

Adapted from Lozupone et al. Nature 2012
Therapeutic interventions targeting the microbiota

Adapted from Lozupone et al. Nature 2012

Normal microbiota

IBD associated dysbiosis

Antibiotics

Devastation
Therapeutic interventions targeting the microbiota

Adapted from Lozupone et al. Nature 2012
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Adapted from Lozupone et al. Nature 2012

Normal microbiota

IBD associated dysbiosis

Antibiotics

Probiotics

Prebiotics

Other

Fecal Transplantation

Restored ecosystem

Devastation
<table>
<thead>
<tr>
<th>Condition</th>
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<tbody>
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</table>
Prevention of post operative recurrence in CD

- **Nitroimidazoles are effective but is not well tolerated**

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Treatment</th>
<th>Control</th>
<th>Risk ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
</tr>
<tr>
<td>Rutgeerts 1995</td>
<td>2</td>
<td>30</td>
<td>31.8%</td>
</tr>
<tr>
<td>Rutgeerts 2005</td>
<td>3</td>
<td>40</td>
<td>68.2%</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>5</td>
<td>70</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 0.14, df = 1 (P = 0.71); I² = 0%
Test for overall effect: Z = 3.18 (P = 0.001)

- **Probiotics are not effective so far** (but high heterogeneity between studies)

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Probiotic</th>
<th>Placebo</th>
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<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
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<tr>
<td>Marteau 2006</td>
<td>4</td>
<td>48</td>
<td>37.2%</td>
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<tr>
<td>Prantera 2002</td>
<td>3</td>
<td>23</td>
<td>25.9%</td>
</tr>
<tr>
<td>Van Gossum 2007</td>
<td>4</td>
<td>34</td>
<td>36.9%</td>
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<tr>
<td>Total (95% CI)</td>
<td>105</td>
<td>108</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Total events: 11
Heterogeneity: Chi² = 0.00, df = 2 (P = 1.00); I² = 0%
Test for overall effect: Z = 0.77 (P = 0.44)
Therapeutic interventions targeting the microbiota

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**S. boulardii does not prevent relapse of CD**

Boureille et al. CGH 2013

S. boulardii 1g/j vs placebo

N=165 patients
Steroid- or salicylate-induced remission
Therapeutic interventions targeting the microbiota

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Rifaximin-Extended Intestinal Release Induces Remission in Patients With Moderately Active CD

402 patients with moderately active CD

400, 800, and 1200 mg rifaximin-EIR, x2/d for 12 weeks.
## Therapeutic interventions targeting the microbiota

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2-week triple antibiotic therapy (amoxicillin, tetracycline, and metronidazole) produced improvement, remission, and steroid withdrawal in active UC.

Patients with active ulcerative colitis (Mayo scores of 6 – 12).

Okhusa et al. AJG 2010
2-week triple antibiotic therapy (amoxicillin, tetracycline, and metronidazole) produced improvement, remission, and steroid withdrawal in active UC.

Patients with active ulcerative colitis (Mayo scores of 6 – 12).

Steroid withdrawal rates in steroid-dependent cases ($n = 100$).
Therapeutic interventions targeting the microbiota

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**Pouchitis**

+ +
Therapeutic interventions targeting the microbiota

Normal microbiota

Antibiotics

Devastation

IBD associated dysbiosis

Other

Probiotics

Prebiotics

Restored ecosystem

Fecal Transplantation

Adapted from Lozupone et al. Nature 2012
Fecal transplantation

Healthy microbiota

IBD-associated microbiota
# Fecal transplantation

## Uncontrolled observations

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>IBD Type</th>
<th>Administration</th>
<th>Effects on IBD</th>
<th>Follow up</th>
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<tbody>
<tr>
<td>Bennet 1989</td>
<td>1</td>
<td>UC</td>
<td>enema</td>
<td>stopped</td>
<td>resolution</td>
</tr>
<tr>
<td>Borody 1989</td>
<td>2</td>
<td>UC</td>
<td>NR</td>
<td>stopped</td>
<td>resolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD</td>
<td>NR</td>
<td>stopped</td>
<td>resolution</td>
</tr>
<tr>
<td>Borody 2011</td>
<td>3</td>
<td>UC</td>
<td>enema</td>
<td>stopped</td>
<td>resolution</td>
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<tr>
<td>Borody 2003</td>
<td>6</td>
<td>UC</td>
<td>enema</td>
<td>stopped</td>
<td>resolution</td>
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<td>Grehan 2010</td>
<td>1</td>
<td>CD</td>
<td>Colonoscopy</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Borody 2011</td>
<td>1</td>
<td>UC</td>
<td>NR</td>
<td>NR</td>
<td>Reduction</td>
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<td>Vermeire 2012</td>
<td>4</td>
<td>CD</td>
<td>Nasojejunal</td>
<td>unchanged</td>
<td>unchanged</td>
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<tr>
<td>Angelberger 2012</td>
<td>5</td>
<td>UC</td>
<td>Nasojejunal</td>
<td>NR</td>
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</tr>
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</table>

23 cases in the littérature :

→ 14 improved
→ 6 unchanged
→ 2 worsen
→ 1 ?
Fecal transplantation

Prospective Pilot study:
- 10 children between 7 and 21 (1 not analyzed)
- Mild to moderate active UC (PUCAI: 15 to 65)
- Stable disease activity and medical treatment for 2 months
- FMT by enema without colon cleansing
Fecal transplantation

Prospective Pilot study:
- 10 children between 7 and 21 (1→ not analyzed)
- Mild to moderate active UC (PUCAI: 15 to 65)
- stable disease activity and medical treatment for 2 months
- FMT by enema without colon cleansing

→ Clinical response: 78% at 1w, 67% at 1 month
→ Remission: 33% at 1w and maintained at 1 month
→ Globally well tolerated
Fecal transplantation

Randomized control studies needed
→ define procedures
→ define indications
Fecal transplantation

Randomized control studies needed
→ define procedures
→ define indications

ClinicalTrials.gov  9 ongoing studies (4 RCT)
Fecal transplantation

Randomized control studies needed
→ define procedures
→ define indications

ClinicalTrials.gov

9 ongoing studies (4 RCT)

Start of a pilot RCT in Crohn’s disease soon in our Department in St Antoine Hospital
Therapeutic interventions targeting the microbiota

Adapted from Lozupone et al. Nature 2012

Normal microbiota → Devastation → Other

IBD associated dysbiosis

Antibiotics → Probiotics → Prebiotics → Fecal Transplantation → Restored ecosystem
Future therapeutics

- Counterbalancing dysbiosis
- Recombinant probiotics
- Other
Future therapeutics

Counterbalancing dysbiosis

Pro-inflammatory Bacteria

Anti-inflammatory Bacteria
Future therapeutics

Counterbalancing dysbiosis

The case of *Faecalibacterium prausnitzii*

**Bar Chart**
- **Faecalibacterium prausnitzii** levels in different conditions:
  - Self limited colitis
  - Active IBD
  - IBD in remission
  - Healthy subjects

**Graphs**
- Comparison between Control and Ileal CD
- **Pro-inflammatory Bacteria** vs. **Anti-inflammatory Bacteria**

Sokol et al. IBD 2009; Morgan, Tickle, Sokol et al. Genome Biology 2012
Future therapeutics

Counterbalancing dysbiosis

*Faecalibacterium prausnitzii* has anti-inflammatory effects

*In vitro*

- Caco-2 cells
- NFkB
- IL8

*In vivo*

- TNBS colitis

Sokol et al. PNAS 2008
Future therapeutics

Counterbalancing dysbiosis

*Faecalibacterium prausnitzii* has anti-inflammatory effects

- Give *F. prausnitzii* to IBD patients
- Identify and give active *F. prausnitzii* products

Ongoing ...

Caco-2 cells

Sokol et al. PNAS 2008
Recombinant Probiotics:
Use good bugs to deliver proteins of health interest
Future therapeutics

Recombinant Probiotics: IL-10

Use good bugs to deliver proteins of health interest

- *L. lactis* delivering IL-10
  - Efficient in DSS-induced colitis and in spontaneous colitis in IL10 KO mice
  - Good signal in Phase I clinical trial in CD

- Effective Biocontainment (auxotroph strain)

Steildler et al. Science 2000
Future therapeutics

Recombinant Probiotics: IL-10

Use good bugs to deliver proteins of health interest

- *L. lactis* delivering IL-10
  - Efficient in DSS-induced colitis and in spontaneous colitis in IL10 KO mice
  - Good signal in Phase I clinical trial in CD

- Effective Biocontainment (auxotroph strain)

- Results of phase 2A clinical trial disappointing (not published)

Steildler et al. Science 2000
Future therapeutics

Recombinant Probiotics: Elafin

High Proteolytic Activity in IBD

Future therapeutics

Recombinant Probiotics: Elafin

High Proteolytic Activity in IBD

Low antiprotease expression in IBD (Elafin)

Future therapeutics

Recombinant Probiotics: Elafin

High Proteolytic Activity in IBD

Low antiprotease expression in IBD (Elafin)

Counterbalancing this defect with probiotics delivering Elafin (antiprotease expressed in healthy intestinal mucosa)

Future therapeutics

Recombinant Probiotics: Elafin

Elafin expressing lactic acid bacteria is efficient in colitis model:

Elastolytic activity

Future therapeutics

Recombinant Probiotics: Elafin

Elafin expressing lactic acid bacteria is efficient in colitis model:

Future therapeutics

Recombinant Probiotics: Elafin
Elafin expressing lactic acid bacteria is efficient in colitis model:

Construct biologically contained strains

Clinical trial in IBD patients

Future therapeutics

Other tracks...

- **Synthetic stool / simplified microbiota**
  - Consortium of gut bacteria (already used in Cdiff)

- **Manipulation of the microbiota**
  - **Phagotherapy**
  - **Use of quorum sensing-derived molecules**

Tvede et al. Lancet 1989
The gut microbiota

Why targeting the gut microbiota in IBD?

Therapeutic interventions targeting the microbiota

Microbiota as a biomarker
20 patients with active CD, requiring ileo-caecal resection

M0
Surgical resection

M6
colonoscopy

Still in remission
or
Endoscopic recurrence

FISH analysis of biopsies

- Eub338 (Eubacteria)
- Bac303 (Bacteroides-Prevotella)
- Ent1458 (Enterobacteria)
- Erec482 (Clostridium coccoides)
- Lab158 (Lactobacillus-Enterococcus)
- Bif164 (Bifidobacterium)
- Fprau645 (F. prausnitzii)

Dapi + Erec-Cy3

Sokol et al.  PNAS 2008
20 patients with active CD, requiring ileo-caecal resection

Surgical resection

M0

F. prausnitzii (M0)

3.3%±3.4 → Remission at M6

Remission at M6

0.3%±0.5 → recurrence at M6
(p=0.027)

Endoscopic recurrence

F. prausnitzii at M0
20 patients with active CD, requiring ileo-caecal resection

F. prausnitzii level at surgery: predictive of endoscopic recurrence at 6 months?

0.3%±0.5 → recurrence at M6 (p=0.027)
STORI study

CD patients treated for at least one year by IFX + immunosuppressant

IFX discontinuation

Relapse?
CD patients treated for at least one year by IFX + immunosuppressant

IFX discontinuation

Relapse?

Relapse at 1 year 43.9%
CD patients treated for at least one year by IFX + immunosuppressant

IFX discontinuation

Relapse?

Risk factors for relapse
- male sex
- absence of surgical resection
- leukocyte counts >6.0 × 10^9/L
- hemoglobin ≤145 g/L
- C-reactive protein ≥5.0 mg/L
- fecal calprotectin ≥300 μg/g.
STORI study: Relapse rate according to Gut Microbiota at IFX discontinuation
STORI study: Relapse rate according Gut Microbiota at IFX discontinuation

- **F. prausnitzii level:**
  - High
  - Low

- **Relapse free Survival**
  - 0.0
  - 0.1
  - 0.2
  - 0.3
  - 0.4
  - 0.5
  - 0.6
  - 0.7
  - 0.8
  - 0.9
  - 1.0

- **Jour**
  - 50
  - 100
  - 150
  - 200
  - 250
  - 300
  - 350

- **p = 0.02**
STORI study: Relapse rate according Gut Microbiota at IFX discontinuation

- **F. prausnitzii level:**
  - High
  - Low

- **E. coli level:**
  - High
  - Low

Jour

Relapse free Survival

$p=0.02$

$p=0.2$
STORI study: Relapse rate according Gut Microbiota at IFX discontinuation

- F. prausnitzii level:
  - High
  - Low
  \( p = 0.02 \)

- E. coli level:
  - High
  - Low
  \( p = 0.2 \)

- All bacteria level:
  - High
  - Low
  \( p = 0.7 \)

- Bifidobacteria level:
  - High
  - Low
  \( p = 0.9 \)

Rajca et al. In prep
UC: Relapse rate according Gut Microbiota composition

116 UC patients in remission → fecal microbiota analysis & follow up
UC: Relapse rate according Gut Microbiota composition

116 UC patients in remission → fecal microbiota analysis & follow up

Varela et al. APT 2013
116 UC patients in remission → fecal microbiota analysis & follow up
116 UC patients in remission → fecal microbiota analysis & follow up

Microbiota (metagenomics features) are very promising biomarkers to predict:
- Relapse
- Response to treatment
- Possibly other complications

< 12 months (n = 65)  > 12 months (n = 51).
The gut microbiota is both a target and a biomarker in IBD

Until now: Probiotics and antibiotics have either limited effects or tolerability issues

Microbiota-derived bacteria or molecules
Recombinant probiotics
Fecal transplantation

→ Indications to be defined
→ RCT results needed

Current effort to go to clinic
Beyond FMT: bacteriotherapy in intestinal disease

Dr Harry Sokol, Paris, France