Better Food for Better Health
Microbiota and Health: the challenges of a promising approach
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Treatment options: pre and probiotics for treatment of malnutrition and cachexia

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I declare no conflict of interest related to this presentation
1. Human cells: 30,000 genes (genome)
2. Bacterial cells: 1,600 genes (microbiome)
3. The gut microbiota contains up to 10,000 species (10^14 cells) dominated by:
   - Firmicutes (E. rectale - Clostridium, Coccoides, Clostridium leptum)
   - Bacteroidetes (Bacteroides, Prevotella)
   - Actinobacteria (Bifidobacterium)
4. Bacteria are classified as gram negative (LPS) or gram positive

The Gut Microbiota: an internal organ we feed everyday

- 10^{14} bacterial cells in the gut
- Hundreds of metabolites, most with unknown function
- 100 fold more gene in the microbiome than in the human genome
- Contains potentially « harmful » components (1g LPS)

Importance of the gut barrier to keep Microbes « at bay »

Dysbiosis: alterations of gut microbiota composition/function
Linked to host inflammation and/or energy metabolism
A role for gut microbiota in cancer-related malnutrition?

- Cancer cachexia: loss of muscle and fat mass, with consequence on lifespan and quality of life.
- Not only due to radio-chimio-therapy, or appetite loss; also linked to inflammation.
- Frequent; 50-80% cancer patients; associated with colon cancer and acute non-lymphocytic leukemia and chronic myeloid leukemia.

A link between gut microbial dysbiosis and cancer cachexia?

- Tumor
- Inflammation
  - Anorexia
  - Fat mass loss
  - Muscle atrophy
- ↑ Bacterial compounds
- ? Gut barrier function
- ?
Link between gut microbiota in cancer cachexia
Experimental approach

- Community-wide approach to characterize the gut microbiota in two mouse models of cancer cachexia (ectopic tumor transplantation)
- Rapid tumor development, linking to weight loss, with critical outcome from day 12-13
Cancer cachexia C26 model

C26 cells

Muscles

Weight (mg)

D0

D11

Bindels et al., The ISME J, 12/2015
Cancer cachexia C26 model

C26 cells

Muscles

Weight (mg)

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FITC-dextran (µg/ml)

D0

D11

PLASMA dextran FITC after gavage: gut barrier alterations

FITC-dextran (µg/ml)

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Bindels et al ISME J. 2015
Cancer cachexia C26 model

**SMALL INTESTINE : gut function and immunity**

**mRNA levels (relative expression)**

- ZO-1
- occludin
- Muc2
- TCF4
- CD3\(^\gamma\)
- CD11c

**FITC-dextran (µg/ml)**

- Plasma dextran FITC after gavage: gut permeability

- Muscles

- Weight (mg)
  - gastrocnemius
  - tibiais
Cancer cachexia C26 model

**SMALL INTESTINE**

- mRNA levels (relative expression)
  - ZO-1
  - occludin
  - Muc2
  - TCF4
  - CD3γ
  - CD11c

**LIVER : (LPS) inflammation**

- mRNA levels (relative expression)
  - LBP
  - CD14
  - TLR2

**PLASMA dextran FITC after gavage**

- FITC-dextran (µg/ml)
  - gastrocnemius
  - tibiales

Changes in gut microbiota?
Dysbiosis in cancer cachexia

**Decreased diversity**

- **C26 cells**
- Decreased diversity
- Increased «pathobionts»
- Increased Lactic acid producers

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*Bindels et al, Isme J 2015*
Cancer cachexia linked to leukemia model

Bindels et al, Plos ONE 2012
« Probiotic approach » *Lactobacillus reuteri* 100-23 + *Lactobacillus gasseri* 311476 5x10⁸ cfu (BaF3-Lrg group) improves muscle atrophy and modulates systemic inflammation.
Probiotics & Prebiotics in cancer cachexia

**Probiotics:** live microorganisms which, when administered in adequate amounts, confer a health benefit to the host.

i.e. *Lactobacilli*

FAO 2001; Hill et al, Nat Rev Gastroenterol Hepatol 2014

**Prebiotics:** non digestible compounds which stimulate the growth/activity of bacteria that confer health benefits to the host.

i.e. Inulin-type fructans: non digested, fermented by bacteria expressing beta-fructosidase (Bifidobacteria) into gaz and short chain fatty acids

Roberfroid et al, Br J Nutr 2010; Bindels et al, Nat Rev Gastroenterol Hepatol 2015
Prebiotic approach: inulin-type fructans (ITF) added in the diet (5%)

Day 0    Day 1    Day 13

BaF3 cells with Bcr-Abl

ITF

ITF has no effect on lactobacilli level, and does not change muscle atrophy but ….

Decreases cancer cell proliferation in the liver

Decreases systemic inflammation

Increases portal propionate

Bindels et al Br J Cancer 2012
Selected synbiotic approach

Bcr-Abl-expressing BaF3 cells

D0

D1

D13

L. reuteri 100-23

†

ITF

Cachexia

Gut microbiota

Cancer progression

Portal metabolome

Survival

Barrier function

Bindels et al., The ISME J, 2015
Paneth cell differentiation and antimicrobials

mRNA levels (relative expression)

TCF4  Lysozyme  α-defensins  Reg3γ  Pla2g2

- CT
- BaF
- BaF-Lrl

Permeability  Immune system  Antimicrobial peptides
Benefits of the « synbiotic » approach

- **Leukemic cell marker**
  - Liver: mRNA levels (relative expression)
  - Bcr-Abl

- **Muscle weight**
  - Organ weight (% body weight)
  - Cathepsin L

- **LC3**
  - mRNA levels (relative expression)

- **Morbidity score**
  - Score

- **Survival**
  - Fraction survival

Bindels et al., The ISME J, 2015
Modulation of gut microbiota by probiotic and prebiotic controls cancer cachexia in a model of leukemia

L. reuteri 100-23

Bcr-Abl-expressing BaF3 cells

Survival

Gut microbiota

Cachexia

Tumor Proliferation In the liver

Barrier function

Bindels et al., The ISME J, 12/2015
Novel prebiotics (pecto-oligosaccharides POS) avoid fat mass loss in cancer cachexia

Day 0  Day 1  Day 15

ITF 5%

STOP

BaF3 cells with Bcr-Abl

or POS 5%

Figure 1. Structure primaire d’un homogalacturonane — Primary structure of a homogalacturonan.

Bindels, Neyrinck et al. Plos One 2015
Summary, future prospects

• In models of cancer cachexia, common bacterial changes are observed (increase in Enterobacteriaceae, Parabacteroides goldsteinii, decrease in Lactobacilli, in richness and evenness, those changes being independent on food intake.
• Disturbances of the gut barrier function (incl. immunity), which could participate to the systemic inflammation and thereby influence host health.
• Experimental studies support the interest of probiotic and prebiotic approaches in this context.
• Future projects: focus on dysbiosis and inflammation in patients presenting acute myeloïd leukemia – association with cachexia.
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