The tissue microbiota: the paradigm shift to metabolic diseases.
Les Pensières, Juin 2013

REMY BURCELIN
Metabolic diseases: the origins

Nutritional

Genetic

Metagenomic

Metabolic diseases
Gut microbiota: the scientific interest

ARTICLE

A metagenome-wide association study of gut microbiota in type 2 diabetes

Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes

Cell Metabolism

Resource

Genetic Control of Obesity and Gut Microbiota Composition in Response to High-Fat, High-Sucrose Diet in Mice
To make a long story short!
Key questions?

1. Which bacteria as Biomarkers?
2. Which genes targeted?
3. Which regulatory mechanisms?
Model

- Prediabetic 1 week
- Established 4 weeks
- Pre+probiotic (synbiotic)
- Glycemia

HIGH FAT DIET

2
Quantification of bacterial mucosal adherence

2h post-gavage?

GFP-\textit{E. coli}  Mucosa/luminal ratio in CFU

- Controls
- Prediabetic
- Diabetic

Increased bacterial mucosal adherence

(Amar J. Chabo C. et al., EMBO Mol Med 2011)
Regulatory mechanism: the transepithelial bacterial translocation

2. Genes of bacterial recognition and targets?

1. Phenotype of the immune system involved?

Philippe Sansonetti: Institut Pasteur, Paris
C57BL/6
10 weeks

HFD (70%)
NC

Day 0 10 days 30 days
HFD start Immune intestinal cell analyses

Hematopoïetic cells
Phagocytes/Lymphocytes

CD45
Dye 1
Dye 2

Experimental design
Intestinal immune features:
Submitted!
Not shown
An increased intestinal bacterial adherence and an impaired intestinal immune system at the onset of the disease

And what is next!
Where are the bacteria going,
What are they doing?
Intestinal bacterial translocation

Transepithelial translocation of bacteria

(Amar J. Chabo C. et al., EMBO Mol Med 2011)
Bacterial DNA into the lamina propria: FISH Analyses

16S rRNA DNA (FISH)
DAPI
Biotin

(Amar J. Chabo C. et al., EMBO Mol Med 2011)
Bacterial translocation from the intestine to tissues: step by step...

1: Intestinal bacterial adherence
2: Intestinal bacterial recognition by dendritic cells
3: Intestinal bacterial phagocytosis
4: Intestinal bacterial translocation through the epithelium
5: Intestinal immune cells carrying bacteria
6: Bacterial DNA within immune cells
6: Bacteria carried by immune cells within adipose tissue ...

⇒ regulation of gene expression !!!!!

Tissue microbiota Microbiota : metafactors and inflammation
A paradigm shift for the discovery of new therapies for cardiometabolic diseases

Our tissues carry bacterial genes that control our genomes and can lead to pathologies....

# colony forming units (Living bacteria) per 100 mg of tissue: Anaerobes

![Graph showing the number of colony forming units (Living bacteria) per 100 mg of tissue for different tissues: Sub cut AT, Muscle, Mes AT, and Mes Lymph node. The graph compares controls and diabetics.]
Cells from the adipose stroma vascular do carry fully alive bacteria

A tissue microbiota is established!

Lolmed, Burcelin, Bouloumie, Schertzer et al submitted
How do the cells get through?
What is the genetic control?
Molecular candidates

Intestinal lumen

Change in diet

Change in microbiome

Translocation

Metabolites SCFA...

FIAF

Bacterial antigens
- LPS
- PGN
- Pilin/flagellae
- Fimbriae
- DNA

Intestinal immune cell

PRRs
- TLR2, TLR5, TLR4, NLRP
- TLR6, TLR9, NOD1/2

Myd88/NFκB

Metabolic inflammation

Host intestinal tissue
• Pattern recognition receptors: Point of convergence of bacterial and nutrient sensing
  • TLRs, PKR, NLRs

Schertzer et al. *Diabetes*, 2011
PGN causes insulin resistance

Hyperinsulinemic euglycemic clamp 6 hours post ligand injection

Schertzer et al. *Diabetes*, 2011
NOD1<sup>−/−</sup> = Insulin Sensitization

Schertzer et al submitted

Amar, Chabo et al EMBO MM 2011
NOD2\textsuperscript{−/−} = HFD sensitive

**B**

Glycemia (mg/dL)

- Nod2\textsuperscript{−/−} NC
- Nod2\textsuperscript{−/−} HFD 4 weeks
- WT NC
- WT HFD 4 weeks

Time (min)

Same phenotypes Myd88 KO
Molecular mechanisms:

• NOD1 and CD14 favor bacterial translocation, inflammation ➡ metabolic diseases
• NOD2 and Myd88 protects against HFD-induced metabolic diseases and controls bacterial translocation and inflammation.
• Leptin regulates immune cells and protects

Schertzer et al Diabetes 2011   Amar, Chabo et al EMBO MM 2011
Is there a molecular crosstalk between both genomes?

Tissue Transcriptomic + Tissue metagenomic analyses:

Primary component analyses

→ biostatistic conclusions
A change in the Proteobacteria to Firmicutes phylum ratio of the stromal vascular fraction (svf) of the adipose depot characterizes obesity.

Serino, Burcelin, Bouloumie, in press
Proteobacteria in human adipose tissue

Ralstonia
Gram neg
LPS+
Methods

Transcriptomics

Glycemia

Biostatistics

Metagenomics

Identify genes of the host to microbiota relationship
PCA microarray: adipose tissue

Pre diabetic & Diabetics

Controls

Diabetic treated
PCA metagenomic: adipose tissue

Liver

Mesenteric adipose tissue

Diabetic treated

Prediabetic

Diabetic

Controls

Prediabetic

Diabetic

Controls
Figure: Principal component analysis (PCA) of metagenomics data. The taxonomic profile illustrated by PCA of the metagenomics data shows bacterial communities clearly separated for NC (circles, red circling) and/or HFD4S (triangles, blue circling) mice in each tissue. HFD1 (squares) and HFD4 (crosses) mice taxonomic profiles overlap in all tissues.
Correlation 1 gene expression with 1 bacteria

Gene A
Arbitrary units

Bacteria A
Arbitrary units
Host genes controlled by tissue bacteria: correlated with glycemia

Matrix of genes positively and negatively correlated with tissue bacteria

Tissue transcriptomic

Tissue bacteria: metagenomics

Negatively

Positively

Non related!
Conclusions

• A high-fat diet:
  – impairs intestinal immune functions before the onset of diabetes and obesity
  – Increase intestinal bacterial adherence
  – Favors translocation of bacteria to tissues to generate a tissue microbiota
  – Induces tissue microbiota dysbiosis

• Tissue microbiota regulates gene expression ➔ putative targets of the host to microbiota relationship.
The Team!
In florae veritas est!

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Metabolic disease in 2013 → an immuno-microbio-metabolic relationship

Inflammation, fat overload
Insulin resistance

Predictive Causal?

Innate immune system

Adaptive

HF diet