L’Homme et son Microbiote Intestinal:
Guerre et Paix aux Surfaces Muqueuses
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Fighting ignorance, learning tolerance, responding to threats, the paradox that forged the immune system.

Commensals
Human colon: $10^{11}$ CFU / g feces
Up to 30,000 species...

Microbes
Pathogens
Possibly small infectious dose

Recognition network:
TLRs, NLRs, Rig1, MDA5...
Danger signals:
(uric acid, ATP, cytochrome C, etc.)

Innate immunity
Surveillance/Tolerance
- Rupture of homeostasis = IBD
- Dysbiosis = obesity, diabetes, metabolic syndrome
(Turnbaugh PJ & Gordon JI. 2009. J.Physiol., 587:4153-4158)

Inflammation
Microbe & tissue destruction
Amplification loop:
TREM, HMGB1, Gal3, etc..

Regulation
Loss of control
Severe sepsis
Septic shock

Adaptive immunity
Pathogens recognition, capture, completion of eradication process, protection:
scavenging receptors, C-type lectins, etc..

Sansonetti & Di Santo, 2007, Immunity
Pédron & Sansonetti, 2008, Cell Host and Microbe
Sansonetti & Medzhitov, 2009, Cell
War and Peace at mucosal surfaces

In the symbiotic relationship between resident bacteria and the eukaryotic host, bacteria profit by acquisition of a stable environment (temperature, oxygen, pH, nutrients supply), eukaryotic hosts gain extended metabolic and digestive ability and benefit from competitive inhibition of pathogenic microbes (barrier effect). Co-evolution has created an « immunological paradox » or « physiological inflammation » in mammalian hosts that forces to conjugate TOLERANCE to commensal bacteria and quick and efficient recognition and elimination of bacterial pathogens resulting in INFLAMMATION.

This « immunological paradox » needs to be deciphered.

Major clues reside in understanding how bacterial pathogens in healthy individuals, or commensal bacteria in genetically susceptible individuals, cause uncontroled inflammation.
Commensalism

*com mensa*: sharing a table.

Symbiose

Mutualism
Man is a primate-microbes hybrid

**Concept of HOLOGENOME / SUPERORGANISM**

Stomach: $10^{-10^3}$ bact./ml,
*Lactobacillus*, *Streptococcus*, *Staphylococcus*, Entérobactéries, *Helicobacter*, levures

Duodenum et jejunum: $10^2$-$10^5$ bact./ml,
*Lactobacillus*, *Streptococcus*, *Bifidobacterium*, Entérobactéries, *Staphylococcus*, levures

Ileum et caecum: $10^3$-$10^9$ bact./ml,
*Bifidobacterium*, *Bacteroides*, *Lactobacillus*, *Streptococcus*, Entérobactéries, *Staphylococcus*, *Clostridium*

Colon (500-1000 species, possibly more ?): $10^{10}$-$10^{12}$ bact./g,
*Bacteroides*, *Eubacterium*, *Clostridium*, *Peptostreptococcus*, *Bifidobacterium*, *Fusobacterium*, *Lactobacillus*, Entérobactéries, *Staphylococcus*, levures

Resident bacteria outnumber human somatic and germinal cells tenfold and represent a combined microbial genome well in excess (x150) of the human genome (Shanahan, 2002).

The intestinal flora has a collective metabolic activity equal to a virtual extra « organ» (Bocci, 1992).
The majority of intestinal bacteria belong to Firmicutes, particularly clusters XIVa and IV that comprise gram positive anaerobes with low GC % (extremely oxygen sensitive, EOS), essentially uncultivable, and to Bacteroidetes (gram negative anaerobes).
A human gut microbial gene catalogue established by metagenomic sequencing.


To understand the impact of gut microbes on human health and well-being it is crucial to assess their genetic potential. Here we describe the Illumina-based metagenomic sequencing, assembly and characterization of 3.3 million non-redundant microbial genes, derived from 576.7 gigabases of sequence, from faecal samples of 124 European individuals.

The gene set, approximately 150 times larger than the human gene complement, contains an overwhelming majority of the prevalent (more frequent) microbial genes of the cohort and probably includes a large proportion of the prevalent human intestinal microbial genes.

The genes are largely shared among individuals of the cohort. Over 99% of the genes are bacterial, indicating that the entire cohort harbours between 1,000 and 1,150 prevalent bacterial species and each individual at least 160 such species, which are also largely shared.

We define and describe the minimal gut metagenome and the minimal gut bacterial genome in terms of functions present in all individuals and most bacteria, respectively.
Functions of the gut resident microbiota

<table>
<thead>
<tr>
<th>Anaerobic genera</th>
<th>Aerobic genera</th>
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<tbody>
<tr>
<td>Bifidobacterium</td>
<td>Escherichia</td>
</tr>
<tr>
<td>Clostridium</td>
<td>Enterococcus</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>Streptococcus</td>
</tr>
<tr>
<td>Eubacterium</td>
<td>Klebsiella</td>
</tr>
</tbody>
</table>

**Protective functions**
- Pathogen displacement
- Nutrient competition
- Receptor competition
- Production of anti-microbial factors e.g., bacteriocins, lactic acids

**Structural functions**
- Barrier fortification
- Induction of IgA
- Apical tightening of tight junctions
- Immune system development

**Metabolic functions**
- Control IEC differentiation and proliferation
- Metabolize dietary carcinogens
- Synthesize vitamins e.g., biotin, folate
- Ferment non-digestible dietary residue and endogenous epithelial-derived mucus
- Ion absorption
- Salvage of energy

Commensal bacteria

IgA

Short-chain fatty acids

Mg^{2+}, Ca^{2+}, Fe^{2+}

Vitamin K, Biotin, Folate
Through production of Short Chain Fatty Acids (SCFA), commensal bacteria positively influence differentiation and proliferation of Intestinal epithelial cells (IEC) and mediate other metabolic effects.

> 50% of carbon source for colonic IEC is from SCFA produced by commensals.

Butyric acid
Analytical methods in the mouse

Massive parallel sequencing with second generation sequencing technology

Phylogenetic tree analysis: composition and diversity

- Species Group
- Phylum
- Bacteroidetes
- Firmicutes
- Actinobacteria
- Proteobacteria
- Cyanobacteria
- Mollicutes
- Euryarchaeota
- Verrucomicrobia

Other DNA-based techniques:
- microarray
- DGGE
- qPCR
- FISH

Inferred metabolic capabilities
- Immunofluorescence

In vivo bioluminescence imaging

16S rDNA

Multiphoton microscopy and live intravital imaging

Villi

Crypts

Fixed samples microarray qPCR 16S rDNA

Laser capture microdissection
Barrier effect against niche occupancy by pathogens


Pathogens alter/eliminate the resident microflora to occupy the intestinal niche (mostly via inflammation: ROS, NOS, etc.):
- Mouse gut infection by *Citrobacter rodentium* and *Campylobacter jejuni*
- Mouse experimental enterocolitis caused by DDS/TNBZ
Both conditions cause « retraction » of microbial diversity, particularly massive deletion of Firmicutes, but also large increase in aerobic bacteria, particularly Enterobacteriaceae (Lupp et al., Cell Host Microb., 2007)

Pathogens benefit from inflammation, if properly « mastered »:
Mouse model of *Salmonella typhimurium* enteritis shows similar retraction of the resident flora plus evidence for pathogens acquiring nutrients thanks to inflammation (Stecher et al., PLoS Biol., 2007)

*Shigella flexneri* suppress expresssion of intestinal antimicrobial peptides (β-defensin hBD3 and cathelicidin LL-37 (Sperandio et al., J. Exp. Med., 2008)
Barrier effect of the commensal microbiota

Ileo-colitis in Streptomycin-pretreated mice infected with Salmonella typhimurium

Barthel et al., 2003, Infect. Immun.
Diseases (possibly) associated with gut microbiota distortions
« dysbiosis-related diseases »
Mai V & Draganov PV. World J. Gastroenterol., 2009

(Increase in energy-harvesting bacterial populations)

(Carbohydrate intake and glycemic control, insulin resistance)

Chronic inflammatory diseases
cardio-vascular diseases (+ cholesterol dysregulation)
Bäckhed F. Clin.Exp.Immunol., 2010
inflammatory bowel diseases  Peterson et al. Cell Host and Microbe, 2008

Cancers
(chronic inflammation)
Host-Flora communication at the mucosal surface

- Host defence requires accurate interpretation of its microbial microenvironment to discriminate between established commensal bacteria and episodic pathogens and react in a manner proportional to the threat: concept of « physiological inflammation ».

- The epithelium itself provides the first sensory line through active sampling of commensals, pathogens and other antigens, in cooperation with dendritic cells and resident macrophages and T cells.

- PAMPs/MAMPs (Pathogen/Microbe-associated molecular patterns) - PRRs (PAMPs-recognition receptors) interactions are in front line of the dialogue.
Sampling of commensal bacteria through the intestinal epithelium

(From Niess & Reinecker, 2006, Cell.Microbiol.)

Other mechanisms:
- IEC apoptosis? Sampling by IEC
  Niess et al., 2005, Science
- DC-mediated sampling of commensal bacteria
- Other mechanisms: IEC apoptosis? Sampling by IEC
**PATHOGEN RECOGNITION RECEPTORS (PRRs) & PAMPs**

- Acide lipoteichoïque (LTA)
- Lipoarabinomannane
- PGN Gram+
- Zymosan
- LPS “atypique”

- Diacyl Lipopeptides
- Triacyl Lipopeptides
- LPS HSP60
- Flagellin
- MD2

- TLR2
- TLR1
- TLR4
- TLR5
- TLR6
- TLR10
- TLR11 (souris)

- Nods (Nod1 and Nod2)
- PGN
- Rip2

- MyD88
- IRAKs
- TRAF6
- TAK

- IkB
- NF-κB

- endosome
- cytoplasme

- Bactéries uropathogènes

**Examples of PAMPs:**
- Acide lipoteichoïque (LTA)
- Lipoarabinomannane
- PGN Gram+
- Zymosan
- LPS “atypique”

**TLRs:**
- TLR1, TLR2, TLR4
- TLR5, TLR6, TLR10, TLR11

**Adapters:**
- MyD88
- TRAF6

**Signaling Pathways:**
- NF-κB
- IKK
- IκB
- JNK

**Endosome:**
- dsRNA
- ssRNA
- CpG DNA
Peptidoglycane (Gram +)

Peptidoglycane (Gram -)

MurNAc-L-Ala-D-Glu

MurNAc-L-Ala-D-Glu-mesoDAP


Girardin et coll., Science, 2003
Avoiding inappropriate immune activation, maintaining homeostasis
The little secrets of physiological inflammation

Bacteria:
• Commensals (i.e. Bacteroidetes, Firmicutes) in general poorly detected by innate defence mechanisms (*Bacteroides* pentacylated lipidA, poor TLR4 agonist, even antagonist)

• Commensals/probiotics express intrinsic anti-inflammatory properties (i.e. *Lactobacillus* spp., *Bacteroides tetraiotamicron*, *Bacteroides fragilis* zwitterionic EPS (Mazmanian et al., 2008, Nature)

• Barrier effect against niche occupancy by pathogens, and more « aggressive » commensals (i.e. Enterobacteriaceae)
1 - Lipid A of gram-negative anaerobic commensals (Bacteroidetes) usually pentacylated, thus not agonist of TLR4 (even antagonist), unlike endotoxic hexacylated Lipid A of commensal Enterobacteriaceae and gram-negative pathogens (Munford & Varley, PLoS Path., 2006).

2 - Intestinal Alkaline Phosphatase (APase) detoxifies Lipid A and prevents inflammation in Zebrafish in response to the gram-negative resident flora. (Bates et al., Cell host Microb., 2007).

3 - Peptidoglycans ? Flagellins ?
Avoiding inappropriate immune activation, maintaining homeostasis
The little secrets of physiological inflammation

Epithelium
- Mucus, antimicrobial peptides (AMPs), and associated molecules (Expression/secretion stimulated by resident flora, even more by pathogens)
- Sequestration of innate receptors
- Early tolerization to LPS following birth (cross tolerization to other PAMPs ?)
Thickness of mucus layer depending on level in gut

- Alterations in mucin homeostasis associated with IBD.
- Ulcerative colitis characterized by depleted goblet cells and reduced mucus layer.
- Aberrant Muc2 assembly in mice causes endoplasmic reticulum stress and inflammation resembling ulcerative colitis (Heazlewood et al., PLoS Med., 2008)
Antimicrobial peptides

Crypts
- α-defensin 5 (Paneth cells)
- α-defensin 6 (Paneth cells)

Villi
- β-defensin 1 (epithelial cells)
- β-defensin 2 (epithelial cells)
- β-defensin 3 (epithelial cells)
- cathelicidin LL-37 (epithelial cells)

Ganz, 2003
Bacterial life at mucosal surfaces
« Seating on a volcano »

- Anti-microbial peptides
- Others: lysozyme, proteases, lectins, phospholipases

- hBD3
- Mucus
- Epithelium

O2
NO
ROS
Anti-microbial peptides
Others: lysozyme, proteases, lectins, phospholipases
Sensing of the luminal flora. Not all epithelial cells are equal (Abreu et al., 2005): (1) Sequestration/weak expression of sensing molecules (and cofactors) on surface epithelium. (2) Expression in crypts (maintaining crypts as +/- sterile sanctuaries?)
Avoiding inappropriate immune activation, maintaining homeostasis
The little secrets of physiological inflammation

Subepithelial tissues (*lamina propria*)
- Immune network oriented toward tolerance (i.e. epithelium, resident macrophages and dendritic cells, regulatory T cells)
- Resident flora essential to maintain tolerogenic signalling (via epithelial signals)
- Resident flora essential to maintain gut homeostasis (Myd88-dependant)
Limited expression of pro-inflammatory cytokines

Production / maintainance of T regulatory lymphocytes achieving sustainable tolerance to the luminal flora

Recirculation, mucosal "homing" of IgA plasmocytes
Concentration gradient of antimicrobial molecules (AMPs, lysozyme…)

Biofilm

Muropeptides

fMLP

H+

Quorum sensing QSMs)

C12 homoserine-lactone

H+

Mucus

PepT1

L-Ala-D-Glu...

OCTN2

Muropeptides

H+

Antimicrobial peptides (AMPs)

Antimicrobial molecules

Mucins, TFFs,

Tolerogenic molecules

TLSP, TGFβ, IL-10...

Stress

« Physiological inflammation »

Vavricka et coll., 2004, Gastroenterology

Fujiiya et coll., 2007, Cell Host Microbe

Kravchenko et coll., 2008, Science
Bacteroides fragilis - capsule - EPS
COMMENSAL/SYMBIOTIC BACTERIA
Absence (limitation) of virulence factors
PAMPs less agonist?
Sequestration, weak activity of TLRs
Life in biofilms on mucus surface
Controlled diffusion and sampling of PAMPs and prokaryotic signalisation molecules
Small intestine
Duodenum, jejunum, ileum

Large intestine
Caecum
Colon

Antigènes alimentaires

E. coli, Enterococcus spp., Lactobacillus spp., Proteus spp., Staphylococcus spp., Bacteroides spp., fusiform bacteria

SFM
Helicobacter spp.
Segmented filamentous bacteria
COMMENSALS + PATHOSYMBIONTS

Gaboriaux et al., 2009, Immunity
Ivanoff et al., 2009, Cell
Increased mucosally-associated Enteobacteriaceae in mucosal biopsies of Crohn’s disease (CD) patients

G = goblet cells, BL = Basal lamina, N = Nucleus

Swidsinski et al., 2002
PATHOGENS
Mucinases
Adhesins
Invasins
Type III / IV secretory systems
Hemolysins
Massive engagement of TLRs
Massive engagement of NLRs
Eradication of commensal microbiota (niche occupancy)
Pathogenic properties perceived as **danger signals**: an addition to the Janeway’s PAMPs-PRRs(TLRs) paradigm
NLR : « chiens de garde » intracellulaires

- Presence/recruitment of signaling molecules at engaged membrane: Gal-3, Nod1/2, NEMO...
- Meso-DAP 
- Peptidoglycan 
- NOD1 
- NOD2 
- MDP 
- Inflammasome: NALP3, ASC, Caspase-1 
- Uric acid 
- ATP 
- Bacterial RNA 
- Flagellin 
- Salmonella {legionella} 

Key:
- CARD
- NOD
- LRR
- Kinase
- PYRIN
- BIR
- Caspase

ProIL-1β → IL-1β
Secretion