Microbiota interactions with the host immune system
Analysis using gnotobiotic mouse models

Nadine Cerf-Bensussan
Inserm U989
WHICH TRADE-OFF MAINTAINS MUTUALISTIC RELATIONSHIPS BETWEEN HOSTS AND THEIR MICROBIOTA?


**Bacterial commensals of the human body**

- **10^{14} bacteria: symbionts**
- **1000 species/ind**
- **7 (2) phyla**
- **3 millions genes**

Metabolic advantages increasing the fitness of each partner

**Distal intestine**

**INNATE**

**ADAPTIVE**

ARBITER: HOST IMMUNE SYSTEM

*Chloridion bacteria*

*Bacteroides*

*Mycobacterium*

*Streptomyces*

*Escherichia*

*Salmomella*

*Yahira*

*Helicobacter*
Flora-driven maturation of gut immune responses
CAN INDIVIDUAL BACTERIA EXERT DISTINCT MODULATORY EFFECTS ON HOST IMMUNE RESPONSES BEYOND THE GUT


Can recent changes in the microbiota participate in the «epidemics» of allergic and autoimmune/inflammatory diseases?

“Innate immunity and intestinal microbiota in the development of Type 1 diabetes”

2008 Nature

Li Wen1*, Ruth E. Ley2*, Pavel Yu. Volchkov3*, Peter B. Stranges3,4, Lia Avanesyan3,4, Austin C. Stonebraker4, Changyun Hu5, F. Susan Wong3, Gregory L. Szot6, Jeffrey A. Bluestone6, Jeffrey I. Gordon2 & Alexander V. Chervonsky3,4
Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns

Maria G. Dominguez-Bello⁰,¹,², Elizabeth K. Costello⁡,¹,³, Monica Contreras⁴, Magda Magris⁵, Glida Hidalgo⁶, Noah Fierer⁰⁴, and Rob Knight⁰,⁴

vaginally delivered infants acquired bacterial communities resembling their own mother’s vaginal microbiota, dominated by Lactobacillus, Prevotella, or Sneathia spp., and C-section infants harbored bacterial communities similar to those found on the skin surface, dominated by Staphylococcus, Corynebacterium, and Propionibacterium spp.

Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo⁰, Duccio Cavalieri⁰, Monica Di Paola⁵, Matteo Ramazzotti⁶, Jean Baptiste Poulet⁴, Sebastien Massart⁴, Silvia Collini⁵, Giuseppe Pieraccini⁶, and Paolo Lionetti⁰,⁴

BF children showed a significant enrichment in Bacteroidetes and depletion in Firmicutes (P < 0.001), with a unique abundance of bacteria from the genus Prevotella and Xylanibacter, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, completely lacking in the EU children.
Analysis of local versus peripheral responses to complex simplified microbiota, individual bacteria

In wild type mice
In modified mice
either by inducing an experimental disease
and/or by using genetically modified mice rendered GF
1- Nature of immune responses to the microbiota in the intestine beyond intestine

2- Evidence of distinctive effects of individual members of the microbiota in the intestine beyond intestine?

3- Mechanisms of the distinctive effects of the microbiota
   Bacterial specific features
   Host factors

4- Clues to apprehend the role of the microbiota in the pathogenesis of intestinal and systemic diseases?
Mouse colonisation by a complete mouse flora induces a large spectrum of IR which balance each other.

SIgA

TH1, TH2, TH17

Treg (IL10, Foxp3)

Innate IR genes (ileum)

Gaboriau-Routhiau et al. Immunity 2009
DISTINCT MICROBIOTA (mouse/human) INDUCE IMMUNE TRANSCRIPTOMIC RESPONSES OF HIGHLY VARIABLE INTENSITY IN THE MOUSE INTESTINE

Cell process

- Immune response
- Metabolism
- Response to extracellular stimulus
- Cell cycle
- Transcription
- Signal transduction
- Chemotaxis
- Cell adhesion
- Transport
- G-protein signaling
- Neurophysiological process
- Apoptosis and survival
- Translation
- Transcription
- Cytoskeleton remodeling
- Cell cycle
- Response to extracellular stimulus
- Metabolism
- Immune response
- Oxidative stress
- Muscle contraction
- Global ileal transcriptome compared to GF mice

Mice colonized from birth by a mouse flora (CV)
Mice colonized at adult age by a mouse flora (Cvd)
Mice colonized at the adult age by a human flora (Hum)

Gaboriau-Routhiau et al Immunity 2009
No significant ileal mRNA response with:

**Individual bacteria:** *Bacteroides thetaiotaomicron*  
*Bacteroides vulgatus*  
3 Clostridium from Schaedler’s flora  
*E. coli MG1655/LF82*

**Complex flora:** *Schaedler’s flora*  
*whole human flora*

**Weak & inconstant ileal induction of FoxP3/IL10 mRNA:**

*Culturable fraction of mouse flora*

**Strong mRNA induction recapitulating the effect of the whole mouse microbiota:**

*Sporulated fraction of mouse flora*  
enriched in non culturable Clostridium spp

Gaboriau-Routhiau et al 2009
Hypothesis: the inducing effect of the microbiota on post-natal maturation of gut-associated immune system depends on one or several non culturable *Clostridium*-related member(s) of the mouse microbiota

*Segmented filamentous bacterium?*
The **SEGMENTED FILAMENTOUS BACTERIUM** (SFB)

An attractive candidate …

SFB is a non culturable commensal spore-forming *Clostridium*-related species conserved during evolution (termites, fruit, chicken, rodents)

(Snel et al. 1995)

SFB settles in the rodent intestine at time of weaning …

(// maturation of the gut adaptive immune system)

With host-specific adherence to the ileum

(Davis & Savage 1974, Tannock et al. 1984, Okada et al. 1994)

Non-culturable bacteria but purified by passage in germ-free mice…

(Klaasen et al. 1991)

In monocolonized mice, SFB induces a strong IgA response and the recruitment and activation of CD8+ TCRαβ intraepithelial lymphocytes

(Klaasen et al. 1993, Talham et al. 1999, Umesaki et al. 1995)
SFB: a key driver of gut immune responses?

Whole mouse flora

- SFB

Innate gut IR (ileum)

- Proinflammatory Cytokines/chemokines
  - Microbicide barrier RegIIIγ, MMP7

Adaptive gut IR

- CD4+ LPL (TH1,2,17, Treg)
- SlgA
- CD8 IEL

Talham et al. 1999
Umesaki et al. 1995

Gaboriau-Routhiau et al
Immunity 2009
using C3H mice
SFB: a key driver of gut immune maturation in mice but what happens with the other bacteria?

1- No adaptive response?

2- IgA but no T cell response?

3- A prominent regulatory T cell response which may hide the pro-inflammatory response
ADAPTIVE RESPONSES TO THE MICROBIOTA COME ON DIFFERENT FLAVOURS

1- SFB (and other SFB-like?)
   Strong coordinate innate and adaptive IgA & T cell responses
   → Specificity ??

2- Bacteria such as E. coli K12 (others?)
   IgA response
   No innate nor T cell response? Weak responses?

3- Bacteria able to induce IgA+ regulatory T cell responses

Culturable fraction of mouse flora → Inconstant ileal induction of FoxP3/IL10 mRNA
MANY MICROBIOTA MEMBERS INIVIDUALLY OR COLLECTIVELY INDUCE REGULATORY RESPONSES

A microbial symbiosis factor prevents intestinal inflammatory disease
Sarkis K. Mazmanian\textsuperscript{1,*}, June L. Round\textsuperscript{1,*} & Dennis L. Kasper\textsuperscript{2,3}

\textit{Faecalibacterium prausnitzii} is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients
Harry Sokol\textsuperscript{1}, Benédicte Pigneur\textsuperscript{1}, Laurie Watterlot\textsuperscript{1}, Omar Lakhdir\textsuperscript{1}, Luis G. Bermúdez-Humarin\textsuperscript{1}, Jean-Jacques Gracie\textsuperscript{1}, Sébastien Blugeon\textsuperscript{1}, Chantal Bardin\textsuperscript{1}, Jean-Pierre Furet\textsuperscript{1}, Gérard Corblot\textsuperscript{1}, Corinne Grangette\textsuperscript{1}, Nadia Vasquez\textsuperscript{1}, Philippe Pochart\textsuperscript{1}, Germain Trugnan\textsuperscript{1}, Ginette Thomas\textsuperscript{1}, Herve M. Blottière\textsuperscript{1}, Josi Dore\textsuperscript{1}, Philippe Marteau\textsuperscript{1}, Philippe Seksik\textsuperscript{1,4}, and Philippe Langella\textsuperscript{1,5}

A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota
Yingzi Cong\textsuperscript{1,*}, Ting Feng\textsuperscript{1,*}, Kohtaro Fujihashi\textsuperscript{\textsuperscript{b}}, Trenton R. Schoeb\textsuperscript{5}, and Charles O. Elson\textsuperscript{1,6}

Induction of Colonic Regulatory T Cells by Indigenous \textit{Clostridium} Species
Koji Atarashi\textsuperscript{1,*}, Takeshi Tanoue\textsuperscript{1,*}, Tatsuchiro Shima\textsuperscript{2}, Akemi Inaoka\textsuperscript{2}, Tomomi Kuwahara\textsuperscript{3}, Yoshika Momose\textsuperscript{4}, Genhong Cheng\textsuperscript{6}, Sho Yamasaki\textsuperscript{7}, Takashi Saito\textsuperscript{7}, Yusuke Ohba\textsuperscript{9}, Tadatsugu Taniguchi\textsuperscript{1}, Kiyoshi Takeda\textsuperscript{5}, Shohei Hori\textsuperscript{8}, Ivaylo I. Ivanov\textsuperscript{10}, Yoshinori Umesaki\textsuperscript{2}, Kikuiji Ito\textsuperscript{4}, Kenya Honda\textsuperscript{1,11†}

Intestinal Bacterial Colonization Induces Mutualistic Regulatory T Cell Responses
Markus B. Gouking\textsuperscript{1,2,*}, Julia Cahenzli\textsuperscript{1,2}, Melissa A.E. Lawson\textsuperscript{1,2}, Derek C.K. Ng\textsuperscript{2}, Emma Slack\textsuperscript{1,2}, Siegfried Hapfmeier\textsuperscript{1,2}, Kathy D. McCoy\textsuperscript{1,2,3}, and Andrew J. Macpherson\textsuperscript{1,2,3,4}
Induction of SlgA
keeping bacteria in the lumen and mucus
preventing host immunostimulation
T or non T dependent?

Induction of Treg
avoiding excessive host responses
deleterious for both bacteria and hosts

Treg may also promote IgA responses

*Tsuji et al Science 2009
*Cong et al PNAS 2009
DISTINCT TRADE-OFF BETWEEN INDIVIDUAL MEMBERS OF THE MICROBIOTA AND IMMUNOCOMPETENT HOSTS

Bulk of bacteria thriving in mucus

Bacteria needing intimate contact with their host

« specific role in shaping local host defenses ? »

DISTINCT TRADE-OFF BETWEEN INDIVIDUAL MEMBERS OF THE MICROBIOTA AND IMMUNOCOMPETENT HOSTS

Bulk of bacteria thriving in mucus

Bacteria needing intimate contact with their host

« specific role in shaping local host defenses ? »
**QUESTION: WHICH TRADE-OFF FOR SFB?**

SFB: a symbiont that needs host epithelial cells for growth?  

1974: Davis & Savage

SFB: pathogen-like behaviour (EC adhesion) resulting in strong innate and adaptive IR

Host immune responses (innate, IgA, T) may then:
- prevent excessive growth of SFB
- but also play a barrier effect  

Ivanov et al Cell 2009
LACK OF INTESTINAL T CELL RESPONSES RESULTS IN SEVERE LOCAL AND SYSTEMIC SEPSIS

No elimination of atypical *mycobacteria* by LP macrophages
Systemic dissemination

Epithelial barrier/defensins/SlgA

Mucosal Macrophages

Phagocytosis

IL-22

IL-17

IFN-γ

PMNs

T cells

CD4

CD4

Epithelial barrier/defensins/SlgA

flora

No elimination of atypical *mycobacteria* by LP macrophages
Systemic dissemination
An innocuous (or even friendly) bacterium can become a dangerous foe in predisposed or modified hosts.

Severe sepsis in Immunodeficient hosts

*B. fragilis*

Inflammation in hosts with defective immunoregulation

Mouse models Humans
Intestinal Bacterial Colonization Induces Mutualistic Regulatory T Cell Responses

Geuking et al Immunity 2011

Strong induction of IL-10 mRNA in CD4+ (Foxp3+?) LPL upon colonisation by Schaedler flora

Protection against DSS colitis

IL-17 and IFNγ production are turned on if IL-10 signaling is blocked

Enhanced severity of DSS colitis
INFANTS LACKING A FUNCTIONAL IL-10 PATHWAY DEVELOP SEVERE COLITIS

IL8 production by LPS-stimulated PBMC+/- IL10

No inhibition by IL-10

Patient 3 stop codon exon 3 rIL10b
Patient 11 point mutation rIL10a

qRT-PCR colonic biopsies

Glocker et al NEJM 2009
Begue et al AJG 2011
THE OUTCOME OF HOST IMMUNE RESPONSES TO THE MICROBIOTA DEPENDS ON HOST FACTORS

*E. Coli LF82* found in normal healthy human gut *but enteroinvasive* In Crohn’s disease patients  (Darfeuille Michaud et al)


Severe colitis in Tg mice with colonic expression of *Ceacam6* receptor for LF82 pili

Inadvertent adhesion to the mucosa can transform an inocuous (or even friendly) bacterium into a dangerous foe

Induction of *Ceacam6* in the human intestine results the production of inflammatory cytokines in genetically predisposed individuals

(Gaboriau-Routhiau et al, unpublished data)
HOST FACTORS PREDISPOSING TO INFLAMMATORY INTESTINAL RESPONSES TO THE MICROBIOTA IN HUMANS
QUESTIONS

1- Nature of immune responses to the microbiota
   in the intestine
   beyond intestine

2- Evidence of distinctive effects of individual members of the microbiota
   in the intestine
   beyond intestine

3- Mechanisms of the distinctive effects of the microbiota
   Bacterial specific features
   Host factors

4- Clues to apprehend the role of the microbiota in the pathogenesis
   of intestinal and systemic diseases?
Microbiota and systemic immune responses: Compartmentalization or not...

Innate and Adaptive Immunity Cooperate Flexibly to Maintain Host-Microbiota Mutualism

Emma Slack,1,5* Siegfried Hapfelmeier,1,5 Bärbel Stecher,2 Yuliya Velykoredko,1 Maaïke Stoel,1 Melissa A. E. Lawson,1 Markus B. Geuking,1 Bruce Beutler,3 Thomas F. Tedder,4 Wolf-Dietrich Hardt,2 Premysl Bercik,1 Elena F. Verdu,1 Kathy D. McCoy,1 Andrew J. Macpherson1,5*

No peripheral specific adaptive responses to the microbiota in immunocompetent host as an efficent immune barrier opposes bacterial translocation and recognition
Microbiota and systemic immune responses: Compartmentalization or not?

BUT

An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System: T cell maturation in the spleen

Sarkis K. Mazmanian,1,3,* Cui Hua Liu,1,2 Arthur O. Tzianabos,1,3 and Dennis L. Kasper1,3,*

Cell 2005

Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity

Thomas B Clarke1, Kimberly M Davis1, Elena S Lysenko1, Alice Y Zhou1, Yimin Yu2 & Jeffrey N Weiser1,3
2010 NATURE MEDICINE

Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota

Patrice D Cani and Nathalie M Delzenne

Current Opinion In Pharmacology 2009, 9:737–743

Translocation of bacterial products can exert non specific pro inflammatory or perhaps regulatory effects on peripheral immune cells (monocytes/DC, polymorphonuclears/ T& B cells
## Contradictory roles of the microbiota on systemic immune-mediated diseases

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<th>Protocol to alter microbiota</th>
<th>Observed effects</th>
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<td><strong>Allergy</strong></td>
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<td>IgE-mediated food allergy to peanut allergen</td>
<td>Weanling C3H mice or C57BL/6 mice</td>
<td>Oral cocktail of antibiotics for 3 weeks</td>
<td>Induction of anaphylactic symptoms, increased production of IgE and IL-13</td>
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<td>IgE-mediated allergic airway disease induced by <em>Aspergillus fumigatus</em> spores or ovalbumin</td>
<td>BALB/c and C57BL/6 mice</td>
<td>Oral cefoperazone (Cefobid, Pfizer; Cefazone, Pharo B International) for 5 days and a single oral gavage of <em>Candida albicans</em></td>
<td>Increase in pulmonary eosinophils and enhanced synthesis of IgE, IL-5 and IL-13</td>
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<td>Aire−/− mice</td>
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<td>No change in autoimmunity</td>
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<td>Spontaneous gastritis</td>
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<td>Colonization with probiotic <em>Lactobacillus</em> spp.</td>
<td>No disease</td>
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<td>Colonization with a mixture of <em>Bacteroides</em>, <em>Enterococcus</em>, <em>Veillonella</em> and <em>Staphylococcus</em> spp.</td>
<td>Disease restored</td>
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<tr>
<td>Spontaneous arthritis</td>
<td><em>Il1Ra−/−</em> BALB/c mice</td>
<td>Germ-free mice</td>
<td>No disease</td>
<td>60</td>
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<td></td>
<td><em>Il1Ra−/−</em> × <em>Tlr4−/−</em> mice</td>
<td>Colonization with <em>Lactobacillus bifidus</em></td>
<td>Disease restored</td>
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<tr>
<td></td>
<td><em>Il1Ra−/−</em> × <em>Tlr2−/−</em> mice</td>
<td>None</td>
<td>Same disease incidence, decreased severity</td>
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<td></td>
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<td>Increased severity</td>
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Cerf-Bensussan & Gaboriau-Routhiau, Nat Immunol Rev 2010
Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis

Yun Kyung Lee, Jusilene S. Menezes, Yoshinori Umesaki, and Sarkis K. Mazmanian

Adjuvant effect of microbiota/SFB on IP immunisation by MBP?

Gut-Residing Segmented Filamentous Bacteria Drive Autoimmune Arthritis via T Helper 17 Cells

Hsin-Jung Wu, Ivaylo I. Ivanov, Jaime Darce, Kimie Hattori, Tatsuihiro Shima, Yoshinori Umesaki, Dan R. Littman, Christophe Benoist, and Diane Mathis

Inducing effect of SFB on TH17 responses in mice lacking appropriate immunoregulation and in a TH17-driven disease?

Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice

Martin A. Kriegel, Esen Sefik, Jonathan A. Hill, Hsin-Jung Wu, Christophe Benoist, and Diane Mathis

Many infectious stimuli can protect NOD mice from diabetes
THE OUTCOME OF HOST IMMUNE RESPONSES TO THE MICROBIOTA DEPENDS ON BACTERIA AND HOST FACTORS

Cerf-Bensussan & Gaboriau-Routhiau Nat Rev Immunol 2010