Intestinal microbiota – a microbial ecosystem between health and disease

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Intestinal microbiota as a complex microbial ecosystem with implications for health and disease

**Intestinal Microbiota**

- 10-times more cells
- 150-times more genes

1. Colonization resistance
2. Metabolic function
3. Immune education
Modulation of the intestinal microbiota by exogenous and endogenous factors

Modulation of the Intestinal microbiota

1. Life course
2. Environment (Diet, …)
3. Host (Genotype)
4. Diseases

Number

- Stomach: $10^1 - 10^3$ CFU/ml
- Duodenum: $10^2 - 10^3$ CFU/ml
- Jejunum: $10^4 - 10^5$ CFU/ml
- Ileum: < $10^8$ CFU/ml
- Colon: $10^{12}$ CFU/ml
Phylogenetic and metagenomic analysis of the intestinal microbiota – from composition to function

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**Biopsies and Feces**
- **Plating**
- **Extraction**
  - **16S rDNA**
  - **Genomic DNA**

**Diversity**

**Metagenome**

~30-40%
Microbe-host interactions at the gut mucosal interface set the tone for local and systemic immune responses.

Intestinal microbes are implicated in the pathogenesis of autoimmune encephalomyelitis – the brain case

Linking commensals with autoimmunity

Autoimmune disorders develop under the combined influence of environmental and genetic factors. A recent study published in Nature reports that both commensal bacteria and self-antigen recognition are required for the initiation of autoimmune responses in relapsing-remitting experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis.

The intestinal microbiota actively regulates local immune responses, partly through the induction of interleukin-17 (IL-17)-producing T cells and regulatory T cells, but its contribution to immune responses at distal sites is largely unknown. Relapsing-remitting EAE develops spontaneously in transgenic mice of the SJL/J background in which a proportion of CD4+ T cells are engineered to express a T cell receptor that is specific for the self-antigen myelin oligodendrocyte glycoprotein (MOG). As disease incidence in these transgenic mice varies among different animal facilities, the authors asked whether the composition of the intestinal microbiota might have a role in EAE development.

Indeed, whereas 80% of the transgenic mice housed under specific-pathogen-free (SPF) conditions developed EAE, transgenic mice housed under germ-free conditions showed no disease symptoms. This effect was not due to differential maturation of the immune system, as active immunization with recombinant MOG induced autoimmune responses in the transgenic mice irrespective of the presence of the intestinal microbiota. Moreover, colonization of germ-free transgenic mice with commensal bacteria was sufficient for EAE development.

Further analysis indicated that, in accordance with previous findings, the percentage of IL-17-producing CD4+ T cells in the lamina propria and Peyer’s patches was higher in SPF transgenic mice than in germ-free transgenic mice. Moreover, splenic T cells from SPF transgenic mice produced higher levels of interferon-γ and IL-17 in vitro than splenic T cells from germ-free transgenic mice. Finally, adoptively transferred MOG-specific or polyoval CD4+ T cells were shown to proliferate locally in the lamina propria of SPF mice, and antibiotic treatment abolished this effect.

Together, these findings indicate that a first step in the initiation of autoimmunity might be the activation of autoreactive CD4+ T cells by commensal bacteria in the gut and the subsequent proliferation of these T cells.

In addition to the increased activation of autoreactive CD4+ T cells, B cells are crucial for disease development. Serum from SPF transgenic mice contained higher levels of MOG-specific IgG2a antibodies than serum from germ-free transgenic mice. However, the induction of MOG-specific B cell responses in SPF transgenic mice did not rely on molecular mimicry between MOG epitopes and epitopes provided by commensal bacteria, but was dependent on self-antigen expression, as MOG-specific autoantibodies were absent from SPF transgenic mice that were deficient for MOG.

Interestingly, the cervical lymph nodes of young, healthy SPF transgenic mice contained germinal centres and germinal centre B cells. This was not observed in germ-free transgenic mice or SPF wild-type mice, indicating that autoreactive T cells previously activated by commensal bacteria initiate MOG-specific germinal centre reactions in a MOG-dependent manner. The elucidation of the compositions of intestinal microorganisms that result in increased susceptibility to autoimmunity through this mechanism will be of therapeutic value.

Marta Bagnis, University of Florence

Berer, …, Krishnamoorthy 2011 Nature
Gene-environment interactions in chronic inflammatory diseases: a forward look

- Inflammatory Bowel Diseases
- Colorectal Cancer
- Immune-mediated diseases
- (Allergic) Asthma
- Metabolically-driven diseases
- Multiple sclerosis
- Alzheimer's disease
- Type 1 diabetes
- Obesity, Type 2 diabetes, Atherosclerosis

My personal hit list of key questions ...

- Prenatal environment
- Postnatal environment
- Diet
- Inflammation
- Microbiota
- Diet

Understanding causality and being able to translate basic mechanisms into clinical relevance ... the vision!

- Animal models
- Mechanisms
- Selected bacteria or structures
- Intestinal microbiota
- Facility microbiota (SPF) and transplants
- Association studies
- Human studies
- Gnotobiology
- Intervention studies
What do we learn from population or disease association studies?
Phylogenetic evaluation to the variability of microbial compositions in larger cohorts of humans

N=161

Claesson, …, O’Toole 2011 PNAS
Clustering the microbiota into functional groups (enterotypes) according to correlations with diet

*Enterotypen sind assoziiert mit:*

- **Bacteroides** (Protein und Fett)
- **Prevotella** (Kohlenhydrate)
Patterns of microbiota cluster around co-abundance groups (*Bacteroides, Prevotella, Rumiococcus, Oscillobacter, Alistepes, Odoribacter*)

- **Long-stay Community**
- **Young healthy**
- **Day hospital**
- **Rehabilitation**

CCI, Charlosn Co-Morbidity Index; GDT, Geriatric Depression Test; MNA, Mini Nutritional Assessment; FIM, Functional Independence Measure; MMSE, Mini-mental State Exam

Long-stay Residency (Frailty)
- Alistepes
- Oscillobacter

Community Subjects (Health)
- Prevotella
- Ruminococcus

N=178
Age = 78

Claesson, …, O’Toole 2012 Nature
Reduced diversity in a monozytotic twin cohort discordant for ulcerative colitis

Lepage, …, Schreiber 2011 Gastroenterology
Recruitment of the intestinal microbiota at early life stages may be influenced by the host genotype.
Understanding causality and being able to translate basic mechanisms into clinical relevance … a long way?!

Intestinal microbiota

Complexity

Relevance

Human studies

Association studies

Intervention studies
Fäkaltransplantation als “Proof-of-Concept” zur Bedeutung der Mikrobiota – metabolisches Syndrom

Vriese, …, Nieuwdrop 2012 Gastroenterology
What do learn from gnotobiotic animal studies?
Gnotobiologie in Tiermollen für chronische Entzündung im Darm

Keimfrei (Steril)  
Normaler Darm

Colitis Modelle  
IL-10^{KO}  
HLA-B27 TG  
CD45RB^{hi} SCID  
N-Cadherin^{KO}  
IKK^{ΔEC}  
DSS  
…

Komplexe Mikrobiota  
Definierte Bakterien

Ileitis Modelle  
TNF^{ΔARE}  
SAMP1/Yit Fc

Chronische Entzündung
Humanizing the mouse at the level of the microbiota
... a word of caution!

Chung, …, Kasper 2012 Cell (Ferreira and Veldhoen 2012 Cell Preview)
Diet-mediated effects in the selection process of colitogenic pathobionts ... an indirect effect

Devkota, ..., Chang 2012 Nature
Selection of disease-associated microbial structures in monoassociated mice … (over) simplification of problems

**Histology**

- **Germfree**
- **OG1RF**
- **∆GelE**

**E-Cadherin**

- **Modellorganismen**
  - OG1RF
  - ∆GelE

- **Morbus Crohn**
  - CD11
  - CD18.1

- **Colitis ulcerosa**
  - UC7
  - UC28.1

- **Healthy**
  - AH114

**Gene Expression**

- **orf1**
- **fsrA**
- **fsrB**
- **fsrC**
- **gelE**
- **sprE**

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Steck, …, Haller 2011 Gastroenterology
Identification of novel probiotic structures ... now is time to validate the relevance

PrtP-encoded lactocepin (L. paracasei, L. lactis)

PrtH-encoded lactocepin (L. helveticus)

PrtB-encoded lactocepin (L. bulgaricus)

PrtS-encoded lactocepin (S. thermophilus)

PrtR-encoded lactocepin (L. rhammnosus)

Histology score of cecum:
* - L.c
+ L.c prtdis
RAG2-/-
CD4+(IL-10-/-)
RAG2-/-

Von Schillde, …, Haller 2012 Cell Host Microbe
Understanding causality and being able to translate basic mechanisms into clinical relevance ... a long way?!

Facility microbiota (SPF) and transplants

Animal models

Mechanisms

Selected bacteria or structures

Intestinal microbiota

Complexity

Simplification

Relevance

Gnotobiology

Intervention studies

Human studies

Intervention studies
Calls for Proposals

No 32  12 June 2012

Priority Programme “INTESTINAL MICROBIOTA – a Microbial Ecosystem at the Edge between Immune Homeostasis and Inflammation” (SPP 1656)

Koordinatoren: Haller (TUM) und Autenrieth (Tübingen)

Koordinationsoffice: Haller (TUM)
Laufzeit: 2013 - 2019
Key questions of priority program
SPP 1656

- Understanding the physiology of a bi-directional interaction between the intestinal microbiota and the mucosal immune system at early life stages and in response to diet and host genotypes.

- Understanding the role of microbe-host interactions in the patho-physiological transition from immune homeostasis to infectious and chronic inflammatory disorders.

- Establishing mechanistic concepts for pre-clinical efficacy and risk evaluation of probiotic intervention and fecal transplantation in infectious and chronic inflammatory disorders.

- Developing an infrastructure for gnotobiotic research
Where do we stand …

Knowledge

Gut

Microbiota

Immune system

Skin

Gut

Immune system

Metabolism

Brain

Microbiota

Time

1990

2000

2011

low

moderat
**Glossar**

**Intestinal Microbiota** – comprises all microorganisms that inhabit the intestinal tract (formerly designated as “microflora”)

**Intestinal Metagenome** – comprises the collective genomes of the intestinal microbiota

**Metagenomics** – describes the functional and sequence-based (meta)analysis of the collective microbial genomes

**Intestinales Mikrobiom** – a term to describe all microorganisms that inhabit the intestinal tract (microbiota) in combination with their collective genomes (metagenome) and their interaction with the host

**Gnotobiology** – *(gnotos greek “erkennen”)* studies organisms (mostly mice) that are either free of bacteria (germ-free) or associated only with known or specified bacteria