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Bacterial Flora: Role in Health and Disease

Gerhard Rogler, Klinik für Gastroenterologie und Hepatologie, UniversitätsSpital Zürich
• The normal microbiota of humans consists of a few eukaryotic fungi, viruses and some Archaea that colonize the lower intestinal tract
• Bacteria are the most numerous and obvious microbial components of the normal flora.
Complexity of Intestinal Microbiota

- Up to 100 Trillion ($10^{14}$) microorganisms per human, with 300-1000 species
- Knowledge on microbial composition has greatly increased with the use of culture independent methods

- So far little is known on the role of environmental factors (nutrition, medication use, way of life, smoking etc.)
Factors influencing the intestinal bacterial flora

- genetics
- age
- sex
- area of living/environment
- stress
- nutrition, diet
- antibiotics
- other drugs
Bacteria have “environmental preferences”

<table>
<thead>
<tr>
<th>BACTERIUM</th>
<th>TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>Throat</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Urogenital epithelium</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>Tooth surfaces</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>Tongue surface</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>Small intestinal epithelium</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Small intestinal epithelium</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Nasal membranes</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>Skin</td>
</tr>
</tbody>
</table>
## Bacteria adhere to specific surfaces or proteins

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Bacterial ligand for attachment</th>
<th>Host cell or tissue receptor</th>
<th>Attachment site</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Protein F</td>
<td>Amino terminus of fibronectin</td>
<td>Pharyngeal epithelium</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em></td>
<td>Glycosyl transferase</td>
<td>Salivary glycoprotein</td>
<td>Pellicle of tooth</td>
</tr>
<tr>
<td><em>Streptococcus salivarius</em></td>
<td>Lipoteichoic acid</td>
<td>Unknown</td>
<td>Buccal epithelium of tongue</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Cell-bound protein</td>
<td>N-acetylhexosamine-galactose disaccharide</td>
<td>Mucosal epithelium</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Cell-bound protein</td>
<td>Amino terminus of fibronectin</td>
<td>Mucosal epithelium</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>N-methylphenylalanine pili</td>
<td>Glucosamine-galactose carbohydrate</td>
<td>Urethral/cervical epithelium</td>
</tr>
<tr>
<td>Enterotoxigenic <em>E. coli</em></td>
<td>Type-1 fimbriae</td>
<td>Species-specific carbohydrate(s) (e.g. mannose)</td>
<td>Intestinal epithelium</td>
</tr>
<tr>
<td>Uropathogenic <em>E. coli</em></td>
<td>Type 1 fimbriae</td>
<td>Complex carbohydrate</td>
<td>Urethral epithelium</td>
</tr>
<tr>
<td>Uropathogenic <em>E. coli</em></td>
<td>P-pili (pap)</td>
<td>Globobiose linked to ceramide lipid</td>
<td>Upper urinary tract</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>Fimbriae (&quot;filamentous hemagglutinin&quot;)</td>
<td>Galactose on sulfated glycolipids</td>
<td>Respiratory epithelium</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>N-methylphenylalanine pili</td>
<td>Fucose and mannose carbohydrate</td>
<td>Intestinal epithelium</td>
</tr>
<tr>
<td><em>Treponema pallidum</em></td>
<td>Peptide in outer membrane</td>
<td>Surface protein (fibronectin)</td>
<td>Mucosal epithelium</td>
</tr>
<tr>
<td><em>Mycoplasma</em></td>
<td>Membrane protein</td>
<td>Sialic acid</td>
<td>Respiratory epithelium</td>
</tr>
<tr>
<td><em>Chlamydia</em></td>
<td>Unknown</td>
<td>Sialic acid</td>
<td>Conjunctival or urethral epithelium</td>
</tr>
</tbody>
</table>

This table summarizes the interactions between various bacteria and their specific attachment sites on host cells or tissues.
The forms of bacterial colonization

- Mutualistic
  Both organisms benefit – “mutually benefical”

- Commensalistic
  One organism benefits, the other is neither helped nor harmed

- Opportunistic
  Under normal conditions, microbe does not cause disease, but if conditions become conductive, it can cause disease
The „in-vironment“

Maintenance of barrier integrity by:
- Gut motility
- Secretion of mucine by goblet cells; chloride secretion
- Luminal flora (products of bacterial metabolism, commensal flora)
- Defensin- and cytokine production
- Cell-cell-contacts
Why do we have bacterial colonization?

• The normal flora synthesize and excrete vitamins in excess of their own needs (vitamin K, vitamin B12, other B-vitamins).

• The normal flora prevent colonization by pathogens by competing for attachment sites or for essential nutrients (most important beneficial effect → *Salmonella* studies)

• The intestinal bacteria produce a variety of substances ranging from peroxides to highly specific bacteriocins, which inhibit or kill other bacteria.
Why do we have bacterial colonization?

- The normal flora stimulate the development of the caecum and certain lymphatic tissues (Peyer's patches) in the GI tract. (the caecum of germ-free animals is enlarged and thin-walled).

- The normal flora stimulate the production of cross-reactive antibodies (IgA, secreted into the gut lumen).
Infection with Salmonella: Role of the “commensal” flora - colonization resistance

normal flora

10^6 pathogenic microbes → GI infection

reduced flora after Streptomycin treatment

10 pathogenic microbes → GI infection
Infection with Salmonella: Role of the “commensal” flora – pathogen clearance

• S. typhimurium causes self-limiting (40 days) gut infection in streptomycin-treated mice.

• Pathogen clearance from the gut lumen is was mediated by the resident microbiota.

• 'L-mice' harbor a low complexity gut flora, lack colonization resistance and fail to clear S. typhimurium from the gut lumen.

• Pathogen clearance was re-achieved by transferring a normal complex microbiota.
Reduced protection under pathophysiological conditions
Bacteria colonize different niches in health and disease

Control-patient

CD patients

Kosovac et al, Inflamm Bowel Dis 2010
Growing list of non-infectious diseases associated with bacterial flora:

- IBD
- Metabolic Syndrome
- IBS
Inflammatory Bowel Diseases (IBD)

- Main forms: Crohn’s Disease (CD) and Ulcerative Colitis (UC)
- Chronic or relapsing intestinal inflammation
- Environmental and genetic risk factors
- CD: segmental, transmural inflammation of the whole gastrointestinal tract
- UC: continuous mucosal inflammation of the colon always starting in the rectum
IBD is a multifactorial disease: important role for bacteria

Modified from: Artis (2010)
Crohn’s disease – Dysregulation of autoprotective mechanisms

**Luminal microflora: „in-vironment“**

1. **Alterations and changes of predominant species of fecal bacteria** in the colon of CD patients
   (Kleesen et al., 2002; Swidsinski et al., 2002; Seksik et al., 2003)

2. **Reduced** expression and biochemical changes of **mucins** in the colon of CD patients; **reduced production of defensins**
   (Shirazi et al., 2000; Wehkamp et al., 2004)

3. **Increase of bacterial colonisation**; reduced mucosa thickness and **changed biodiversity**
   (Swidsinski et al., 2005)

**Consequence: Dysbalanced GALT**

- **Peptido glycans**
- **Toxins**
- **Food antigens**

- **Defensins**

**Immunological Response**

- **NF-κB**
- **TNF**
- **IL-12**
- **IL-18**
- **IFN-γ**
- **NF-κB**

**Th1**

- **Th2**

**Macrophage**

**Non-tolerogenic immune response to microbial - (commensal-) and food antigens**

**Chronic inflammation**
CD Susceptibility Genes

- Pattern Recognition Receptors (PRRs): NOD2, TLR4, CARD8, CARD9, NLRP3
- Autophagy genes: ATG16L1, IRGM, LRRK2
- Antibacterial response: defensins
- Maintenance of epithelial barrier integrity (IBD5, DLG5, PTGER4, ITLN1, DMBT1, XBP1)
- Differentiation of Th17-lymphocytes: IL-23R, JAK2, STAT3, CCR6
- Orchestration of the secondary immune response (HLA-region, TNFSF15/TL1A, IRF5, PTPN2, PTPN22, NKX2-3, IL-12B, IL-18RAP, MST1)
Key pathways in IBD

INNATE IMMUNITY
- NOD2
- MDP
- NF-κB
- IL1β

DEFECTIVE BARRIER
- ECM1
- CDH1
- LAMB1
- HNF4A
- GNA12

Autophagy
- ATG16L1
- IRGM
- ATG5

Autophagosome
- Phagophore

IL10 signalling
- IL22, IL26

ADAPTIVE IMMUNITY
- IL23R
- IL12B
- JAK2
- STAT3
- TNFSF15
- CCR6
- IL27
- REL

IL23

Th17
- IL17
Disease overlap

C W Lee et al., New IBD genetics: common pathways with other diseases, *Gut* 2011;60:1739-1753
Impaired intracellular recognition of invasive bacteria in CD patients

GENETIC BACKGROUND: NOD2

Function
- **2001**: Identification as *susceptibility gene* for Crohn’s disease on chromosome 16
- expressed by phagocytes and epithelial cells; Increase of expression by proinflammatory cytokines (TNF, IFN-γ)
- Ligand: muramyldipeptide (MDP)
- signalling to: **NF-κB**-activation
- regulatory function:
  - synthesis of antimicrobial α-defensins
  - interference with TLR-mediated signal pathways (TLR2 ↓; TLR4: NOD2 induces MyD88)

Structure
- **SNP8** (Arg702Trp)
- **SNP12** (Gly908Arg)
- **SNP13** (Leu1007fsinsC)

mutations
- 40% of all CD patients
- Disease risk:
  - 2-4fold increased in heterozygous patients
  - 20-40fold increased in homozygous patients

NOD2/CARD15-mutations impair the intracellular recognition of bacterial invasion
Accumulation of endotoxin in mucosa in CD

Kosovac et al, Inflamm Bowel Dis 2010
NOD2 variants correlate with severe GvHD

Cumulative Incidence Plot

GvHD grade III/IV

R & D mut

R wt,D mut

R mut,D wt

R & D wt

** p 0.02

*** p 0.002

Holler, Rogler, Herfarth, Blood 2004
Bacteria trigger intestinal inflammation

Holler et al; Blood 2006
Intestinal microbiota and metabolic syndrome

World-wide pandemia of obesity – “globesity”

- CDC-Data: 68% of US adults >20 years are overweight or obese

Role of the microbiota in the pathogenesis of obesity

- Increase in *Firmicutes* and *Actinobacteria* and decrease in *Bacteroidetes* in obese vs. lean human and mice

- Obese phenotype has been shown to be transmissible via transplantation of an “obese microbiota” in mice

- Induction of lean phenotype has not been performed so far - nevertheless: modification of gut microbiota already discussed as a future treatment strategy for obesity

1. Sherry B. CDC-Website, 2010
4. Ley, R.E., PNAS, 2005
Intestinal microbiota and metabolic syndrome

Mechanisms linking bugs and kilos...? (I)

- Increase in energy extraction in Firmicutes-rich gut microbiota\(^1\)
- Relationship most likely more complex than just a ratio including Firmicutes:
- No correlation to abundance of major phyla in structured weight loss program in humans\(^2\)
- Success in weight loss higher in individuals rich in Bacteroides fragilis, Lactobaillus and Bifidobacterium

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2. Santacruz A., Obesity, 2009
Intestinal microbiota and metabolic syndrome

Mechanisms linking bugs and kilos...?

- Transcriptional response of epithelial genes
- Increase in gene expression\(^1\)
  - Nutrient absorption
  - Mucosal barrier fortification
  - Xenobiotic metabolism
  - Angiogenesis
  - Postnatal intestinal maturation

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\(1. \) Hooper L.V., Science, 2005

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold (\Delta) (relative to germ-free)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Na}^+)/glucose cotransporter (SGLT-1)</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>Colipase</td>
<td>6.6 ± 1.9</td>
</tr>
<tr>
<td>Liver fatty acid–binding protein (L-FABP)</td>
<td>4.4 ± 1.4</td>
</tr>
</tbody>
</table>
Intestinal Microbiota and IBD

Strategies to manipulate the microbiota in IBD – Efficacy?

- Antibiotics in IBD – recent Meta-Analysis
  - Significant effect in active CD (no remission: 0.85; CI = 0.73 – 0.99)
  - Quiescent CD (relapse risk: 0.62; CI = 0.46-0.84)
  - Active UC (no remission: 0.64; CI = 0.43-0.96)
- E. Coli Nissle, VSL#3
- Rifaximin in CD

2. Kruis W., Gut, 2004
3. Mimura T., Gut, 2004
4. Prantera C., Gastroenterology 2012
Gut Microbiota – Smoking - IBD

Background of our Study

• Average weight gain after smoking cessation: 7-8kg\(^1\)
  • Just an increase of food?
  • Multiple Risk Factor Intervention Trial: quitters gained weight, although the consumed less calories and a healthier diet than continuing smokers or recidivist!\(^2\)

• Smoking and IBD:
  • Smoking has detrimental effects in Crohn’s disease (CD)\(^3\)
  • In UC smoking seems to be protective
    • Lower incidence, especially early onset UC\(^4\)
    • More severe disease course after cessation\(^5\)

Does smoking status influence intestinal microbial composition ???

3. Cosnes J., Gastroenterology, 1996
Gut Microbiota – Smoking - IBD

- 10 healthy smoking subjects, 10 subjects control group (smoking and non-smoking, 5 each)
- Observational period: 9 weeks (5 study visits); collection of stool samples
- Intensive counseling (physicians, psychologists)
- control: food diary, verification of strict adherence to smoking cessation

Methods: 16s rRNA-gene based analyzes of abundant bacterial lineages

- Terminal Restriction Fragment Length Polymorphism (T-RFLP)
- 454-Pyrosequencing
Shift of Phyla: Pyrosequencing

- the big four phyla: 97.3% of all sequences
- Intervention Group - after smoking cessation:
  - Increase of *Firmicutes* and *Actinobacteria*
  - Decrease of *Proteobacteria* and *Bacteroidetes*
- Stable microbiota in both control groups
Shift of Phyla: Pyrosequencing

- Creation of a **sample distance matrix**, taking the phylogenetic distance of every pair of sequences into account.

- Performance of phylogeny-based **PCA**, including 9 environments with **Fast UniFrac**

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C. diff Colitis – clinical importance

- most frequent form of hospital-acquired diarrhea\(^1\)
- Estimated costs: > \textbf{1.100.000.000} $ per year \(^2\)
- toxin A: enterotoxin; permeability, secretion \(\uparrow\)
- toxin B: cytotoxin; inflammation
- new, more \textbf{virulent strain} (BI/NAP1/027 & Co.), Quinolon-resistance, gene-deletion: toxin-production \(\uparrow\)\(^3\)
- US numbers 2008 – \textbf{mortality}:
  - 6x more deaths than all other enteropathogens

3. O’Connor JR; Gastroenterology 2009
Relapsing C. diff. Colitis

- Definition: recurrence of symptoms within 8 weeks after successful antibiotic-therapy\(^1\)
- Clinical definition (no toxin or culture necessary)
- recurrence – a frequent problem?
  - „only“ 10-30%...
  - ...however after 1x relapse: 40-60% further relapses\(^2,3\)

2. Kelly CP.; NEJM 2008
## Therapy recommendations - C. diff colitis

### CDC practice guidelines for treating *Clostridium difficile* infection

<table>
<thead>
<tr>
<th>Initial episode, mild or moderate</th>
<th>Supportive clinical data</th>
<th>Recommended treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. difficile infection</td>
<td></td>
<td>Vancomycin 1250 mg three times per day for 10–14 days</td>
</tr>
<tr>
<td>First recurrence, severe</td>
<td></td>
<td>Metronidazole 500 mg four times per day for 10–14 days</td>
</tr>
<tr>
<td>Second recurrence, severe, complicated, or fulminating</td>
<td></td>
<td>Vancomycin 1250 mg every 8 hours</td>
</tr>
</tbody>
</table>

**Note:** For treatment of initial episode, severe, consider adding rectal vancomycin or oral rifaximin. For first recurrence, severe, consider adding rectal vancomycin or oral rifaximin, or increasing vancomycin dosing. For second recurrence, severe, consider adding rectal vancomycin or oral rifaximin, or increasing vancomycin dosing.

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**ADAPTED FROM**

**FMT for C. diff colitis**

**Antibiotic therapy for C difficile infection** suppresses active infection, but the organism can form resistant spores. In addition, antibiotics further disrupt the normal intestinal flora. As a result, *C difficile* infection often recurs.

**Fecal microbiota transplantation** involves instilling fecal material from a healthy donor to **restore the normal intestinal flora.**
FMT for C. diff - colitis

Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile

43 Underwent randomization

17 Were assigned to receive donor-feces infusion
  1 Was excluded
  16 Completed evaluation

13 Were assigned to receive vancomycin
  1 Died
  12 Completed evaluation

13 Were assigned to receive vancomycin and bowel lavage
  13 Completed evaluation

The NEW ENGLAND JOURNAL of MEDICINE

2. Van Nood E; NEJM 2013
Initially, the inclusion of 40 patients per study group was planned. Because most patients in both control groups had a relapse, the data and safety monitoring board performed the interim efficacy analysis and advised termination of the trial, as
FMT – How to do it?
Thank you for your attention