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The role of the intestinal microbiota in pneumonia and sepsis

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The gut microbiota in internal medicine: implications for health and disease

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Abstract

The human gut microbiota may be viewed upon as an organ, executing numerous functions in metabolism, development of the immune system and host defense against pathogens. It may therefore be involved in the development of a range of diseases like gastrointestinal infections, inflammatory bowel disease, allergy and diabetes mellitus. Reversely, certain therapies that are often used, such as antibiotics and chemotherapy, may negatively affect the composition and function of the gut microbiota and thereby the well-being of patients.   As the microbiota research field is currently moving from association studies to intervention studies and even clinical trials, implementation of this new knowledge in clinical practice is coming near. Several therapeutic interventions that target the gut microbiota are being evaluated, ranging from supplementation of food components to transplantation of fecal microbiota. In this review we provide an overview of current literature on the gut microbiota in both a healthy state and a range of diseases that are relevant for internal medicine. In anticipation of gut microbiota-targeted therapies, it is important to realize the key function of the gut microbiota in physiological processes and the collateral damage that may be caused when disrupting this ecosystem within us.

Introduction

The field of microbiota research has exploded in recent years. In the first six months of 2014, over 100 peer-reviewed articles were published on this topic on each consecutive day. The awareness that commensal microorganisms are not simple bystanders in our bodies, but instead play key roles in physiology and pathology has excited scientists and clinicians in almost every discipline of medicine [1-3]. The unprecedented success of fecal transplantation as a potential cure for recurrent *Clostridium difficile* infection has become the gem of the microbiota research field [4]; still, the gut microbiota is suspected of having an important role in a whole range of diseases [5].

"The last human organ" plays a major role in the development of the immune system, the defense against pathogens and the metabolism of fatty acids, glucose and bile acids (*figure*) [6]. Of special interest is the degradation of otherwise non-fermentable dietary fibre such as resistant starch into short-chain fatty acids (SCFA), mainly by bacteria from the Bacteroidetes phylum [7]. These SCFA - particularly acetic, propionic and butyric acid - have anti-inflammatory and immune-signaling properties and are an energy source for epithelial cells [8].

Our gut contains ten times more bacteria than our bodies contain cells of our own [9]. Moreover, the collective microbiome is 150 times larger than the human genome, indicating the astonishing number of processes that the intestinal microbes are involved in [9]. It remains to be determined, however, if this new wealth of knowledge will affect clinical practice medicine. In this review we provide an overview of the current literature on the role of the gut microbiota in health and disease from the perspective of the internist.
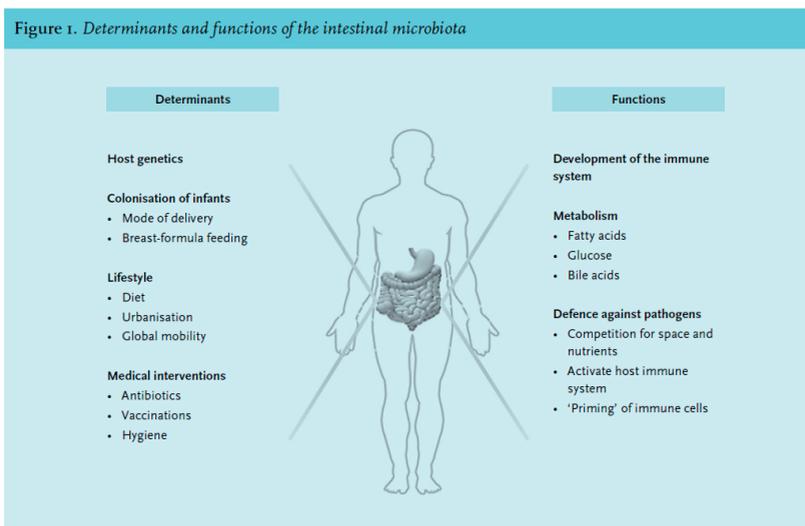

Figure 1. Determinants and functions of the intestinal microbiota

Composition of the gut microbiota

Determining the exact composition of the gut microbiota has been difficult due to the limited success of culturing (often anaerobic) bacteria. With the development of specialized microarrays and high-throughput sequencing techniques, our knowledge on this subject has started to expand quickly (*textbox*) [9, 10]. The new insights gathered with these new techniques start right at birth: we now know that we are not born sterile, as low levels of bacteria are detected in meconium and umbilical cord blood [11]. Major colonization, however, starts after birth and is influenced by many factors such as genetics of the host, mode of delivery, breast- or formula feeding, nutrition and antibiotics. The diversity of the gut microbiota increases in the first years of life [11], after which the core composition appears to remain relatively constant during adult life [12, 13]: 60% of all gut microbiota strains in healthy adults remain detectable over a period of 5 years, following a power law that suggests that this core is present for a much longer period [13, 14].

Investigating the gut microbiota

Techniques
Classic microbiological culturing techniques have proved inadequate in characterizing the gut microbiota as a whole; sequencing techniques are therefore essential. High throughput methods have been introduced, sequencing either total DNA ("shotgun metagenomics") or 16S ribosomal RNA- genes [9, 10]. 16S rRNA contains nine sequences that are highly variable between species and are well conserved during evolution; therefore it is suitable as a key identifier of bacteria.

Two major reference databases serve as the basis of our knowledge of the structure and function of the human gut microbiota. The U.S. Human Microbiome Project (HMP; www.hmpdacc.org) is a consortium that has reported the structure and function of the human microbiome in 242 healthy individuals at 18 body sites from a single time point, using 16S ribosomal RNA gene pyrosequencing [10]. Similarly, the European Metagenomics of the Human Intestinal Tract (MetaHIT; www.metahit.eu) project has studied fecal samples from 124 healthy, overweight and obese Europeans, as well as IBD patients, by total DNA metagenomic sequencing [9].

Analysis
One of the major outcome measurements is species diversity, which is defined as effective number of different species that are represented in dataset. Results are often expressed as:
1. Species richness and species evenness, that together make up diversity (also termed alfa diversity – see below)
- Species richness: number of different species represented in an ecological community
- Species evenness: the relative abundance with which each species is represented in the community. An ecosystem where all the species are represented by the same number of individuals has high species evenness.

2. Alfa and beta diversity
- Alfa diversity: within-sample species diversity
- Beta diversity: similarity between samples (from one individual at different time points or between samples from different subjects)

3. Different indexes, such as:
- Shannon index: quantifies the degree of uncertainty when predicting an individual's type within a particular dataset
- Simpson index: quantifies the probability that two entities taken at random from the dataset represent the same type

Each individual has their own unique microbiota. Even between healthy persons the composition may vary strongly as was shown in over 1000 adults [10, 15]. However, when looking at a functional level, most people carry equal amounts of bacterial genes involved in metabolic pathways. This suggests that the microbiota as a functional organ is similar among human beings [10]. Diet is one of the most important determinants of the microbial composition [16]. A study in healthy adults showed that an extremely fat-rich diet is capable of changing the microbiota in only several days [17]. Still, pinpointing causal relationships between the absence or overabundance of bacterial strains and clinical observations is difficult. Few prospective- and intervention studies have been performed hitherto [13, 17, 18]. With older age, inter-individual differences in gut microbiota composition become larger. For instance, long-term care residency is associated with lower bacterial diversity [19]. Still, the largest and most direct effects on microbial composition are achieved by antibiotics.

Influence of antibiotics

A simple antibiotic course, prescribed to kill just one or a few pathogens, will quickly result in major disturbances of the gut microbiota (*table 1*) [20]. Recovery and stabilization of its diversity level may take months, and the new composition of bacteria may significantly differ from pre-treatment. Not only bacteria that fall within the spectrum of the antibiotic are affected; species that are dependent on the ones being killed may disappear as well. Vancomycin for example does not only lead to disappearance of Gram-positive species, but also Gram-negative species and results in more dramatic shifts than amoxicillin [21, 22]. Antibiotics may thus have negative effects on the health of patients, for example on their metabolic state. Use of antibiotics has been associated with the development of obesity, diabetes mellitus and asthma [2].

The role of the gut microbiota in disease

Gastrointestinal infections

A healthy gut microbiota protects the host against pathogens like *C. difficile* directly, by competition for nutrients, space and binding spots on the epithelium and production of bacteriocins, but also indirectly, by activation of the host immune system resulting in release of IgA, cytokines and antimicrobial peptides [23]. Depletion of the microbiota by antibiotics therefore creates an opportunity for *C. difficile* to proliferate.

Multi-drug resistant organisms are a rapidly increasing problem around the world. Vancomycin-resistant enterococci (VRE) a now a leading cause of bloodstream infections in hematopoietic stem cell transplant recipients [24]. In both mice and men, domination by VRE (being at least 30% of the microbiota) correlates with subsequent development of a bloodstream infection [25] and in mice can be reversed by fecal microbiota transplantation

(FMT) [24]. This suggests that fecal transplantation therapy might be effective in both prevention and treatment of VRE infections. Assessment of fecal microbiota may be used to identify those patients at highest risk for bloodstream infection [25].

The first randomized clinical trial for FMT in recurrent *C. difficile* infection was stopped prematurely because of clear advantage in the FMT arm: 15 of 16 patients had complete resolution of disease, compared to 4 of 13 patients in the vancomycin-arm and 3 of 13 patients in the vancomycin + bowel lavage arm.[4] European recommendations now include FMT for patients with multiple relapses of *C. difficile* infection [26].

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is hypothesized to be either an aberrant immune response against normal commensal bacteria, or a normal immune response against abnormal gut microbiota, both driven by an autoimmune genetic background [27]. Indeed, IBD patients have a significantly lower microbial diversity compared to healthy controls [28] as well as a lower abundance of immune-modulating bacteria such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* [5, 29]. *F.prausnitzii* and *A. muciniphila* produce butyrate and propionate, respectively: both SCFA that are thought to protect the mucosal barrier. A meta-analysis of 41 cases in which FMT was used for ulcerative colitis found that 63% of patients entered remission and 76% could stop medications [28]. Obviously, these numbers may suffer from publication bias. Thirteen clinical trials are currently ongoing to evaluate the effect of FMT in IBD (*table 2*).

Transplant immunology

Intestinal inflammation secondary to graft-versus-host disease (GVHD) is likely to be influenced by the gut microbiota. A mouse model of GVHD caused by allogeneic stem cell transplantation (SCT) showed that mice developing intestinal GVHD have a decreased bacterial diversity compared to those that do not [30]. A greater proportion of bacteria consisted of Lactobacillales, while Clostridiales were decreased. The same pattern was found in 31 patients receiving allogeneic SCT - probably due to chemotherapy, irradiation and antibiotics. The percentage *Enterococcus faecium* and *Enterococcus faecalis* (of the order Lactobacillales) in the microbiota was 21% in patients who did not develop intestinal GVHD, 46% in those that subsequently developed GVHD and 74% at the time of actual GVHD [31]. The diversity level at the time of engraftment seems to predict outcome: after classifying 80 SCT-patients into low, intermediate, and high diversity-groups, overall three-year survival was 36%, 60%, and 67% respectively [32].

Colorectal cancer and response to chemotherapy

The intestinal microbiota play a key role in mucosal immune responses, epithelial cell homeostasis, barrier function and metabolism – all important to tumour formation. For example, the microbiota produce (precursors of) carcinogenic substances, such as nitrate

[5]. A strictly animal-based diet (meats, eggs and cheeses) of only five days in healthy volunteers increased bile acids produced by the microbiota, including the DNA damage- and hepatic carcinoma- promoting deoxycholic acid [17]. Accordingly, bile-tolerant bacteria such as *Bilophila wadsworthia* were increased, which in mice has been described to induce IBD by producing hydrogen sulfide [17]. This supports the association between diets that are rich in red meat and the development of colorectal cancer [33]. Fecal transplantation from colon tumour-developing mice to germ-free mice resulted in a higher incidence of colon tumours in the latter, compared to transplantation from control mice – most likely by inducing inflammation prior to induction of tumorigenesis by dextran sodium sulfate in drinking water [34].

The gut microbiota also influences the effectiveness of anticancer therapy [35-37]. In different cancer mouse models, it was shown that in absence of a healthy microbiota, tumour-infiltrating myeloid cells are less capable of producing cytokines and inducing cytotoxicity upon immuno- or platinum chemotherapy, resulting in decreased necrosis of tumours [35]. Both cytotoxic drugs and irradiation may cause translocation of bacteria or bacterial particles to lymph nodes, thereby enhancing immune responses directed against malignant cells [36, 37]. Destruction of the microbiota by antibiotics and other drugs may therefore reduce the efficacy of these treatments [35-37].

Allergic and infectious airway diseases

Low diversity of the gut microbiota, whether caused by low exposure to environmental microbes or (pre- or postnatal) use of antibiotics, is associated with increased risk of allergic airway disease [38, 39]. The intestinal microbiota is postulated to enhance the hematopoiesis of dendritic cell precursors in bone marrow by production of SCFAs [40]. Intriguingly, dendritic cells from mice that have received propionate exhibit an impaired ability to activate T-helper2 cells in the lung, thus preventing allergic airway inflammation [40]. Supplementation of dietary fibre or SCFA may therefore prevent or reverse allergic airway disease.

A similar "priming" effect by healthy gut microbiota has been observed in murine models of pneumonia and peritonitis [41-43]. Depletion of the gut microbiota by antibiotics in neonatal mice appears to hamper neutrophil production by the bone marrow, thus leading to increased counts of pathogenic bacteria and decreased survival [43]. It is suggested that lipopolysaccharide from gut bacteria is the priming component, although another role for SCFA is not excluded. For now, evidence for this so called gut-lung axis in humans is lacking.

Metabolic disorders

Obesity has been associated with a lower microbial diversity in the intestine [44, 45]. Studies have reported an association between obesity and increased levels of Firmicutes,

combined with decreased Bacteroidetes (the two most dominant phyla), although this could not be replicated in all cohorts [5, 46]. Overweight and obese individuals have higher levels of SCFA, without a different intake of dietary fiber, reflecting either an increased production or decreased absorption by the gut and utilization by bacteria [46].

In both mice and men, there is evidence that the obese microbiota is more effective at harvesting energy from food, as suggested by enriched biochemical pathways [5, 47]. Accordingly, transplantation of gut microbiota from obese to germ-free mice leads to more weight gain than transplantation of microbiota from control mice [47]. Transplantation of feces from lean human donors into patients with metabolic syndrome decreases peripheral insulin resistance six weeks after transplantation. This is potentially caused by increased levels of butyrate-producing bacteria (*Roseburia intestinalis*) [48]. By contrast, among 20 children of 3-5 years of age, levels of butyrate-producing bacteria were also found to be higher in those that subsequently developed type 1 diabetes [49]. Of interest, seven days of vancomycin therapy resulted in decreased peripheral insulin sensitivity in 10 males with metabolic syndrome [22]. This was associated with a decrease in Firmicutes bacteria and decreased metabolism of bile acids.

From theory to therapy

Although the role of the gut microbiota in the pathophysiology of disease may be easy to imagine, translation of this knowledge into new therapeutic strategies represents a tremendous challenge. Changing one's intake of specific nutrients would be the most natural way to prevent diseases. Consumption of dietary fibre seems one of the best solutions, since they are fermented into SCFA that may have beneficial effects. Both extreme diets [17] and the use of various fibers were shown to modify the human intestinal microbiota, although the latter not in all subjects [50].

Probiotics that include a variety of lactic acid bacteria are by now commonplace in both the supermarket and the clinic, but their effectiveness is highly debatable [51]. Moreover, higher mortality in patients treated with lactic acid bacteria during severe pancreatitis compared to placebo treatment has led to increased caution regarding these bacteria, notably in a clinical setting [52].

The successful trial on severe *C. difficile* infection has put the spotlight on FMT [28]. However, we currently do not know how to select a microbiota that is best to transplant. In addition, the possibility of transplanting pathogenic microorganisms, or the possible risk of metabolic- and autoimmune diseases, might be a reason for caution. So far, no serious (long-term) adverse events have been reported.

Transplanting a cocktail of bacteria (the synthetic microbiota) that can be cultured or isolated easily, may be a key objective the coming years [53]. First steps were taken already

in 1989, when ten selected intestinal strains that showed inhibitory effects against *C. difficile* cured five patients with *C. difficile* infection [53, 54]. More recently, two *C. difficile* infected patients were transplanted with 33 cultured bacterial species, isolated from healthy donor feces – both resulting in resolution of disease and no relapse in 24 months [23]. These results encourage the development of synthetic, standardized microbiota.

Conclusion

The booming popularity of the gut microbiota in biomedical research seems justified; in fact, the interest in host-microbiota interactions and their potential value as a therapeutic target seems rather belated in view of our ancient commensal relationship. Obviously, results from animal studies cannot be translated directly to human health and disease. Still, some of the possible microbiota-disease interactions will prove to be relevant and applicable in everyday clinical practice. The great possibilities offered by this new field of research provide hope for new, relatively straightforward and inexpensive therapies. Still, most research is currently in a preclinical phase, and causative relationships remain to be established. Moving from correlation to causality will be particularly difficult for phenotypically and/or genetically heterogeneous disorders, such as diabetes, IBD and GVHD [55]. In anticipation of gut microbiota- targeted therapies, it seems important to realize the key function of the gut microbiota in physiological processes and the potential collateral damage we cause when disrupting this well-balanced ecosystem.

Table 1. Overview of studies on the effect of antibiotics on the human intestinal microbiota

Author	Antibiotic regimen	Subjects	Short term results (days, weeks)	Long term results (months, years)
De la Cochetiere et al., 2005 [56]	Amoxicillin, orally, 5 days	6 adult healthy volunteers	Major shift in dominant species after 24 hours. Average similarity (compared to pre-treatment) 74% after 4 days	1-2 month: average similarity 88-89%
Penders et al., 2006 [57]	Mostly amoxicillin	28 pediatric patients (1 month old)	Decreased counts of *Bifidobacteria* and *B. fragilis*-species compared to non-treated children	None reported
Jernberg et al., 2007 [58]	Clindamycin, orally, 7 days	4 adult healthy volunteers (vs 4 controls)	Day 7, 21: large and persistent shift in composition	3, 6, 9, 12, 18 and 24 months: large and persistent shift in composition
Dethlefsen et al., 2008 [59]	Ciprofloxacin, orally, 5 days	3 adult healthy volunteers	Abundance of about a third of the bacterial taxa in the gut affected; decreased taxonomic richness, diversity, and evenness of the community	1 and 6 months: richness, diversity and evenness comparable to pre-antibiotic state, some long-term losses
Dethlefsen et al., 2010 [20]	Ciprofloxacin, orally, 5 days; after 6 months again 5 days	3 adult healthy volunteers	Loss of diversity and shift in composition within 3–4 days; 7 days after end of a course, communities start returning to initial state; often incomplete	10 months: composition stabilized but altered; long-term losses of some taxa
Jakobsson et al., 2010 [60]	Metronidazole + clarithromycin, orally, 7 days	3 adult healthy volunteers (vs 3 controls)	Dramatic decline in diversity, especially loss of Actinobacteria, in both throat and feces	1 year: diversity levels recovered to pre-treatment states
Fouhy et al., 2012 [61]	Parenteral antibiotic treatment with ampicillin and gentamicin (within 48 h of birth)	9 pediatric patients (plus 9 untreated infants)	4 weeks after treatment, antibiotic-treated infants had higher proportions of Proteobacteria and lower proportions of Actinobacteria as well as the genus *Lactobacillus*	2 months: Proteobacteria levels remained higher, but Actinobacteria and *Lactobacillus* levels had recovered
Panda et al., 2014 [62]	Different broad-spectrum antibiotic regimens, orally	21 adult patients	Fluoroquinolones and b-lactams decreased microbial diversity by 25% and reduced the number of taxa from 29 to 12; Increase in proportion Bacteroidetes taxa B-lactams increased the average microbial load two-fold	None reported

Table 2. Current status of microbiota-related therapies on clinicaltrials.gov and trialregister.nl (September 2014)

Gastrointestinal infections	First randomized trials in *C. difficile* completed, with promising results for FMT in severe recurrent infections 21 clinical trials for FMT in *C. difficile* infections registered First case report describing FMT clearing Extended Spectrum beta-Lactamase producing Escherichia coli (ESBL) colonization in end stage renal disease patient [63]
Inflammatory bowel disease	Thirteen clinical trials currently registered for FMT in IBD (both Crohn's disease and ulcerative colitis)
Transplant immunology	No microbiota-related clinical trials being conducted yet
Colorectal cancer	No microbiota-related clinical trials being conducted yet
Allergic airway diseases	>50 trials registered for pre- or probiotics for treatment or prevention of allergic airway disease, mostly in infants
Metabolic disorders	Two randomized trials for FMT in metabolic syndrome registered, one completed One randomized trial for FMT in recently diagnosed diabetes mellitus type 1 registered One randomized trial for FMT in diabetes mellitus type 2 registered

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