Host-microbiota interactions in the digestive tract

Wirt-Mikrobiota Interaktionen im Verdauungstrakt

M. Blaut* – Nuthetal

The intestinal tract: a microbial ecosystem: The digestive tract of mammals represents an ideal environment for a wide range of microorganisms as it provides a continuous supply of nutrients and a constant temperature facilitating microbial growth. However, intestinal microorganisms are challenged by the peristaltic gut movements which transport them along with the digesta through the intestinal tract from which they finally become expelled. Only microorganisms that adhere to the surface of the intestinal tract or that are capable of proliferating at a rate that equals their removal from the ecosystem are not washed out. They also need to be resistant toward bile acids, which are effective detergents, and they have to cope with the highly proteolytic environment created by the secretion of a wide range of proteases and other digestive enzymes.

The gut is not a homogeneous habitat but consists of various niches that differ in their physicochemical properties. Both the anatomy and the physiology of the respective animal species are major determinants for the conditions that prevail in the intestinal tract. Important parameters that influence the growth of intestinal bacteria include pH, oxygen tension, redox potential and available substrates. The intestinal tract of a given animal species reflects its adaptation to its typical diet. Differences between herbivorous, carnivorous and omnivorous animal species affect microbiota composition (Ley et al. 2008). Interestingly bacterial diversity was found to be higher in herbivores than in omnivores or carnivores, with the latter displaying the lowest bacterial diversity. It is generally assumed that the gut microbiota co-evolved and co-diversified with their hosts so that each animal species harbors a characteristic microbiota. Host anatomy and host physiology have been demonstrated to shape the intestinal microbiota (Rawls et al. 2006). Transplantations of the microbiota from mice to germfree zebrafish and vice versa revealed that the microbiota transplanted from one host to the other led to changes in the relative abundance of major community members. The donor microbiota in the recipient host changed such that it resembled the typical microbiota of the recipient host as far as possible with the given inoculum.

Even though considerable differences in microbiota composition exist between different mammals at genus and species levels, there are also a number of common features including the representation of major bacterial lineages in the ecosystem. The main bacterial phyla representing more than 90% of intestinal bacterial cells in humans, pigs and rodents are the Firmicutes and the Bacteroidetes. These phyla are complemented by varying proportions of Proteobacteria, Actinobacteria, and Verrucomicrobia (Table 1). Additional phyla may be present but they usually account for only small proportions of the community. It should also be mentioned that fungi and methanogenic archaea are also often found.

Impact of diet on intestinal microbiota

Diet affects the intestinal microbiota on both the community level and the cellular level. This is evident from the drastic changes in microbiota composition that can be observed after weaning in humans. The microbiota of exclusively breast-fed infants is characterized by the predominance of bifidobacteria and a low bacterial diversity. Bacterial diversity in the infant's intestinal tract increases in response to the introduction of additional foods. In addition the dominance of bifidobacteria is usually lost after weaning. A very recent human study demonstrated considerable changes in the microbiome in response to a switch from a mixed baseline diet to either a plant-based diet or an animal-based diet (David et al. 2014). The latter diet led to an increase in bile-acid tolerant bacteria such as *Bacteroides, Alistipes* and *Bilophila* within 2-3 days while the plant-based diet led to an increase in taxa that are known to play a major role in the breakdown of dietary fiber: e.g., *Roseburia, Eubacterium rectale, Ruminococcus bromii.* The changes in microbial community structure observed

Table 1: Microbial taxa encountered in intestinal tract of human and other mammals

Domain	Phylum	Order	Genus
Archaea	Euryarchaeota	Methanobacteriales	*Methanobrevibacter*
Bacteria	Actinobacteria	Bifidobacteriales	*Bifidobacterium*
		Coriobacteriales	*Adlercreutzia*
			Atopobium
			Collinsella
			Coriobacterium
			Eggerthella
			Slackia
	Bacteroidetes	Bacteroidales	*Alistipes*
			Bacteroides
			Prevotella
			Porphyromonas
	Firmicutes	Bacillales	*Staphylococcus*
		Clostridiales	*Anaerostipes*
			Blautia
			Butyrivibrio
			Clostridium
			Coprococcus
			Dorea
			Eubacterium
			Faecalibacterium
			Finegoldia
			Lachnospira
			Lactonifactor
			Roseburia
			Ruminococcus
			Subdoligranulum
		Erysipelotrichales	*Coprobacillus*
			Holdemania
			Catenibacterium
		Lactobacillales	*Enterococcus*
			Lactobacillus
			Lactococcus
			Streptococcus
	Fusobacteria	Fusobacteriales	*Fusobacterium*
	Proteobacteria	Enterobacteriales	*Escherichia*
			Enterobacter
		Desulfovibrionales	*Bilophila*
	Verrucomicrobia	Verrrucomicrobiales	*Akkermannsia*
Eukarya	Ascomycota	Saccharomycetales	*Candida*

in response to the animal-based diet were accompanied by increased gene expression of bile salt hydrolases

and sulfite reductase. Sulfite may arise from the cleavage of the taurocholate, which stimulates the growth of these bacteria because they can take advantage of the sulfite, which is derived from taurine, as an electron acceptor (Devkota et al. 2012).

Changes in response to diet may also occur on cellular level. This was demonstrated in a simplified model of host-microbiota interaction. Mice monoassociated with *Escherichia coli* MG1655 were fed either a diet rich in starch, protein or lactose (Rothe and Blaut 2013). Protein expression of *E. coli* isolated from small intestinal and cecal contents of mice fed the diet rich in lactose and protein was compared with that of *E. coli* obtained from mice fed the starch diet. Several *E. coli* proteins were up-regulated in response to the lactose diet, including those required for the utilization of lactose. Several of the up-regulated proteins belong to the *oxyR* regulon including the DNA protection during starvation protein (Dps), the alkylhydroperoxide reductase (AhpR), and the ferric uptake Regulatory Protein (Fur). This regulon has previously been known to help *E. coli* in defending itself against oxidative stress. However, further analysis revealed that also osmotically active nutritional factors lead to an up-regulation of *ahpCF* and *dps*. Inactivation of the *oxyR* or *ahpCF* genes led to growth retardation in the presence of sucrose. These results demonstrate that genes usually protecting *E. coli* against oxidative stress also play an important role in the adaptation of this intestinal bacterium to osmotically active nutrition factors (Rothe and Blaut 2013). Other enzymes up-regulated in intestinal *E. coli* from the mice fed the lactose-rich diet compared to *E. coli* from mice fed a diet rich in protein included 2-deoxy-D-gluconate 3-dehydrogenase (KduD) and 5-keto 4-deoxyuronate isomerase (KduI). In vitro experiments demonstrated that galacturonate and glucuronate induced kduD and kduI gene expression and that KduI facilitates the breakdown of these hexuronates. In *E. coli*, galacturonate and glucuronate are normally degraded by altronate hydrolase (UxaA), altronate oxidoreductase (UxaB), uronate isomerase (UxaC), mannonate dehydratase (UxuA) and mannonate oxidoreductase (UxuB). However, osmotic stress represses the expression of the corresponding genes in an OxyR-dependent manner. When grown in the presence of galacturonate or glucuronate, kduID-deficient *E. coli* displayed a considerably lower maximal cell density and extended doubling times under osmotic stress conditions than wild type *E. coli*. Growth on lactose promoted the intracellular formation of hexuronates, which possibly explain the induction of KduD on a lactose-rich diet. These results indicate a novel function of KduI and KduD in *E. coli* and demonstrate the crucial influence of host diet on gene expression in an intestinal bacterium (Rothe et al. 2013).

Importance of the intestinal microbiota for the host

In particular herbivorous and mammals depend to a large extent on the ability of their intestinal microbiota to degrade plant fibers. This is particularly evident for ruminants which harbor complex microbial communities in their digestive system which afford the conversion of complex polysaccharides to short chain fatty acids (SCFA). SCFA are subsequently utilized by the host for energy generation and/or for neoglucogenesis. Even though humans, pigs or rodents do not depend to such a large on their respective intestinal microbiota as ruminants do, the gut microbiota exerts profound effects on digestion, gut function and immunity.

Role of intestinal microbiota in conversion of dietary fibers

Typical diets of herbivores and omnivores contain considerable amounts of polysaccharides that escape digestion because the enzymes required for their degradation are missing. The intestinal microbiomes of humans and mice encompass a wealth of enzymes that catalyze the depolymerization of complex polysaccharides referred to as dietary fibers. Indigestible polysaccharides are components of plant-based diets. They are embedded in complex plant structures such as plant cell walls. Typical plant-derived polysaccharides include cellulose, pentosans, hexosans, xylanoglycans, pectin, inulin and resistant starch. They vary with respect to solubility, viscosity, molecular mass and three-dimensional configuration. Their high degree of structural variability is based on the variability of the monomeric components and how these are linked and branched. Dietary fiber represents the main source of carbon and energy for intestinal microorganisms. Owing to the structural diversity of plant polysaccharides, intestinal bacteria need to express a large spectrum of depolymerizing enzymes to take advantage of dietary fiber. Accordingly the human colonic metagenome is enriched with genes involved in the depolymerization

and utilization of complex carbohydrates while genes involved in energy production or lipid metabolism are underrepresented (Gill et al. 2006). More than 80 different glycoside hydrolase families have been found in the human gut microbiome. The human genome lacks the majority of these enzymes rendering the host dependent on the presence of the intestinal enzymes to take advantage of such carbohydrates, which otherwise would be excreted. By converting dietary fiber into nutrients the microbiota helps the host to extract more energy and nutrients from the diet. The main fermentation products of dietary fiber are SCFA such as acetate, propionate, and butyrate, all of which can be utilized by the host. In addition, carbon dioxide, molecular hydrogen and methane are produced to some extent.

Various genera including *Bacteroides, Ruminococcus, Butyrivibrio, Eubacterium, Clostridium*, and *Bifidobacterium* contribute to the breakdown of dietary fiber. *B. thetaiotaomicron*'s ability to utilize a large variety of carbohydrates is also reflected by its genome which contains various sets of genes predicted to encode a wide range of glycosylhydrolases including amylases, a- and b-glucosidases, a- and b-galactosidases, a-mannosidases, b-glucuronidases, b-fructofurano-sidases, and endo-1,2-b-xylanases (Xu et al. 2003). Dietary polysaccharides are the preferred growth substrates for the majority of intestinal bacteria. However, it has been demonstrated for *Bacteroides thetaiotaomicron* that this organism is sufficiently flexible to switch to host glycans contained in glycoproteins when dietary polysaccharides are not available (Sonnenburg et al. 2005).

The conversion of complex polysaccharides to SCFA occurs in several steps, the first one leading to the formation of oligomeric and monomeric carbohydrates (Figure 1). The ensuing degradation of these oligo- and monosaccharides leads to the formation lactate, succinate, ethanol, and formate. These intermediates may serve as substrates for other bacterial genera such as butyrate-producing *Anaerostipes caccae*, *Faecalibacterium prausnitzii* and *Roseburia intestinalis* (Duncan et al. 2004) or propionate-producing *Veillonella* and *Megasphaera*. Formate and molecular hydrogen in conjunction with carbon dioxide can be used by methanogenic archaea such as *Methanobrevibacter smithii* for methane formation and by acetogenic bacteria such as *Eggerthella lenta* or *Blautia producta* for the formation of acetate via the Wood-Ljundahl pathway.

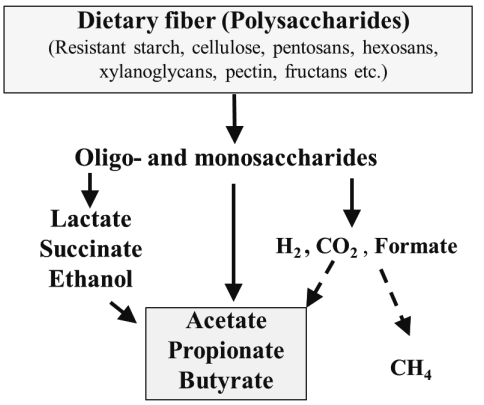

Figure 1: Various steps in the microbial breakdown of dietary fiber in the digestive tract

The core microbiome encompasses essential functions of the microbiota

The breakdown of dietary fiber represents one of the key functions of the intestinal microbiome. Metagenomic analyses revealed that the relative abundance of genes reflecting different functions was similar among human individuals even though the same individuals differed considerably in their microbiota composition (Turnbaugh et al. 2009). It may be concluded that a given function may be fulfilled by different bacteria in the gut microbiota of different individuals. It has been proposed that important functions that are found in every individual represent the core microbiome. Such functions include fiber degradation, conversion of bile acids and various types of interactions with the host (Turnbaugh et al. 2007). Intestinal bacterial activities that can only be found in a certain proportion of human individuals do not belong to the core microbiome. Examples of activities that cannot be found in the microbiome of every individual include the ability to activate isoflavones such as daidzein and genistein, which are present in soy. This activity is limited to individuals that harbor bacteria that are capable of converting isoflavones. Isoflavones have been implicated in the prevention of hormone-related cancers, cardiovascular diseases and the alleviation of menopausal symptoms. In the case of daidzein, these effects have mainly been attributed to one of its bacterial transformation products, namely equol, which undergoes urinary excretion. Every third to every other healthy adult who consumed soy excreted

equol in his/her urine (Atkinson et al. 2004). It may be surmised that those individuals who excreted equol harbored Adlercreutzia equolifaciens or Slackia isoflavoniconvertens, both of which convert daidzein to the biologically active equol (Maruo et al. 2008; Matthies et al. 2009).

Another example of an activity that is restricted to a certain human population concerns the ability of the microbiota to degrade the sulfated polysaccharide porphyran from marine red algae of the genus Porphyra (Hehemann et al. 2010). Metagenome analyses revealed that the corresponding porphyranases are frequently found in the microbiomes of Japanese but not in those of North American individuals. Bacteroides plebeius, which was isolated from the microbiota of Japanese individuals, harbored the corresponding genes. Several lines of evidence indicate that they were acquired from Zobellia galactanivorans a member of the marine Bacteroidetes, which grow on marine algae. The latter supports the widely accepted view that diet rather than phylogenetic position of an animal drives the evolution of the corresponding microbiota.

Impact of the gut microbiota on host physiology

The pioneering studies by Hooper et al. demonstrated that the association of germfree mice with a common inhabitant of the intestinal tract, Bacteroides thetaiotaomicron, resulted in changes in the expression of more than 100 different host genes in intestinal tissue (Hooper et al. 2001). Mice monoassociated with B. thetaiotaomicron displayed higher mRNA levels in ileal tissue of genes involved in nutrient uptake, including Na+/glucose cotransporter(SGLT-1), pancreatic lipase-related protein-2 (PLRP-2), colipase and liver fatty acid-binding protein (L-FABP) suggesting that this intestinal bacterium improved the ability of the host to extract energy from the diet. The threefold increase in expression of the high-affinity epithelial copper transporter (CRT1) in the colonized mice compared to the germfree mice suggested that B. thetaiotaomicron improved the absorption of micronutrients from the intestinal tract. Another set of ileal genes found to be up-regulated in mice monoassociated with B. thetaiotaomicron relates to an improvement of gut barrier function. For example, mRNA levels of small proline-rich protein-2 (sprr2a) were 280-fold higher in the monoassociated mice than in the germfree mice. Sprr has been implicated in fortification of the cell envelope by acting as a cross-bridging protein. Similarly, gene expression of decay-accelerating factor (DAF), an apical epithelial inhibitor of complement-mediated cytolysis, and polymeric immunoglobulin receptor (pIgR), which transports IgA across the epithelium, were up-regulated. This indicates that the colonization of the mouse intestinal tract prompts the host to take measures against the translocation of intestinal bacteria. The third category of ileal genes, whose expression changed in response to the presence of B. thetaiotaomicron, concerns enzymes involved in the detoxification of drugs and certain dietary components. For example, mRNA levels of glutathione S-transferase and multidrug resistance protein 1A (Mdr1a) were found to be reduced in mice monoassociated with B. thetaiotaomicron compared to germfree mice (Hooper et al. 2001).

Another study revealed that colonization of germfree mice with B. thetaiotaomicron or with a complex microbiota results in the formation of Angiogenin 4 (Ang4). Ang4 is produced by Paneth cells and has a selective microbiocidal activity against certain bacterial species such as Listeria monocytogenes and Enterococcus faecalis but not against others (Hooper et al. 2003). By inducing Ang4 in Paneth cells of mice, B. thetaiotaomicron and other commensal bacteria may contribute to the defense against pathogens. Intestinal bacteria have been demonstrated to influence the differentiation of epithelial cells in the ileum of mice (Bry et al. 1996). After birth, both germfree and conventional mice express α-1,2 and α-1,6 fucosyltransferases which catalyze the fucoslyation of ileal epithelial cells. In conventional mice, fucosylation of the epithelial surface is complete four weeks after birth. In contrast, the fucosylation program, which starts right after birth, is discontinued in germfree mice so that their ileal epithelial cells are devoid of fucosyl residues at four weeks of age. This fucosylation program could be re-initiated by association of the mice with a conventional microbiota or with B. thetaiotaomicron. It turned out that the re-induction of fucosylation by B. thetaiotaomicron was dose-dependent. At least 104 cells/ml were required to induce fucosylation in germfree mice and 107 cells/ml had the same effect on fucosylation as observed in conventional mice. Interestingly, a mutant of B. thetaiotaomicron devoid of fucose utilization genes was unable to induce α-1,2 and α-1,6 fucosyltransferases in germfree mice. This indicates that an intricate relationship exists between this representative of the microbiota and the host. By inducing the

fucosylation on ileal epithelium B. thetaiotaomicron triggers the host to provide a growth substrate to this commensal bacterium. At the same time B. thetaiotaomicron modifies the surface of its intestinal epithelium, which in turn possibly affects the ability of pathogens to adhere to the epithelium.

Pathogens, commensals and pathobionts

Pathogens represent a continuous threat because they are capable of causing various types of infections. How severe an infection in the intestinal tract may become depends on the virulence of the pathogen and on the ability of the host to mount an effective defense. Components of the defense include commensal microorganisms that provide a barrier against incoming bacteria by curtailing their proliferation. This is accomplished by subjecting invading bacteria to a fierce competition for nutrients and by preventing their binding to the gut epithelium. The mucus layer covering the epithelium represents another important barrier against intruding pathogens. However, the immune system represents the core of the defense against infectious agents. The innate immune system affords a quick response based on its ability to recognize cell constituents of microorganisms and to mount an inflammatory response to intruders, while the acquired immune system gives rise to the formation of antibodies and cytotoxic cells which in concert with a plethora of regulatory immune cells aim at the elimination of the pathogen. Commensal bacteria contribute to the prevention of intestinal infections by creating unfavorable conditions for pathogens and by modulating the host immune response, for instance by inducing the formation of bacteriocidal substances such as Ang4, which is formed in Paneth cells (as described above).

Some bacteria in the intestinal tract do not do any harm unless they reach certain concentrations or unless the host defense is impaired. One recent study revealed that a diet rich in saturated fat may lead to the proliferation of bacteria that are normally present at concentrations that do not cause disease (Devkota et al. 2012). Interleukin (IL)10/ mice, which are prone to gut inflammation, had a considerably higher incidence of gut inflammation and higher inflammatory score when fed a diet rich in saturated milk fat, compared to IL10/ mice fed an isocaloric diet, in which the milk fat was replaced by unsaturated fats. Further analyses revealed that the diet containing the saturated fat led to changes in the relative proportions of bile acids. The most obvious change in response to the milk fat diet was an increase in the relative proportion of taurocholate. The taurine moiety of taurocholate was demonstrated to promote the growth of intestinal bacteria capable of utilizing the sulfite moiety of taurine as an electron acceptor. One group of organisms whose growth was stimulated under these conditions was Bilophila wadsworthia. This organism is a member of the normal microbiota but is usually present at low numbers only or even undetectable. At higher concentrations this bacterium induces gut inflammation in IL10/ mice as observed in response to a diet rich in saturated fats. The term “pathobiont” has been coined for organisms such as B. wadsworthia.

Another example is Akkermansia muciniphila, which is a normal member of the human gut microbiota, and therefore considered to be a commensal organism (Derrien et al. 2008). It colonizes the human gut within the first year after birth and is known for its ability to degrade mucins. In rodents and humans the population size of A. muciniphila inversely correlates with body weight (Everard et al. 2013) and has therefore been proposed to have health-promoting properties. However, under certain circumstances this bacterium may also have adverse effects. In a gnotobiotic mouse model of intestinal inflammation, in which gut inflammation is caused by Salmonella enterica serotype Typhimurium the concomitant presence of A. muciniphila caused an exacerbation of inflammatory and infectious symptoms (Ganesh et al. 2013) as evident from significantly increased histopathology scores and increased gene expression levels of interferon-gamma, interferon-gamma-induced protein 10, tumor necrosis factor alpha, interleukin (IL)-12, IL-17 and IL-6 in cecal and colonic tissue. In addition, S. Typhimurium cell numbers in mesenteric lymph nodes of the gnotobiotic mice associated with A. muciniphila and S. Typhimurium were tenfold higher compared to the gnotobiotic mice with S. Typhimurium but without A. muciniphila. Since Akkermansia is a mucin-degrading organism, mucus producing goblet cells were investigated. The number of mucin-filled filled goblet cells was 2- to 3- fold lower in cecal tissue of gnotobiotic mice containing both A. muciniphila and S. Typhimurium compared to mice containing either one or none of these two organisms. These differences were accompanied by a drastic change in the composition of the