World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i47.17727

World J Gastroenterol 2014 December 21; 20(47): 17727-17736
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (17): Intestinal microbiota

Impact of the gut microbiota on rodent models of human disease

Axel Kornerup Hansen, Camilla Hartmann Friis Hansen, Lukasz Krych, Dennis Sandris Nielsen

Axel Kornerup Hansen, Camilla Hartmann Friis Hansen, Section of Experimental Animal Models, Department of Veterinary Disease Biology, University of Copenhagen, 1871 Frederiksberg, Denmark
Lukasz Krych, Dennis Sandris Nielsen, Department of Food Science, Faculty of Sciences, University of Copenhagen, 1958 Frederiksberg, Denmark
Author contributions: All authors contributed to this paper equally.
Correspondence to: Axel Kornerup Hansen, Professor, DVSc, DVM, DipECLAM, Section of Experimental Animal Models, Department of Veterinary Disease Biology, University of Copenhagen, 57 Thorvaldsensvej, 1871 Frederiksberg, Denmark. akh@sund.ku.dk
Telephone: +45-353-32726 Fax: +45-353-32755
Received: February 27, 2014 Revised: September 30, 2014
Accepted: November 18, 2014
Published online: December 21, 2014

Abstract

Traditionally bacteria have been considered as either pathogens, commensals or symbionts. The mammal gut harbors 10^{14} organisms dispersed on approximately 1000 different species. Today, diagnostics, in contrast to previous cultivation techniques, allow the identification of close to 100% of bacterial species. This has revealed that a range of animal models within different research areas, such as diabetes, obesity, cancer, allergy, behavior and colitis, are affected by their gut microbiota. Correlation studies may for some diseases show correlation between gut microbiota composition and disease parameters higher than 70%. Some disease phenotypes may be transferred when recolonizing germ free mice. The mechanistic aspects are not clear, but some examples on how gut bacteria stimulate receptors, metabolism, and immune responses are discussed. A more deeper understanding of the impact of microbiota has its origin in the overall composition of the microbiota and in some newly recognized species, such as *Akkermansia muciniphila*, Segmented filamentous bacteria and *Faecalibacterium prausnitzii*, which seem to have an impact on more or less severe disease in specific models. Thus, the impact of the microbiota on animal models is of a magnitude that cannot be ignored in future research. Therefore, either models with specific microbiota must be developed, or the microbiota must be characterized in individual studies and incorporated into data evaluation.

Key words: Animal models; Gut microbiota; Diabetes; Obesity; Cancer; Allergy; Behavior; Colitis

Core tip: Full characterization of the gut microbiota of animal models has revealed that animal models within different research areas, such as diabetes, obesity, cancer, allergy, behavior and colitis, are highly affected by their gut microbiota. The mechanistic aspects are not clear; however, the impact of the microbiota on animal models is of a magnitude that cannot be ignored in future research. Therefore, either models with specific microbiota must be developed, or the microbiota must be characterized in individual studies and incorporated into data evaluation.

Hansen AK, Friis Hansen CH, Krych L, Nielsen DS. Impact of the gut microbiota on rodent models of human disease. *World J Gastroenterol* 2014; 20(47): 17727-17736 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i47/17727.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i47.17727

INTRODUCTION

Host-microbiota relationship

The gut is an ideal incubation chamber for bacteria adapted to the mammal body temperature and the anaer-

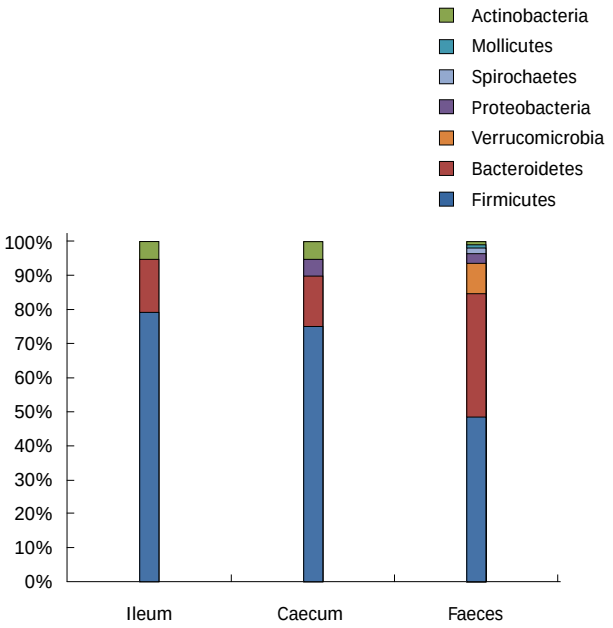

Figure 1 The approximate composition of the gut microbiota in the ileum, cecum and feces of mice[3,7,8,11,16].

obic environment. Thousands of years of co-existence has led to such adaptation, and the mammal gut harbors 10^{14} organisms dispersed over approximately 1000 different species, dependent on how the cut-offs are set for similarity. Within the traditional approach to laboratory animal bacteriology, bacteria have been considered as either pathogens, commensals or symbionts; however, there seems to be a need for a broader understanding of this. When first inside the gut, the bacteria will be fed and will be allowed to propagate, while the host organism will benefit from otherwise unavailable products of microbial digestion. Generally, pathogenicity is not in the interest of the microorganism, because it induces a strong and eradicating immune response from the host, and even in the case of microbial victory in this battle, the end result may be the death of the host and the need for the microbe to relocate to a new habitat. The host immune system, on the other hand, needs to protect the host from invasion without being so aggressive that it loses the microbe and thereby all its benefits.

Complexity of microbial impact on the host

A more advanced understanding of the impact of the microbiota takes into consideration both the overall composition and the balance between the members of the microbiota, as well as some newly recognized species, which, by themselves, seem to have an effect on the specific models. Some of these have a symbiotic effect, while others push disease development in a more detrimental direction. However, same species may act in favor of the development of one disease, while being more protective against another disease, and the mechanistic potential of the species may differ between different parts of the gut. For most of these bacteria, it is their abundance, rather than their qualitative presence or absence, which are responsible for their effect on the host[1-4]. The microbiota is normally not very diverse in the upper part of the gut, *e.g.* in the ileum, where there is a huge accumulation of lymphatic tissue available for stimulation[3,5-10]. It gradually becomes more diverse as the gut contents pass through the large intestine and become feces (Figure 1)[3,5-11]. In both man and mouse, a microbiota with a low diversity is indicative of an increased risk of developing inflammatory disease[12,13]. Furthermore, in animals, a microbiota that is roughly similar in the upper part of the gut, may differ substantially in the lower part of the gut and *vice versa*[3,14]. Finally, there might be essential differences between the effects of the various species at different ages of the animals, which may explain why some species favor the development of one disease, while protecting against another.

Modern microbiological identification techniques

Over recent decades, new methods based upon molecular biology diagnostics have been developed. Such methods, which include quantitative real-time polymerase chain reaction (qPCR) assays[15], pyrosequencing[16] and metagenomic sequencing, have permitted identification of close to 100% of the gut's operational taxonomic units (OTU), which include both cultivable and non-cultivable bacterial species, and in principle, viral, eukaryotes and Archea[17], although they are seldom specifically tested for at present. In contrast, previous cultivation techniques only allowed cultivation and identification of 10%-20% of the bacterial species present in the gut[18]. These molecular biology-based tools have enabled detailed correlation studies. Such studies have revealed that a range of animal models within a range of different research areas are affected by their gut microbiota[19].

GENERAL MECHANISMS UNDERLYING THE GUT MICROBIOTA EFFECT

As described below, the impact of the microbiota on animal models is well documented, while the mechanisms underlying this are less clear. Some hypotheses, though, make more sense than others. As techniques for the full characterization of the microbiota have been developed over the last decade, we are only now beginning to achieve an understanding of how the microbiota actually exerts its effect on the host; however, some examples can be given.

Window of opportunity

In early life, there is a window for the induction of oral tolerance in the gut[20]. This seems essential to avoid inflammatory disease later in life[21]. Molecular structures in bacteria known as microbial-associated molecular patterns (MAMP) stimulate pattern-recognition receptors (PRR) in the host, thereby inducing innate responses[22]. Among the most important PRRs are the toll-like receptors (TLR), which are present in different types on a range of different cell types[22-29] (Figure 2). An impor-

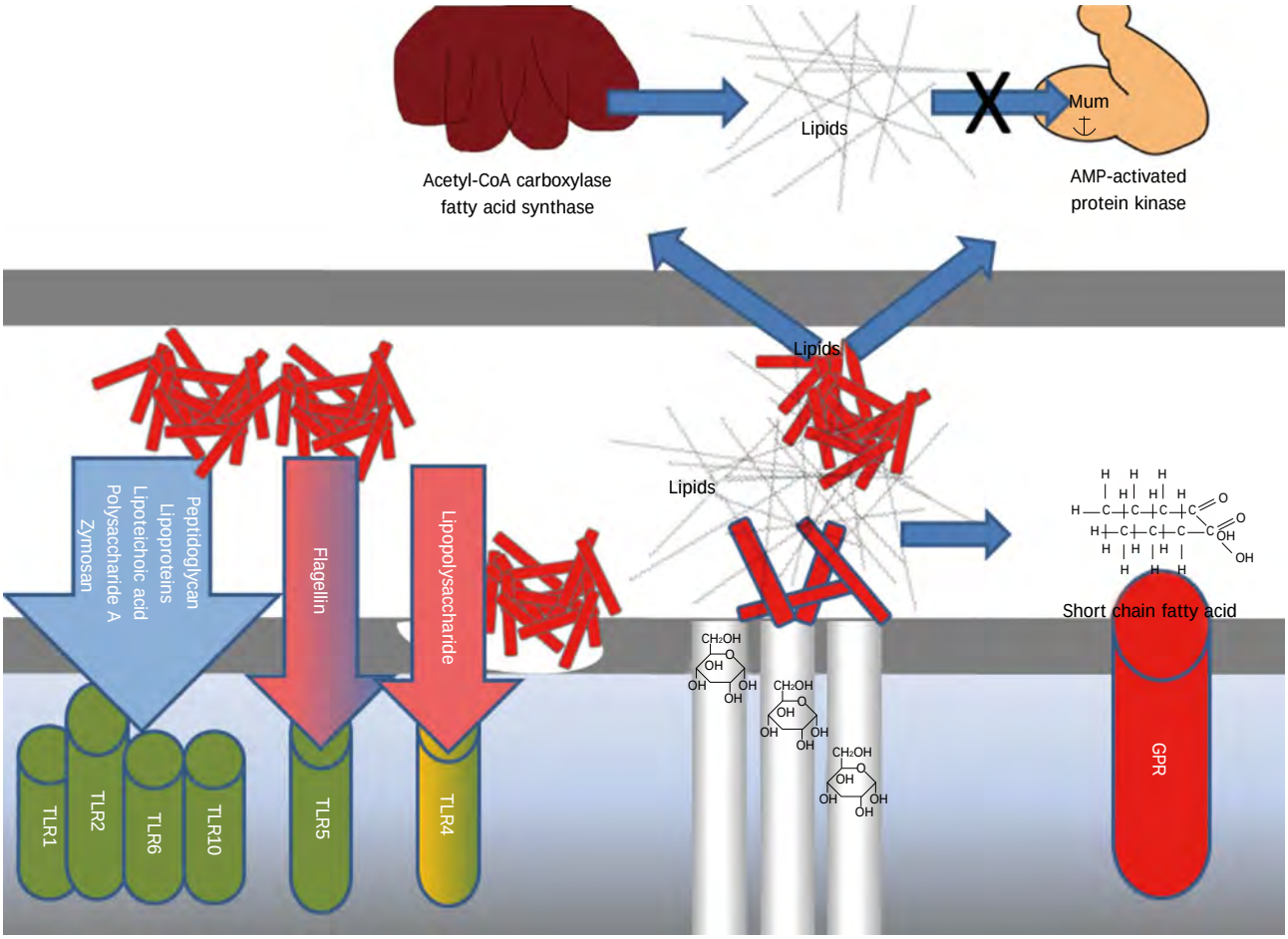

Figure 2 Examples of some theories on potential pathways for the impact of gut microbiota on animal models of human disease. Bacterial colonization may double the density of capillaries in the small intestinal epithelium, thereby promoting intestinal monosaccharide absorption[28]. Undigested food components may be fermented into SCFAs and subsequently act as signals for GPRs of importance for the development of obesity[26,29]. Bacteria may express several key enzymes relevant for hepatic lipogenesis[27,50], and hepatic and muscular fatty acid oxidation[31]. Molecular structures in the cell walls of bacteria may act as MAMPs, which stimulate TLRs on the host cells to induce innate immune responses. The complex of TLR1, TLR2, TLR6 and TLR10 is expressed on a range of cell types such as enterocytes, macrophages, dendritic cells, natural killer cells, mast cells, T cells, B cells, neutrophilic cells and Schwann cells, and may be stimulated by various MAMPs, *e.g.* peptidoglycan, from Gram positive bacteria cell types[21,23-25,31-33]. TLR4, expressed by *e.g.* macrophages, dendritic cells, mast cells, natural killer cells and enterocytes, is stimulated by lipopolysaccharides from Gram negative bacteria[30], while flagellin from various bacteria may stimulate TLR5 expressed by *e.g.* mucosal dendritic cells and macrophages[33]. Mucin-degrading *Akkermansia muciniphila* may reduce the mucus layer to increase TLR-stimulation[79]. SCFAs: Short chain fatty acids; GPR: G-protein receptor; MAMP: Microbial-associated molecular pattern; TLR: Toll-like receptor.

tant example of a MAMP is lipopolysaccharides (LPS), which are important parts of the cell wall of Gram negative bacteria[30], such as Proteobacteria[31], from which it stimulates TLR4. Another important example is peptidoglycan, found in the cell walls of Gram positive bacteria, which stimulates TLR2[32] and flagellin deriving from flagellated bacteria, leading to stimulation of TLR5[33]. Therefore, as different types of MAMPs stimulate different TLR's dispersed on a variety of different cell types[23], and as MAMPs are also dispersed and shared between members of the microbiota[22], there is a vast range of innate host responses to bacteria.

Adult life stimulation

The age of the animal also makes a difference. For example, stimulation of TLR1, TLR2 and TLR4 in early life leads to higher production of interleukin (IL)-6 than stimulation later in life[34]. Germ free animals have more T-helper cells type 2 (T_H2) and less T_H1 cells[35], as the stimulation of the gut lamina propria dendritic cells, *e.g.* by polysaccharide A (PSA) from *Bacteroides fragilis,* induces IL-12 secretion, which favors T_H1 at the cost of T_H2[36]. Host-bacterial interactions, probably mediated through glucagon-like peptide 2 (GLP-2), seem to control the gut barrier function[37]. Metabolic endotoxaemia is responsible for the phenomenon whereby excess intake of dietary fat increases plasma LPS levels[38,39], which in mice, is a sufficient molecular mechanism to trigger metabolic diseases, such as obesity and diabetes[40].

EXAMPLES OF SOME ANIMAL MODELS UNDER IMPACT OF THE GUT MICROBIOTA

Impact of germ free status

The clearest documentation of a general microbial impact on rodent models is observed when comparing a conven-

Table 1 Examples of rodent models in which germ free status has a documented impact

Model	Disease
Models with increased disease incidence or severity	
β-lactoglobulin induced mouse[51]	Allergy
NOD mouse[42]	Type 1 diabetes
MyD88 KO NOD mouse[42]	Type 1 diabetes
Restrained mouse[43]	Stress
Models with decreased disease incidence or severity	
Ovalbumin-specific TCR TG mouse[44]	Allergy
Swiss-Webster mouse[45]	Anxiety
Collagen induced rat[52]	Arthritis
HLA-B27 TG rat[53]	IBD
IL-2 KO mouse[54,55]	IBD
IL-10 KO mouse[56]	IBD
TCRα KO mouse[57]	IBD
Dextran sulfate sodium induced mouse[46]	IBD
SAMP1/Yit mouse[47]	IBD
Adoptive T-cell transfer in the mouse[48]	IBD
Carrageenan, LPS, or formalin induced mouse[49]	Inflammatory pain
C57BL/6 mouse[65]	Obesity
C57BL/6 mouse[65]	Type 2 diabetes

NOD: Non-obese diabetic; MyD88: Myeloid differentiation primary response gene 88; KO: Knockout; TCR: T cell receptor; TG: Transgenic; HLA-B27: Human leucocyte antigen subtype B27; IL-2: Interleukin 2; SAMP1/Yit: Senescence accelerated mice prone line 1 Yakult; LPS: Lipopolysaccharide. IBD: Inflammatory bowel disease.

tional model with a microbiota with a germ free version. In several studies, this has revealed essential differences in disease expression (Table 1)[22,41-57]. Although germ free mice eat more, they are leaner, and they have less body fat compared with conventional mice because they are less efficient in extracting energy from their diet[50]. Germ free mice have increased expression of obesity-related peptides, such as glucagon-like peptide 1 (GLP-1) in the brain[58], which is relevant, because central GLP-1 reduces food intake in rats[59]. Germ free mice also behave differently from microbiota-harboring mice and this behavior may be normalized by colonization[43]. However, for this phenotype there also seems to be an important time window in early life[60]. Germ free mice with a mutation causing a defect in the skin barrier suffer from a more severe B-lymphoproliferative disorder, because they express significantly higher levels of the proinflammatory cytokine thymic stromal lymphopoietin[61]. Inflammatory bowel disease (IBD) occurs either because of a T_H1/T_H17 response (Crohn's disease) or a T_H2 response (ulcerative colitis) to gut commensals[62]. Therefore, IBD under germ free conditions does not develop at all in, *e.g.* Human Leucocyte Antigen subtypes B27 (HLA-B27) transgenic rats[53] and IL-10 knockout mice[56]. For the IL-10 knockout mice[63] it does not occur even under barrier protected conditions (Table 1). IL-2 knockout mice may, under germ free conditions, show mild focal intestinal inflammation[64] (Table 1).

Impact of fluctuations in the gut microbiota composition

Within animal models of the metabolic syndrome, there seems to be an association between the gut microbiota and at least some of the metabolic parameters. For example, in leptin-deficient obese mice, there is a strong correlation between glycated hemoglobin levels and the composition of the gut microbiota[1]. Further, these mice have significantly more Firmicutes and fewer Bacteriodetes members compared with their wild-type and heterozygous litter mates[10]. Their obese phenotype may be transferred with the microbiota by recolonizing germ free lean wild-type mice[65]. In C57 Black substrain 6 (C57BL/6) mice on both high and low calorie diet, continuous oral ampicillin improves glucose tolerance[66,67]. However, this effect is mainly caused by an early life impact on glucose tolerance, and the effect ceases immediately after termination of treatment; thereafter, the glucose tolerance may even decrease[68,69]. Several studies describe cross-talk between the brain and the gut through both the vagal system and the hypothalamus-pituitary-adrenal (HPA) axis[70]. Stressing animal models changes their microbiota[71], and the composition of the gut microbiota has an impact on responses in rodent stress tests[72,73]. Innate immune system cytokines, such as IL-1, IL-6 and tumor necrosis factor α (TNFα), which may originate from a gut microbiota provocation, induce "sickness behavior", changing the priorities of the organism to enhance recovery and survival[74]. However, metabolites formed by microbial decomposition in the gut may also have a direct impact on the brain[75]. In mouse models of atopic dermatitis, more than 70% of the variation observed in the local tissue cytokine response may be shared with the variation in gut microbiota[76]. Changes in the structure of the microbial community seem to reduce the number, as well as the size, of tumors in azoxymethane/dextran sodium sulfate (AOM/DSS) colon cancer-induced mice, and tumor induction may be achieved by colonizing germ free mice with microbiota from induced mice[77].

EXAMPLES OF THE IMPACTS OF SPECIFIC BACTERIAL SPECIES

Verrucomicrobioa

Akkermansia muciniphila (*A. muciniphila*) is a Gram negative bacterium, which in mice is the only species belonging to the phylum Verrucomicrobia[78]. It interacts *via* its mucin degrading capabilities with enteroendocrine cells to modulate gut barrier function, and it is capable of producing certain short chain fatty acids (SCFA's) with a direct action on the receptor G-protein receptor 43 (GPR43)[79]. Abundance of *A. muciniphila* is reduced in mice with obesity and type 2 diabetes[80], and it gradually disappears as aging leptin deficient obese mice develop insulin resistance[1]. In non-obese diabetic (NOD) mice it becomes more abundant when mice are fed a gluten-free diet, which decreases the incidence of type 1 diabetes[81]. Early life treatment with vancomycin in NOD mice allows *A. muciniphila* to become a dominant gut microbiota member, which reduces the incidence of type 1 diabetes[3], but enhances susceptibility to allergic asthma[82], which

is in accordance with other studies showing allergy and diabetes to counteract one another in NOD mice[83,84]. Induction of IBD in mice with dextran sodium sulfate (DSS) reduces the number of extracellular vesicles derived from *A. muciniphila*, and feeding DSS induced mice such vesicles reduces the severity of IBD[85], which fits well with observations in humans[4]. However, it not only reduces the severity of diseases: its presence is correlated with higher severity when infecting mice with *Salmonella typhimurium*[86], and AOM/DSS colon cancer-induced mice have an increased abundance of *A. muciniphila*[77], which may be explained by its ability to downregulate the natural killer cell receptor, NKG2D, which is part of the anti-carcinogenic defense[87].

Firmicutes

Segmented filamentous bacteria (SFB's) are clostridia-related Gram-positive bacteria[88]. The term has been applied for decades to describe intestinal bacteria of a uniform morphology[89]. However, today the term refers to one single species, also known as *Candidatus Savagella*[90]. SFBs induce secretion of the pro-inflammatory cytokine IL-17 from TH17 cells[91], which in the adult mouse is correlated with a low number of regulatory T cells[92]. The presence of SFB's differs between mice from different vendors[92], and SFB positive NOD mice have a significantly lower incidence of type 1 diabetes compared with SFB negative ones[93]. In the adoptive transfer severe combined immune deficiency (SCID) mouse model of IBD, SFBs are essential for the induction of severe inflammation[48]. Furthermore, SFBs and the induced TH17 are important in the defense against intestinal pathogens. For example, mice infected with *Citrobacter rodentium*, a potent murine colon pathogen, exhibit severe symptoms if they lack SFBs[91].

IBD in IL-10 knockout mice is enhanced by *Enterococcus fecalis*[94,95], which is probably linked to its production of gelatinase[96].

Faecalibacterium prausnitzii (*F. prausnitzii*) is a clostridia-related bacterium[97] linked to a protective effect against human Crohn's disease[98]. Oral feeding of *F. prausnitzii* reduced the severity of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice, and some studies indicated that this may also be the case in both multidrug resistance gene deficient (*mdr1a* knockout)[99] and in the DSS-induced mouse models of colitis[100].

High abundances of *Lactobacillus* spp. and bifidobacteria are correlated strongly with low levels of inflammation in mice[101] and leptin in rats[102], which also fits well with these bacteria acting protectively against IBD in IL-10 knockout mice[103], allergic sensitization in mice[104], and myocardial infarction in rats[102]. *Lachnospiraceae* seems quantitatively correlated to improved glucose tolerance in leptin-deficient obese mice[1].

In stressed mice, there is correlation between their Firmicutes levels and their responses in the stress tests[73]. Ingestion of *Lactobacillus rhamnosus* in mice regulates their emotional behavior and central γ-aminobutyric acid (GABA) receptor expression *via* the vagus nerve[72].

Bacteroidetes

A high abundance of the Gram negative family Prevotellaceae, perhaps restricted to one unclassified genus, in the gut of leptin-deficient obese mice correlated with impaired glucose tolerance[1].By contrast, in AOM/DSS induced colon cancer mice, a high abundance of Prevotellaceae correlated with a low tumor burden[77]. *P. copri*, which has been correlated with the development of arthritis in humans, seems to increase the severity of DSS induced colitis in mice[5]. *Caspase-3* knockout mice exhibit a lower inflammatory response to DSS induction of colitis compared with wild-type mice; however, this protective effect of the mutation is decreased by cohousing knockout mice with wild-type mice, which significantly increases the abundance of *Prevotella* spp. in the knockout mice[105].

Bacteroides vulgatus seems to enhance IBD in HLA-B27 transgenic rats[106] and IL-10 knockout mice[95], and in the Bio Breeding (BB) rat, a spontaneous type 1 diabetes model. The fecal microbiota differ and contain an increased number of *Bacteroides* spp. before onset of diabetes[107]. As in all other mammals, *Bacteroides* spp. form an important part of the Bacteroidetes fraction of the rodent gut[16]. These Gram negative bacteria are important for the processing of complex molecules to simpler ones in the gut[108]: complex glycans are their key source of energy[109]. *B. fragilis* toxins cause symptoms of diarrhea and IBD in germ-free mice[110], and they induce colonic tumors strongly in multiple intestinal neoplasia (MIN) mice[111]. On the other hand, *B. fragilis* PSA, which is important for the inflammatory gut response to pathogens[36], also protects against *Helicobacter hepaticus*-induced colitis in mice; probably *via* the prevention of IL-17 secretion[112]. Feeding the maternal immune activation (MIA) mouse model with *B. fragilis* reduces symptoms of autism, which is probably linked to the normalization of the levels of a specific gut metabolite[113].

The abundance of *Alistipes* spp., a bacterium of the Rikenellaceae family, seems to increase when mice are stressed by grid floor housing[73].

Proteobacteria

Escherichia coli (*E. coli*) enhances IBD in HLA-B27 overexpressing rats[106], although *E. coli* Nissle stabilizes the enteric barrier in mice[114]. When reducing type 1 diabetes by pre-weaning treatment of NOD mice with vancomycin, a vast increase in the abundance of Proteobacteria in the pups was observed[3].

Actinobacteria

Bifidobacterium spp. in rodents have a positive impact on the regulatory and innate immunity[101,115]. Perinatal supplementation of *B. longum* reduced TH1 and TH2 responses in allergen sensitized mice[104]. On the other hand, their numbers are also increased in gluten-fed NOD mice with a high incidence of type 1 diabetes compared with NOD

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.