REVIEW ARTICLE

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The Human Intestinal Microbiome in Health and Disease

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Human-associated microbes have primarily been viewed through the lens of a single species and its environment. Advances in culture-independent technologies have shown the enormous diversity, functional capacity, and age-associated dynamics of the human microbiome (see the Glossary). A large number of diverse microbial species reside in the distal gastrointestinal tract, and gut microbiota dysbiosis — imbalances in the composition and function of these intestinal microbes — is associated with diseases ranging from localized gastroenterologic disorders to neurologic, respiratory, metabolic, hepatic, and cardiovascular illnesses. Much effort is currently concentrated on exploring potential causality and related microbiota-mediated disease mechanisms, with the hope that an improved understanding will fuel the conception and realization of novel therapeutic and preventive strategies.

Until recently, our view of human microbiology was largely shaped by culture-based studies of single microbes (bacteria, archaea, fungi, and viruses), frequently isolated from patients who had acute infection or chronic disease. However, several decades ago, environmental microbial ecologists recognized that the diversity of microbes observed by microscopy far exceeded that of organisms recovered with the use of traditional culture-based approaches.[1] A variety of culture-independent molecular assays (Table 1) for detecting and classifying microorganisms (microbiota) and assessing their encoded genes (microbiome) and gene products showed

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Glossary

Biomarker sequencing: The process of cataloguing microbes in a mixed-species community through analysis of sequence variation in a single ubiquitous gene.

Holobiont: The totality of organisms in a given ecosystem (e.g., the shared human and microbial ecosystem); also called a superorganism.

Metabolome: The complete set of small-molecule chemicals found in a biologic sample.

Metagenome: All the genetic material present in an environmental sample, consisting of the genomes of many individual organisms.

Methanogenic archaea: Methane-producing microbes of the ancient Archaea kingdom.

Microbiome: The collection of all genomes of microbes in an ecosystem.

Microbiota: The microbes that collectively inhabit a given ecosystem.

Pathobionts: Typically benign endogenous microbes with the capacity, under altered ecosystem conditions, to elicit pathogenesis.

Prebiotics: Nutritional substrates that promote the growth of microbes that confer health benefits in the host.

Probiotics: Live microbes that confer health benefits when administered in adequate amounts in the host.

Synbiotics: Formulations consisting of a combination of prebiotics and probiotics.

Table 1. Tools for Analyzing Microbiota.

Approach	Data	Platform	Pros and Cons
Biomarker sequencing (e.g., 16S rRNA gene or internal transcribed spacer region)*	Community composition	Next-generation sequencing	Is cost-effective, is semiquantitative, permits resolution of genus level and in some cases species level; shorts reads may make accurate classification difficult
Metagenomics	Generation of draft genomes, functional capacity, growth dynamics	Next-generation sequencing	Has capacity for strain-level reconstruction, is quantitative, allows for functional annotation with pathway predictions; is currently very costly, has community coverage that may be relatively shallow in more complex assemblages
Metatranscriptomics (RNA sequencing)	Gene expression	Next-generation sequencing	Highly expressed genes are more likely than others to be detected, depletion of human transcripts is possible, requires immediate preservation or processing of fresh or snap-frozen intestinal specimens
Metaproteomics	Protein expression	Liquid or gas chromatography–mass spectrometry	Primarily detects dominant proteins; makes removal of host-derived proteins impossible
Metabolomics	Metabolic productivity	Liquid or gas chromatography–mass spectrometry or magnetic resonance spectroscopy	Is semiquantitative; can be targeted or untargeted; detects metabolites that are platform- and database-dependent; detects metabolites that may originate from microbes, diet, or host

* The term rRNA denotes ribosomal RNA.

that microbes rarely exist in isolation. Instead, they subsist in complex, interactive, interkingdom, multispecies microbial communities within a habitat. As the field has developed, it has become apparent that virtually every habitat, and every organism, on earth has its own microbiota. This includes the human holobiont, a conglomerate of mammalian and multispecies microbial cells in spatially segregated ecosystems, the genomic content of which is influenced by both topography and biologic individuality.[2]

In humans, the gastrointestinal tract represents a large microbial ecosystem, housing several trillion microbial cells. An integrated catalogue of the human fecal microbial metagenome, based on data from 1200 persons in the United States, China, and Europe, identified an aggregate 9.9 million microbial genes across these fecal microbiomes.[3] More than a billion years of mammalian–microbial coevolution has led to interdependency. As a result, the intestinal microbiota play a critical role in the maturation and continued education of the host immune response[4]; provide protection against pathogen overgrowth[5]; influence host-cell proliferation[6] and vascularization[7]; regulate intestinal endocrine functions,[8] neurologic signaling,[9] and bone density[10]; provide a source of energy biogenesis[11] (5 to 10% of daily host energy requirements); biosynthesize vitamins,[12] neurotransmitters,[9] and multiple other compounds with as yet unknown targets; metabolize bile salts[13]; react to or modify specific drugs; and eliminate exogenous toxins[14] (Fig. 1). The relevance of these microbial activities to health probably varies across the human population. Given the diverse functional repertoire of the gut microbiota, it is not surprising that they are the focus of research into a broad range of chronic diseases, including cancer and diseases with inflammatory, metabolic, cardiovascular, autoimmune, neurologic, and psychiatric components.

GUT MICROBIOTA ACROSS THE AGES

The in utero environment has, until relatively recently, been considered sterile. However, DNA-based microbiota studies have detected bacterial species in the placentas of healthy mothers,[15] in amniotic fluid of preterm infants,[16] and in meconium.[17] At parturition, the mode of delivery influences postnatal microbial exposure.[18,19] A study

of fecal samples (collected 4 days, 4 months, and 12 months after birth) from Swedish infant–mother dyads showed that the gut microbiota of vaginally delivered neonates is taxonomically similar to the maternal gut and vaginal microbiota,[19] though strain-level analyses are required to confirm the exact source of neonatal gut microbial diversity. This study also showed that the composition of the gut microbiota in infants changes to resemble adult microbiota in association with the cessation of breast-feeding (not the introduction of solid food).[19] During the first postnatal years, bacterial diversity and functional capacity expand,[12] an observation that is consistent with improved cysteine metabolism and augmented fermentation pathways (encoded by lactic acid bacteria acetolactate decarboxylase [EC4.1.1.5] and 6-phosphogluconate dehydrogenase [EC1.1.1.44]), as well as more efficient bacterial foraging of intestinal mucosal mucins[12] (a capacity that confers colonization advantages[20]).

The rapid rate of expansion in bacterial diversity that is observed in infancy slows in early childhood (between 1 and 5 years of age) (Fig. 2),[21] and gut microbial diversity remains lower in children than in adults.[21] In childhood, the composition of gut microbiota becomes more stable, with multiple members of Bacteroidetes, including those with butyrate-producing capacity, establishing a presence.[21] By preadolescence (7 to 12 years of age), although the number of bacterial taxa and functional genes present in the gut microbiome is similar to that in adulthood,[22] the age-differentiated microbial communities are taxonomically and functionally distinct. In preadolescents, as compared with adults, the gut microbiota are enriched in anaerovorax, bifidobacterium, faecalibacterium, and Lachnospiraceae and for pathways involved in vitamin B_{12} and folate biosynthesis[22]; folate biosynthesis is also characteristically increased in babies as compared with adults.[12,19] Healthy adult gut microbiota are dominated by Bacteroidetes and Firmicutes but also include smaller proportions of Actinobacteria, Proteobacteria, and Verrucomicrobia,[23] as well as methanogenic archaea (primarily *Methanobrevibacter smithii*), Eucarya (predominantly yeasts), and multiple phages.[24] At the bacterial phyla level, the gut microbiota in adults, as compared with those in infants, are stable, but the specific microbial species and subspecies (strains) and their proportions vary enormously from one person to another.[12] In fact, the microbial collection in each person is unique. Despite this taxonomic interindividual variation, the functional capacity of the adult gut microbiota is relatively consistent across healthy persons,[25,26] with pathways involved in metabolism,[12] fermentation, methanogenesis,[12] oxidative phosphorylation, and lipopolysaccharide biosynthesis.[22] In the elderly, the gut microbiota become compositionally unstable and less diverse, events that are associated with coexisting conditions and age-related declines in immunocompetence[27] (Fig. 2).

INFLUENCES ON THE GUT MICROBIOTA

Endogenous and exogenous factors influence the gut microbiota,[28,29] including mode of delivery of a neonate,[19] host genetic features,[30] host immune response,[31] diet[32] (including dietary supplements, breast-feeding, and formula-feeding), xenobiotics (including antibiotics) and other drugs,[10,33] infections,[34] diurnal rhythm,[35] and environmental microbial exposures,[36] several of which are established risk factors for childhood diseases such as obesity[37] and allergy.[38] The relative influence of these factors on the composition and function of the human gut microbiota, as well as the

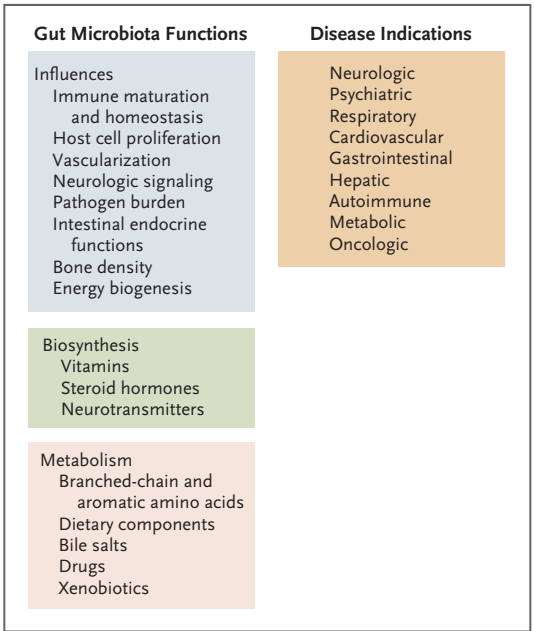

Figure 1. Some Functions of the Gut Microbiota and Disease Associations.

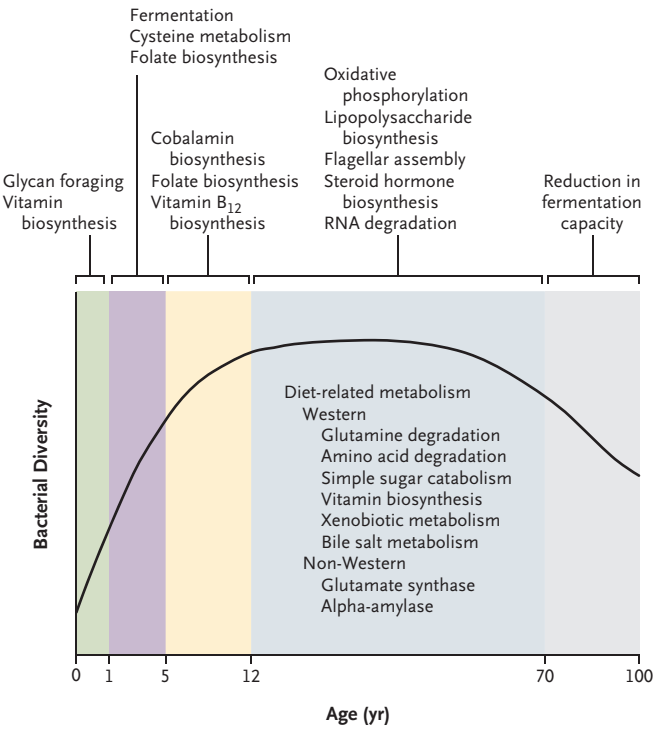

Figure 2. Temporal Development of the Gut Microbiota in Humans.

persistence of these effects, varies substantially. Sex, age, diet, exposure to antimicrobial agents, and stool consistency have been shown to exert large effects on the gut microbiota.[12,28,29,33] The host genome has been shown to be associated with the heritability of specific bacterial families such as Christensenellaceae, which forms a co-occurrence network with other heritable bacteria and methanogenic archaea, and is associated with leanness and healthy metabolism.[30,39] Disease-associated single-nucleotide polymorphisms of the human genome have been associated with the enrichment of specific bacterial taxa in the gut microbiota of persons with inflammatory bowel disease.[40] However, specific nongenetic factors, including the use of antimicrobial and immunosuppressant drugs and the site of intestinal biopsy sample collection, have had larger effects on the composition of the gut microbiota, indicating the need to control for such variables.[40]

IMMUNITY

Studies of mice rendered deficient for specific genes have shown that sensing of microbes by regulatory T (Treg) cells promotes mucosal tolerance and prevents overgrowth of segmented filamentous bacteria by triggering intestinal development and synthesis of Treg cells and secretion of antimicrobial IgA.[31] Recently, three compositionally, functionally, and metabolically distinct gut microbiota states were described in babies who were approximately 1 month of age; one of these conferred a significantly higher relative risk of allergy in 2-year-old children and of asthma in 4-year-old children. In ex vivo assays, the associated products of the high-risk microbiota state induced an increase in CD4+ cells that produce interleukin-4 , increased interleukin-4 production, and reduced the number of CD4+CD25+FOXP3+ cells, indicating that perturbation of early-life gut microbiota may contribute to subclinical inflammation that precedes childhood disease development.[41]

DIET AND OTHER ENVIRONMENTAL INFLUENCES

Dietary habits strongly influence the selection of gut microbiota.[12] Short-term intervention studies involving healthy adults exposed to diets restricted to meat or vegetable intake have shown rapid and reproducible gut microbiota responses, with meat consumption selectively enriching for bile-metabolizing microbiota, the expansion of which is associated with inflammatory bowel disease, and vegetable consumption increasing plant polysaccharide-fermenting organisms.[32] It has also been reported that persons have very different metabolic responses to identical meals. The results of a machine-learning approach applied to the integration of blood glucose levels, dietary habits, and gut microbiome data, among other factors, predicted personalized postprandial glycemic responses to real-life meals, indicating that personalized diets may be used to successfully modify blood glucose levels.[42] Emerging data also indicate that the microbiota is distinct in house dust from residences associated with protection against, or development of childhood allergic disease.[43-45] Moreover, either oral supplementation or nasal exposure of mice to protective house dust prevents airway sensitization,[36,45,46] indicating that exposure to environmental microbes modulates mucosal immunity. Other influences on the gut microbiota include pathogenic infection. For example, *Vibrio cholerae* initially dominates fecal bacterial communities during a cholera infection,[34] and clinical recovery is associ-

ated with a restoration of the preinfection composition of the gut microbiota resembling that observed in infancy. In contrast, even if drug-induced viral suppression of human immunodeficiency virus (HIV) replication is successful, the gut microbiota of patients with HIV infection frequently remain perturbed,[47,48] a feature related to the degree of peripheral immune activation.[47]

DYSBIOSIS OF GUT MICROBIOTA

Association studies in humans and rodents[49-53] have shown disease-related dysbioses across a wide spectrum of common chronic disorders, including atherosclerosis,[54-56] metabolic disorders,[57-60] asthma,[41,61] and autism spectrum disorder.[62] Some of these observations have been combined with experimental studies, prospective studies, or both to identify putative microbiota-derived molecular mediators of pathogenic mechanisms. Many clinical studies have used targeted sequencing of 16S ribosomal RNA (rRNA), which although economical, is limited to assessment of bacterial taxonomic composition. However, PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States),[63] an algorithm that uses 16S rRNA sequence data to predict conserved bacterial functional capacity, permits in silico bacterial metagenomic analyses. Studies have applied high-throughput methods of untargeted DNA sequencing in conjunction with the newly expanded human microbial gene catalogues,[3,25] draft genomes,[64] and new genome assembly, permitting microbial species-level and strain-level resolution and detailed functional annotations of microbial communities.[58-60,65-75] Such innovative approaches have facilitated the use of machine-learning algorithms to identify microbial gene- or taxon-based signatures as disease biomarkers.

Although many novel insights have been gained from these explorations, the study of the gut microbiome in human health and disease remains fraught with challenges. These include major intraindividual variability of the microbiome with changes in lifestyle, reproducibility issues, and statistically underpowered case–control studies, in addition to studies in which the cases and controls are phenotypically, etiologically, and microbiologically heterogeneous. These issues are compounded by a lack of stratification based on drug treatment, which potentially confounds analytical outcomes,[72] and a lack of statistically powered longitudinal and interventional studies involving study participants with well-defined diseases or preclinical at-risk conditions in order to explore causality. Despite evidence linking dysbiosis of the gut microbiome with disease manifestations at sites distant from the gut, most studies have not explored mechanisms outside the affected site, nor have they considered the effect of the microbiome and its varied products on the multitude of molecular pathways potentially involved. Stool samples are often used as proxies for the microbial content of the entire gastrointestinal tract, which covers more than 30 luminal intestinal square meters and contains distinct macroecosystems and microecosystems. Moreover, since bacterial genome databases are incomplete, the majority of genes in human gut microbiomes cannot be functionally assigned, a problem that is exacerbated by our lack of knowledge of both the dynamic transcriptional and translational activities of the gut microbiome and the biologic effect of the enormous numbers of polymorphisms and other structural variations of the microbiome. Finally, most studies have focused primarily on bacterial species rather than on the functional interplay among bacteria, archaea, viruses, fungi, and eukaryotes throughout the human gastrointestinal tract.

There have been few demonstrations of causality for which human studies correlating gut microbial dysbiosis with distinct clinical states have been complemented by mechanistic studies. These studies have addressed human obesity,[76] kwashiorkor,[77] childhood asthma,[41] massive weight loss after bariatric surgery,[65] and the insulin-resistant state of third-trimester pregnancy.[78] In addition, a study combining analyses of human host insulin sensitivity, fasting serum metabolome, and the gut microbiome with findings from experiments in mice suggests that specific bacteria may cause insulin resistance.[79] Also, transplantation of fecal microbiota from healthy lean human donors to obese patients with insulin resistance is associated with improvement in whole-body insulin sensitivity in the recipients,[80] further supporting the notion that microbiota-associated phenotypes may be transferred and reproduced, at least to some extent, in a genetically susceptible recipient.

A "common ground" hypothesis (Fig. 3), which

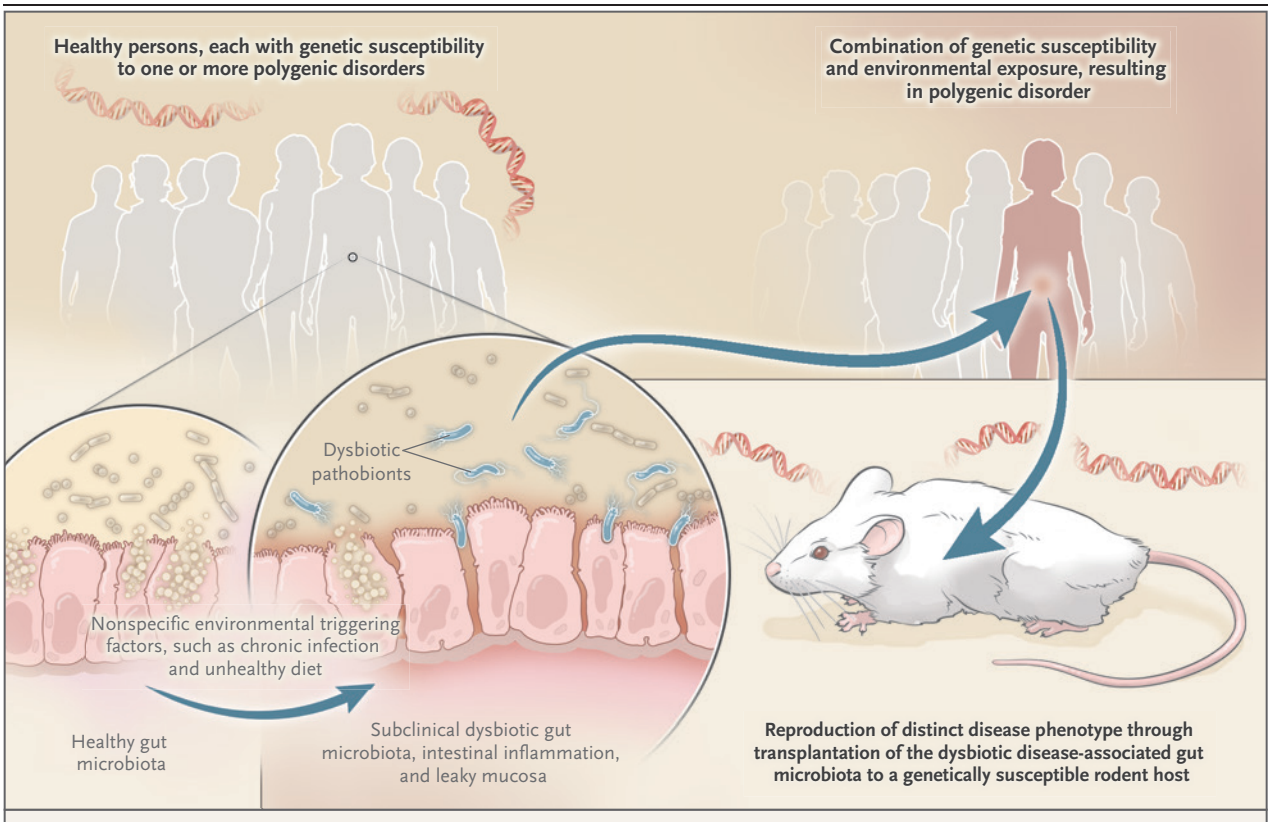

Figure 3. "Common Ground" Hypothesis of Pathogenesis Caused by Dysbiotic Gut Microbiota.

has yet to be rigorously examined, has been proposed[81] to explore the question of whether imbalances of gut microbial communities are a consequence or a cause of chronic polygenic diseases. This hypothesis posits, first, that various endogenous or exogenous factors, or combinations of such factors, trigger an increase in gut permeability ("leaky mucosa") or mucosal inflammation either directly or through selective pressure on the gut microbiota; second, that in persons who are genetically susceptible to one or more chronic disorders, the subclinical intestinal abnormalities favor the expansion of opportunistic microbes and the transition to pathobionts; third, that microbial gene products from the dysbiotic pathobiont gut communities promote local or systemic morphologic and functional changes that are pathogenic; and finally, that once disease-associated gut microbiota have been expressed in a genetically susceptible person, they can be transferred from that person to a genetically sensitive recipient, acting as a continual and contributing pathogenic mechanism.

THERAPEUTIC AND PREVENTIVE OPPORTUNITIES

INFECTION WITH *CLOSTRIDIUM DIFFICILE*

Fecal microbial transplantation for severe cases of recurrent diarrhea caused by antibiotic-resistant *C. difficile* infection is efficacious in approximately 90% of affected patients. This finding remains the prime proof of principle that healthy gut microbiota can reproducibly correct a severe and specific microbial dysbiosis and that transplantation of healthy microbiota is therefore medically actionable.[82] For chronic inflammatory bowel diseases, clinical remission is less predictable, and success rates are more modest.[83] Because many persons dislike aspects of fecal microbial transplantation and, more important, because transplantation carries the potential risk of transferring to recipients infections and other phenotypes that are clinically silent in donors, several preclinical and clinical initiatives[84,85] are under way. These investigations test and develop single commensals, mixtures of defined species or

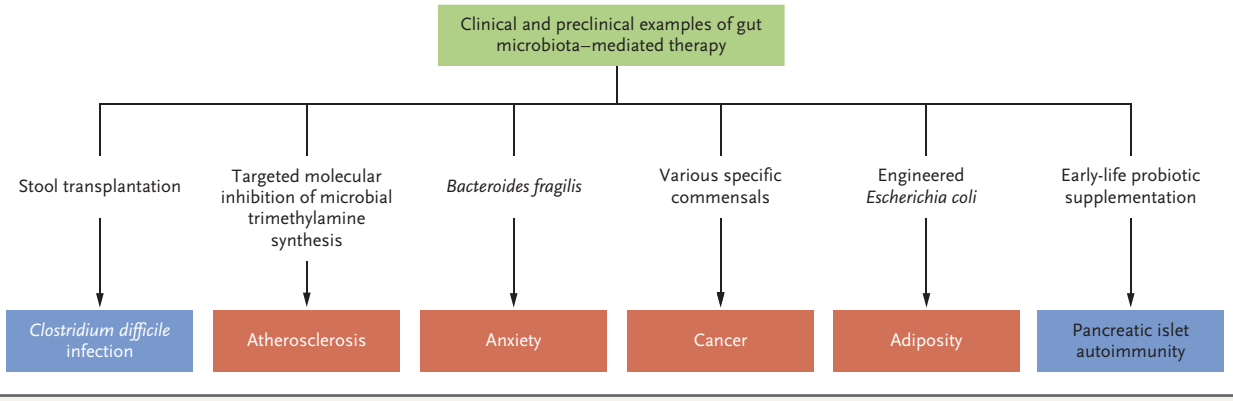

Figure 4. Gut Microbiota and Specific Commensals as Potential Preventive or Therapeutic Agents.
Clinical studies are indicated in blue, and preclinical (mouse) studies in red.

strains, or cocktails of microbiota-derived molecules targeting specific microbial species or pathways that are enriched in the disease state, in an effort to treat or prevent various common disorders (Fig. 4). The outcomes of selected preclinical studies are discussed below. The findings suggest that microbial-based therapeutics or preventives may have an advantage over interventions with synthetic drugs. They may be less likely to have severe side effects, given the coevolution of human-derived microbial strains and humans.

ATHEROSCLEROSIS AND PRODUCTS OF THE MICROBIOTA

Studies have shown that gut microbiota metabolism of dietary phosphatidylcholine and L-carnitine produces trimethylamine, which subsequently undergoes flavin monooxygenase 3–dependent oxidation to trimethylamine-*N*-oxide (TMAO); elevated circulating levels of TMAO appear to be a strong risk factor for atherosclerosis in humans and animals.[55,56] A study of a mouse model showed how oral application of a structural analogue of choline, 3,3-dimethyl-1-butanol, inhibited commensal microbial trimethylamine production, lowered plasma TMAO levels, and prevented atherosclerosis without apparent side effects, despite a pro-atherosclerosis diet.[86]

Bacteriocins represent another class of products of the mammalian gut microbiota. These high-potency peptide toxins may offer leads for the development of species-specific or strain-specific alternatives to current antibiotics.[87] For example, screening in human stool has identified 13 bacteriocin-producing bacterial strains.[88] Identifying target microbial species that exclude pathogens is equally important. With the use of mathematical modeling of gut microbiota, *C. scindens*, a secondary bile acid–producing bacterium, was identified as promoting resistance against *C. difficile* colonization.[89] Given the interactive nature of microbiomes, therapies targeting a network of interacting organisms that provide protection against or precipitate disease are likely to be more efficacious than therapies targeting a single species.

BEHAVIOR

Exploration of the therapeutic potential of microbial species at the preclinical level has been undertaken in several mouse models, including the maternal immune activation (MIA) model. The offspring of MIA mice exhibit autistic-like behavior, gut microbiota dysbiosis, increased gut mucosa permeability, and an altered serum metabolome, with an increase in 4-ethylphenylsulfate (4EPS) by a factor of 46.[62] Injection of this neurotoxin into the blood of healthy mice resulted in an anxiety phenotype. Feeding MIA mice a strain of *Bacteroides fragilis* ameliorated the intestinal dysbiosis, restored the integrity of the mucosal barrier, and diminished behavioral abnormalities in conjunction with significant decreases in circulating 4EPS levels.[62] Similar studies have been performed in mouse models of allergic airway disease in which oral supplementation with *Lactobacillus johnsonii*, a human vaginal commensal species, provides airway protection against both allergen challenge and respiratory viral infection.[36]

CANCER

Gut microbial species are being explored in the field of oncology. Of specific interest is the capacity of some commensal bacteria to modulate the tumor microenvironment and anticancer therapies.[90,91] It is well known that T-cell infiltration of solid tumors is associated with a positive therapeutic response. A recent study comparing melanoma growth in mice with distinct gut commensals support the hypothesis that certain microbes enhance the efficacy of cancer immunotherapy.[92] Oral administration of a mixture of bifidobacterium species modulates the activation of dendritic cells, which in turn helps improve the effector function of tumor-specific CD8+ T cells. Bifidobacterium supplementation improved tumor control to the same degree as anti–PD-L1 (programmed cell death ligand 1) therapy (checkpoint blockade) in an animal model, and combination treatment (bifidobacterium supplementation and anti–PD-L1 therapy) almost completely eliminated tumor expansion.[92] Similarly, studies in both humans and mice have shown that the antitumor effect of treatment with antibodies against cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) is potentiated by specific members of the gut microbiota. T-cell responses specific for *B. thetaiotaomicron* or *B. fragilis* were associated with the efficacy of CTLA-4 blockade, and the introduction of *B. fragilis* to tumorigenic, germ-free mice, which do not have a response to CTLA-4 treatment, brought about a response.[93] The discovery of a tumor-inhibiting molecule produced by a probiotic strain was recently made. *L. casei* strain ATCC 334 produces ferrichrome, which has been shown to inhibit progression of colon cancer by means of apoptosis mediated through the c-Jun *N*-terminal kinase pathway.[94]

INFLAMMATORY AND METABOLIC DISEASES

Given the tight interplay between gut microbes and host immunity,[95,96] efforts have focused on the isolation of human gut microbial species with therapeutic potential in inflammatory disorders. A mixture of 17 human clostridium strains has been shown to diminish the severity of experimentally induced allergic colitis in rodents through mechanisms that promote the expansion and activity of Treg cells, although it remains to be determined which bacterial molecules mediate these effects.[97] More recently, a substantially broader range of human gut bacterial strains were shown to promote the expansion of Treg cells.[98] In addition, *Lactococcus lactis* expressing interleukin-10, an antiinflammatory cytokine, has been shown to be safe in a phase 1 clinical trial[99] and was effective in reducing inflammation in mouse models of colitis[100] and allergic airway inflammation.[101]

Within the area of metabolism, studies suggest that proteins secreted by *Escherichia coli*, including ClpB, a chaperone protein and a mimic of alpha-melanocyte–stimulating hormone, affect food intake and meal patterns in rodents, with the magnitude of the effect depending on the bacterial growth phase. *E. coli* proteins stimulated intestinal hormones, glucagon-like peptide 1 (a potent antihyperglycemic hormone), and peptide YY (produced in the ileum in response to feeding) and activated anorexigenic pathways in the brain, inducing those that mediate satiety.[102] Bioengineered commensals may also have a role in future microbiota-mediated therapy.[99,103] In a preclinical setting, an *E. coli* strain was genetically manipulated to biosynthesize precursors of the anorexigenic *N*-acylethanolamides, which are produced in the ileum in response to feeding and serve to reduce food intake and, thus, obesity. Introduction of this engineered strain in obese mice fed a high-fat diet resulted in lower food intake, an increased basal metabolic rate, and a pronounced loss of adiposity, which endured for 4 weeks after cessation of bacterial supplementation.[104] Similarly, in studies of rats with diabetes, commensals engineered to synthesize and release glucagon-like peptide 1 have been shown to stimulate epithelial secretion of insulin, thereby improving carbohydrate metabolism.[105,106]

CAUTION IN EXTRAPOLATING BASIC FINDINGS

The examples discussed above involve introduction of human commensals or their products in isogenic rodents under highly controlled conditions during defined stages of pathogenesis. Predictions of similar effects in humans with preclinical or overt disease should be viewed with caution. Controlled clinical trials have shown relatively modest therapeutic effects of traditional probiotics in adults with various established disorders.[107] In contrast to these findings in adults, a recent prospective study showed the effect of probiotics on predisease autoimmune signatures in newborns in a large birth cohort that was followed to the start of school age.[108] In this study, neonatal probiotic supple-

mentation (administered between 0 and 27 days after birth) was associated with a 60% reduction in the risk of pancreatic islet autoimmunity before school age, as compared with either no supplementation or intervention after 27 days, with the association accounted for by high-risk children with the HLA-DR3/4 genotype. This finding raises the question of whether the introduction of more targeted formulations in neonates who are at increased risk for autoimmunity would prevent the subsequent development of autoimmune disorders.

DIETARY INTERVENTIONS TARGETING GUT MICROBIOTA

On the basis of studies in both animals and humans, dietary intake appears to be a major short-term and long-term regulator of the structure and function of gut microbiota.[32,42,109] Still, only a relatively small number of randomized, clinically controlled dietary interventions targeting the gut microbiota have been reported in humans, and these show that energy restriction and diets rich in fiber and vegetables are associated with gut microbial changes that, in turn, are associated with a health benefit.[70,110-112] Although investigation of the relevance of the gut microbiome to health and disease is in an early phase, the findings, in aggregate, support the view that specific dietary regimens, used alone or combined with the administration of mixtures of microbial species that have been validated and approved by regulatory authorities (next-generation synbiotics),[111] may hold potential for enhancing public health.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES

1. Staley JT, Konopka A. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu Rev Microbiol 1985;39:321-46.
2. Oh J, Byrd AL, Deming C, Conlan S, Kong HH, Segre JA. Biogeography and individuality shape function in the human skin metagenome. Nature 2014;514:59-64.
3. Li J, Jia H, Cai X, et al. An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol 2014;32:834-41.
4. Fulde M, Hornef MW. Maturation of the enteric mucosal innate immune system during the postnatal period. Immunol Rev 2014;260:21-34.
5. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 2013;14:685-90.
6. Ijssennagger N, Belzer C, Hooiveld GJ, et al. Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. Proc Natl Acad Sci U S A 2015;112:10038-43.
7. Reinhardt C, Bergentall M, Greiner TU, et al. Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling. Nature 2012;483:627-31.
8. Neuman H, Debelius JW, Knight R, Koren O. Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS Microbiol Rev 2015;39:509-21.
9. Yano JM, Yu K, Donaldson GP, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015;161:264-76.
10. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012;488:621-6.
11. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 2015;11:577-91.
12. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222-7.
13. Devlin AS, Fischbach MA. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. Nat Chem Biol 2015;11:685-90.
14. Haiser HJ, Gootenberg DB, Chatman K, Sirasani G, Balskus EP, Turnbaugh PJ. Predicting and manipulating cardiac drug inactivation by the human gut bacterium Eggerthella lenta. Science 2013;341:295-8.
15. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med 2014;6:237ra65.
16. DiGiulio DB, Romero R, Kusanovic JP, et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. Am J Reprod Immunol 2010;64:38-57.
17. Gosalbes MJ, Llop S, Vallès Y, Moya A, Ballester F, Francino MP. Meconium microbiota types dominated by lactic acid or enteric bacteria are differentially associated with maternal eczema and respiratory problems in infants. Clin Exp Allergy 2013;43:198-211.
18. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA 2010;107:11971-5.
19. Bäckhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 2015;17:852.
20. Martens EC, Chiang HC, Gordon JI. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 2008;4:447-57.
21. Cheng J, Ringel-Kulka T, Heikamp-de Jong I, et al. Discordant temporal development of bacterial phyla and the emergence of core in the fecal microbiota of young children. ISME J 2016;10:1002-14.
22. Hollister EB, Riehle K, Luna RA, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome. Microbiome 2015;3:36.
23. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science 2005;308:1635-8.
24. Reyes A, Haynes M, Hanson N, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 2010;466:334-8.
25. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 2010;464:59-65.
26. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
27. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488:178-84.
28. Zhernakova A, Kurilshikov A, Bonder MJ, et al. Population-based metagenomics

analysis reveals markers for gut microbiome composition and diversity. Science 2016;352:565-9.
29. Falony G, Joossens M, Vieira-Silva S, et al. Population-level analysis of gut microbiome variation. Science 2016;352:560-4.
30. Goodrich JK, Waters JL, Poole AC, et al. Human genetics shape the gut microbiome. Cell 2014;159:789-99.
31. Wang S, Charbonnier LM, Noval Rivas M, et al. MyD88 adaptor-dependent microbial sensing by regulatory T cells promotes mucosal tolerance and enforces commensalism. Immunity 2015;43:289-303.
32. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559-63.
33. Maurice CF, Haiser HJ, Turnbaugh PJ. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. Cell 2013;152:39-50.
34. Hsiao A, Ahmed AM, Subramanian S, et al. Members of the human gut microbiota involved in recovery from Vibrio cholerae infection. Nature 2014;515:423-6.
35. Thaiss CA, Zeevi D, Levy M, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell 2014;159:514-29.
36. Fujimura KE, Demoor T, Rauch M, et al. House dust exposure mediates gut microbiome Lactobacillus enrichment and airway immune defense against allergens and virus infection. Proc Natl Acad Sci USA 2014;111:805-10.
37. Yallapragada SG, Nash CB, Robinson DT. Early-life exposure to antibiotics, alterations in the intestinal microbiome, and risk of metabolic disease in children and adults. Pediatr Ann 2015;44(11):e265-9.
38. Johnson CC, Ownby DR, Alford SH, et al. Antibiotic exposure in early infancy and risk for childhood atopy. J Allergy Clin Immunol 2005;115:1218-24.
39. Beaumont M, Goodrich JK, Jackson MA, et al. Heritable components of the human fecal microbiome are associated with visceral fat. Genom Biol 2016;17:189.
40. Knights D, Silverberg MS, Weersma RK, et al. Complex host genetics influence the microbiome in inflammatory bowel disease. Genome Med 2014;6:107.
41. Fujimura KE, Sitarik AR, Havstad S, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. Nat Med 2016;22:1187-91.
42. Zeevi D, Korem T, Zmora N, et al. Personalized nutrition by prediction of glycemic responses. Cell 2015;163:1079-94.
43. Fujimura KE, Johnson CC, Ownby DR, et al. Man's best friend? The effects of pet ownership on house dust microbial communities. J Allergy Clin Immunol 2010;126(2):410-2.e3.
44. Lynch SV, Wood RA, Boushey H, et al. Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children. J Allergy Clin Immunol 2014;134(3):593-601.e12.
45. Stein MM, Hrusch CL, Gozdz J, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. N Engl J Med 2016;375:411-21.
46. Ege MJ, Mayer M, Normand A-C, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med 2011;364:701-9.
47. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med 2013;5:193ra91.
48. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE. HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. Gut Microbes 2014;5:562-70.
49. Marchesi JR, Adams DH, Fava F, et al. The gut microbiota and host health: a new clinical frontier. Gut 2016;65:330-9.
50. Ha CW, Lam YY, Holmes AJ. Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health. World J Gastroenterol 2014;20:16498-517.
51. Walsh CJ, Guinane CM, O'Toole PW, Cotter PD. Beneficial modulation of the gut microbiota. FEBS Lett 2014;588:4120-30.
52. Wang WL, Xu SY, Ren ZG, Tao L, Jiang JW, Zheng SS. Application of metagenomics in the human gut microbiome. World J Gastroenterol 2015;21:803-14.
53. Wu H, Tremaroli V, Bäckhed F. Linking microbiota to human diseases: a systems biology perspective. Trends Endocrinol Metab 2015;26:758-70.
54. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57-63.
55. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19:576-85.
56. Wilson Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575-84.
57. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature 2009;457:480-4.
58. Le Chatelier E, Nielsen T, Qin J, et al. Richness of human gut microbiome correlates with metabolic markers. Nature 2013;500:541-6.
59. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012;490:55-60.
60. Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 2013;498:99-103.
61. Arrieta MC, Stiemsma LT, Dimitriu PA, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med 2015;7:307ra152.
62. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013;155:1451-63.
63. Langille MG, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013;31:814-21.
64. Nielsen HB, Almeida M, Juncker AS, et al. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. Nat Biotechnol 2014;32:822-8.
65. Tremaroli V, Karlsson F, Werling M, et al. Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. Cell Metab 2015;22:228-38.
66. Feng Q, Liang S, Jia H, et al. Gut microbiome development along the colorectal adenoma-carcinoma sequence. Nat Commun 2015;6:6528.
67. Yu J, Feng Q, Wong SH, et al. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. Gut 2015 September 25 (Epub ahead of print).
68. Zeller G, Tap J, Voigt AY, et al. Potential of fecal microbiota for early-stage detection of colorectal cancer. Mol Syst Biol 2014;10:766.
69. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med 2015;21:895-905.
70. Cotillard A, Kennedy SP, Kong LC, et al. Dietary intervention impact on gut microbial gene richness. Nature 2013;500:585-8.
71. Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014;513:59-64.
72. Forslund K, Hildebrand F, Nielsen T, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 2015;528:262-6.
73. Graessler J, Qin Y, Zhong H, et al. Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters. Pharmacogenomics J 2013;13:514-22.
74. Karlsson FH, Fåk F, Nookaew I, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat Commun 2012;3:1245.
75. Sharon I, Morowitz MJ, Thomas BC, Costello EK, Relman DA, Banfield JF.

Time series community genomics analysis reveals rapid shifts in bacterial species, strains, and phage during infant gut colonization. Genome Res 2013;23:111-20.
76. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341:1241214.
77. Subramanian S, Huq S, Yatsunenko T, et al. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature 2014;510:417-21.
78. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470-80.
79. Pedersen HK, Gudmundsdottir V, Nielsen HB, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature 2016;535:376-81.
80. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143(4):913-6.e7.
81. Hansen TH, Gøbel RJ, Hansen T, Pedersen O. The gut microbiome in cardiometabolic health. Genome Med 2015;7:33.
82. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108:500-8.
83. Colman RJ, Rubin DT. Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis 2014; 8:1569-81.
84. Lawley TD, Clare S, Walker AW, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. PLoS Pathog 2012; 8(10):e1002995.
85. Tvede M, Tinggaard M, Helms M. Rectal bacteriotherapy for recurrent Clostridium difficile-associated diarrhoea: results from a case series of 55 patients in Denmark 2000-2012. Clin Microbiol Infect 2015;21:48-53.
86. Wang Z, Roberts AB, Buffa JA, et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. Cell 2015;163:1585-95.
87. Cotter PD, Hill C, Ross RP. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 2005;3:777-88.
88. Lakshminarayanan B, Guinane CM, O'Connor PM, et al. Isolation and characterization of bacteriocin-producing bacteria from the intestinal microbiota of elderly Irish subjects. J Appl Microbiol 2013; 114:886-98.
89. Buffie CG, Bucci V, Stein RR, et al. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. Nature 2015;517: 205-8.
90. Iida N, Dzutsev A, Stewart CA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013;342:967-70.
91. Viaud S, Saccheri F, Mignot G, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013;342:971-6.
92. Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015;350:1084-9.
93. Vétizou M, Pitt JM, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015;350:1079-84.
94. Konichi H, Fujiya M, Tanaka H, et al. Probiotic-derived ferrichrome inhibits colon cancer progression via JNL-mediated apoptosis. Nat Commun 2016 August 10 (Epub ahead of print).
95. Cebula A, Seweryn M, Rempala GA, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. Nature 2013;497:258-62.
96. Dasgupta S, Erturk-Hasdemir D, Ochoa-Reparaz J, Reinecker HC, Kasper DL. Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. Cell Host Microbe 2014;15:413-23.
97. Atarashi K, Tanoue T, Oshima K, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 2013;500:232-6.
98. Ahern PP, Faith JJ, Gordon JI. Mining the human gut microbiota for effector strains that shape the immune system. Immunity 2014;40:815-23.
99. Braat H, Rottiers P, Hommes DW, et al. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol 2006;4: 754-9.
100. Steidler L, Hans W, Schotte L, et al. Treatment of murine colitis by Lactococcus lactis secreting interleukin-10. Science 2000;289:1352-5.
101. Marinho FA, Pacífico LG, Miyoshi A, et al. An intranasal administration of Lactococcus lactis strains expressing recombinant interleukin-10 modulates acute allergic airway inflammation in a murine model. Clin Exp Allergy 2010;40:1541-51.
102. Breton J, Tennoune N, Lucas N, et al. Gut commensal E. coli proteins activate host satiety pathways following nutrient-induced bacterial growth. Cell Metab 2016;23:324-34.
103. Westendorf AM, Gunzer F, Deppenmeier S, et al. Intestinal immunity of Escherichia coli NISSLE 1917: a safe carrier for therapeutic molecules. FEMS Immunol Med Microbiol 2005;43:373-84.
104. Chen Z, Guo L, Zhang Y, et al. Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. J Clin Invest 2014;124:3391-406.
105. Duan F, Curtis KL, March JC. Secretion of insulinotropic proteins by commensal bacteria: rewiring the gut to treat diabetes. Appl Environ Microbiol 2008;74: 7437-8.
106. Duan FF, Liu JH, March JC. Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes. Diabetes 2015;64:1794-803.
107. Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. Genes Nutr 2011;6:209-40.
108. Uusitalo U, Liu X, Yang J, et al. Association of early exposure of probiotics and islet autoimmunity in the TEDDY Study. JAMA Pediatr 2016;170:20-8.
109. Shoaie S, Ghaffari P, Kovatcheva-Datchary P, et al. Quantifying diet-induced metabolic changes of the human gut microbiome. Cell Metab 2015;22:320-31.
110. Macfarlane S, Cleary S, Bahrami B, Reynolds N, Macfarlane GT. Synbiotic consumption changes the metabolism and composition of the gut microbiota in older people and modifies inflammatory processes: a randomised, double-blind, placebo-controlled crossover study. Aliment Pharmacol Ther 2013;38:804-16.
111. Neef A, Sanz Y. Future for probiotic science in functional food and dietary supplement development. Curr Opin Clin Nutr Metab Care 2013;16:679-87.
112. Brahe LK, Le Chatelier E, Prifti E, et al. Dietary modulation of the gut microbiota — a randomised controlled trial in obese postmenopausal women. Br J Nutr 2015; 114:406-17.