Microbes and Cancer*

Amiran Dzutsev, Jonathan H. Badger, Ernesto Perez-Chanona, Soumen Roy, Rosalba Salcedo, Carolyne K. Smith, and Giorgio Trinchieri

Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, email: trinchig@mail.nih.gov

Annu. Rev. Immunol. 2017. 35:199–228

First published online as a Review in Advance on January 30, 2017

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-051116-052133

Keywords

microbiota, cancer, carcinogenesis, metabolism and cancer, cachexia, chemotherapy, immunotherapy

Abstract

Commensal microorganisms (the microbiota) live on all the surface barriers of our body and are particularly abundant and diverse in the distal gut. The microbiota and its larger host represent a metaorganism in which the cross talk between microbes and host cells is necessary for health, survival, and regulation of physiological functions locally, at the barrier level, and systemically. The ancestral molecular and cellular mechanisms stemming from the earliest interactions between prokaryotes and eukaryotes have evolved to mediate microbe-dependent host physiology and tissue homeostasis, including innate and adaptive resistance to infections and tissue repair. Mostly because of its effects on metabolism, cellular proliferation, inflammation, and immunity, the microbiota regulates cancer at the level of predisposing conditions, initiation, genetic instability, susceptibility to host immune response, progression, comorbidity, and response to therapy. Here, we review the mechanisms underlying the interaction of the microbiota with cancer and the evidence suggesting that the microbiota could be targeted to improve therapy while attenuating adverse reactions.

INTRODUCTION

Microbial communities have been living on the surface of the Earth for three-fourths of its history. They have developed a diversity of specialized lineages to adapt to different habitats and have been instrumental in shaping the evolution of modern life (1). When eukaryotes and multicellular organisms appeared over a billion years ago, their close interaction with microbes necessitated their coevolution, establishing persistent relationships including mutualism, commensalism, parasitism and a dependency on each other for survival and control of homeostasis; this history is clearly written in the genomes of eukaryotic hosts and their associated microbiota. The phagocytic capability of eukaryotic cells was preserved from the most primitive organisms to the highest forms of animal life and was required for both nutrition and defense against pathogens. Phagocytosis was probably the key trait that eventually gave rise to eukaryotes, by enabling the acquisition of bacterial endosymbionts that evolved into mitochondria (2, 3). Eventually, mobile phagocytic cells in higher organisms developed as specialized cell types of the myeloid lineage (granulocytes, monocytes, macrophages, dendritic cells) that play a central role in wound repair, innate resistance to infections, and promotion of adaptive immunity (4, 5). Commensal microorganisms, including eubacteria, archaea, protists, fungi, and viruses, inhabit all the epithelial barrier surfaces of our body, where bacteria, in particular, are as numerous as human cells (6–8). The unique microbial genes in our body outnumber human genes by a factor of at least 100, although many microbial genes are functionally redundant (6). Thus, we are symbionts or metaorganisms composed of host and microbial cells (9–11). The microbial genome is an integral part of the metaorganism genetic framework (metagenome), and the wealth of metabolic processes and products (metabolome) it encodes profoundly influence all the physiological functions of the body and alter its pathology. Both microbial and human cells act as sensors for chemical, physical, and biological environmental cues. Through reciprocal communications they detect homeostatic changes and modify their composition and/or function accordingly (12). The signaling pathways by which human and microbial cells in the metaorganism communicate involve small molecule metabolites, nucleic acids, and physical protein-protein interactions. This cross talk includes molecular and cellular mechanisms that are also key to innate resistance mediated by myeloid/phagocytic cells (5). Both commensal microbiota and many pathogens produce "danger molecules" recognized by the immune system to elicit a reaction. However, tolerance to microbiota is maintained, despite a substantial translocation of microbial products, and cells remain in steady state until pathogenic microorganisms breach the epithelial barriers and then an immune response is mounted (13, 14).

METHODS OF MICROBIAL COMPOSITION IDENTIFICATION AND FUNCTIONAL ANALYSIS

Elucidation of the interaction between the host and commensal microbiota has been hampered, until recently, by the fact that only a small fraction of microbial cells could be isolated, cultured in vitro, and analyzed (15). Although the leading laboratories have made great progress in culturing previously uncultivable bacterial species (16), the real revolution in microbiota studies over the last few years has been due to the widespread availability of next-generation sequencing technology. This has allowed many investigators to analyze the composition of the commensal microbiota in an unbiased way by sequencing all or part of their genome. The standard method of identifying bacterial and archaeal taxa in the microbiota is to sequence one or more variable regions of the gene encoding 16S rRNA (17), although the optimal region may vary by body site. Fungal composition analysis relies on sequencing of the internal transcribed spacer (ITS) region between the 18S and 28S rRNAs (18). Analysis of the protist fraction of the microbiota is in its early stages, but 18S rRNA (which also has variable regions, as in 16S) appears to be the preferred

method (19). Alternatively, the microbial metagenome can be sequenced using standard shotgun sequencing protocols. A further degree of information is available with metatranscriptomic data, where not only the functional potential of the metagenome is revealed, but also the genes that are actively expressed. Both metatranscriptomic and metagenomic information could be used to infer relationships with host transcriptomes or proteomes by building trans-kingdom networks (20).

MICROBIAL CONTRIBUTION TO HOST HOMEOSTASIS

In the metaorganism, cross talk between the commensal microbes and the host is essential for maintenance of physiological homeostasis, response to environmental changes and survival. Evidence accumulating in the last few years suggests that the composition of the microbiota at the epithelial barrier affects systemic functions including metabolism, energy balance, central nervous system physiology including cognitive functions, cardiovascular functions, nutrition, circadian rhythm, inflammation, innate resistance, and adaptive immunity (21–23). Microbes reside along the gastrointestinal tract, with the largest microbial population in the colon, ranging between 10^{13} to 10^{14} bacteria (24). Approximately 10^{12} bacteria live on the skin in various communities depending on factors such as sebum or dryness of the environment (25). The composition of the microbiota at various anatomical sites is controlled by host genetics, particularly by the polymorphisms in immune-related genes, as well as by environmental factors, such as lifestyle and nutrition (26). The initial composition of a newborn's microbiota largely resembles either the vaginal microbiota or, if the method of delivery was cesarean section, the skin and environmental microbiota (27). The gut microbiota matures during the first three years of life. Concurrently, large changes in the expression of genes related to vitamin biosynthesis and metabolism are also observed. Throughout adulthood, the microbiota is relatively stable, although disease onset, use of antibiotics, and changes in alimentation affect its composition (28). In individuals older than 60–70 years, the composition of the microbiota gradually changes; a striking negative association is observed between frailty in elderly individuals and microbial diversity (29, 30).

Many of the effects of the microbiota occur at local barrier sites. For example, in the gut, the microbiota modulates nutrient absorption, synthesis of vitamins, metabolism of bile and hormones, and fermentation of carbohydrates. In addition, it contributes to barrier fortification and the establishment of mucosal immunity (22, 31, 32). However, the microbiota also exerts systemic effects, including modulation of metabolism, inflammation, and immunity (32). The microbiota trains myeloid and lymphoid cells by providing instructive signals to regulate epigenetic modifications that calibrate the immune response to inflammatory stimuli, infections, vaccines, and autoimmunity (33–36). In addition to the gut mucosa, commensals at other epithelial barriers such as the skin also control local immune homeostasis, protective responses, and tissue pathology (32). A compartmentalized control of immunity by the skin microbiota has been clearly defined (37). Although many of the cellular and molecular mechanisms mediated by vicinal microbiota on barrier homeostasis have been identified, the exact method of systemic influence is yet to be determined. Traditionally the epithelial barriers and their immune system were believed to prevent, in healthy organisms, the translocation of bacteria into internal tissues. However, it is now becoming evident that bacteria can penetrate the gut mucosa and skin, diffuse to the draining lymph nodes, and disseminate systemically with protective rather than pathogenic effects (13, 38, 39). This translocation is amplified in conditions of immune deficiency or during diseases that compromise the integrity of the epithelium, such as colitis or atopic dermatitis (38, 39). Other mechanisms also contribute to the systemic effects conferred by the microbiota: diffusion of bacterial products, host growth factors, cytokines, chemokine-induced migration of barrier immune cells, and release of vesicles or exosomes from bacteria or immune cells.

The effect of the microbiota on innate resistance and immunity is, in part, dependent on its effect on hematopoiesis. Germfree mice are deficient in bone marrow–derived peripheral myeloid populations (40). Recolonization of these mice with microbes provides cues from microbe-associated molecular patterns (MAMPs) and short-chain fatty acids (SCFAs) to promote hematopoietic homeostasis by activating innate immune receptors (40–42). The maturation of the yolk sac–derived central nervous system microglia and their ability to respond to infections was also defective in germfree mice. This deficiency was similarly reproduced by treating conventionally raised mice with antibiotics, showing that a complex microbiota is required to maintain the functions of tissue-resident myeloid cells (43). The functional maturation of circulating neutrophils and their ability to form extracellular traps is also regulated by the microbiota (44). Diurnal fluctuations of circulating inflammatory monocytes are regulated by synchronous oscillations of gut microbial composition and through the level of expression of Toll-like receptors (TLRs) on gut epithelial cells, which control the expression of the circadian gene *Bmal1* (45, 46). Alteration of the circadian clock (e.g., due to jet lag) induces pathological alterations to the microbiota, termed dysbiosis, promoting a metabolic syndrome that is transferable by fecal transplantation to germfree mice (47).

Bacterial products can induce expression of type II interferon (IFN-γ), which is known to affect neutrophil survival and activation (48, 49). This suggests that activated barrier myeloid cells, among others, can reenter circulation and contribute to systemic microbiota effects (50). Interleukin-12 (IL-12) produced during intestinal *Toxoplasma gondii* infection affects monocyte precursor differentiation in the bone marrow by inducing IFN-γ production by natural killer (NK) cells (51). Low concentrations of MAMPs, such as lipopolysaccharide, can also change myeloid cell activation thresholds for an enhanced inflammatory response, also known as priming (52). These mechanisms likely contribute to the microbiota's influence on the responsiveness of peripheral and tumor-infiltrating myeloid cells (34, 53) and to the generalized Shwartzman reaction, which also depends on the priming of myeloid cells by low doses of lipopolysaccharide and IL-12 (54).

MICROBIOTA ASSOCIATION WITH CANCER

Although tumors grow locally before invading other tissues or metastasizing to other parts of the organism, cancer should be considered a systemic disease affected by and altering the homeostatic mechanisms that control the physiology of the metaorganism. The growth characteristics of the tumor cells are dependent on the genetic alterations resulting in the activation of oncogenes, the silencing of tumor suppressor genes, and other classical hallmarks of cancer (55). However, it is becoming evident that transformed cells cannot effectively grow without a suitable microenvironment (56). The microbiota, with its ability to modulate the metaorganism's physiology, contributes to the establishment of a pro- or antitumor inflammatory milieu (**Figure 1**). Two findings exemplify the requirement of a suitable microenvironment for tumor growth. Rous sarcoma virus, the first-discovered oncovirus, induces tumors in adult birds at the site of injection or injury, but not in sterile embryos. This is true even if the infected cells in the embryo express the *v-Src* oncogene and show a transformed phenotype in vitro (57). The other example is the finding that more than one-quarter of the skin cells in aged, sun-exposed eyelid carry clonally expressed cancer-causing driver mutations similar to those found in squamous cell carcinoma, yet they maintain the physiological differentiation and functions of normal skin without evolving into cancer (58). Although evidence for the impact of the skin microbiota on the malignant progression of keratinocyte carcinomas is still lacking, normal tissue homeostasis and architecture are known to restrain cancer so that changes in the microenvironment are required for tumor progression (59). Indeed, the microbiota affects tissue metabolism as well as the differentiation and function of immune cells

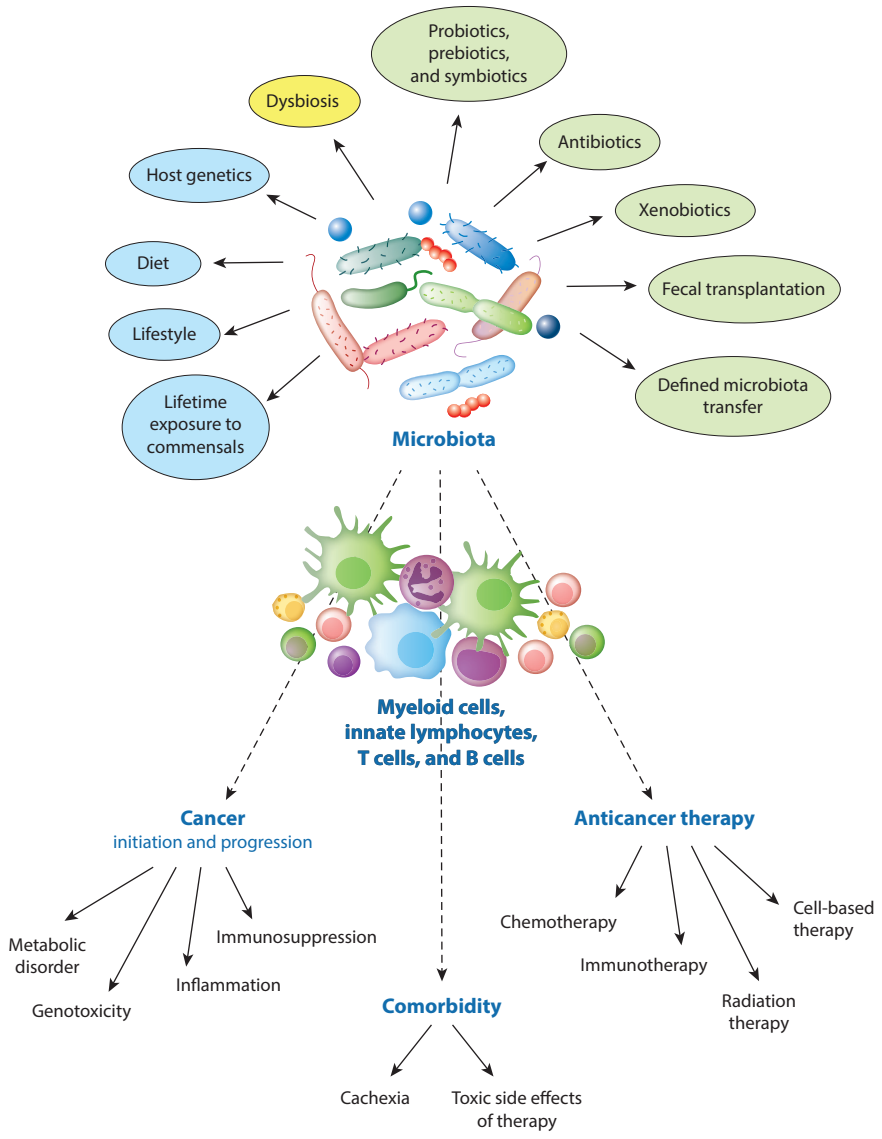


Figure 1

Microbiota-dependent regulation of cancer development, progression and treatment. The microbiota plays a dynamic role, from maintenance of healthy host physiology to disease development. Physiological factors (*blue*) such as lifetime exposure to commensals, diet, lifestyle, and host genetics shape the composition of the microbiota in each individual, which affects response to disease and therapy. Under pathogenic conditions, changes in microbiota composition (dysbiosis; *yellow*) may contribute to disease. The microbiota regulates cancer initiation and progression, comorbidity, and anticancer therapy in part by priming myeloid cells and (directly or indirectly) lymphoid cells that mediate innate resistance and adaptive immunity. Treatments targeting microbiota composition (*green*), such as probiotics, prebiotics, symbiotics, antibiotics, xenobiotics, transplantation of fecal or defined microbiota, have potential to modulate cancer progression, cancer-associated comorbidity, response to therapy, and adverse reactions.

in the tumor microenvironment, which may foster skin carcinogenesis. In turn, host genetics and environmental factors may also affect the tumor microenvironment by modifying the composition and diversity of the microbiota (12, 26). The incidence of keratinocyte cancer and cancer at other barrier surfaces exposed to the microbiota is elevated in immunosuppressed recipients of organ transplants (60), likely owing to changes in the microbiota composition at these sites and defective tumor immunosurveillance.

Epidemiological cancer studies based on the analysis of oral, fecal, and tissue samples to evaluate the role of the microbiota and dysbiosis have mostly been limited to gastrointestinal and lung carcinomata (61). These studies have confirmed the role in stomach cancer of *Helicobacter pylori*, the only bacterial species recognized as a group 1 human carcinogen by the International Agency for Research on Cancer (IARC) (62, 63). These studies also identified other emerging candidate oncogenic bacteria, such as *Fusobacterium*, enterotoxigenic *Bacteroides fragilis* (ETBF), and *pks*$^+$ strains of *Escherichia coli* (64–67). Traditional epidemiological data showing an association of the presence of certain species with cancer incidence provide important clues but are in many cases difficult to interpret because evidence of causality is often lacking. The presence of dysbiosis or shifts in the abundance of specific microbes may, in fact, be a consequence rather than a cause of cancer. The microorganisms responsible for cancer initiation may no longer be present when the patients are analyzed, and dysbiosis may cause homeostatic alterations or epigenetic effects that contribute to cell transformation and tumor promotion (68, 69). Definitive evidence for the role of particular species in cancer pathogenesis will come from more molecular-based epidemiological studies as well as identification of the mechanisms involved in both clinical studies and experimental animal studies.

ROLE OF THE MICROBIOTA IN DIET- AND OBESITY-ASSOCIATED CANCER AND CANCER COMORBIDITY

High body mass index (BMI) is a risk factor at the population level for diabetes, cardiovascular disease, and many common cancers, including colon, liver, gallbladder, postmenopausal breast, uterus, and kidney cancers (70). High BMI is associated with the pathological inflammation responsible for insulin resistance and altered energy metabolism (71). Colon cancer and gut dysbiosis, too, are linked to obesity. High-fat diet (HFD) increases tumorigenicity of intestinal cell precursors by activating peroxisome proliferator-activated receptor delta (72). In hepatocarcinoma, carcinogenesis is associated with *Clostridium* cluster XI– and cluster XIVa–induced production of the secondary bile acid product deoxycholic acid, which induces DNA damage and senescence in hepatic stellate cells (73, 74). Although it has been estimated for over 30 years that one-third of all cancers and the majority of gastrointestinal cancers may be due to dietary factors (75), the epidemiological evidence associating HFD with cancer is modest, unlike the experimental data demonstrating that HFD causes cardiovascular disease and diabetes (74, 76). Conversely, high-fiber diets increase the microbe-driven metabolism of SCFAs, such as butyrate, which have anti-inflammatory properties. In addition, they may protect against gastrointestinal cancers and lymphomas through the modulation of epigenetic changes by inhibiting histone deacetylases (74, 77). Exposure of female mice to HFD during pregnancy induced obesogenic and diabetogenic traits in two generations of offspring and was associated with the development of lung and liver cancers (69, 78). This phenotype was linked to epigenetic changes in genes encoding adiponectin and leptin, and administration of a normal diet abolished it in three generations. Cancer susceptibility was also induced in recipients of the microbiota from HFD-fed females but was reversed by treatment with the beneficial probiotic species *Lactobacillus reuteri* (69, 78). The use of antibiotics can persistently reduce bacterial diversity, deplete protective niches that otherwise prevent

overgrowth of pathogens and pathobionts, and induce gut dysbiosis that may increase the risk of disease (79, 80). The repeated use of antibiotics in young children resulted in an increased risk of childhood obesity (81) and up to sevenfold increase in the risk of developing inflammatory bowel disease (82); thus, it may also increase the risk of colitis-associated cancer. In breast cancer, a meta-analysis of five case-control studies showed that antibiotic use was associated with a slightly elevated risk of breast cancer, though this association is controversial (83). Mammary tissue is not sterile and as the bacterial composition changes in the presence of cancer, the local microbiota modulates antitumor immunity in the tumor microenvironment (84). The long-term effects of antibiotics and diets such as HFD and high fiber diet, however, alter the microbial composition and confound the identification of specific cancer-causing microbes.

Exposure to microbes during natural birth may help the newborn establish a proinflammatory environment that favors tumor immunosurveillance (85, 86). Data from murine studies have provided insight into the balance of microbial exposure and immune cell activity. One study showed that acute intestinal infection induced intolerance to commensals because of dysbiosis-driven alterations in mucosal integrity and immunity (14, 87). However, this resulted in the accumulation of Th1-biased, microbe-specific memory T cells that also facilitated protective responses to subsequent infections and tumor growth (14).

Whereas obesity may initiate certain cancers, anorexia-cachexia syndrome is a cancer-associated comorbidity observed in a large majority of patients with late-stage, advanced cancer. This wasting of muscle and adipose tissue is also triggered by infection, kidney and intestinal damage, and chemotherapy (88). It dramatically affects quality of life, decreases the efficacy and tolerability of anticancer treatments, and is the predominant cause of cancer-associated comorbidity and mortality (88). Cancer-associated cachexia is linked to systemic inflammation and characterized by elevated levels of chemokines and cytokines such as IL-1β and IL-6 (89). Cancer cells induce cachexia through the secretion of proinflammatory cytokines and factors such as parathyroid hormone–related protein (90). Different microbial species dominate in patients with metabolic dysfunctions: the archaeon *Methanobrevibacter smithii* in patients with anorexia nervosa and *Lactobacillus* spp. in the obese patients (91). Indeed, mice treated with the probiotic *L. reuteri*, prebiotics, or both combined (symbiotics) were protected against cancer-associated cachexia, likely because of enhanced anti-inflammatory, mucosa-protective effects (92, 93). In experimental models of intestinal damage or of infection-induced cachexia, *E. coli* O21:H^+, present in the gut, protected against cachexia (94). *E. coli* colonized white adipose tissue, where it activated the NLRC4 inflammasome and induced production of insulin-like growth factor 1, a hormone that prevents muscle degradation (94). Changes in the microbial composition of the gut may be a cause or consequence of cancer cachexia. Regardless, multimodal strategies, including the use of beneficial microbes, aimed at fortifying barrier function may help reduce the incidence of cachexia (95). Therefore, further investigation using experimental models may shed light on the role of the gut microbiota in cancer anorexia/cachexia.

HUMAN CARCINOGENIC MICROORGANISMS

Infection-induced cancer accounts for approximately 16% of the global burden of all human cancers, corresponding to approximately 2 million new cases per year (96). The frequency varies by region, with lower percentages, on average, in more developed countries (7.4%) compared to less developed countries (22.9%) (96). The IARC classifies 10 microbial agents (7 viruses, 2 parasites, and 1 bacterium) as group 1 human carcinogens; i.e., their status as carcinogens is based on either strong evidence in humans, or limited evidence in humans supported by strong evidence in animals (97). *H. pylori*, hepatitis B and C viruses (HBV, HCV), and human papillomaviruses (HPV)

together are responsible for over 90% of all infection-attributed cancers (96). The herpesvirus Epstein-Barr virus (EBV) is the causative agent of Burkitt lymphoma, nasopharyngeal carcinoma, and a subset of gastric carcinomas, whereas the Kaposi sarcoma–associated herpesvirus (HSV8) causes Kaposi sarcoma and other pathologies in immunosuppressed or elderly individuals (98). HBV and HCV are associated with hepatocellular carcinoma (HCC) (99). High-risk oncogenic strains of HPV (HPV16, HPV18, and 11 others) are associated with anogenital cancers, a subset of head and neck cancers, and skin cancers (100, 101). Human T cell lymphotropic virus type 1 is associated with the T cell lymphomas prevalent in certain geographical regions (102). Merkel cell polyomavirus is the first human virus discovered by metagenomic sequencing and is associated with the majority of cases of Merkel cell carcinoma, an aggressive skin cancer observed in immunosuppressed individuals (103).

With the exception of HCV, all human oncogenic viruses encode at least one oncogene and may directly induce cell transformation (104). However, factors such as infection-associated genital tract inflammation and vaginal dysbiosis likely play a role in cancer progression even for viruses such as HPV that have a potent transforming ability (105). HBV and HCV establish chronic liver infection and account for over 80% of HCCs (106). In some patients, the immune response to chronic viral infection progresses to fibrosis and cirrhosis and ultimately HCC. Whereas the initial innate response to HBV infection is modest, HCV actively evades innate responses by inhibiting both the production and the signaling of type I and type III interferon (106, 107). Unlike HCV, HBV may directly transform hepatocytes. However, for both viruses, the pathogenesis of HCC is dependent on immune-related inflammation (106). A higher degree of chronic HBV and liver pathology correlates with greater gastrointestinal richness of *Candida* spp. and *Saccharomyces cerevisiae* and with a less abundance and diversity of *Bifidobacterium* spp. (108, 109). The role of the gut microbiota in regulating liver pathology and progression to HCC in mice has been clearly documented (110). Interestingly, young mice, similarly to neonates or young children, fail to clear HBV infection in a hydrodynamic transfection model until an adult-like gut microbiota is established (111). Oncogene-carrying viruses (HPVs and HBV) require inflammation to promote tumorigenesis, but this may indeed apply to all oncogenic viruses (112), including the previously mentioned Rous sarcoma virus (57). As the limited data hitherto published suggest that the commensal microbiota may be involved in regulating the response to infection and progression to cancer, these represent possible targets for preventive and therapeutic interventions for cancer.

Gastric infection with *H. pylori* is strongly associated with noncardiac gastric carcinoma and lymphoma (62). Yet, approximately half of the world population is infected with *H. pylori*, and in most cases this infection only develops into well-tolerated gastritis. Only rarely does *H. pylori* infection progress to serious lesions such as atrophy, metaplasia, and cancer (62). The virulence and carcinogenicity of various strains of *H. pylori* have been associated with the variable expression of two cytotoxin-encoding genes: cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin gene A (*vacA*) (113). *H. pylori* infection, before any damage to the gastric mucosa occurs, cooperates with the gut microbiota to control energy homeostasis by affecting circulating metabolic gut hormones (114). *H. pylori* infection has profound effects on the host immune response. It activates the TLR4 and TLR2 receptors as well as the NLRP3 inflammasome, thus inducing the secretion of both IL-1β and IL-18. This promotes the activation of both Th1 cell and regulatory T cell (Treg) responses to protect against asthma, chronic inflammatory diseases, and tuberculosis (115, 116). Whereas *H. pylori* directly affects gastric epithelial cells and can compromise genetic integrity by inducing DNA damage, gastric carcinogenesis requires exposure to the bacterium over multiple decades, with an initial inflammatory response, epithelium injury and atrophy, reduction in acid secretory functions, and intestinal metaplasia (117, 118). Often, *H. pylori* is no longer detectable in the stomach of seropositive individuals with atrophic body gastritis, suggesting that deterioration

of the gastric niche and the lowering of acidity due to long-term *H. pylori* infection cause gastric dysbiosis dominated by the presence of cancer-provoking species of oropharyngeal or intestinal origin (119). In developed countries, the incidence of *H. pylori* infection is decreasing owing to frequent use of antibiotics, improved hygiene, and eradication protocols using broad-spectrum antibiotics and proton pump inhibitors (120). Although this decrease correlates with a lower incidence of gastric inflammation and carcinogenesis, the eradication protocols cause perturbation of the gut microbiota with possible side effects (120). Also, the absence of *H. pylori* may remove some of the beneficial effects of the infection, thereby increasing susceptibility to asthma and obesity as well as gastroesophageal reflux with increased risk of esophageal and gastric cardia carcinoma (63). This hypothesis, however, has been challenged by the observation that the incidence of these pathologies is not increased in certain ethnic Malaysian populations that have a low natural incidence of *H. pylori* infection and generally poor sanitation (85). Thus, these findings may suggest that the relationship between the absence of *H. pylori* infection and the increased incidence of these pathologies is not universal and that, in some populations, *H. pylori* infection may be a marker of poor hygiene that has a protective effect on asthma, obesity, and esophageal carcinoma (85).

MICROBIOTA AND CANCER AT THE EPITHELIAL BARRIERS

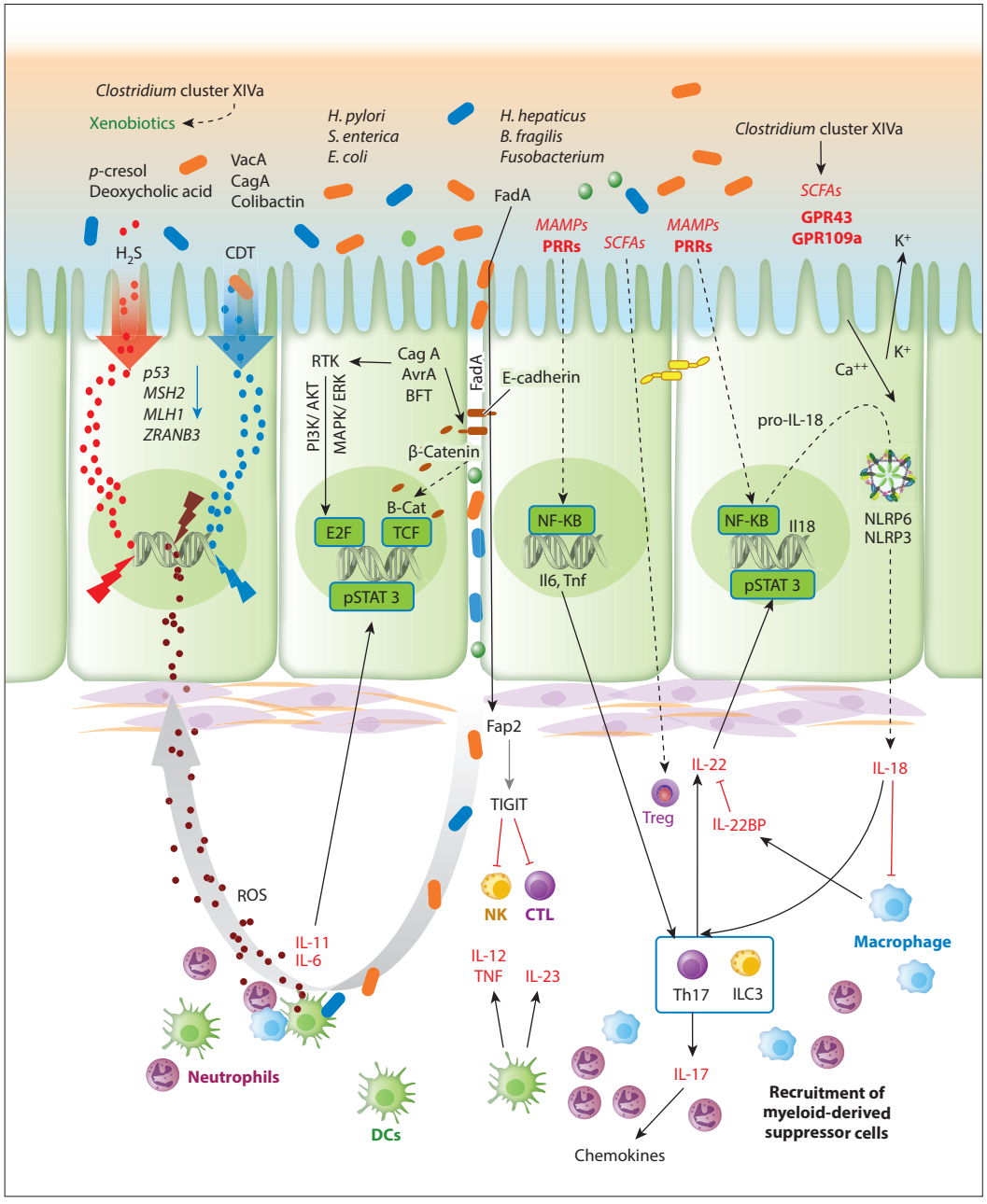
At the epithelial barrier surfaces, the primary contact point between host commensals, the composition of the microbiota or the abundance of particular species affects both inflammation and immunity, as well as the homeostasis of epithelial and stromal cells (22, 121). Although this cross talk occurs at all barriers (32), it is particularly evident at the level of the lower gastrointestinal tract, where the most bacteria are present (24) (**Figure 2**). Direct interactions of bacterial structural components and their metabolites, for example, hydrogen sulfide and *p*-cresol, with epithelial, stromal, and hematopoietic cells may have direct genotoxic effects and promote cancer progression (122–126).

Mice deficient in genes controlling host-microbe cross talk have been extensively used for studying the mechanisms by which the microbiota affects cancer. In particular, mice deficient in immunologically relevant genes, such as *Tlr5*, *Il10*, *Tbx1*, and *Rag2*, are not only susceptible to colitis and colon carcinogenesis, but are also characterized by gut dysbiosis. This susceptibility to cancer can be transferred to healthy mice by cohousing, fostering, or fecal transplant (65, 127, 128). These findings are relevant to humans because polymorphisms in immunologically relevant genes affect human microbiota composition and cancer predisposition (26).

Clinical and epidemiological investigations as well as experimental studies in animal models have already identified bacterial species putatively involved in carcinogenesis on the basis of either their physical association with the neoplastic lesions or a positive correlation of their abundance with cancer risk. Mechanisms of bacteria-mediated carcinogenesis have been well characterized in mice using several microbial species, including *Enterococcus faecalis*, *Streptococcus gallolyticus*, enteropathogenic *E. coli*, ETBF, *Helicobacter hepaticus*, *Salmonella enterica*, and *Fusobacterium nucleatum* (64, 65, 129). The bacterial genera *Odoribacter* and *Akkermansia* are reportedly enriched in colon cancer–bearing mice (130), and archaea, such as the order Methanobacteriales, have been found in the fecal microbiota of patients with colorectal cancer (131).

Fusobacterium spp. are anaerobic, gram-negative bacteria that usually reside in the oropharynx, where they are involved in oral pathology and participate in the formation of dental biofilms (132). However, they are also found in the inflamed colon and are particularly enriched in human colonic adenomas and adenocarcinomas (64, 129). The latter observation may be explained by the binding of the *F. nucleatum* protein Fap2 to the carbohydrate moiety Gal-GalNac that is overexpressed on colonic tumor cells (133). *F. nucleatum* is immunosuppressive, owing to its

ability to recruit tumor-promoting myeloid cells to the intestine of $APC^{Min/+}$ mice and to inhibit human NK and T cell activity via binding of its Fap2 protein to the TIGIT inhibitory receptor (64, 134). It also activates β-catenin/Wnt signaling in epithelial cells by the association of its FadA adhesin to E-cadherin (129). The level of expression of the gene encoding FadA is much higher in colon tissues of patients with colon neoplasia compared to those of healthy individuals, suggesting FadA as a potential diagnostic and therapeutic target (129). Fusobacteria have also been identified



in the biofilms observed in carcinomas of the ascending colon and the tumor-free mucosa of the same patients (135). The biofilms show upregulation of the polyamine metabolite N^1,N^{12}-diacetylspermine, which is likely responsible for the observed enhanced epithelial proliferation, diminished E-cadherin, and activation of STAT3 and IL-6 (135, 136).

Patients with colitis and colon cancer also have an increased abundance of *E. coli* (67). A direct role in mouse carcinogenesis was demonstrated for strains of *E. coli* expressing the *pks* pathogenicity island, which encodes the genotoxin colibactin (67). Both induction of inflammation and a direct effect of the *pks*$^+$ *E. coli* are needed for carcinogenesis (67). Colibactin induces alterations in p53 SUMOylation, inducing cellular senescence associated with the production of growth factors leading to tumor-promoting effects (137). *E. coli*, along with several other microbial species including *Campylobacter jejuni*, *Aggregatibacter actinomycetemcomitans*, *Haemophilus ducreyi*, *Shigella dysenteriae*, *Helicobacter hepaticus*, and *S. enterica*, has another type of genotoxic compound that belongs to the family of cytolethal distending toxins (CDTs). CDT consists of three subunits: CdtA, CdtB, and CdtC. Its CdtB subunit has similarities with DNAse-I-like nucleases and demonstrates potent DNAse activity that causes extensive DNA lesions and apoptosis in target cells (138). Attaching and effacing *E. coli* has also been shown, similarly to attaching and effacing *H. pylori*, to downregulate the key DNA mismatch proteins MSH2 and MLH1, which are also mutated in hereditary nonpolyposis colorectal cancer (139, 140). In addition, enteropathogenic *E. coli* utilizes another pathway to inhibit DNA repair via the secretory cysteine methyltransferase NleE, which blocks the function of DNA annealing helicase and endonuclease ZRANB3 (141).

ETBF is a subclass of the human commensal *B. fragilis*. It is present in the intestine at relatively low abundance; however, it can act as a pathobiont by causing diarrhea and has been associated

Figure 2

The role of prokaryotic microbes in cancer initiation and progression. (*Far left*) Microbial metabolites with direct and indirect genotoxic activity include products of protein (e.g., H_2S, *p*-cresol) and bile (e.g., deoxycholic acid) degradation as well as products of the breakdown of liver-detoxified xenobiotics (e.g., sulfation and glucuronidation conjugates). A number of bacterial species can also produce toxins that are injected into host cells via the type III secretion system. These induce cell toxicity via apoptosis and repression of proton pump expression. Among the toxins that induce DNA damage are colibactin, produced by some strains of *E. coli*, and cytolethal distending toxins (CDTs), produced by *Escherichia coli*, *Campylobacter jejuni*, *Helicobacter hepaticus*, and *Salmonella enterica*. In addition to directly damaging DNA, some bacteria, including *H. pylori* and *E. coli*, downregulate genome stability–related and repair-related genes (e.g., TP53, MSH2, MLH1, and ZRANB3). Bacterial species that translocate through the epithelial barrier induce recruitment of myeloid cells, including reactive oxygen species (ROS)–producing neutrophils that contribute to DNA damage in the host cell. (*Center left*) FadA protein from *Fusobacterium nucleatum*, CagA toxin from *H. pylori*, *Bacteroides fragilis* toxin (BFT), and avirulence protein A (AvrA) from *S. enterica* Typhi can activate the β-catenin pathway by promoting detachment of β-catenin from E-cadherin. Some of the same bacterial species, such as *H. pylori* and *S. enterica*, also activate the PI3K/AKT and MAPK/ERK pathways via receptor tyrosine kinases. (*Center* and *far right*) Cytokines, such as IL-22, IL-11, and IL-6, promote development of colon cancer via activation of STAT3. The intestinal microbiota and transmucosally translocated bacterial species, following mucosal damage or tumor growth, regulate the production of many cytokines—such as IL-6, IL-11, IL-12, IL-18, IL-23, and TNF production by macrophages, dendritic cells (DCs), and epithelial and mesenchymal cells and IL-22 and IL-17 production by T cells and innate lymphocytes. The IL-18/IL-22 axis is particularly important in maintaining mucosal homeostasis, and it is tightly regulated. Microbial products induce epithelial cell production of pro-IL-18, which is cleaved into active IL-18 by inflammasomes. These inflammasomes (NLRP3 and/or NLRP6) are activated by bacteria-derived small-chain fatty acids (SCFAs) via GPR43 and GPR109a receptors and by commensal protists. IL-18 then blocks macrophage production of the soluble IL-22 antagonist IL-22BP, and it is required for IL-22 production by T cells and innate lymphoid cells, thus increasing production and bioavailability of IL-22. IL-22 also induces STAT3 phosphorylation in epithelial cells, promoting proliferation and secretion of antibacterial peptides, and, in a positive feedback loop, enhances production of IL-18. Bacteria also activate immunosuppressive mechanisms: *F. nucleatum* promotes accumulation of immunosuppressive myeloid cells in colonic tumors and produces the Fap2 protein, which activates the inhibitory receptor TIGIT on natural killer (NK) and T cells; SCFAs induce regulatory T cells (Tregs), which inhibit local immune response. Abbreviations: CTL, cytotoxic T lymphocyte; MAMP, microbe-associated molecular pattern.

with inflammatory bowel disease and colorectal cancer (142). *B. fragilis* toxin stimulates intestinal epithelial cell proliferation, which depends in part on E-cadherin degradation and β-catenin activation. ETBF induces mucosa permeabilization and STAT3 activation in both inflammatory and epithelial cells, leading to colon carcinogenesis in $APC^{Min/+}$ mice, which is dependent on simultaneous expansion of both Tregs and Th17 cells (143, 144).

In mouse colon carcinogenesis, the polyp-containing epithelium is more permeable than healthy tissue and allows transmucosal bacterial translocation (145). The translocated bacteria induce the production of proinflammatory IL-6, IL-11, IL-23, IL-17, and IL-22, which are required for cancer progression (145, 146).

Another pro-inflammatory cytokine, IL-18, is a potent inducer of IFN-γ production and, when combined with IL-12, results in type 1 polarization of both innate and adaptive lymphocytes. However, paradoxically, results in IL-18-deficient mice suggest that in the large intestine this cytokine has anti-inflammatory properties and mediates mucosal protective mechanisms (147). Mice deficient in IL-18, IL-18R, and MyD88 as well as those deficient for inflammasome-related genes, which are unable to process pro-IL-18, are more susceptible to dextran sulfate sodium (DSS)-induced colitis; azoxymethane (AOM)/DSS-induced, colitis-associated cancer; and diet-induced, nonalcoholic steatohepatitis (148). These mice also have gut dysbiosis characterized by increased abundance of the phyla Bacteroidetes (*Prevotellaceae*) and TM7 and greater susceptibility to colon carcinogenesis that can be transferred to wild-type mice by cohousing or by fecal transplant (128). Commensal bacteria and protists induce production of IL-18 in intestinal epithelial cells by transcriptionally inducing the production of pro-IL-18, which is then cleaved into biologically active IL-18 by inflammasomes (149, 150). Bacteria-induced SCFAs activate the epithelial cell inflammasome via the metabolite-sensing receptors GPR43 and GPR109A (149). SCFAs also act on macrophages, dendritic cells, and T cells, in particular, causing the expansion of IL-10-producing Tregs that limit colonic inflammation and carcinogenesis (151, 152). Whether NLRP3 and/or NLRP6 are involved in IL-18 induction remains controversial and diametric results have been published by different laboratories (153). The discordant data could be explained by variations in the composition of the microbiota in different animal facilities and by the differential distribution of the two inflammasomes in epithelial and hematopoietic cells.

The ability of IL-18 to protect the mucosa depends on its regulation of IL-22 production and availability (154–156). IL-22 is produced by T cells and innate lymphoid cells in the intestinal lamina propria and, through activation of STAT3, induces epithelial cell proliferation and production of antibacterial peptides (155). Three regulatory mechanisms have been proposed: (*a*) IL-18 inhibits IL-22 binding protein (IL-22BP) production by macrophages; (*b*) IL-18 is required for IL-22 production by T lymphocytes and innate lymphoid cells; and (*c*) in a positive feedback loop, IL-22 induces pro-IL-18 but not its processing by inflammasomes in intestinal epithelial cells (154–156). In different experimental models of colon carcinogenesis, IL-22, which promotes mucosa repair, has been observed to be pro- or anticarcinogenic depending on the extent of mucosal damage in the carcinogenic model (154). The paradoxical ability of the proinflammatory cytokine IL-18 to protect from colitis and colon carcinogenesis (154) may be explained by a recent report showing that IL-18 protects against mucosal inflammation by maintaining a protective microbiota (157). This article reports that IL-18 signaling in intestinal epithelial cells prevented mucus-forming goblet cells from maturing, and mice that were deficient for IL-18 or IL-18R in intestinal epithelial cells and that were cohoused with wild-type mice to equalize the microbiota composition were resistant to DSS-induced colitis (157).

In several experimental models, SCFAs acting through the GPR109A receptors are anti-inflammatory and decrease incidence of colon and mammary cancer (152, 158). Some bacterial species contribute more than others to SCFAs levels, such as the butyrate-producing members of

Clostridium cluster XIVa (159). SCFAs, particularly butyrate and propionate, are also inhibitors of histone deacetylases and downregulate expression of proinflammatory genes such as IL-6 and tumor necrosis factor (TNF) (160). However, reducing the production of SCFAs using either antibiotics or a carbohydrate-poor diet decreases incidence of colonic polyps in *Apc*$^{Min/+}$/*Msh2* $^{-/-}$ mice (161). This procarcinogenic effect of SCFA-producing bacteria was not dependent on inflammation or DNA damage but on induction of hyperproliferation and aberrant β-catenin signaling in *Msh2* $^{-/-}$ cells (161).

Although the microbiota is present on all barrier surfaces, scarce and mostly correlative data are available for the role of the local microbiota in cancer development outside the gastrointestinal tract. More studies deciphering the contribution of the microbiota to cancers of the skin, oropharyngeal cavity, lung, and urogenital tract are needed. Also, relatively little attention has been devoted to microorganisms other than bacteria and viruses, although production of carcinogenic acetaldehyde by fungi has been proposed to play a role in oral cancer in patients with a mutation in the *AIRE* gene and with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (162). Additionally, other members of our microbiota could also contribute to cancer, including protists and helminths, which have been shown to orchestrate gut immunity (150, 163).

The studies described here have allowed investigators to identify many bacterial species with carcinogenic activity; however, with the exception of *H. pylori*, none of them have been formally proven to be a human carcinogen, for example, by disease prevention upon their elimination from the host (65).

EFFECTS OF THE GUT MICROBIOTA ON TUMORS IN DISTANT ORGANS

The microbiota regulates cancer not only at the epithelial barriers that it inhabits but also systemically at distant sterile sites. One example is the regulation by the gut microbiota of metabolism and energy balance, which contributes to cancer-predisposing conditions such as obesity and metabolic disease. Bacteria may also modulate distant malignancies such as endometrial and breast cancer through a noninflammatory pathway by expressing β-glucuronidases and β-glucuronides that participate in estrogen metabolism. Thus, the use of antibiotics and dysbiosis may alter estrogen metabolism and affect the progression of these tumors (119).

The microbiota also metabolizes xenobiotics by transforming heterocyclic aromatic amines from burned meat or environmental pollutants into genotoxic and procarcinogenic metabolites (164). The colonic procarcinogen AOM is processed by P450 enzymes in the liver and in the small intestine into carcinogenic methylazoxymethanol (MAM) that also gets transformed in the liver by UDP-glucuronosyltransferase into inactive MAM-glucuronide. Accumulation of carcinogenic AOM products in the colon and formation of DNA adducts require gut microbial β-glucuronidase (165) that converts inactive MAM-glucuronide back into active methylazoxymethanol. Colon carcinogenesis induced by AOM is prevented by prebiotics that reduce the number of β-glucuronidase-expressing bacteria, or by inhibitors of the enzyme (166, 167). Systemic effects of the microbiota can also be inflammatory. In mice where gut dysbiosis was induced by fungal *Candida* sp. and by *T. gondii* infection, the lung macrophages and bone marrow monocyte precursors, respectively, were polarized into cells with antiallergic and anti-inflammatory characteristics (51, 168).

There are many examples of systemic regulation of carcinogenesis by intestinal microbes in experimental mouse cancer models. Colonic infection with *Helicobacter hepaticus* has contrasting effects on carcinogenesis in the intestine and at distant organs. Colonic *H. hepaticus* infection increases ileal and colonic carcinogenesis in APC$^{min/+}$ mice and in AOM-treated *Rag2*$^{-/-}$ mice by

inducing IL-22 production by innate lymphoid cells (155, 169). It also promotes mammary and prostate carcinoma in $APC^{min/+}/Rag2^{-/-}$ mice (124, 170) and enhances chemical and viral liver carcinogenesis (124, 171). However, in *Rag2*-sufficient animals *H. hepaticus* infection protects against intestinal and mammary carcinogenesis by inducing IL-10-producing Tregs (172). In a different model of mammary carcinogenesis based on expression of SV40 T antigen under the control of sex steroid hormones, infection with *H. hepaticus* increases tumor multiplicity, affecting migration of neutrophils into the mammary gland (173).

Variation in intestinal microbes between different animal facilities or as a consequence of experimental perturbations profoundly affects incidence of lymphoma and survival of *Atm* (ataxia telangiectasia mutated)-deficient mice (174). The gut microbiota contributes to the incidence of thymic lymphoma in these animals by modulating the TNF-regulated inflammatory tone and by inducing oxidative stress as well as genotoxicity in leukocytes and epithelial cells (170, 171, 174, 175). In mice TLR5-mediated recognition of commensal microbiota induces production of IL-6 and recruitment of myeloid-derived suppressor cells, enhancing progression of malignant tumors at distal extramucosal locations (176).

BACTERIA AS ANTICANCER TREATMENT

Following the personal and historical observations of tumor regression associated with acute bacterial infections, at the end of the nineteenth century, the New York surgeon William Coley successfully treated soft tissue sarcoma patients with "Coley's toxins," a combination of *Streptococcus pyogenes* and gram-negative *Bacillus prodigiosus* (*Serratia marcescens*), providing evidence that a severe localized infection may induce a systemic antitumor immune response (177). Subsequently, several bacteria or bacterial preparations, such as *Corynebacterium parvum* and the streptococcal preparation OK-432, were tested in cancer therapy; local treatment with bacille Calmette Guérin, an attenuated strain of *Mycobacterium bovis*, is still a first-line therapy for superficial bladder carcinoma (178). Many genera of bacteria, including *Salmonella*, *Escherichia*, and *Clostridium*, preferentially accumulate in tumors when delivered systemically and have been tested as anticancer agents (179). A major limitation of cancer therapies is that large tumors contain hypoxic, necrotic, and quiescent regions in which the tumor cells do not proliferate. These regions are either inaccessible to drugs or, because of hypoxia, poorly susceptible to DNA damage induced by chemotherapy or radiation (179). Because obligate anaerobic bacteria such as *Clostridium* spp. can only proliferate in hypoxic regions of the tumors, when they are delivered as spores they germinate and proliferate with antitumor effects only in the hypoxic tissues (180). Although the use of spores could be considered for large hypoxic tumors, small tumors or metastases are better oxygenated and might be better treated with facultative anaerobes, such as *Salmonella* and *Escherichia*, that are attracted by small molecules released by tumors (181). The ideal bacterial therapeutic would have the following properties: toxic for tumor cells but not normal cells, selective for the tumor (particularly regions not targeted by conventional therapies), susceptible to immunological clearance but not immediately destroyed by the immune response, proliferative, and genetically modifiable (181). Furthermore, the therapeutic bacteria should be mobile and allow genetic alteration to reduce systemic toxicity and localization in the tumors (182, 183). For example, deletion in *Salmonella* of the Trg gene that encodes a sugar-sensing transmembrane receptor allows the bacteria to deeply penetrate within poorly vascularized and nutrient-poor regions of the tumor (183). Bacterial therapeutics can be used for delivery of antitumor molecules such as toxin, cytokines, antigens, and antibodies, as well as for transfer of genes encoding molecules with antitumor activity (179, 182). The advantages of bacterial cancer therapies include not only their efficacy when other

therapies fail but also their tumor tropism and ability to proliferate and maintain favorable pharmacokinetics for an extended period of time (179).

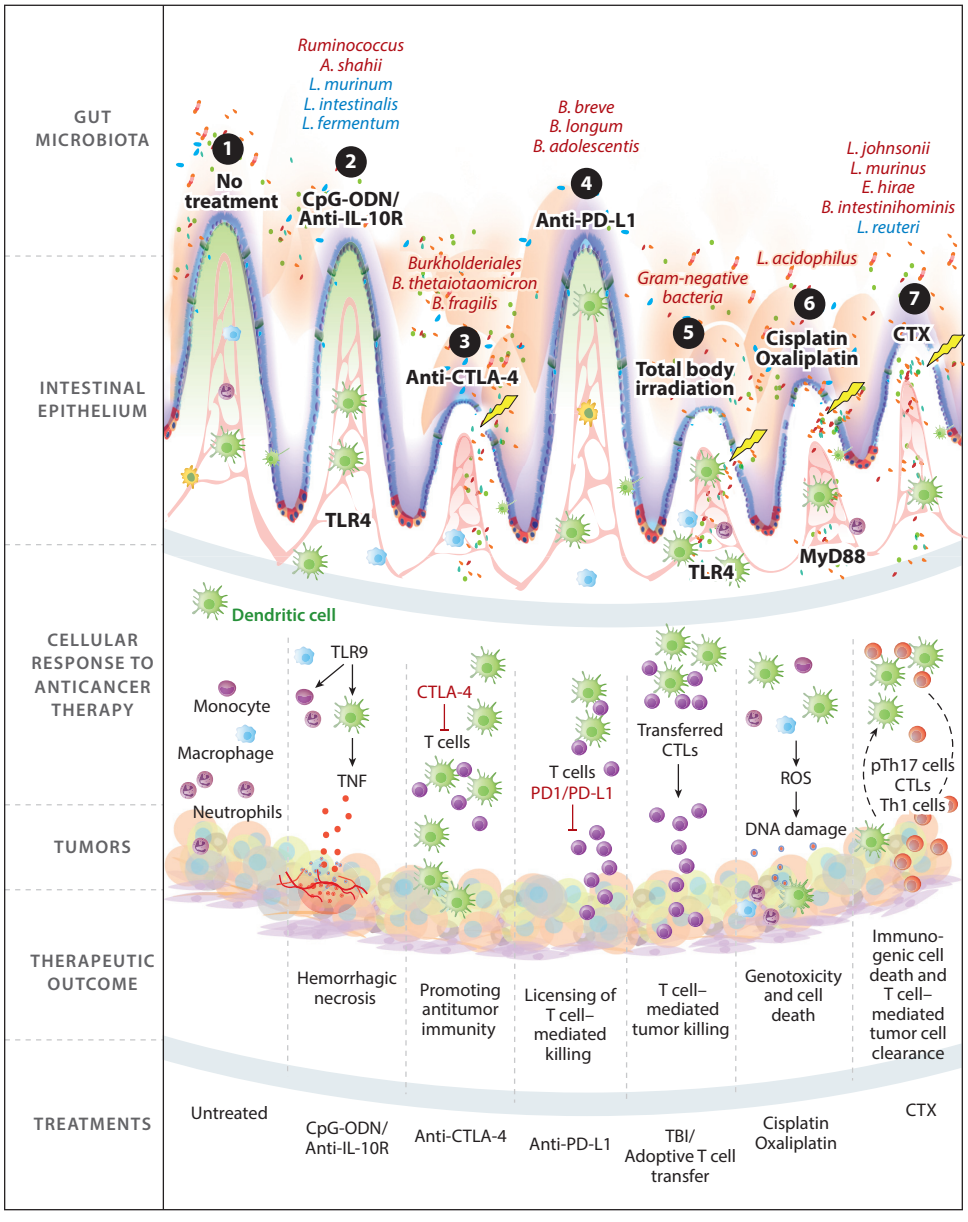
CANCER CHEMOTHERAPY AND THE MICROBIOTA

The microbiota regulates the response of different types of cancer chemotherapy by affecting their pharmacokinetics, mechanism of action, and toxicity (184, 185) (**Figure 3**). Intestinal host and bacterial enzymes control the bioavailability of many oral drugs (186). Exposure to xenobiotics, including chemotherapy agents, alters the composition, physiology, and gene expression of the human gut microbiota (187). The gut microbiota also metabolizes injected drugs after biliary excretion and reabsorption (188). For example, tissue carboxylesterase transforms Irinotecan (CPT-11; an intravenous topoisomerase I inhibitor used for colorectal cancer treatment) into its active form, SN-38, which is then detoxified in the liver by UDP-glucuronosyltransferases, becoming inactive SN-38-G before being secreted in the gut (188). When bacterial β-glucuronidase reconverts SN-38-G in the gut into SN-38, intestinal toxicity and diarrhea are observed (189). β-Glucuronidase activity in the human gut is mostly associated with abundance of Firmicutes, particularly within clostridial clusters XIVa and IV (190). Intestinal inflammation induced by CPT-11 therapy can be successfully treated with antibiotics to decrease the abundance of β-glucuronidase-positive bacteria or with bacterial β-glucuronidase-specific inhibitors (191).

Platinum-based anticancer drugs inhibit DNA replication by forming intrastrand platinum-DNA adducts and double-stranded breaks (192). In addition to inducing tumor cell cytotoxicity and apoptosis, platinum drugs cause severe intestinal toxicity, nephrotoxicity, and peripheral neuropathy that compromise the quality of life (193–195). In germfree mice or in mice depleted of gut commensals using nonabsorbable broad-spectrum antibiotics, the antitumor efficacy of oxaliplatin or cisplatin is dramatically decreased (53). In the absence of commensal microbiota the drugs still reach the tumor and form platinum-DNA adducts, but DNA damage is severely attenuated (53). The microbiota is necessary for training tumor-infiltrating myeloid cells to produce reactive oxygen species (ROS) via NADPH oxidase 2 (NOX2), which is necessary for platinum compound–induced DNA damage (53). Mice deficient for the *Cybb* gene encoding the gp91phox chain of NOX2 and mice treated with antibodies depleting myeloid cells are poorly responsive to oxaliplatin (53). Although ROS were known to be involved in the induction of DNA damage and apoptosis of the tumor cells, the source of ROS was expected to be autocrine (196). However, observations in microbiota-depleted mice are more compatible with paracrine production of ROS by NOX2-expressing and ROS-producing tumor infiltrating myeloid cells (53). Administration of *Lactobacillus acidophilus* to antibiotics-treated mice restores cisplatin antitumor activity (197). How precisely the gut commensals and *L. acidophilus* prime myeloid cells for ROS production in response to platinum drugs is still unclear. Interestingly, *L. acidophilus* also attenuates intestinal toxicity in patients treated with both radiotherapy and cisplatin, suggesting that this probiotic may enhance the antitumor effect while preventing adverse reactions (197, 198). Acetovanillone, an inhibitor of NADPH oxidases, protects mice from cisplatin nephrotoxicity by preventing toxicity of both the ROS produced by kidney tubular cells soon after treatment and the late ROS produced by infiltrating myeloid cells (199). Not surprisingly, these observations indicate that the antitumor effect and the toxicity of platinum compounds are modulated in a similar way by gut commensals. In devising procedures targeting the microbiota to improve therapeutic efficacy while limiting toxicity, it will be important to determine how the drugs and the microbiota intersect in mediating toxicity in tumors and organs and to identify the roles and mechanisms of action of different commensals. Treatment with prebiotics, probiotics, and symbiotics could prevent dysbiosis

after chemotherapy and reduce inflammation in the gut and liver, although data from stringent clinical trials are needed (200).

Antitumor treatment with the alkylating agent cyclophosphamide (CTX) rapidly damages the mouse gut epithelial barrier by increasing mucosal permeability, resulting in transmucosal translocation of gram-positive gut bacteria, such as *Lactobacillus johnsonii*, *Lactobacillus murinus*, and *Enterococcus hirae*, into the mesenteric lymph nodes (201). Within a week, CTX treatment also alters the composition of commensals in the small intestine of mice, similar to what observed in cancer patients (201, 202). CTX treatment selectively reduces Firmicutes (*Clostridium cluster XIVa*, *Roseburia*, *Coprococcus* and unclassified *Lachnospiraceae*) and Spirochaetes (particularly



Treponema genus) phyla in the small intestinal mucosa while it enhances gram-positive bacteria, mainly *L. johnsonii*, *L. murinus*, *E. hirae*, and *L. reuteri*, some of which translocate into mesenteric lymph nodes (201). CTX induces immunogenic tumor cell death that, in concert with the translocated gram-positive bacteria and the accumulation of the gram-negative *Barnesiella intestinihominis* in the colon, activates pathogenic T helper 17 (pTh17) cells and memory Th1 immune responses that mediate an antitumor adaptive immune response (201, 203). The pTh17 responses and the antitumor effect of CTX treatment are reduced in germfree mice and in mice depleted of gram-positive bacteria by antibiotics (201). The antitumor effect of CTX can be restored in microbiota-depleted mice by adoptive transfer of pTh17 cells (201).

IMMUNOTHERAPY AND THE GUT MICROBIOTA

Immunotherapy is one of the greatest successes of cancer research (204). Enduring, complete responses have been obtained in patients with metastatic melanoma and lung cancer even when previous treatments failed. However, the response in different patients and cell types has varied. New evidence that the composition of the gut microbiota modulates the response to immunotherapy opens new possibilities of improving outcomes (53, 205, 206).

An early study showed that in mice preconditioned with total body irradiation (TBI) before receiving adoptive T cell therapy, the antitumor effect was dependent on the presence of gut

Figure 3

Gut microbiota in cancer therapy. Gut commensal microorganisms regulate complex cellular networks, enhancing (*red names*) or attenuating (*blue names*) the efficacy of cancer treatments. Some of the mechanisms by which the microbiota affects the various anticancer therapies (*bottom*) are depicted here:

(❶) An intact, healthy intestinal epithelial cell layer and mucous layer are an efficient barrier for luminal commensal bacteria. Together with well-regulated interactions between the intestinal innate and adaptive immune system, they maintain mucosal homeostasis. Some but not all cancer therapies damage mucosal integrity and allow transmucosal translocation of commensal bacteria.

(❷) Intratumoral treatment with the immunostimulating TLR9 agonist CpG-ODN combined with inhibition of IL-10 signaling (anti-IL10R antibodies) induces rapid tumor hemorrhagic necrosis mediated by TLR9-induced TNF production. The gut microbiota primes (via a TLR4-dependent mechanism) tumor-infiltrating myeloid cells to produce TNF in response to CpG-ODN.

(❸) Immunotherapy with anti-CTLA-4 induces mucosal damage and translocation of *Burkholderiales* and *Bacteriodales*, which promote anticommensal immunity that acts as an adjuvant for antitumor immunity and is required for inducing a positive response to therapy.

(❹) The antitumor effect of anti-PD-L1 therapy, which does not damage the gut epithelia, requires preexisting antitumor immunity that is particularly effective in mice harboring intestinal *Bifidobacterium* spp.

(❺) Preconditioning total body irradiation (TBI) enhances the efficacy of adoptive T cell therapy by inducing mucosal damage, which allows translocation of gram-negative commensals that activate dendritic cells via TLR4 signaling, augmenting proliferation and cytotoxic functions of the transferred T cells in the tumor microenvironment.

(❻) Platinum-based drugs, including cisplatin and oxaliplatin, cause DNA damage in tumor cells that is dependent on both the formation of platinum-DNA adducts and the production of NADPH-oxidase dependent reactive oxygen species (ROS) by tumor-infiltrating myeloid cells that have been primed in a MyD88-dependent way by components of the commensal microbiota.

(❼) Cyclophosphamide (CTX) therapy induces immunologic cell death of tumor cells, which elicits the generation of antitumor pathogenic Th17 (pTh17) cells, Th1 cells, and cytotoxic T lymphocytes (CTLs), leading to tumor destruction. Optimal generation of this antitumor immune response requires the activation of tumor-antigen-presenting dendritic cells by components of the intestinal microbiota that translocate following CTX-induced mucosal damage.

commensals (207). TBI damaged the intestinal mucosa, inducing translocation of bacteria and activation of dendritic cells via TLR4 activation. This improved the proliferation and antitumor effect of the transferred $CD8^+$ T cells (207). The beneficial effect of TBI on therapy efficacy was lost in *Tlr4*-deficient mice and in antibiotic-treated mice (207). Administration of the TLR4 agonist lipopolysaccharide to nonirradiated animals enhanced the efficacy of transferred T cells (207).

Intratumoral administration of the TLR9 agonist CpG oligonucleotide (CpG-ODN) along with IL-10R-blocking antibodies induces a strong inflammatory antitumor response, characterized by secretion of proinflammatory cytokines such as TNF and IL-12 that was potentiated by antagonizing the immunosuppressive role of IL-10 produced by Tregs and myeloid cells (208–210). CpG-ODN/anti-IL-10R treatment induces rapid hemorrhagic necrosis and repolarization of tumor-infiltrating dendritic cells and macrophages to a proinflammatory state. These then promote T cell–mediated antitumor immunity to permanently eliminate the tumors in most mice (208). In germfree mice or mice whose gut microbiota has been depleted by antibiotic treatment, tumor-infiltrating myeloid cell production of proinflammatory cytokines in response to CpG-ODN is poor, preventing the induction of TNF-dependent necrosis and antitumor adaptive immunity (53). Tumors of microbiota-depleted mice have only minor alterations in the number and differentiation of tumor-infiltrating myeloid cells, mostly derived by recruited $Ly6C^+$ circulating inflammatory monocytes. Tumor-infiltrating myeloid cell subsets in microbiota-depleted mice show modest alterations in their gene expression profile compared with conventional mice. This observation contrasts with the major gene expression differences between microbiota-depleted and conventionally raised mice that are observed after CpG-ODN treatment. Myeloid cells from *Tlr4*-deficient tumor-bearing mice are also partially unresponsive to CpG-ODN, and administration of lipopolysaccharide by gavage to microbiota-depleted mice reconstitutes the myeloid cell response to CpG, suggesting that activation of TLR4 by products of commensal bacteria primes tumor myeloid cells for responsiveness to TLR9 (53). These results are reminiscent of the reported inability of mononuclear phagocytes in nonmucosal lymphoid organs of germfree mice to respond to microbial stimulation with transcription of inflammatory genes due to epigenetically closed chromatin conformation at those loci (34). Thus, colonizing the mice postnatally introduces chromatin changes in the myeloid cells, poising them for rapid responses to inflammatory stimuli, similar to the phenomenon of trained innate resistance recently reported for various innate effector cell types (34, 211). However, this epigenetic conformation can be reversed by antibiotic-induced depletion of the commensal microbiota for two to three weeks, suggesting either that this chromatin conformation is not stable and requires continued instructions from the commensal microbiota or that inflammatory monocytes are newly generated in the bone marrow and require training (53). The ability of the tumor-infiltrating myeloid cells from animals with altered microbiota to produce TNF in response to CpG-ODN intratumoral treatment correlated with the abundance of specific bacterial genera. For example, TNF production was positively correlated with the frequencies of gram-negative *Alistipes* spp. and gram-positive *Ruminococcus* spp. in the fecal microbiota. Frequencies of *Lactobacillus* species that are considered to be probiotics with anti-inflammatory properties, including *L. murinum*, *L. intestinalis*, and *L. fermentum*, negatively correlated with TNF production (53). *Alistipes shahii* administration by gavage to mice previously exposed to antibiotics restored TNF production, whereas *L. fermentum* impaired TNF production in conventionally raised mice (53). Thus, the training of myeloid cells for response to CpG-ODN is reversed when the commensal microbiota is depleted, and individual bacterial strains can either increase or attenuate priming for TNF production. Selective antibiotics, prebiotics, or probiotics that modify microbiota composition could be considered for optimizing anticancer immunotherapy.

Immunity plays a dual role in cancer. As part of immunosurveillance, it may prevent or delay the growth of the tumor by destroying tumor cells or creating a hostile microenvironment.

On the other hand, it can also promote tumor initiation and progression by activating an anti-inflammatory microenvironment or selecting cells that are able to evade the antitumor immune response (212). In patients with progressive tumors, anticancer immunity is dormant or suppressed, unable to prevent tumor growth. However, in many patients, it can be reactivated by releasing the immunological brakes responsible for tumor escape (204, 213). Immune checkpoint inhibitors include antibodies against cytotoxic T lymphocyte–associated protein 4 (CLTA-4), expressed on activated T effector cells and Tregs, and programmed cell death protein 1 (PD1) or its ligand PDL-1. These inhibitors have had enduring clinical efficacy in many cancer patients (214). Anti-CTLA-4 antibodies enhance T cell immune responses and proliferation by preventing the T cell–suppressive interaction of CTLA-4 with its CD80 and CD86 ligands (214). Anti-PD1 and anti-PD-L1 antibodies avoid T cell exhaustion and maintain T cell effector functions by preventing the binding of PD1 on activated T cells with PD-L1 that is expressed on the tumor cells and other stromal cells (214). As with many effective anticancer therapies, checkpoint inhibitors may have immune-related adverse effects. Onset of colitis and hypophysitis is mostly observed in response to anti-CTLA-4 antibodies, whereas blocking PD1/PD-L1 interactions can lead to thyroid dysfunctions and pneumonitis (215). Variability in patient response and susceptibility to adverse reactions has been primarily attributed to the genetic characteristics of the tumors (including the number of mutations and possibly the number of neoantigens); however, the immune status of the patient and the possible effect of the microbiota in modulating it are additional important factors (213, 214).

Two recent studies have identified the important role of the microbiota in modulating the efficacy of anti-CTLA-4 and anti-PD-L1 therapy (205, 206). The anti-CTLA-4 treatment was found to be ineffective in antibiotic-treated mice and in germfree mice (205). Treatment of mice with anti-CTLA-4 antibodies consistently induces T cell–mediated mucosal damage in the ileum and colon and alters the proportion of bacterial species both in the intestine and in the feces (205). *Bacteroides thetaiotaomicron* or *B. fragilis*, when orally administered to microbiota-depleted mice, corrected the deficient response to anti-CTLA-4 by activating intratumoral dendritic cells and inducing a Th1 response in the tumor-draining lymph nodes (205). Of particular interest, introducing *B. fragilis* and *Burkholderia cepacia*, combined, not only enhanced the anticancer response but also prevented the intestinal inflammation and colitis induced by anti-CTLA-4 (205). The correlation between adverse effects and microbiota composition was confirmed by clinical trials in which the increased frequency of the Bacteroidetes phylum was found to be correlated with resistance to colitis, whereas underrepresented genetic pathways involved in polyamine transport and B vitamin biosynthesis were associated with increased risk (216). In mice, selective antibiotics alter the composition of the gut microbiota affecting the anti-CTLA-4 response: e.g., vancomycin prevented the loss of Bacteroidales and Burkholderiales and enhanced the efficacy of anti-CTLA-4 therapy (205). Melanoma patients treated with anti-CTLA-4 clustered into three fecal microbiota enterotypes: Cluster A was dominated by *Prevotella* spp. and clusters B and C by *Bacteroides* spp. After treatment, some of the patients in cluster B acquired the cluster C enterotype. When germfree mice were colonized with the patients' fecal microbiota, the mice colonized with cluster C enterotype showed expansion of *B. thetaiotaomicron* or *B. fragilis* and an ability to respond to anti-CTLA-4 treatment (205). Thus, anti-CTLA-4 treatment may, in some cases, alter the composition of the gut microbiota in a direction that favors its antitumor activity. Anti-CTLA-4 elicits, both in mice and in the humans, a Th1 response specific for either *B. thetaiotaomicron* or *B. fragilis*. Microbiota-depleted mice recover their ability to respond to anti-CTLA-4 therapy when *B. fragilis*–specific T cells are adoptively transferred (205). A similar observation was reported for mucosal damage induced by infection that also induces a persistent memory Th1 cell response against commensal bacteria that, upon reinfection at the same anatomical site, prime for a

polarized Th1 response (14). However, in the case of the anti-CTLA-4 treatment, the mucosal response to commensals and the antitumor response are at different anatomical sites and the mechanism regulating migration and tropism of the T cells involved is not yet understood.

Unlike anti-CTLA4, anti-PD-L1 treatment in mice does not induce intestinal damage, and its anticancer effect does not have an absolute requirement for the presence of gut commensals (206). However, B16 melanoma grew faster in C57BL/6 mice purchased from Taconic (TAC) than in those purchased from Jackson Laboratory (JAX) (206). Anti-PD-L1 almost completely arrested growth of the smoldering tumors of JAX mice, whereas it only slowed the progression of the faster-growing tumors of TAC mice (206). More $CD8^+$ T cells infiltrated within tumors in JAX mice compared to TAC mice, suggesting that the slower tumor growth and better responsiveness to anti-PD-L1 in JAX mice was due to a more robust anticancer immune response (206). TAC mice cohoused with JAX mice acquired the same rate of tumor growth, antitumor resistance, and responsiveness to anti-PD-L1 observed in JAX mice (206). The responsiveness of JAX mice to anti-PD-L1 correlated with the fecal abundancy of the *Bifidobacterium* species, including *B. breve*, *B. longum*, and *B. adolescentis* (206). A commercially available probiotic cocktail of *Bifidobacterium* species (including *B. breve* and *B. longum*) administered, alone or with anti-PD-L1, to TAC mice enhanced $CD8^+$ T cell–induced antitumor activity (206). Treatment of TAC mice with *Bifidobacterium* spp. or anti-PD-L1 showed equivalent therapeutic efficacy (206). Thus, unlike anti-CTL-4, anti-PD-L1 treatment does not require an inflammatory immune activation that is supported by the presence of the commensal microbiota. However, the presence of *Bifidobacterium* spp. in the gut microbiota fosters a more robust antitumor immune response that, when enhanced by anti-PD-L1, prevents tumor progression.

CONCLUSIONS

Recent technological advances have revolutionized our understanding of human metaorganism physiology. This has opened new possibilities for basic and clinical science, including precision medicine. Although novel sequencing technologies have contributed most to the success of microbiology studies, progress in other areas has been critical as well: improved bacterial culture methodology, gnotobiotic technology, bioinformatics, computational science (including an exponentially growing number of reference data sets), microscopy, and systems biology. Indeed, we might safely predict that microbiota research will benefit from both experimental and computational advances. For example, there is a need for software that interprets larger metagenomic data sets and presents hierarchically organized functional data such as novel microorganism-centered annotated pathways and other gene function interpretations. Metagenomic and metatranscriptomic analyses will be improved by new reference genomes from human and animal microbiota. This will be aided by new single-molecule sequencing methods that generate reads tens of kilobases long, easing genome assembly of even uncultivable microbes. Single-cell sequencing technologies will also allow us to sequence microbial genomes and transcriptomes without the need of culturing the microorganisms. New computational methods will be developed to better understand the kinetics of microbial communities and to identify keystone species that have greater influence than their abundance may imply. Advances in bacterial culture methods and artificial gut development [e.g., "robogut" technology (217) and "gut on a chip" (218)] will serve to validate computational predictions and identify novel microbe-microbe and host-microbe interactions. Finally, the definition of microbiota will be broadened to include not just prokaryotes, unicellular eukaryotes, and viruses but also small multicellular animals residing in the gut (e.g., helminths) and in the skin (e.g., *Demodex* mites). Our internal ecosystem is much more diverse than previously realized.

The studies reviewed here have led us to conclude that many of the mechanisms of microbe-dependent host physiology and tissue homeostasis are derived from ancestral mechanisms stemming from the earliest interactions between prokaryotes and eukaryotes. Phagocytosis began as a method of obtaining nutrients and probably led to the genesis of mitochondria. Phagocytes and myeloid cells, in particular, communicate with commensal microbiota to dictate responses involving immune defense, tissue homeostasis, repair, cancer development, and response to cancer therapy (219). As phagocytes act as bridges between all the leukocyte subsets involved in response to infection and tissue damage, the cross talk between the host and microbiota transverses both innate and adaptive immunity.

The composition and functional status of different members of the microbial community can modulate or even control cancer initiation and progression, comorbidity, and response to therapy. We are at a critical point when the accumulated knowledge of host-microbe interactions can be used to design new therapeutic strategies. The commensal microbiota is a target for interventions to prevent cancer, control its progression, induce its destruction via genetically engineered bacteria, and prevent cancer-associated comorbidities such as cachexia that can affect treatment success and quality of life. Indeed, recent studies demonstrating that microbes can modulate anticancer therapies offer the prospect that targeting the gut microbiota may improve therapeutic efficacy and attenuate toxicity and adverse reactions. Currently, however, few therapeutic approaches target microbes; clinical applications are still from the dark ages. Probiotics and prebiotics might be a more precise tool for manipulating the gut microbiota than antibiotics. However, until now, there has been little consistent or sufficient evidence of their clinical efficacy in many microbiota-related diseases. An exception is *Clostridium difficile* infection, where crude community-altering therapies, such as fecal transplantation, have been successful (220). Progress in bacterial ecology and in understanding the role of bile acids in controlling *C. difficile* infection has identified bacterial species that for treating *C. difficile* infection (221) as an alternative to fecal transplantation. Therapeutic use of bacterial species presents the challenge of formulation that will achieve efficient colonization in the intestine after oral delivery. Whereas preparations of facultative anaerobic bacteria may be relatively easy to formulate and administer, obligate anaerobes may present more obstacles. Fortunately, many obligate anaerobes, like *Clostridium* spp., form spores that germinate only in hypoxic environments. Spores are durable and survive dry, oxygen-rich environments and therefore could be used as a vector for microbial delivery. These characteristics of the spores also make them attractive for use in anticancer treatments, since they would germinate in the hypoxic environment of large tumors. Looking at the road ahead there is hope that we will overcome the many challenges of using microbes as anticancer therapeutics. In addition to identifying therapeutic microbes, we have to address the temporal and geographical variability of the microbiota in human populations. Through the accumulation of clinical data, we need to identify key metabolic processes of the healthy microbiota and microbial taxa that promote resistance to disease or improve therapeutic outcomes in different clinical situations. As we identify beneficial microbes and metabolic processes that promote resistance to disease or improve efficacy of available treatments, microbiology will likely become an important component of precision and personalized medicine for cancer and other diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI. 2008. Worlds within worlds: Evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* 6:776–88
2. Yutin N, Wolf MY, Wolf YI, Koonin EV. 2009. The origins of phagocytosis and eukaryogenesis. *Biol. Direct* 4:9
3. McInerney JO, O'Connell MJ, Pisani D. 2014. The hybrid nature of the Eukaryota and a consilient view of life on Earth. *Nat. Rev. Microbiol.* 12:449–55
4. Chovatiya R, Medzhitov R. 2014. Stress, inflammation, and defense of homeostasis. *Mol. Cell* 54:281–88
5. Chu H, Mazmanian SK. 2013. Innate immune recognition of the microbiota promotes host-microbial symbiosis. *Nat. Immunol.* 14:668–75
6. Hum. Microbiome Proj. Consort. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486:207–14
7. Sender R, Fuchs S, Milo R. 2016. Are we really vastly outnumbered? revisiting the ratio of bacterial to host cells in humans. *Cell* 164:337–40
8. Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, et al. 2013. An estimation of the number of cells in the human body. *Ann. Hum. Biol.* 40:463–71
9. Bosch TC, McFall-Ngai MJ. 2011. Metaorganisms as the new frontier. *Zoology* 114:185–90
10. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJ, Relman DA. 2012. The application of ecological theory toward an understanding of the human microbiome. *Science* 336:1255–62
11. McFall-Ngai M. 2008. Are biologists in 'future shock'? Symbiosis integrates biology across domains. *Nat. Rev. Microbiol.* 6:789–92
12. Candela M, Biagi E, Maccaferri S, Turroni S, Brigidi P. 2012. Intestinal microbiota is a plastic factor responding to environmental changes. *Trends Microbiol.* 20:385–91
13. Zeng MY, Cisalpino D, Varadarajan S, Hellman J, Warren HS, et al. 2016. Gut microbiota-induced immunoglobulin G controls systemic infection by symbiotic bacteria and pathogens. *Immunity* 44:647–58
14. Hand TW, Dos Santos LM, Bouladoux N, Molloy MJ, Pagan AJ, et al. 2012. Acute gastrointestinal infection induces long-lived microbiota-specific T cell responses. *Science* 337:1553–56
15. Stewart EJ. 2012. Growing unculturable bacteria. *J. Bacteriol.* 194:4151–60
16. Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, et al. 2011. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *PNAS* 108:6252–57
17. Tringe SG, Hugenholtz P. 2008. A renaissance for the pioneering 16S rRNA gene. *Curr. Opin. Microbiol.* 11:442–46
18. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *PNAS* 109:6241–46
19. Parfrey LW, Walters WA, Lauber CL, Clemente JC, Berg-Lyons D, et al. 2014. Communities of microbial eukaryotes in the mammalian gut within the context of environmental eukaryotic diversity. *Front. Microbiol.* 5:298
20. Morgun A, Dzutsev A, Dong X, Greer RL, Sexton DJ, et al. 2015. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. *Gut* 64:1732–43
21. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, et al. 2015. Personalized nutrition by prediction of glycemic responses. *Cell* 163:1079–94
22. Belkaid Y, Hand TW. 2014. Role of the microbiota in immunity and inflammation. *Cell* 157:121–41
23. Cryan JF, Dinan TG. 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13:701–12
24. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, et al. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464:59–65
25. Tsai YC, Conlan S, Deming C, Segre JA, Kong HH, et al. 2016. Resolving the complexity of human skin metagenomes using single-molecule sequencing. *mBio* 7:e01948-15
26. Blekhman R, Goodrich JK, Huang K, Sun Q, Bukowski R, et al. 2015. Host genetic variation impacts microbiome composition across human body sites. *Genome Biol.* 16:191
27. Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. 2015. The infant microbiome development: Mom matters. *Trends Mol. Med.* 21:109–17

28. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, et al. 2012. Human gut microbiome viewed across age and geography. *Nature* 486:222–27
29. Jackson MA, Jeffery IB, Beaumont M, Bell JT, Clark AG, et al. 2016. Signatures of early frailty in the gut microbiota. *Genome Med*. 8:8
30. O'Toole PW, Jeffery IB. 2015. Gut microbiota and aging. *Science* 350:1214–15
31. Brestoff JR, Artis D. 2013. Commensal bacteria at the interface of host metabolism and the immune system. *Nat. Immunol.* 14:676–84
32. Belkaid Y, Naik S. 2013. Compartmentalized and systemic control of tissue immunity by commensals. *Nat. Immunol.* 14:646–53
33. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, et al. 2011. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *PNAS* 108:5354–59
34. Ganal SC, Sanos SL, Kallfass C, Oberle K, Johner C, et al. 2012. Priming of natural killer cells by nonmucosal mononuclear phagocytes requires instructive signals from commensal microbiota. *Immunity* 37:171–86
35. Abt MC. 2016. Acute gastroenteritis leaves a lasting impression. *Cell Host Microbe* 19:3–5
36. Chervonsky AV. 2013. Microbiota and autoimmunity. *Cold Spring Harb. Perspect. Biol.* 5:a007294
37. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, et al. 2012. Compartmentalized control of skin immunity by resident commensals. *Science* 337:1115–19
38. Vaishnava S, Behrendt CL, Ismail AS, Eckmann L, Hooper LV. 2008. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *PNAS* 105:20858
39. Shen W, Li W, Hixon JA, Bouladoux N, Belkaid Y, et al. 2014. Adaptive immunity to murine skin commensals. *PNAS* 111:E2977–86
40. Khosravi A, Yanez A, Price JG, Chow A, Merad M, et al. 2014. Gut microbiota promote hematopoiesis to control bacterial infection. *Cell Host Microbe*. 15:374–81
41. Balmer ML, Schurch CM, Saito Y, Geuking MB, Li H, et al. 2014. Microbiota-derived compounds drive steady-state granulopoiesis via MyD88/TICAM signaling. *J. Immunol.* 193:5273–83
42. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, et al. 2014. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* 20:159–66
43. Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, et al. 2015. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* 18:965–77
44. Zhang D, Chen G, Manwani D, Mortha A, Xu C, et al. 2015. Neutrophil ageing is regulated by the microbiome. *Nature* 525:528–32
45. Mukherji A, Kobiita A, Ye T, Chambon P. 2013. Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. *Cell* 153:812–27
46. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A. 2013. Circadian gene *Bmal1* regulates diurnal oscillations of $Ly6C^{hi}$ inflammatory monocytes. *Science* 341:1483–88
47. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, et al. 2014. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* 159:514–29
48. Perussia B, Dayton ET, Fanning V, Thiagarajan P, Hoxie J, Trinchieri G. 1983. Immune interferon and leukocyte-conditioned medium induce normal and leukemic myeloid cells to differentiate along the monocytic pathway. *J. Exp. Med.* 158:2058–80
49. Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A. 1992. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood* 80:2012–20
50. de Oliveira S, Rosowski EE, Huttenlocher A. 2016. Neutrophil migration in infection and wound repair: going forward in reverse. *Nat. Rev. Immunol.* 16:378–91
51. Askenase MH, Han S-J, Byrd AL, Morais da Fonseca D, Bouladoux N, et al. 2015. Bone-marrow-resident NK cells prime monocytes for regulatory function during infection. *Immunity* 42:1130–42
52. Gottschalk RA, Martins AJ, Angermann BR, Dutta B, Ng CE, et al. 2016. Distinct NF-κB and MAPK activation thresholds uncouple steady-state microbe sensing from anti-pathogen inflammatory responses. *Cell Syst*. 2:378–90
53. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, et al. 2013. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 342:967–70

54. Ozmen L, Pericin M, Hakimi J, Chizzonite RA, Wysocka M, et al. 1994. Interleukin 12, interferon γ, and tumor necrosis factor α are the key cytokines of the generalized Shwartzman reaction. *J. Exp. Med.* 180:907–15
55. Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. *Cell* 100:57–70
56. Fidler IJ. 2003. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat. Rev. Cancer* 3:453–58
57. Dolberg DS, Bissell MJ. 1984. Inability of Rous sarcoma virus to cause sarcomas in the avian embryo. *Nature* 309:552–56
58. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, et al. 2015. Tumor evolution: high burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348:880–86
59. Bissell MJ, Hines WC. 2011. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. *Nat. Med.* 17:320–29
60. Adami J, Gabel H, Lindelof B, Ekstrom K, Rydh B, et al. 2003. Cancer risk following organ transplantation: a nationwide cohort study in Sweden. *Br. J. Cancer* 89:1221–27
61. Vogtmann E, Goedert JJ. 2016. Epidemiologic studies of the human microbiome and cancer. *Br. J. Cancer* 114:237–42
62. IARC Work. Group Evaluation Carcinog. Risks Hum. 1994. *Schistosomes, Liver Flukes and* Helicobacter pylori. IARC Monogr. Evaluation Carcinog. Risks Hum. Vol. 61. Geneva, Switz.: World Health Organ.
63. Atherton JC, Blaser MJ. 2009. Coadaptation of *Helicobacter pylori* and humans: ancient history, modern implications. *J. Clin. Investig.* 119:2475–87
64. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, et al. 2013. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 14:207–15
65. Sears CL, Garrett WS. 2014. Microbes, microbiota, and colon cancer. *Cell Host Microbe* 15:317–28
66. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, et al. 2012. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.* 13:R79
67. Arthur JC, Perez-Chanona E, Muhlbauer M, Tomkovich S, Uronis JM, et al. 2012. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338:120–23
68. Thomas RM, Jobin C. 2015. The microbiome and cancer: Is the 'oncobiome' mirage real? *Trends Cancer* 1:24–35
69. Poutahidis T, Varian BJ, Levkovich T, Lakritz JR, Mirabal S, et al. 2015. Dietary microbes modulate transgenerational cancer risk. *Cancer Res.* 75:1197–204
70. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. 2014. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. *Lancet* 384:755–65
71. Conroy MJ, Dunne MR, Donohoe CL, Reynolds JV. 2016. Obesity-associated cancer: an immunological perspective. *Proc. Nutr. Soc.* 75:125–38
72. Beyaz S, Mana MD, Roper J, Kedrin D, Saadatpour A, et al. 2016. High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* 531:53–58
73. Ohtani N, Yoshimoto S, Hara E. 2014. Obesity and cancer: a gut microbial connection. *Cancer Res.* 74:1885–89
74. Abnet CC, Corley DA, Freedman ND, Kamangar F. 2015. Diet and upper gastrointestinal malignancies. *Gastroenterology* 148:1234–43.e4
75. Doll R, Peto R. 1981. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* 66:1191–308
76. Song M, Garrett WS, Chan AT. 2015. Nutrients, foods, and colorectal cancer prevention. *Gastroenterology* 148:1244–60.e16
77. Wei W, Sun W, Yu S, Yang Y, Ai L. 2016. Butyrate production from high-fiber diet protects against lymphoma tumor. *Leuk. Lymphoma* 57:2401–8
78. Masuyama H, Mitsui T, Nobumoto E, Hiramatsu Y. 2015. The effects of high-fat diet exposure in utero on the obesogenic and diabetogenic traits through epigenetic changes in adiponectin and leptin gene expression for multiple generations in female mice. *Endocrinology* 156:2482–91
79. Modi SR, Collins JJ, Relman DA. 2014. Antibiotics and the gut microbiota. *J. Clin. Investig.* 124:4212–18

80. Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, et al. 2012. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 488:621–26
81. Scott FI, Horton DB, Mamtani R, Haynes K, Goldberg DS, et al. 2016. Administration of antibiotics to children before age 2 years increases risk for childhood obesity. *Gastroenterology* 151:120–129.e5
82. Hviid A, Svanstrom H, Frisch M. 2011. Antibiotic use and inflammatory bowel diseases in childhood. *Gut* 60:49–54
83. Sergentanis TN, Zagouri F, Zografos GC. 2010. Is antibiotic use a risk factor for breast cancer? A meta-analysis. *Pharmacoepidemiol. Drug Saf.* 19:1101–7
84. Xuan C, Shamonki JM, Chung A, Dinome ML, Chung M, et al. 2014. Microbial dysbiosis is associated with human breast cancer. *PLOS ONE* 9:e83744
85. Lee YY, Mahendra Raj S, Graham DY. 2013. *Helicobacter pylori* infection—a boon or a bane: lessons from studies in a low-prevalence population. *Helicobacter* 18:338–46
86. Levkovich T, Poutahidis T, Cappelle K, Smith MB, Perrotta A, et al. 2014. 'Hygienic' lymphocytes convey increased cancer risk. *J. Anal. Oncol.* 3:113–21
87. Fonseca DM, Hand TW, Han S-J, Gerner MY, Glatman Zaretsky A, et al. 2015. Microbiota-dependent sequelae of acute infection compromise tissue-specific immunity. *Cell* 163:354–66
88. Chen JA, Splenser A, Guillory B, Luo J, Mendiratta M, et al. 2015. Ghrelin prevents tumour- and cisplatin-induced muscle wasting: characterization of multiple mechanisms involved. *J. Cachexia Sarcopenia Muscle* 6:132–43
89. de Matos-Neto EM, Lima JDCC, de Pereira WO, Figuerêdo RG, Riccardi DMDR, et al. 2015. Systemic inflammation in cachexia—is tumor cytokine expression profile the culprit? *Front. Immunol.* 6:629
90. Kir S, White JP, Kleiner S, Kazak L, Cohen P, et al. 2014. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. *Nature* 513:100–4
91. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. 2009. Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and *Methanogens* in anorexic patients. *PLOS ONE* 4:e7125
92. Varian BJ, Goureshetti S, Poutahidis T, Lakritz JR, Levkovich T, et al. 2016. Beneficial bacteria inhibit cachexia. *Oncotarget* 7:11803–16
93. Bindels LB, Neyrinck AM, Claus SP, Le Roy CI, Grangette C, et al. 2016. Synbiotic approach restores intestinal homeostasis and prolongs survival in leukaemic mice with cachexia. *ISME J.* 10:1456–70
94. Schieber AMP, Lee YM, Chang MW, Leblanc M, Collins B, et al. 2015. Disease tolerance mediated by microbiome *E. coli* involves inflammasome and IGF-1 signaling. *Science* 350:558–63
95. Klein GL, Petschow BW, Shaw AL, Weaver E. 2013. Gut barrier dysfunction and microbial translocation in cancer cachexia: a new therapeutic target. *Curr. Opin. Support. Palliat. Care* 7:361–67
96. de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, et al. 2012. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol.* 13:607–15
97. IARC Work. Group Evaluation Carcinog. Risks Hum. 2012. *A Review of Human Carcinogens: Part B; Biological agents.* IARC Monogr. Evaluation Carcinog. Risks Hum. Vol. 100B. Geneva, Switz.: World Health Organ.
98. Zhang X, Zhang Z, Zheng B, He Z, Winberg G, Ernberg I. 2013. An update on viral association of human cancers. *Arch. Virol.* 158:1433–43
99. Ng J, Wu J. 2012. Hepatitis B- and hepatitis C-related hepatocellular carcinomas in the United States: similarities and differences. *Hepat. Mon.* 12:e7635
100. Psyrri A, Rampias T, Vermorken JB. 2014. The current and future impact of human papillomavirus on treatment of squamous cell carcinoma of the head and neck. *Ann. Oncol.* 25:2101–15
101. Tommasino M. 2014. The human papillomavirus family and its role in carcinogenesis. *Semin. Cancer Biol.* 26:13–21
102. Ciminale V, Rende F, Bertazzoni U, Romanelli MG. 2014. HTLV-1 and HTLV-2: highly similar viruses with distinct oncogenic properties. *Front. Microbiol.* 5:398
103. Feng H, Shuda M, Chang Y, Moore PS. 2008. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 319:1096–100
104. Zheng ZM. 2010. Viral oncogenes, noncoding RNAs, and RNA splicing in human tumor viruses. *Int. J. Biol. Sci.* 6:730–55

105. Mitra A, MacIntyre DA, Lee YS, Smith A, Marchesi JR, et al. 2015. Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. *Sci. Rep.* 5:16865
106. Rehermann B, Nascimbeni M. 2005. Immunology of hepatitis B virus and hepatitis C virus infection. *Nature Rev. Immunol.* 5:215–29
107. Bender S, Reuter A, Eberle F, Einhorn E, Binder M, Bartenschlager R. 2015. Activation of type I and III interferon response by mitochondrial and peroxisomal MAVS and inhibition by hepatitis C virus. *PLOS Pathog.* 11:e1005264
108. Chen Y, Chen Z, Guo R, Chen N, Lu H, et al. 2011. Correlation between gastrointestinal fungi and varying degrees of chronic hepatitis B virus infection. *Diagn. Microbiol. Infect. Dis.* 70:492–98
109. Xu M, Wang B, Fu Y, Chen Y, Yang F, et al. 2012. Changes of fecal *Bifidobacterium* species in adult patients with hepatitis B virus-induced chronic liver disease. *Microb. Ecol.* 63:304–13
110. Henao-Mejia J, Elinav E, Thaiss CA, Licona-Limon P, Flavell RA. 2013. Role of the intestinal microbiome in liver disease. *J. Autoimmun.* 46:66–73
111. Chou HH, Chien WH, Wu LL, Cheng CH, Chung CH, et al. 2015. Age-related immune clearance of hepatitis B virus infection requires the establishment of gut microbiota. *PNAS* 112:2175–80
112. Moore PS, Chang Y. 2010. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat. Rev. Cancer* 10:878–89
113. Ki MR, Hwang M, Kim AY, Lee EM, Lee EJ, et al. 2014. Role of vacuolating cytotoxin VacA and cytotoxin-associated antigen CagA of *Helicobacter pylori* in the progression of gastric cancer. *Mol. Cell Biochem.* 396:23–32
114. Khosravi Y, Bunte RM, Chiow KH, Tan TL, Wong WY, et al. 2016. *Helicobacter pylori* and gut microbiota modulate energy homeostasis prior to inducing histopathological changes in mice. *Gut Microbes* 7:48–53
115. Perry S, de Jong BC, Solnick JV, de la Luz Sanchez M, Yang S, et al. 2010. Infection with *Helicobacter pylori* is associated with protection against tuberculosis. *PLOS ONE* 5:e8804
116. Koch KN, Hartung ML, Urban S, Kyburz A, Bahlmann AS, et al. 2015. *Helicobacter* urease-induced activation of the TLR2/NLRP3/IL-18 axis protects against asthma. *J. Clin. Investig.* 125:3297–302
117. Kim DJ, Park JH, Franchi L, Backert S, Nunez G. 2013. The Cag pathogenicity island and interaction between TLR2/NOD2 and NLRP3 regulate IL-1β production in *Helicobacter pylori* infected dendritic cells. *Eur. J. Immunol.* 43:2650–58
118. Peek RM Jr., Blaser MJ. 2002. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat. Rev. Cancer* 2:28–37
119. Plottel CS, Blaser MJ. 2011. Microbiome and malignancy. *Cell Host Microbe* 10:324–35
120. Yap TW, Gan HM, Lee YP, Leow AH, Azmi AN, et al. 2016. *Helicobacter pylori* eradication causes perturbation of the human gut microbiome in young adults. *PLOS ONE* 11:e0151893
121. Belkaid Y, Segre JA. 2014. Dialogue between skin microbiota and immunity. *Science* 346:954–59
122. Goldszmid RS, Trinchieri G. 2012. The price of immunity. *Nat. Immunol.* 13:932–38
123. Jobin C. 2012. Colorectal cancer: CRC—all about microbial products and barrier function? *Nat. Rev. Gastroenterol. Hepatol.* 9:694–96
124. Rao VP, Poutahidis T, Ge Z, Nambiar PR, Boussahmain C, et al. 2006. Innate immune inflammatory response against enteric bacteria *Helicobacter hepaticus* induces mammary adenocarcinoma in mice. *Cancer Res.* 66:7395–400
125. Attene-Ramos MS, Wagner ED, Plewa MJ, Gaskins HR. 2006. Evidence that hydrogen sulfide is a genotoxic agent. *Mol. Cancer Res.* 4:9–14
126. Andriamihaja M, Lan A, Beaumont M, Audebert M, Wong X, et al. 2015. The deleterious metabolic and genotoxic effects of the bacterial metabolite *p*-cresol on colonic epithelial cells. *Free Radic. Biol. Med.* 85:219–27
127. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK. 2007. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* 131:33
128. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, et al. 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145:745–57
129. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. 2013. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin. *Cell Host Microbe* 14:195–206

130. Zackular JP, Baxter NT, Iverson KD, Sadler WD, Petrosino JF, et al. 2013. The gut microbiome modulates colon tumorigenesis. *mBio* 4:e00692-13
131. Mira-Pascual L, Cabrera-Rubio R, Ocon S, Costales P, Parra A, et al. 2015. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. *J. Gastroenterol.* 50:167–79
132. Okuda T, Kokubu E, Kawana T, Saito A, Okuda K, Ishihara K. 2012. Synergy in biofilm formation between *Fusobacterium nucleatum* and *Prevotella* species. *Anaerobe* 18:110–16
133. Abed J, Emgard JE, Zamir G, Faroja M, Almogy G, et al. 2016. Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host Microbe* 20:215–25
134. Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, et al. 2015. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 42:344–55
135. Dejea CM, Wick EC, Hechenbleikner EM, White JR, Mark Welch JL, et al. 2014. Microbiota organization is a distinct feature of proximal colorectal cancers. *PNAS* 111:18321–26
136. Johnson CH, Dejea CM, Edler D, Hoang LT, Santidrian AF, et al. 2015. Metabolism links bacterial biofilms and colon carcinogenesis. *Cell Metab.* 21:891–97
137. Dalmasso G, Cougnoux A, Delmas J, Darfeuille-Michaud A, Bonnet R. 2014. The bacterial genotoxin colibactin promotes colon tumor growth by modifying the tumor microenvironment. *Gut Microbes* 5:675–80
138. Nesic D, Hsu Y, Stebbins CE. 2004. Assembly and function of a bacterial genotoxin. *Nature* 429:429–33
139. Kim JJ, Tao H, Carloni E, Leung WK, Graham DY, Sepulveda AR. 2002. *Helicobacter pylori* impairs DNA mismatch repair in gastric epithelial cells. *Gastroenterology* 123:542–53
140. Maddocks OD, Short AJ, Donnenberg MS, Bader S, Harrison DJ. 2009. Attaching and effacing *Escherichia coli* downregulate DNA mismatch repair protein in vitro and are associated with colorectal adenocarcinomas in humans. *PLOS ONE* 4:e5517
141. Yao Q, Zhang L, Wan X, Chen J, Hu L, et al. 2014. Structure and specificity of the bacterial cysteine methyltransferase effector NleE suggests a novel substrate in human DNA repair pathway. *PLOS Pathog.* 10:e1004522
142. Boleij A, Hechenbleikner EM, Goodwin AC, Badani R, Stein EM, et al. 2015. The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin. Infect. Dis.* 60:208–15
143. Wick EC, Rabizadeh S, Albesiano E, Wu X, Wu S, et al. 2014. Stat3 activation in murine colitis induced by enterotoxigenic *Bacteroides fragilis*. *Inflamm. Bowel Dis.* 20:821–34
144. Geis AL, Fan H, Wu X, Wu S, Huso DL, et al. 2015. Regulatory T-cell response to enterotoxigenic *Bacteroides fragilis* colonization triggers IL17-dependent colon carcinogenesis. *Cancer Discov.* 5:1098–109
145. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, et al. 2012. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 491:254–58
146. Ernst M, Putoczki TL. 2013. Targeting IL-11 signaling in colon cancer. *Oncotarget* 4:1860–61
147. Salcedo R, Worschech A, Cardone M, Jones Y, Gyulai Z, et al. 2010. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. *J. Exp. Med.* 207:1625–36
148. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, et al. 2012. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 482:179–85
149. Macia L, Tan J, Vieira AT, Leach K, Stanley D, et al. 2015. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat. Commun.* 6:6734
150. Chudnovskiy A, Mortha A, Kana V, Kennard A, Ramirez JD, et al. 2016. Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. *Cell* 167:444–56
151. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, et al. 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341:569–73
152. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, et al. 2014. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40:128–39

153. Saleh M, Trinchieri G. 2011. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. *Nat. Rev. Immunol.* 11:9–20
154. Huber S, Gagliani N, Zenewicz LA, Huber FJ, Bosurgi L, et al. 2012. IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. *Nature* 491:259–63
155. Kirchberger S, Royston DJ, Boulard O, Thornton E, Franchini F, et al. 2013. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J. Exp. Med.* 210:917–31
156. Munoz M, Eidenschenk C, Ota N, Wong K, Lohmann U, et al. 2015. Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. *Immunity* 42:321–31
157. Nowarski R, Jackson R, Gagliani N, de Zoete MR, Palm NW, et al. 2015. Epithelial IL-18 equilibrium controls barrier function in colitis. *Cell* 163:1444–56
158. Elangovan S, Pathania R, Ramachandran S, Ananth S, Padia RN, et al. 2014. The niacin/butyrate receptor GPR109A suppresses mammary tumorigenesis by inhibiting cell survival. *Cancer Res.* 74:1166–78
159. Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, et al. 2013. Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J.* 7:949–61
160. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. 2014. The role of short-chain fatty acids in health and disease. *Adv. Immunol.* 121:91–119
161. Belcheva A, Irrazabal T, Robertson SJ, Streutker C, Maughan H, et al. 2014. Gut microbial metabolism drives transformation of Msh2-deficient colon epithelial cells. *Cell* 158:288–99
162. Uittamo J, Siikala E, Kaihovaara P, Salaspuro M, Rautemaa R. 2009. Chronic candidosis and oral cancer in APECED-patients: production of carcinogenic acetaldehyde from glucose and ethanol by *Candida albicans*. *Int. J. Cancer* 124:754–56
163. Howitt MR, Lavoie S, Michaud M, Blum AM, Tran SV, et al. 2016. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* 351:1329–33
164. Nowak A, Czyzowska A, Huben K, Sojka M, Kuberski S, et al. 2016. Prebiotics and age, but not probiotics affect the transformation of 2-amino-3-methyl-3H-imidazo[4,5-*f*]quinoline (IQ) by fecal microbiota—an in vitro study. *Anaerobe* 39:124–35
165. Megaraj V, Ding X, Fang C, Kovalchuk N, Zhu Y, Zhang QY. 2014. Role of hepatic and intestinal p450 enzymes in the metabolic activation of the colon carcinogen azoxymethane in mice. *Chem. Res. Toxicol.* 27:656–62
166. Morita N, Walaszek Z, Kinjo T, Nishimaki T, Hanausek M, et al. 2008. Effects of synthetic and natural in vivo inhibitors of β-glucuronidase on azoxymethane-induced colon carcinogenesis in rats. *Mol. Med. Rep.* 1:741–46
167. Vergara-Castaneda HA, Guevara-Gonzalez RG, Ramos-Gomez M, Reynoso-Camacho R, Guzman-Maldonado H, et al. 2010. Non-digestible fraction of cooked bean (*Phaseolus vulgaris* L.) cultivar Bayo Madero suppresses colonic aberrant crypt foci in azoxymethane-induced rats. *Food Funct.* 1:294–300
168. Kim YG, Udayanga KG, Totsuka N, Weinberg JB, Nunez G, Shibuya A. 2014. Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE2. *Cell Host Microbe*. 15:95–102
169. Nagamine CM, Sohn JJ, Rickman BH, Rogers AB, Fox JG, Schauer DB. 2008. *Helicobacter hepaticus* infection promotes colon tumorigenesis in the BALB/c-Rag2$^{-/-}$ Apc$^{Min/+}$ mouse. *Infect. Immun.* 76:2758–66
170. Poutahidis T, Cappelle K, Levkovich T, Lee CW, Doulberis M, et al. 2013. Pathogenic intestinal bacteria enhance prostate cancer development via systemic activation of immune cells in mice. *PLOS ONE* 8:e73933
171. Fox JG, Feng Y, Theve EJ, Raczynski AR, Fiala JL, et al. 2010. Gut microbes define liver cancer risk in mice exposed to chemical and viral transgenic hepatocarcinogens. *Gut* 59:88–97
172. Erdman SE, Poutahidis T, Tomczak M, Rogers AB, Cormier K, et al. 2003. CD4$^+$ CD25$^+$ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. *Am. J. Pathol.* 162:691–702
173. Lakritz JR, Poutahidis T, Mirabal S, Varian BJ, Levkovich T, et al. 2015. Gut bacteria require neutrophils to promote mammary tumorigenesis. *Oncotarget* 6:9387–96
174. Yamamoto ML, Maier I, Dang AT, Berry D, Liu J, et al. 2013. Intestinal bacteria modify lymphoma incidence and latency by affecting systemic inflammatory state, oxidative stress, and leukocyte genotoxicity. *Cancer Res.* 73:4222–32

175. Westbrook AM, Wei B, Hacke K, Xia M, Braun J, Schiestl RH. 2012. The role of tumour necrosis factor-α and tumour necrosis factor receptor signalling in inflammation-associated systemic genotoxicity. *Mutagenesis* 27:77–86
176. Rutkowski MR, Stephen TL, Svoronos N, Allegrezza MJ, Tesone AJ, et al. 2015. Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. *Cancer Cell* 27:27–40
177. Coley WB. 1893. Treatment of malignant tumors by repeated inoculation of erysipelas: with a report of 10 cases. *Am. J. Med. Sci.* 105:487–564
178. Tang DH, Chang SS. 2015. Management of carcinoma in situ of the bladder: best practice and recent developments. *Adv. Urol.* 7:351–64
179. Forbes NS. 2010. Engineering the perfect (bacterial) cancer therapy. *Nat. Rev. Cancer* 10:785–94
180. Bettegowda C, Huang X, Lin J, Cheong I, Kohli M, et al. 2006. The genome and transcriptomes of the anti-tumor agent *Clostridium novyi-NT*. *Nat. Biotechnol.* 24:1573–80
181. Forbes NS. 2006. Profile of a bacterial tumor killer. *Nat. Biotechnol.* 24:1484–85
182. Mlynarczyk GS, Berg CA, Withrock IC, Fick ME, Anderson SJ, et al. 2014. *Salmonella* as a biological "Trojan horse" for neoplasia: future possibilities including brain cancer. *Med. Hypotheses* 83:343–45
183. Zhang M, Forbes NS. 2015. Trg-deficient *Salmonella* colonize quiescent tumor regions by exclusively penetrating or proliferating. *J. Control Release* 199:180–89
184. Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ. 2016. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. *Nat. Rev. Microbiol.* 14:273–87
185. Dzutsev A, Goldszmid RS, Viaud S, Zitvogel L, Trinchieri G. 2015. The role of the microbiota in inflammation, carcinogenesis, and cancer therapy. *Eur. J. Immunol.* 45:17–31
186. Li H, Jia W. 2013. Cometabolism of microbes and host: implications for drug metabolism and drug-induced toxicity. *Clin. Pharmacol. Ther.* 94:574–81
187. Maurice CF, Haiser HJ, Turnbaugh PJ. 2013. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* 152:39–50
188. Fujita K, Sparreboom A. 2010. Pharmacogenetics of irinotecan disposition and toxicity: a review. *Curr. Clin. Pharmacol.* 5:209–17
189. Stringer AM, Gibson RJ, Logan RM, Bowen JM, Yeoh AS, Keefe DM. 2008. Faecal microflora and β-glucuronidase expression are altered in an irinotecan-induced diarrhea model in rats. *Cancer Biol. Ther.* 7:1919–25
190. McIntosh FM, Maison N, Holtrop G, Young P, Stevens VJ, et al. 2012. Phylogenetic distribution of genes encoding β-glucuronidase activity in human colonic bacteria and the impact of diet on faecal glycosidase activities. *Environ. Microbiol.* 14:1876–87
191. Wallace BD, Wang H, Lane KT, Scott JE, Orans J, et al. 2010. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* 330:831–35
192. Galluzzi L, Vitale I, Michels J, Brenner C, Szabadkai G, et al. 2014. Systems biology of cisplatin resistance: past, present and future. *Cell Death Dis.* 5:e1257
193. Roy S, Ryals MM, Van den Bruele AB, Fitzgerald TS, Cunningham LL. 2013. Sound preconditioning therapy inhibits ototoxic hearing loss in mice. *J. Clin. Investig.* 123:4945–49
194. Zhu S, Pabla N, Tang C, He L, Dong Z. 2015. DNA damage response in cisplatin-induced nephrotoxicity. *Arch. Toxicol.* 89:2197–205
195. Park SB, Goldstein D, Krishnan AV, Lin CSY, Friedlander ML, et al. 2013. Chemotherapy-induced peripheral neurotoxicity: a critical analysis. *CA Cancer J. Clin.* 63:419–37
196. Laurent A, Nicco C, Chereau C, Goulvestre C, Alexandre J, et al. 2005. Controlling tumor growth by modulating endogenous production of reactive oxygen species. *Cancer Res.* 65:948–56
197. Gui QF, Lu HF, Zhang CX, Xu ZR, Yang YH. 2015. Well-balanced commensal microbiota contributes to anti-cancer response in a lung cancer mouse model. *Genet. Mol. Res.* 14:5642–51
198. Chitapanarux I, Chitapanarux T, Traisathit P, Kudumpee S, Tharavichitkul E, Lorvidhaya V. 2010. Randomized controlled trial of live *Lactobacillus acidophilus* plus *Bifidobacterium bifidum* in prophylaxis of diarrhea during radiotherapy in cervical cancer patients. *Radiat. Oncol.* 5:31

199. Wang Y, Luo X, Pan H, Huang W, Wang X, et al. 2015. Pharmacological inhibition of NADPH oxidase protects against cisplatin induced nephrotoxicity in mice by two step mechanism. *Food Chem. Toxicol.* 83:251–60
200. Dhiman RK. 2013. Gut microbiota and hepatic encephalopathy. *Metab. Brain Dis.* 28:321–26
201. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillere R, et al. 2013. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 342:971–6
202. Zwielehner J, Lassl C, Hippe B, Pointner A, Switzeny OJ, et al. 2011. Changes in human fecal microbiota due to chemotherapy analyzed by TaqMan-PCR, 454 sequencing and PCR-DGGE fingerprinting. *PLOS ONE* 6:e28654
203. Daillere R, Vetizou M, Waldschmitt N, Yamazaki T, Isnard C, et al. 2016. *Enterococcus hirae* and *Barnesiella intestinihominis* facilitate cyclophosphamide-induced therapeutic immunomodulatory effects. *Immunity* 45:931–43
204. Couzin-Frankel J. 2013. Breakthrough of the year 2013: cancer immunotherapy. *Science* 342:1432–33
205. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, et al. 2015. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 350:1079–84
206. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, et al. 2015. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* 350:1084–89
207. Paulos CM, Wrzesinski C, Kaiser A, Hinrichs CS, Chieppa M, et al. 2007. Microbial translocation augments the function of adoptively transferred self/tumor-specific $CD8^+$ T cells via TLR4 signaling. *J. Clin. Investig.* 117:2197–204
208. Guiducci C, Vicari AP, Sangaletti S, Trinchieri G, Colombo MP. 2005. Redirecting in vivo elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. *Cancer Res.* 65:3437–46
209. Vicari AP, Chiodoni C, Vaure C, Ait-Yahia S, Dercamp C, et al. 2002. Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody. *J. Exp. Med.* 196:541–49
210. Stewart CA, Metheny H, Iida N, Smith L, Hanson M, et al. 2013. Interferon-dependent IL-10 production by Tregs limits tumor Th17 inflammation. *J. Clin. Investig.* 123:4859–74
211. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, et al. 2016. Trained immunity: a program of innate immune memory in health and disease. *Science* 352:aaf1098
212. Schreiber RD, Old LJ, Smyth MJ. 2011. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 331:1565–70
213. Page DB, Postow MA, Callahan MK, Allison JP, Wolchok JD. 2014. Immune modulation in cancer with antibodies. *Annu. Rev. Med.* 65:185–202
214. Sharma P, Allison JP. 2015. The future of immune checkpoint therapy. *Science* 348:56–61
215. Teply BA, Lipson EJ. 2014. Identification and management of toxicities from immune checkpoint-blocking drugs. *Oncology* 28(Suppl. 3):30–38
216. Dubin K, Callahan MK, Ren B, Khanin R, Viale A, et al. 2016. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. *Nat. Commun.* 7:10391
217. Santiago-Rodriguez TM, Ly M, Daigneault MC, Brown IH, McDonald JA, et al. 2015. Chemostat culture systems support diverse bacteriophage communities from human feces. *Microbiome* 3:58
218. Kim HJ, Li H, Collins JJ, Ingber DE. 2016. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *PNAS* 113:E7–15
219. Goldszmid RS, Dzutsev A, Viaud S, Zitvogel L, Restifo NP, Trinchieri G. 2015. Microbiota modulation of myeloid cells in cancer therapy. *Cancer Immunol. Res.* 3:103–9
220. Kelly CR, Ihunnah C, Fischer M, Khoruts A, Surawicz C, et al. 2014. Fecal microbiota transplant for treatment of *Clostridium difficile* infection in immunocompromised patients. *Am. J. Gastroenterol.* 109:1065–71
221. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, et al. 2015. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature* 517:205–8