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REVIEW ARTICLE

The mutual interplay of gut microbiota, diet and human disease

Placido Illiano[1], Roberta Brambilla[1,2,3] and Cinzia Parolini[4]

1 The Miami Project to Cure Paralysis, Department of Neurological Surgery, University of Miami Miller School of Medicine, FL, USA
2 Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark
3 Department of Clinical Research, BRIDGE-Brain Research-Inter-Disciplinary Guided Excellence, University of Southern Denmark, Odense C, Denmark
4 Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Italy

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Correspondence
C. Parolini, Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, via Balzaretti 9, 20133 Milano, Italy
Tel: +390250318328
E-mail: cinzia.parolini@unimi.it

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The intestinal milieu harbours the gut microbiota, consisting of a complex community of bacteria, archaea, fungi, viruses and protozoans that bring to the host organism an endowment of cells and genes more numerous than its own. In the last 10 years, mounting evidence has highlighted the prominent influence of the gut mutualistic bacterial communities on human health. Microbial colonization occurs alongside with immune system development and plays a role in intestinal physiology. The community of the gut microbiota does not undergo significant fluctuations throughout adult life. However, bacterial infections, antibiotic treatment, lifestyle, surgery and diet might profoundly affect it. Gut microbiota dysbiosis, defined as marked alterations in the amount and function of the intestinal microorganisms, is correlated with the aetiology of chronic noncommunicable diseases, ranging from cardiovascular, neurologic, respiratory and metabolic illnesses to cancer. In this review, we focus on the interplay among gut microbiota, diet and host to provide a perspective on the role of microbiota and their unique metabolites in the pathogenesis and/or progression of various human disorders. We discuss interventions based on microbiome studies, that is faecal microbiota transplantation, probiotics and prebiotics, to introduce the concept that correcting gut dysbiosis can ameliorate disease symptoms, thus offering a new approach towards disease treatment.

Introduction

Daily, our body is exposed to a wide variety of foods. Similarly to drugs, these materials are recognized as 'not-self' and may represent a potential source of toxicity [1].

To assess adherence to dietary guidelines meant to promote health and prevent chronic disease, the Healthy Eating Index (HEI) was developed in the United States. The most updated version of the HEI

Abbreviations
AD, Alzheimer's disease; AhR, aryl hydrocarbon receptor; Angptl4, angiopoietin-like protein 4; ASD, autism spectrum disorder; BP, blood pressure; CNS, central nervous system; CRC, colorectal carcinoma; CVD, cardiovascular disease; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; FMO3, flavin monooxygenase 3; FMT, faecal microbial transplantation; FXR, farnesoid-X-receptor; GI, gastrointestinal; GOS, galacto-oligosaccharides; GUS, microbial beta-glucuronidase; HEI, Healthy Eating Index; IEC, intestinal epithelial cells; IP, intestinal permeability; LPS, lipopolysaccharide; MS, multiple sclerosis; NEC, neonatal-necrotizing enterocolitis; PD, Parkinson's disease; SCFAs, short-chain fatty acids; TC, total cholesterol; TGR5, membrane-bound G protein-coupled bile acid receptor; Th, T helper; TLRs, Toll-like receptors; TMA, trimethylamine; TMAO, trimethylamine N-oxide.

focused on food quality, including healthy choices such as whole grains, seafood and plant proteins [2]. What emerged from recent prospective studies is that the majority of the population in the United States achieved only an intermediate or poor HEI, as reported in the National Health and Nutrition Examination Survey [3].

Bacteria, the main types of microorganisms, together with archaea, fungi, viruses (especially bacteriophages) and protozoans are found in the mammalian intestine [4]. These latter groups of microorganisms likely modulate the activities of the host and may be as important as bacteria [5,6]. By definition, the collective microbial community present inside and on the surface of the human body constitutes our microbiota, whereas the term microbiome refers to the genes expressed by the microbiota [7]. The stomach and small intestine have a bacterial density of about 10^3–10^5 organisms/g in mice. The ileum, which corresponds to the distal portion of the small intestine, has a higher bacterial density (10^8/g) and species diversity. The highest density of colonization is found in the colon (10^{10}–10^{12}/g), with a spectrum of at least 400 bacterial species. In the lower intestine, anaerobes are predominant, particularly *Bacteroides*, *Bifidobacteria*, *Fusobacteria* and *Peptostreptococci*. On the other hand, aerobes and facultative aerobes, including *Enterobacteria* and *Lactobacilli*, are present at only moderate density [8]. The microbiome composition is unique to each individual, rapidly developing throughout early childhood to become established in adulthood. Modifications in microbial composition depend on both genetic and environmental factors including diet, geographical location, toxin/carcinogen exposure and hormones [9].

This review focuses on the connections among gut microbiota (mainly the bacterial fraction), diet and human illnesses. Specifically, we discuss the role of gut bacteria and diet in the pathophysiology of cardiovascular disease (CVD), central nervous system (CNS) syndromes and cancer.

The gut microbiota system

The intestinal microbial community that inhabits the human gut counts more than 100 trillion microbial cells ($\sim 4 \times 10^{13}$), lives in a mutualistic relationship with its host and is a key contributor to host metabolism, for instance by producing vitamins and others metabolites necessary to the host's physiology. For this reason, dramatic changes in the composition and function of intestinal microorganisms, defined as gut microbiota dysbiosis, are associated with gastroenteric disorders, as well as neurologic, respiratory, metabolic, hepatic and CVD [10]. Bacteria also inhabit extraintestinal organs such as skin, oral and nasal cavities, and vagina, but their number in these compartments does not exceed 10^{12}/g [11].

Shotgun metagenomics sequencing through random sequencing of all genes established that the bacterial microbiome of the human gut is dominated by *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria* and *Verrucomicrobia* phyla. *Firmicutes* and *Bacteroidetes* represent 90% of the gut microbiota [7]. Arumugam *et al*. proposed that the gut microbiota is grouped into three enterotypes or clusters, namely *Bacteroides*, *Prevotella* and *Ruminocccus* [12]. However, further studies showed that discrete clustering methodologies could be sensitive to sampling and selection bias, providing evidence that enterotypes are fluid entities rather than discrete community types [13]. Recently, Costea *et al*.[14] concluded that, even though an appropriate statistical description of the microbiome remains elusive, the enterotype composition is still relevant in various clinical settings, ranging from direct disease associations to personalized dietary interventions.

The intestine is colonized by microorganisms prenatally, reaching a steady state between 2 and 5 years of age. Several studies have demonstrated that the foetus lives in a nonsterile environment and the microbes that colonize the foetus could influence both the pregnancy outcome and later on the health status of the infant [15,16]. Following birth, various microbes colonize the human intestine, and factors like gestational age, mode of delivery, diet (breastmilk vs. formula), sanitation and antibiotic treatment are known to affect this process [17]. In the preterm neonate, the microbiota have reduced diversity with lower numbers of *Bifidobacterium* and *Bacteroides* and higher levels of *Enterococcus* and *Proteobacteria* compared to full-term children. Of note, high concentrations of *Proteobacteria* have been recognized as a risk factor for the development of neonatal-necrotizing enterocolitis (NEC) [18]. Maternal vaginal and faecal bacteria, including *Lactobacillus* and *Bifidobacterium*, colonize vaginally delivered infants [19]. *Enterobacteriaceae* represent the main group of microorganisms transferred from mother to child through faeces [20]. Neonates born via C-section, thus not directly exposed to maternal microorganisms, are colonized by microbes coming from the skin and the hospital environment. The main consequence is that C-section delays *Bacteroidetes* colonization and reduces microbiota diversity. However, as more recent studies report conflicting results, it remains unclear whether disruption of mother-to-infant transmission of microbiota through C-section occurs and whether it affects human physiology early on [21,22]. Besides,

long-term follow-up trials based on larger cohorts, high-resolution multi-omics analyses and detailed immunological screening are necessary to unravel the relationships between mode of delivery and health status [23].

Infant feeding methods, namely breastmilk and formula, dramatically shape the gut microbiota in early life. Human milk contains proteins, fats, carbohydrates (mainly oligosaccharides), immunoglobulins and endocannabinoids. The milk oligosaccharides reach the colon where are fermented, mainly by *Bifidobacterium*, to produce short-chain fatty acids (SCFAs), mainly acetic, propionic and butyric acids [17,24,25]. A direct correlation between oligosaccharides present in the human milk and number of *Bifidobacterium* has been found, showing that these milk components act as probiotics by selectively promoting the formation of *Bifidobacterium*-rich microbiota [26]. Based on these data, milk formula has been optimized by adding certain types of oligosaccharides, making it possible for infants to establish *Bifidobacterium*-rich microbiota [27]. Breastmilk contains also immunoglobulins (IgA and IgG), lysozyme, lactoferrin, immune-regulatory cytokines (e.g. TGF-β and IL-10) and lymphocytes expressing gut homing markers [28,29]. For this reason, intestinal bacterial colonization dramatically influences the maturation and physiology of the immune system in early life and affects adult's health and disease, as pointed out by the loss of immune function in germ-free mice [30]. Various studies have also demonstrated that intestinal bacteria and their metabolites, including SCFAs, play a key role in the proliferation and differentiation of T and B cells [29,31,32].

A marked transformation in the gut microbial community occurs after weaning from the mother and the introduction of solid foods, with an increase in the number of butyrate producers, that is *Bacteroides* and *Clostridium* species [33]. Antibiotics use significantly impacts the evolution of the infant gut microbiota by increasing *Proteobacteria* and lowering *Actinobacteria* populations [34], decreasing the overall diversity and selecting for drug-resistant bacteria. Moreover, some epidemiological studies correlated the antibiotic consumption in early life with the increased risk of allergic disease (i.e. asthma, atopic dermatitis, eczema) and type-1 diabetes later on in life [35].

Compared to infants, the gut microbiome of children is characterized by a higher degree of stability and a reduced interindividual variance. In childhood, gut microbiota are influenced by geography and food culture and differ between developed and developing countries, as well as between industrialized and rural areas [15]. For example, it has been reported that the gut microbiota of children from Burkina Faso were enriched of *Bacteroides*, while *Enterobacteriaceae*, pathogenic intestinal microbes causing diarrhoea, were significantly lower compared to Italian children [36]. A meta-analysis of metagenomic datasets obtained from faecal samples of healthy adults living in 13 different industrialized regions, as well as two preagricultural communities, indicated that the urbanization process has significantly shaped the gut microbiota, thereby potentially impacting the overall functionality of the gut microbiome [37]. Besides, epidemiologic studies have suggested that the incidence of autoimmune disorders is increasing in industrialized countries potentially reflecting environmental/dietary/microbial population changes [38]. In adulthood, the gut microbiota community is to some extent stable. However, a higher level of interindividual variability has been observed in the gut microbiota composition of aged people compared to younger adults [39]. Centenarians had reduced concentrations of *Bacteroides*, *Bifidobacterium* and *Enterobacteriaceae*, while *Clostridium* species concentrations were increased compared with younger adults. A correlation between gut microbiota composition, diet and institutional or community living was also reported. It will be necessary to devise new dietary intervention studies to fully understand and elucidate the clinical relevance of these modifications. Specifically, it should be important to establish if a controlled variation on the dietary habits of aged individuals could positively impact their gut microbiota composition in a context of global ageing population [40,41].

Diet

Changes in socio-economic status, cultural traditions, population growth and employment impact dietary habits worldwide. Finding ways to increase the production and availability of healthy foods is a major need, giving the exponential growth of the global population, whose size is predicted to expand to 9 billion by 2050 [42,43]. Various studies have demonstrated that the nutritional value of food is partially affected by the composition of the individual's gut microbiota, and that food, in turn, moulds both the microbiota and the microbiome [44,45]. In line with these data, we have to keep in mind that also plant hormones (such as abscisic acid, auxins and salicylates) and polyphenols (such as curcumin, lignans and cinnamic acid) can influence microbial richness, diversity and composition [46,47]. Besides, abscisic acid, salicylates and curcumin exert positive effects against chronic and degenerative disease, including diabetes and cancer [48–50]. Finally,

answering the question of how food processing by the gut microbiota influences our immune system will give us the whole picture of this complex network.

It is still unclear what defines a 'healthy' microbiota. We know that 30–40% of the gut microbiota in the adult can be modified during the lifetime, and diet is one of the most powerful instruments to do so. For example, the *Bacteroides* enterotype is found in individuals consuming fat and protein-rich diet, whereas the *Prevotella* enterotype is often present in the gut microbiota of persons eating a fibre's rich diet. It has been estimated that about 90–95% of dietary fats are absorbed in the small intestine, which may explain why fats dramatically impact the gut microbiota composition [51,52]. High-fat diets are indeed associated with low SCFAs and low *Bifidobacterium* concentrations [53]. Mice fed a palm oil-based diet show an increase in the ratio of *Firmicutes* to *Bacteroidetes*, an elevation in *Clostridium* species, and a reduction of overall microbiota diversity [51]. Proteins are an integral part of a healthy diet, but it has been shown that the relationship between protein intake and health follows a U-shaped curve [54,55]. Specifically, intakes below the minimum are associated with undernutrition states, and intakes above the tolerable limit are associated with overnutrition illnesses [56]. Clinical studies indicate a linear correlation between the levels of protein intake and the long-term risk of kidney disease in people with below normal kidney function, and a nonlinear relationship with long-term mortality risk regardless of kidney dysfunction [56]. It has been demonstrated that dietary proteins reaching the colon (about 10%) act both as a substrate for proteolytic bacteria and as a source of nitrogen for saccharolytic species [57]. The products of protein and amino acid breakdown are SCFAs, branched-chain fatty acids (BCFAs; isobutyrate, isovalerate and 2-methylbutyrate), phenol compounds (phenylpropionate, phenylacetate, p-cresol, indole propionate and indole acetate), amines, sulphides and ammonia (Fig. 1). Microorganisms involved in deamination are *Clostridium*, *Bacteroides* and *Enterobacterium* species [58]. For example, the acetate-producing commensal *Bacteroides thetaiotaomicron* and the *Faecalibacterium prausnitzii* induce goblet cell proliferation and mucus production, both effects able to maintaining gut homeostasis and epithelial integrity [59]. On the other hand, depletion of acetate-producing *Bifidobacteria* in mice increased susceptibility to infections and promoted intestinal inflammation [54]. Moreover, proteins are the source of L-carnitine and choline [60,61], which can be fermented by bacteria to trimethylamine (TMA) and subsequently oxidized to trimethylamine N-oxide (TMAO) via flavin monooxygenase 3 (FMO3) (Fig. 1) [62]. Metabolomic studies suggested that high TMAO concentrations are positively correlated with cardiovascular events such as death, myocardial infarction (MI) and stroke [63]. In line with these data, in *Apoe*-deficient mice fed a TMAO-rich diet increased macrophage foam cell formation and aortic atherosclerotic plaque development was observed [28,64]. Besides, dietary L-carnitine and choline were similarly associated with atherosclerosis worsening and reduction of reverse cholesterol transport in mouse models with an intact gut microbiota [65–67]. Furthermore, high L-carnitine and TMAO levels were correlated with increased risk for MI or death in a clinical study with a 3-year follow-up [68].

Dietary fibres are polysaccharides divided into resistant starches and nonstarch polysaccharides, which are not digested by human enzymes and are able to enlarge the faecal mass and increase the motility of the intestine [69,70]. The nonstarch polysaccharides include cellulose (a substrate for SCFAs synthesis) and

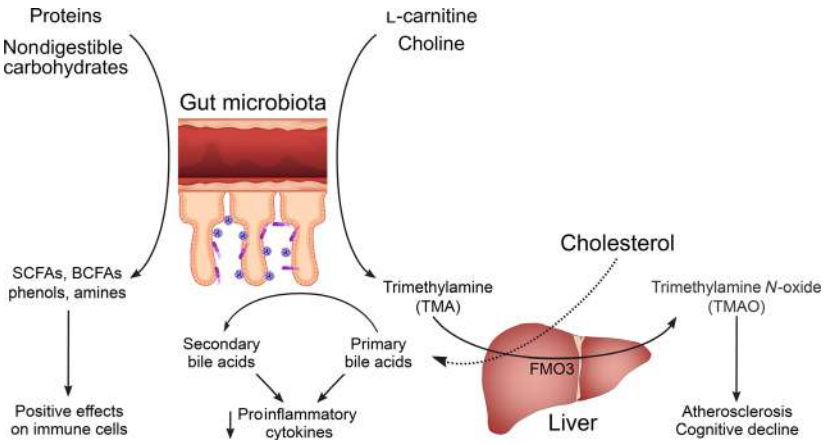

Fig. 1. Microbial metabolite-driven pathways. Intestinal bacteria convert dietary nutrients to metabolites able to modulate the immune system and alter risk of developing atherosclerotic cardiovascular disease and cognitive decline.

noncellulose fibres, that is pectins, gums, glycosaminoglycans, alginates, carrageenans, chitosans and fucoidans. A significant reduction of the *Roseburia/ eubacterium* rectale group and of the faecal levels of SCFAs has been correlated with the consumption of a high-fibre's diet (Fig. 1). Several tryptophan metabolites (e.g. indole-3-carbinol and indole [3,2b] carbazole) and xenobiotics (e.g. 2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) are ligands of the aryl hydrocarbon receptor (AhR), which has been shown to significantly influence host metabolism and immunity [71]. *Ahr*-deficient mice display various immunological deficits, including a higher sensitivity to *Listeria monocytogenes* or *Citrobacter rodentium*-induced infections [72,73]. Experimental studies have demonstrated that IL-22 plays a key role in the protection against this kind of infections by stimulating (a) the secretion of antimicrobial peptides from epithelial cells, (b) the production of mucins and (c) the proliferation of intestinal goblet cells. Besides, microbiota-derived tryptophan metabolites have the ability to modulate the AhR-IL-22 axis, thus impacting mucosal immune homeostasis in the gut [74]. Importantly, high expression of IL-22 has been detected in tumour tissues and it has been correlated with cancer progression and poorer patient survival in pancreatic ductal adenocarcinoma [75]. Therefore, dietary-induced modifications of host metabolomics and the presence of environmental contaminants may alter physiological homeostasis, impacting host fitness and leading to inflammatory responses.

Finally, gut microorganisms also metabolize molecules that are secreted by the host into the gastrointestinal (GI) lumen. Primary bile acids, such as glycocholic and taurochenodeoxycholic acid, are released into the duodenum from the gall bladder to facilitate the absorption of dietary lipids and lipophilic vitamins. In the small intestine and, predominantly, in the colon they are converted to secondary bile acids (such as deoxycholic and lithocholic acids) by the local microbiota (Fig. 1). This involves multiple steps, with the initial deconjugation of glycine or taurine, followed by dehydroxylation [76]. The enzymes involved in these unique microbial modifications are encoded by the gut microbiome, thus the bile acids profile excreted in faeces, mainly composed by secondary bile acids, largely depends on the gut microbiota composition [77]. On the other hand, without the microbial contribution, the host bile acid signature is perturbed resulting in several GI, metabolic and inflammatory disorders [78,79]. Primary and secondary bile acids function as signalling molecules for various cells of the immune system through interaction with host bile receptors, such as ligand-activated nuclear receptors such as the farnesoid-X-receptor (FXR) and the vitamin D receptor, as well as the membrane-bound G protein-coupled bile acid receptor (TGR5). Importantly, these receptors are ubiquitously distributed in several tissues and have different affinity for individual bile acids. For instance, TGR5 recognizes both conjugated and free secondary bile acids [80], whereas the primary bile acid chenodeoxycholic acid is the most potent ligand for FXR [81]. The consequence is that different microbial communities can differentially affect bile signalling and determine the degree of activation of these receptors with a concomitant impact on host metabolism. Interacting with their receptors, they reduce the expression of proinflammatory cytokines from monocytes, macrophages, dendritic cells (DCs) and hepatic macrophages known as Kupffer cells [82]. Furthermore, it has been demonstrated that free taurine itself can enhance the activation of the NLRP6 inflammasome, thereby increasing the production of IL-18 by the intestinal epithelium, which supports epithelial barrier function and maintenance [83]. This novel gut microbiota-bile acid-host signalling triangle represents the starting point for microbiota-based therapeutic approaches to manage diseases linked to alteration of bile acids metabolism. Indeed, a cholesterol-lowering effect was observed after administration of a *Lactobacillus* strain to mice [84] as well as to hypercholesterolaemic subjects [85], whereas administration of epigallocatechin-3-gallate regulated bile signalling and reduced the development of obesity [86].

Functional foods

Probiotics

Several bacterial species, that is *Lactobacillus* and *Bifidobacterium* species, have been used to improve human health. The concept of probiotics evolved from a theory first proposed by Nobel Prize laureate Eli Metchnikoff, who suggested in 1908 that the long life of Bulgarian peasants depended on their consumption of fermented milk products [87]. Organizations and agencies such as Codex (which comes under the FAO/WHO umbrella), Health Canada, the World Gastroenterology Organisation, the European Food Safety Authority (EFSA) and the Institute of Food Technologists use the following definition when referring to probiotics: 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.' This definition describes the philosophy behind the term 'probiotics,' that is microbial, viable and

beneficial to health [88,89]. The beneficial effects of probiotics have been demonstrated in diarrhoea, allergies, inflammatory bowel disease, lactose malabsorption and NEC [90].

Prebiotics

The term 'prebiotic' was introduced in the 1980s and refers to 'non-digestible food ingredients that beneficially influence the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, thus improving host health' [91]. SCFAs, peptidoglycans, polysaccharide A (PSA) and various oligosaccharides belong to the prebiotics family. Their main effect is on microbial metabolism, and SCFAs are the most studied. Indeed, if dietary fibres are present in the colon, the anaerobic bacteria extract energy from the fermentation of the carbohydrate component, producing SCFAs, which are not toxic to the host. Besides being an energy source, SCFAs have various important physiological functions including maintaining the luminal pH, inhibiting pathogens' growth, influencing bowel motility and potentially reducing colon cancer by causing cancer cell apoptosis [89]. Moreover, inulin, galacto-oligosaccharides (GOS), fructo-oligosaccharides, soybean oligosaccharides and xylo-oligosaccharides are the oligosaccharides considered prebiotics [92]. GOS and short-chain trisaccharides (such as sialyllactose or fucosyllactose) present in human milk are usually the first prebiotics used by humans to promote the growth and the activity of *Bifidobacterium* and *Lactobaccillus* species in infants [93]. Prebiotics act as regulatory factors of the immune system due to their ability to directly stimulate toll-like receptors (TLRs) on intestinal epithelial cells (IEC) and host immune cells inducing the expression of anti-inflammatory cytokines (i.e. IL-10 and TGFβ). In addition, SCFAs and other bacterial metabolites might stimulate the expression of G protein-coupled receptors, such as Gpr41 and Gpr43, on the IEC to limit host inflammatory responses [90,94].

Ten years ago, Gibson introduced the concept of synbiotics, a combination of prebiotics and probiotics meant to strengthen the effects of probiotics administered alone [95]. Synbiotics are used to improve survival of live microbial dietary supplements in the GI tract and to selectively stimulate the growth and/or activate the metabolism of health-promoting bacteria [96].

In summary, while the effectiveness of these agents seems promising, additional studies are needed to establish recommendations for most clinical settings.

Faecal microbial transplantation

In addition, faecal microbial transplantation (FMT) from healthy donors into the bowel of patients suffering from recurrent diarrhoea due to the antibiotic-resistant *Clostridium difficile* has emerged as an alternative option to treat severe cases of *C. difficile* infections [97]. The efficacy rate for these patients is about 90%, dramatically higher than with antibiotic therapies [98]. Based on these data, FMT is now included in the standard practice for treating recurrent and refractory *C. difficile* infections, the only indication approved by the Food and Drug Administration (FDA) since 2013 (FDA-2013-D-0811-0022) [99]. The mechanisms underlying probiotics function have been the object of intensive investigation and include the following: (a) increased integrity and enhancement of the epithelial barrier; (b) increased adhesion to the intestinal mucosa and concomitant inhibition of pathogen adhesion; (c) competitive elimination of pathogens; (d) production of antimicrobial substances; (e) modulation of DCs; (f) effect on T-cell polarity; and (g) modulation of the immune system and the inflammatory response [90]. Even though probiotics have an excellent overall safety record, for selected groups of patients, particularly those with severe immunodeficiency, malnutrition, cancer or preterm neonates, caution has to be taken. Indeed, bacteraemia, sepsis and cholangitis induced by *Bacillus subtilis* or fungal sepsis caused by *Saccharomyces boulardii* have been reported [100].

Gut microbiota and human disease

Cardiovascular disease

In 2011, the American Heart Association committed that by 2020 the cardiovascular status of Americans should improve by 20%, aiming at reducing by the same magnitude the deaths from CVD and stroke. These goals became the foundation of the 'Health Campaign for Life's Simple 7' where 'ideal cardiovascular health' is described by the lack of clinically manifest CVD combined with optimal indexes for seven major metrics, including (a) not smoking, (b) healthy diet, (c) daily physical activity, (d) body weight, (e) total cholesterol (TC), (f) blood pressure (BP) and (g) fasting blood glucose, in the absence of any drug treatment [101–103].

A recent meta-analysis of 12 878 individuals provided data on the importance of cardiovascular health metrics on risk for clinical events. This meta-analysis indeed inversely correlated the cardiovascular health

metrics and the risk of stroke, CVD, cardiovascular mortality and all-cause mortality [103,104]. Up to now, about 92.1 million adults in the United States have at least one type of CVD, and by 2030, 43.9% of the US adult population is predicted to have some form of CVD [105]. Moreover, the direct costs (cost of physicians, hospital services, prescribed medications and home health care) and indirect costs (loss of productivity) of CVD and stroke in the United States were $316.1 billion in 2013, and by 2030, it is projected to increase to $918 billion [103,106]. It is important to note that 80% of CVD deaths take place in low- and middle-income countries and occurs almost equally in males and females. Indeed, a survey comparing the availability of aspirin, β-blockers, angiotensin-converting enzyme inhibitors and statins in 18 countries showed that these medications were potentially unaffordable for 60% and 30% of households in low- and in middle-income countries, respectively, compared with only 0.14% of households in high-income countries [107–109]. This dramatic scenario appears to be dependent on the fact that, with greater industrialization, Western-type diets instead of traditional plant-rich diets have become the most consumed. As a consequence, a significant increase in human pathologies such as obesity and type-2 diabetes has been recorded [110]. These metabolic diseases are now considered as a chronic low-grade inflammatory disease, but the underlying mechanisms have not been clearly elucidated [111,112].

Studies show that dietary habits influence various 'old' cardiovascular risk factors, including BP, TC, glucose levels and obesity/weight gain, as well as 'novel' risk factors, that is inflammation, cardiac arrhythmias, endothelial cell function, triglyceride levels, lipoprotein[a] levels and heart rate [113–115]. A clinical study demonstrated that greater adherence to a Mediterranean diet characterized by higher intakes of vegetables, legumes, fruits, whole grains, fish and lower intakes of red meat was associated with a 22% reduction in cardiovascular mortality (RR, 0.78; 95% CI, 0.69–0.87) [116–118]. These findings were confirmed by a randomized secondary prevention trial enrolling patients with recent MI and by the PREDIMED trial [103,119]. This latter clinical study demonstrated a 30% reduction in the risk of stroke, MI and death due to cardiovascular causes in those patients randomized to Mediterranean-style diets rich in extravirgin olive oil or mixed nuts [119].

Numerous studies have shown that atherosclerosis, the dominant cause of CVD, is influenced by the immune system with cytokines involved in all stages of the disease [120–122]. Specifically, chronic inflammation triggers the migration and proliferation of monocyte-derived macrophages and T lymphocytes within the plaques. It has been demonstrated that human atherosclerotic plaques are characterized by a predominance of T helper (Th) cells secreting Th1 cytokines, that is IFN-γ and TNF-α, and few clones producing Th2 cytokines. This Th1 milieu may contribute to the thrombogenicity of the lesions by increasing the tissue factor production, which associates with platelet activation and endothelial dysfunction [123]. The Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) trial demonstrated that anti-inflammatory therapy targeting the IL-1β pathway markedly reduced plasma levels of IL-6 and of C-reactive protein [124], lowering the rate of recurrent cardiovascular events compared with placebo, independently of lipid-level lowering [125].

Several evidences suggested that the gut microbiota regulates inflammation by affecting cytokine production and haematopoiesis, as well as by affecting the differentiation of inflammatory cell types [32,126,127]. An increase in lipopolysaccharide (LPS) plasma levels, likely derived from gut bacteria [6,128], has been associated with inflammatory cell infiltration in adipose tissues, liver and pancreatic islets. LPS is a major component of the outer membrane in Gram-negative bacteria and its plasma levels are correlated with changes in intestinal microbiota where the Gram-negative-to-Gram-positive ratio is increased by high-fat feeding [129]. Besides, atherosclerotic plaques contain bacterial DNA, and the bacterial taxa observed in atherosclerotic lesions are also present in the gut of the same individuals. This indicates that the gut microbiota could be a source of bacteria in the plaque, which may affect plaque stability and the incidence of CVD. In line with this hypothesis, a link between the gut microbiota and the severity of MI has been reported in an experimental model [130]. Also, the high blood concentration of the microbiota-dependent metabolite TMAO has been linked to an increased risk of atherosclerosis, indicating a pivotal role for the gut microbiota in atherogenesis (Fig. 1) [66]. In addition, germ-free *Apoe*-deficient mice showed lower circulating LPS levels, reduced systemic inflammation and decreased atherogenesis compared with conventionally raised *Apoe*-deficient animals [131,132]. Together, these findings suggest a link between gut microbiota, host immunity and atherogenesis (Fig. 2). This is supported by a recent study by Brandsma *et al.* showing that proinflammatory microbiota accelerated atherosclerosis development in *LDLr*-deficient mice fed a high-fat/high-cholesterol diet. Mice were also characterized by increased proinflammatory plasma cytokines, and

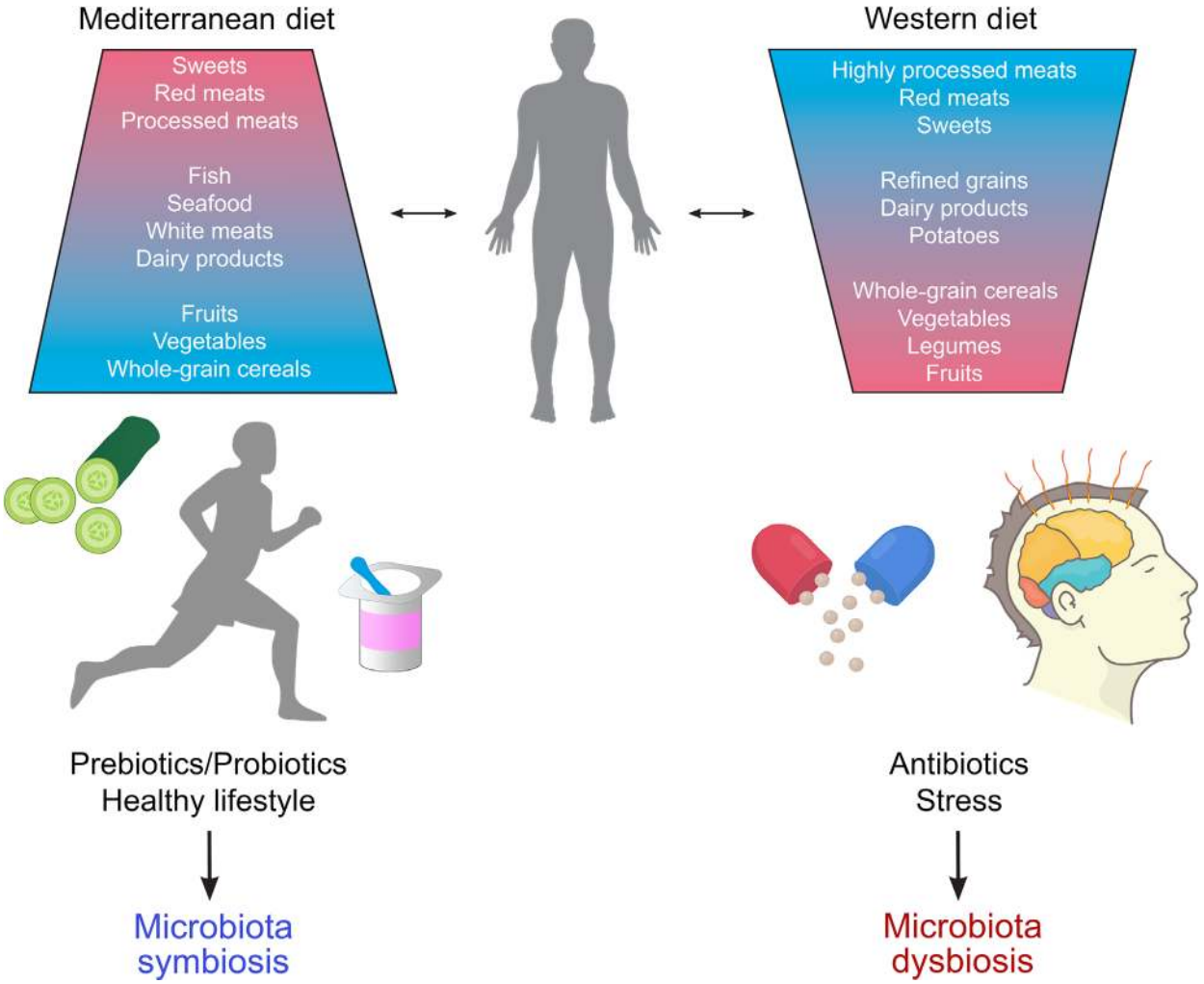

Fig. 2. Major mechanisms involved in the cross-talk between microbes and host. The balance between healthy and pathological conditions depends on different factors, including genes, food (Mediterranean vs. Western diet), prebiotic/probiotic intake, stress and antibiotic treatments.

circulating and in-plaque monocytes and neutrophils, as well as reduced levels of SCFAs in the caecum and of SCFA-producing taxa [133,134].

The bacterium *Akkermansia muciniphila*, the most abundant species in the human intestinal microbiota, has been associated with anti-inflammatory functions. Indeed, in genetically and diet-induced diabetic mice, the levels of *A. muciniphila* were inversely correlated with body mass, inflammation index, insulin resistance and glucose tolerance [135]. Prebiotic feeding markedly increased the abundance of *A. muciniphila* and reduced fat mass, insulin resistance and liver steatosis. In line with these results, the administration of *A. muciniphila* protects against the development of atherosclerosis in *Apoe*-deficient mice [72,136]. Also, metformin-treated diabetic patients reported beneficial effects on metabolic parameters linked to CVD risk [137]. It is possible that the beneficial action of *A. muciniphila* is due to the protein Amuc_1100, localized on the membrane of the bacteria, which has been shown to play immuno-modulatory roles both *in vitro* and *in vivo* [138].

A leaky gut and alterations in gut microbiota composition can lead to the transfer of endotoxins into the circulation promoting systemic inflammation and development of obesity and related metabolic disease [139]. Backhed *et al.* demonstrated that germ-free animals are protected from diet-induced obesity by two main mechanisms that result in increased fatty acid metabolism: (a) elevated levels of the fasting-induced adipose factor, also called angiopoietin-like protein 4 (Angptl4), in the intestinal epithelium and (b)

increased AMP-activated protein kinase activity [140]. These data were not confirmed by Fleissner *et al.* [141]. A recent study showed higher expression of the *Angptl4* gene in the small intestine of germ-free mice compared with conventionally raised animals. *Angptl4* expression was decreased upon gut recolonization [142]. These conflicting data warrant further investigations into the role of *Angptl4* in the intestinal epithelium. Various clinical studies indicate that gut microbiota play a key role in adiposity and glucose metabolism, demonstrating that subjects with obesity and type 2 diabetes have an altered microbiota composition [143]. Additionally, FMT from obese mice to recipient germ-free mice significantly increased body fat content and insulin resistance, compared to those receiving FMT from lean mice [144]. Based on these experimental results, several studies have evaluated the possibility of altering the gut microbiome, that is by FMT, as a potential therapy for obesity and the metabolic syndrome [99]. In a small clinical study, improved peripheral insulin sensitivity was associated with increased levels of butyrate-producing intestinal microbiota 6 weeks after FMT from lean donors into patients with metabolic syndrome [145].

Mouse and human gut bacterial species are composed for the most part (about 90%) of *Bacteroidetes* and *Firmicutes* phyla. Homozygous genetically obese (ob/ob) mice, carriers of a single autosomal recessive mutation on the leptin encoding gene (also known as the obese gene), are lacking functional leptin despite showing high levels of leptin mRNA in adipocytes [146]. Compared to their lean counterparts, ob/ob mice have early-onset obesity, decreased abundance of *Bacteroidetes* and increased presence of *Firmicutes* [147]. Enrichment of hydrogen-oxidizing methanogens has been observed in obese compared to normal weight individuals. These microorganisms are critical for fermentation since hydrogen accumulation in the colon inhibits fermentation. Many of the hydrogen-oxidizing methanogens detected in the human gut belong to the *Firmicutes* phylum, and this may partly explain why an increase in *Firmicutes* has been observed in obesity [148].

Central nervous system disease

The 'gut–brain axis,' consisting of a series of integrated physiological functions (neural, endocrine) based on the bidirectional interaction of intestine and CNS, is well established (Fig. 3) [149]. It has become increasingly evident that, through various mechanisms, gut microbiota are active elements of the gut–brain axis. The CNS can influence the gut microbiota through metabolic and endocrine pathways and via the release of signalling molecules such as cytokines and peptides [150]. On the other hand, the microbiota can influence CNS function in several ways, including the release of molecules that, once in circulation, can

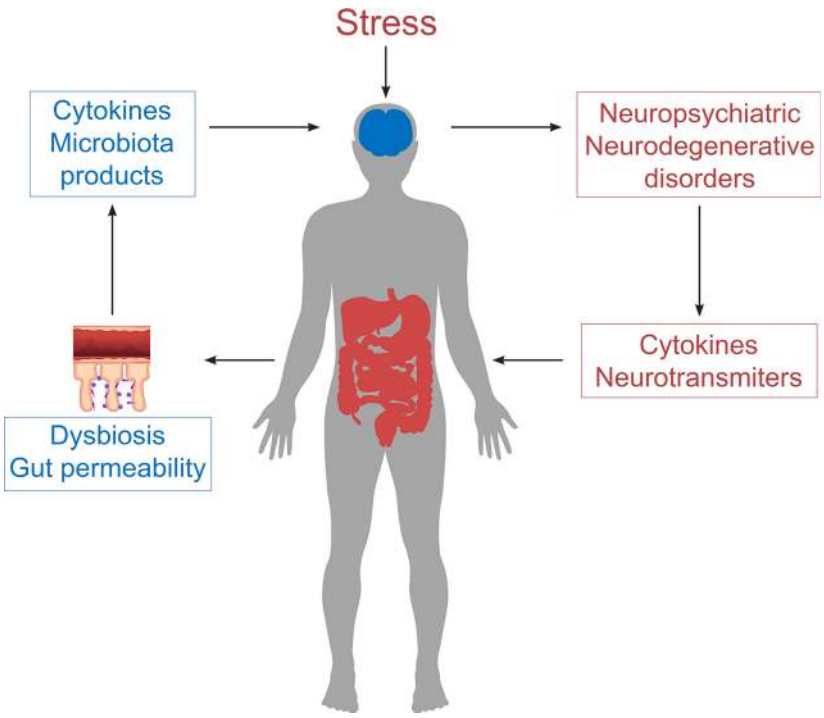

Fig. 3. The gut–brain axis. Brain and gut communicate bidirectionally. Stress conditions trigger CNS responses that may lead, chronically, to the development of neuropsychiatric and neurodegenerative disorders. This causes aberrant release of soluble mediators, including cytokines and neurotransmitters, which affect the functionality of the gut and the gut microbiota. On the other hand, microbiota products produced by a dysbiotic intestinal flora can cause gut permeability, leaking bioactive molecules into circulation. As they reach the brain, they can affect brain function exacerbating CNS disease.

reach the CNS and activate specific receptors on neural cells (Fig. 3). This bidirectional interaction between microbiota and CNS occurs from development through adult life. It is known, for example, that exposure to early life stress is able to trigger disturbances in the composition of the adult gut microbiota, and this can be counteracted by probiotic administration [151]. Stress-induced modifications in the gut microbiota composition have been shown in preclinical models as well, where animals developing stress-induced despair behaviour show alterations in microbiota composition and function, specifically in the *Lactobacillus* genus. Reactive oxygen species produced by *Lactobacillus* species negatively affect the metabolism of kynurenine, a serotonin precursor, thus possibly affecting brain function [152].

The stability of the gut microbiota has been associated with neuropsychiatric diseases. It is worth mentioning that also blood circulating microbiota have been suggested to play a role in neuropsychiatric disorders [153]. In a study on major depressive disorder, faecal samples from affected patients displayed increased *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*, whereas *Firmicutes* levels were significantly reduced compared to age-matched controls, and these alterations positively correlated with the severity of depressive symptoms [154]. Furthermore, FMT from depressed patients into germ-free mice induced depressive- and anxiety-like behaviours, possibly through glucocorticoid signalling, thus correlating the gut microorganisms with the development of depression [155]. Alterations in gut microbiota have also been identified in patients with autism spectrum disorder (ASD). It is well established that individuals with ASD suffer from GI tract complications. Though the cause is still unclear, this appears to be related to abnormal gut flora and/or excessive use of antibiotics that contribute to further alter the gut microbiota [156,157]. ASD severity has been strongly correlated with GI symptoms, as well as alterations in the gut bacterial species *Bifidobacter* and *Lactobacillus* [157]. Prebiotic intervention in ASD paediatric patients lowers the abundance of *Bifidobacterium* species and *Veillonellaceae* family bacteria and concomitantly increases *F. prausnitzii* and *Bacteroides* species levels. This regimen proves to be beneficial, triggering changes in faecal metabolites and improvements in GI symptoms as well as in antisocial behaviours, one of the hallmarks of ASD [158]. Recent studies highlighted that ASD patients may also display alterations in the GI bacterial phyla *Bacteroidetes* and *Firmicutes*, with higher abundance of *Sutterella*, *Odoribacter* and *Butyricimonas*, and lower abundance of *Veillonella* and *Streptococcus* genera [159].

Evidence of altered gut microbiota in individuals diagnosed with bipolar disorder has been found. Patients show increased levels of *Flavonifractor Bacterium*, *Actinobacteria* and *Coriobacteria* phyla, which may induce oxidative stress and inflammation in the host, although other factors, such as smoking, may also contribute to the dysbiosis [160].

Alterations in the gut microbiota have been reported in Parkinson's disease (PD), the most common movement disorder [161]. Although motor dysfunction is central in PD, nonmotor symptoms have received growing attention in the last decade [162]. Gut dysbiosis has been associated with the severity of PD symptoms. Indeed, the abundance of *Prevotellaceae* in faeces of PD patients was shown to be reduced by 77.6% compared with unaffected individuals, and the relative abundance of *Enterobacteriaceae* was positively associated with the severity of postural instability and gait difficulty [163]. Furthermore, GI disorders, possibly associated with dysbiosis, are known to be prominent and disabling in PD patients [164] and have been suggested to precede the onset of motor symptoms by years [165]. Lewy bodies, α-synuclein aggregates characteristic of PD neuropathology [166], are also found in the GI system of PD patients both undergoing treatment and newly diagnosed, thereby never exposed to PD drugs [167]. This has prompted intensive investigations into the profiling of the gut microbiome in newly diagnosed PD patients, as it may lead to the identification of reliable early biomarkers that can allow precocious therapeutic intervention to slow or stop the course of the disease [161].

Alzheimer's disease (AD) is the primary cause of dementia in the ageing population and is characterized by the progressive decline of cognitive functions [168]. Neuropathological hallmarks of the AD brain are neurofibrillary tangles of Tau protein aggregates, and plaques of amyloid beta peptide [169]. It has been shown that diet and specific nutrients can modify the composition of the gut microbiota influencing the production and/or aggregation of amyloid proteins through mechanisms of molecular mimicry [170]. Computational studies also associated cognitive decline in AD with metabolites such as succinic acid, mannitol, 4-hydroxybenoic acid (DOPAC) and TMAO, the latter being a gut microbial metabolite of dietary meat and fat (Fig. 1) [171]. Clinical studies performed on cerebrospinal fluid samples have demonstrated that TMAO may be relevant to the neurodegenerative changes in AD-related tau pathology, thus confirming the role of the gut–brain axis in the pathophysiology of AD [172].

Comprehensive 16s rRNA sequencing of stool samples of AD patients demonstrated their microbiome has decreased microbial diversity, with an increased relative abundance of *Lactobacillales*, and decreased abundance of *Bacteroidales* and *Selenomonadales* [173].

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the CNS. Though its aetiology is unknown, MS is initiated and sustained by the synergistic contribution of dysregulated immunity, genetic susceptibility and environmental factors. Hallmarks of MS are white and grey matter demyelinating lesions in the brain and spinal cord, where demyelination occurs through the concerted action of immune cells (T cells, B cells, macrophages) infiltrating into the CNS and CNS-resident cells, mostly microglia and astrocytes [174]. Studies on the gut microbiota in MS have provided evidence of alterations at the levels of phyla and genera in MS patients, such as an increase in *Methanobrevibacter* and *Akkermansia* and decrease in *Butyricimonas*. Compared with untreated patients, patients on treatment have increased abundance of *Prevotella* and *Sutterella* [175]. Microbial dysbiosis was also confirmed in another study on patients affected by relapsing–remitting MS displaying increases in *Psuedomonas*, *Mycoplana*, *Haemophilus*, *Blautia* and *Dorea* genera [176]. Modifications of the gut microbiota have been detected in early-onset paediatric MS, where patients showed enrichment in microorganisms involved in glutathione metabolism, suggestive of a proinflammatory environment [177]. Moderate dysbiosis was also reported in a study on a small cohort of Japanese relapsing–remitting MS patients. In this study, 20 patients were analysed (12 who were under therapeutic regimen and eight that never received any type of treatment) and compared to control individuals. Faecal samples were collected during the remission phase. The mixed MS group exhibited alterations of intestinal taxa comprised primarily of *Clostridium* species belonging to *Clostridium* clusters XIVa and IV and *Bacteroidetes* [178].

From a preclinical standpoint, various studies in the experimental autoimmune encephalomyelitis (EAE) model of MS have highlighted the role of the gut microbiota in the development of motor symptoms. Ochoa-Reparaz *et al.*[179] have shown that oral treatment with antibiotics can induce the accumulation of $FoxP3^{+}$ Treg cells in distal peripheral lymph nodes and reduce the severity of EAE. This indicates that modifications of gut commensal bacteria can modulate peripheral immune tolerance that ultimately confers protection from EAE in mice. Studies from the same group have underscored the protective role of PSA, a nontoxic component of the human commensal microbiota produced by *Bacteroides fragilis*. Oral administration of purified PSA produced by *B. fragilis* induced protection from EAE in mice, reducing its severity [180]. In a large-scale screen of MS patients, increases in *A. muciniphila* and *Acinetobacter calcoaceticus* taxa have been identified. These have been shown to trigger proinflammatory responses and maintain the proinflammatory environment. Indeed, FMT from MS patients into germ-free mice induced more severe EAE symptoms compared to mice colonized with microbiota from healthy age-matched controls. This indicated a causal role for the gut microbiota in MS development [181].

Cancer

Cancer is the second leading cause of death worldwide, resulting from the random accumulation of spontaneous mutations during DNA replication, together with environmental exposure and lifestyle habits, both able to dramatically affect cancer risk [182–184]. In recent years, accumulating evidence highlighted the role of commensal bacteria as key determinants of health or pathologic conditions, including cancer [185,186]. Various experimental studies performed on germ-free animals demonstrated that gut microorganisms promote carcinogenesis in various organs such as lungs, skin and breast [187], and there is an inverse relationship between cancer development and intestinal microbiota depletion by antibiotics [188]. In addition, accumulating evidence indicated that dysbiosis is associated not only with tumours arising in the affected organs but also in distant organs [189,190]. The high degree of variability on both the number of microorganisms and their diversity in different organs and from individual to individual could be responsible for the above-described phenomenon [191].

The tumour microenvironment, where the neoplastic and immune cells interact with microorganisms and vice versa, is dramatically affected by genetic and epidemiological factors [192,193]. The communication between the gut microbiota system and the body organs is controlled by the intestinal permeability (IP). Importantly, mechanisms regulating IP control the exchange between the intestinal content and the portal blood. Food, intact bacteria and bacterial components, that is bacterial DNA, peptidoglycans (molecules belonging to the class of pathogen-associated molecular patterns) can reach the liver in large amount depending upon the degree of IP [194]. Indeed, IP degree is highly variable and results from the combination of several factors: diet, gene expression, intestinal/liver pathology, surface mucus, tight junction integrity

and production of immunoglobulins [195]. The microbiota can contribute to carcinogenesis both by enhancing or diminishing the risk through three main mechanisms: (a) altering the balance of cell proliferation and death; (b) modulating immune system function; and (c) affecting the metabolism of ingested foods, host-produced factors and drugs. In addition, it is important to remember that the host immune system supports the gut microorganisms while protecting against pathogenic microbes. In essence, human gut microbiota carry out a pivotal role in cancer initiation, development and its response to cancer therapy (Fig. 4) [196].

Carcinogenic microorganisms include *Helicobacter pylori* and Epstein–Barr virus for gastric carcinoma [197]; hepatitis B and C viruses for hepatocellular carcinoma [198]; human herpesvirus-8 for Kaposi's sarcoma [199]; human papilloma virus for uterine cervical cancer [200]; and *Fusobacterium nucleatum* for colorectal carcinoma (CRC) [201]. By quantitative PCR analysis, high prevalence of *F. nucleatum* sequences in tumour vs. normal tissue was detected and correlated with lymph node metastasis [202]. It has been hypothesized that these *F. nucleatum* sequences promote carcinogenesis by stimulating the Wnt signalling cascade in CRC [203,204]. The correlation between abdominal infections and increased risk of CRC development reinforces the clinical correlation between dysbiosis and carcinogenesis. Alteration in the gut microbiota community directly modulates the probability of developing CRC in genetic and mutagen-induced animal models of carcinogenesis [205]. Experimental studies performed in germ-free or cohoused mice, as well as in gnotobiotic models and mice treated with antibiotics, strengthen the role played by the intestinal microorganisms in CRC and hepatocellular carcinoma [206,207]. Dysbiosis in patients with CRC has been detected in several studies [208,209]. Besides, *F. nucleatum* could be also regarded as a marker of CRC susceptibility [210], being abundantly found in patients suffering from chronic gut inflammation, that is inflammatory bowel disease [211,212]. It has been shown that the adhesion molecule FadA from *F. nucleatum* attaches to E cadherin leading to activation of β-catenin in CRC tumours, promoting inflammation and tumour development [213]. Coherent with these data, FadA is elevated in human CRC samples.

Besides their tumour-inducing actions, microbiota have also been reported to display anticancer activities [187]. For instance, TLRs and NOD-like receptors-based treatments promote the antitumor effects of microbiota, as the innate immune system could activate the anticancer responses [214,215]. Indeed, a reduction of cell growth and cancer risk was demonstrated in *Trl-4*-deficient mice and with FMT from wild-type into *Nod-2*-deficient mice, respectively [216,217]. Similarly, microbial-derived SCFAs are able to inhibit host's tumour cell histone deacetylase causing antitumour effects, as shown in both CRC and lymphoma [218,219]. These data indicate that several commensal bacteria could potentially be used for their probiotic activity, that is protecting against gut dysbiosis and/or enhancing host immune defence mechanisms, in combination therapies. One example is *Lactobacillus casei*, which can trigger tumour apoptosis via JNK pathway activation on host's immune cells, that is NK cells and DCs, leading to the suppression of cancerous or precancerous cells [220,221]. Various clinical investigations have been designed to address the potential of single strain probiotics at improving antitumour immune response [222,223]. Probiotics or synthetic stools have sought to recapitulate the benefits of FMT in delivering a diverse ecosystem in a product easy to manufacture and with

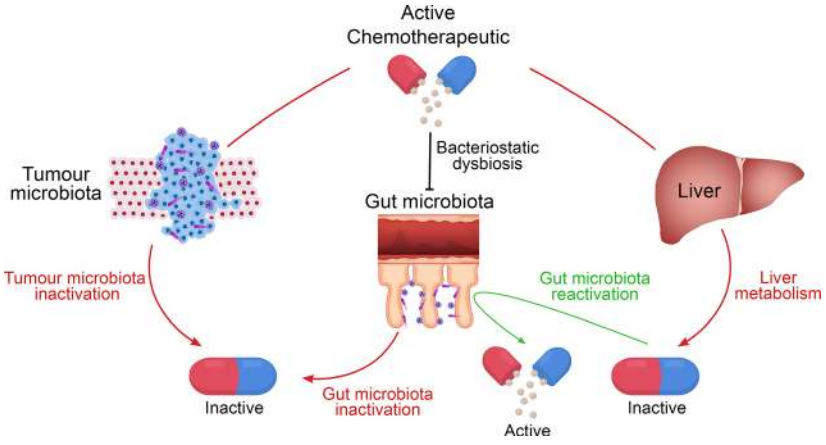

Fig. 4. Interactions between gut microbiota and cancer therapies. Chemotherapeutic agents can undergo metabolic inactivation by bacterial present in the GI tract (gut microbiota) or within the tumour (intratumour microbiota). As an alternative, gut microbiota can reactivate the drugs leading to treatment-related systemic toxicity. On the other hand, cancer therapy can exert a bacteriostatic effect on the gut microbiota causing dysbiosis.

minimal lot-to-lot variation (i.e. consistent composition). Preliminary results obtained with these products in recurrent *C. difficile* infections demonstrated stable engraftment of a diverse microbial community [224]. Many foods or food components have been associated with increase or decrease in the risk of cancer. Nowadays, the idea is that different dietary ingredients work together to generate a meta-inflammatory milieu responsible or not of tumour progression. Nevertheless, whether specific dietary components might favourably alter the microbiota is still an open question. Data from short-term dietary studies showed that a dramatic shift in diet could equally impact on the microbiota as well as on cancer biomarkers, that is cellular proliferation [225–227].

Cancer therapy

Data from preclinical and human studies have coherently indicated that both the tumour microenvironment and the gut microbiota may influence therapeutic efficacy and tolerability of anticancer therapy by affecting microbial diversity and the abundance of specific taxa (Fig. 4) [228–230].

Specifically, microbial beta-glucuronidase (GUS) enzymes have been linked to the GI side effects of drugs, tumour progression and increased incidence of Cohn's disease and colitis [231]. GUS enzymes play a key role in the metabolic fate of molecules important in host health and disease, including bilirubin, hormones, neurotransmitters, bile acids, fatty acids and many anticancer agents. Indeed, bacterial GUS enzymes regenerate the original drug inactivated by the host, increasing its half-life and sometimes its toxicity. For this reason, 89% of glucuronated-chemotherapic drugs exhibit GI toxicity that is mediated, at least in part, by bacterial GUS activity, resulting in a parasitic symbiosis in which bacteria receive sugar from drug glucuronides and the host retains toxic metabolites [232]. For example, SN-38, the active form of irinotecan, which is used to treat colorectal and pancreatic cancers, is inactivated in the liver by conversion into SN-38-glucuronide [232]. In the gut, microbial GUS enzymes recreate SN-38 and cause severe GI toxicity in the form of dose-dependent diarrhoea that is reduced by the selective inhibition of GUS enzyme activity [215].

Although insight has been gained on the role of the gut microbiota in response to cancer therapy, more studies are needed to fill significant gaps of knowledge in the components of the human gut microbiota and how they interact and influence one another and the whole immune response.

Conclusion

The importance of the human gut microbiota in health and disease has emerged over the past decade. Each person's microbiome is unique, rapidly developing throughout early childhood and relatively stable but susceptible to changes in adulthood. These variations in microbial composition can be influenced by both genetic and environmental factors, including diet, geographical location, toxin/carcinogen exposure and hormones. Dysbiosis, meaning imbalances in the composition and function of the intestinal microbes, is associated with various human diseases. Despite current unresolved questions that limit interpretative and translational capabilities, the possibility of correcting gut dysbiosis by microbiota-based interventions represents an intriguing approach in preventing and treating human pathologies.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

CP designed the manuscript. PI, RB and CP wrote and critically examined the manuscript. PI and CP designed the graphical artwork. PI, RB and CP read and approved the final version.

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