CARMELA LOGUERCIO

GUT MICROBIOTA AND GASTROINTESTINAL TRACT, LIVER AND PANCREAS

From physiology to pathology

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PREFACE

ABOUT GUT MICROBIOTA: WHY?

The human organism is colonized by an enormous number of microbes, both on its surface and its inside. The set of microorganisms living in our gut is defined gut microbiota, which is considered now a functional organ of our body. Gut microbiota plays a key role in regulating both health and disease; its quantitative and/or qualitative variations, by altering the homeostasis between microbes and host, may lead to the onset of various diseases. Disorders associated with the impairment of gut microbiota include functional gastrointes tinal diseases, intestinal infectious diseases, inflammatory bowel disease (IBD), liver and pancreatic diseases, gastrointestinal malignancies, but also metabolic syndrome and diabetes and obesity, allergic diseases, autism, and others. Intestinal microbiota development is a complex process that begins in utero and continues for the first 2-3 years of life. Several factors influence the microbiome over these critical early months and years of life and by 2 to 3 years of age, the child's microbiota has come to closely resemble that of an adult in terms of composition. Thereafter, the microbiota is thought to remain relatively stable until old age when changes are seen, possibly related to alterations in digestive physiology and diet. Antibiotic and other exposures during the early years of life, when the microbiome is in evolution, may be especially deleterious and could result in metabolic and inflammatory disorders later in life. The intestinal microbiota is considered a signaling hub that integrates environmental inputs, such as diet, with genetic and immune signals to affect the host's metabolism, immunity, including autoimmunity and autoinflammation and elaborate networks linked to several organs. For example, it is long time recognized the importance of gut micro-organisms as a whole essential metabolic organ in biotransformation of bile acids and breakdown of otherwise indigestible dietary components, such as plant polysaccharides and production of short-chain fatty acids, a major energy source for colonic epithelium, from fermentable carbohydrates. In this setting, microbiota promotes absorption of monosaccharides from the gut lumen, with consequent induction of hepatic lipogenesis; moreover, it suppresses fasting-induced adipocyte factor, a lipoprotein lipase inhibitor, allowing the deposition of triglycerides in the adipocytes. Gut microbiota may interfere in host metabolism by the modulation of inflammatory pathways. Enrichment in Gram-negative species results in an increase of lipopolysaccharide absorption, which leads to the so-called "metabolic endotoxemia." In addition, farnesoid X receptor regulates the synthesis, transport and enterohepatic circulation of bile acids (BA) by modulating the expression of related genes in the liver and small intestine. Other activities promoted by our intestinal microbes include synthesis of biotin, folate, and vitamin K. Intestinal microbiota has been linked to enteric bacterial enzymes such as azoreductase to convert prodrugs such as sulfasalazine to active drug metabolites (*e.g.*, aminosalicylate). Other examples of bacterial action on drug bioavailability include the metabolism of l-dopa to dopamine and degradation of digoxin. Although bacteria probably degrade some carcinogens, they also might promote

the production of carcinogens from dietary procarcinogens. Gut microbiota is strictly involved in the development of the mucosa- or, gut-associated immune system (mucosa-associated lymphoid tissue or gut-associated lymphoid tissue), immunologic tolerance, epithelial and barrier function, motility, and vascularity. Finally, a growing body of evidence points to the microbiota playing a significant role in modulating brain function and behaviour and that the "microbiota-gut-brain axis" poised as a bidirectional communication pathway enabling gut microbes to communicate with the brain, and the brain with the gut. The mechanisms of communication include immune, neural, endocrine and metabolic pathway.

Based on these recent knowledges, there is increasing interest on gut microbiota modulation as both a preventive strategy or as a therapeutic option in different gastrointestinal, hepatic and systemic diseases. Many studies have tried to find out the impact of diet, prebiotic, probiotic and antibiotics on gut microbiota composition and function, obtaining very heterogeneous and sometimes conflicting results, especially as regards the effect on clinical outcomes. In 2016 an european consensus conference on fecal transplantation in clinical practice was hold in Rome.

The continuous use of words such as gastrointestinal microbiome, probiotics, prebiotics, fecal microbiota transplantation also through the media communications, suggests that this monography, which is totally dedicated to the main relationships between gut echological system and diseases based on the most recent literature, could be a useful instrument both for research and for clinical practice in medicine and biology.

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1 THE GUT MICROBIOTA: ITS ANATOMY AND PHYSIOLOGY OVER A LIFETIME

G. Gibiino, G. Ianiro, G. Cammarota, A. Gasbarrini

Gut microbiota is the wide assemblage of microorganisms that inhabit our gastrointestinal tract, ranging from bacteria, archaea, yeasts,[1] and single-celled eukaryotes to helminth parasites and viruses, including bacteriophage. Though often used as synonymous, the "microbiome" is more precisely the whole range of microbes, along with their genes and genomes. Growing interest in this symbiotic cohabitation allowed also the study of "Metagenomics" considering the definition of the gene content and encoded functional attributes of the gut microbiome in healthy humans. Metabolomics is the quantitative measurement of the multiparametric (time-related) metabolic responses.[2]

The composition of the microbiota varies quantitatively and qualitatively over the longitudinal and the cross-sectional axes of the alimentary tract. Beyond the oral cavity, which harbors approximately 200 different bacterial species, the size and diversity of the microbiota increase distally along the digestive tract. Gastric acid restricts bacterial numbers within the stomach to fewer than 103 colony-forming units (CFU)/mL.[3, 4] The gradient in bacterial density is greatest across the ileocecal valve, with approximately 108 bacteria per gram of ileal contents and up to 1012 bacteria per gram of colonic contents, comprising more than 1000 different bacterial species. More than 99% of the culturable bacteria in the ileum and the colon are obligate anaerobes, but the composition of the flora at the mucosal surface differs from that within the lumen; ratios of anaerobes to aerobes are lower at mucosal surfaces.[5]

The microbiota of the proximal small intestine consists predominantly of Gram-positive facultative bacteria, although enterobacteria and *Bacteroides* species also may be present. Peristalsis is the principal factor restricting bacterial numbers in the small intestine. In the distal small intestine, the composition of the microflora resembles that of the colon, with a preponderance of Gram negative anaerobes. The most prominently represented genera in the distal bowel include *Bacteroides*, *Clostridium*, *Lactobacillus*, *Fusobacterium*, *Bifidobacterium*, *Eubacterium*, *Peptococcus*, and *Escherichia* species. National or multinational consortia [5-9] provide the spectrum of composition and functions of normal gut microbiota. European consortium indicated that, at a higher level of organization, some general patterns can be identified across populations. They identified 3 broad groupings ("enterotypes") driven by the predominance of certain species: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2), and *Ruminococcus* (enterotype 3). Enterotype prevalence seemed independent of age, Body Mass Index, or geographic location but might have been driven by differing dietary habits.[7, 8] Each of them has specific metabolic patterns and is modulated by dietary habits: a carbohydrate-rich diet, indeed, exposes to a *Prevotella* enterotype, while having a diet rich in proteins and fats promotes the development of enterotypes 1 and 3.[8, 10]

The number of studies investigating the microbiome has exploded since the technological advances in high-throughput sequencing that facilitate culture- and cloning-independent analysis.[11] These technical advances

have been paradigm shifting since the majority (>90%) of microbial species cannot be readily cultured using current laboratory culture techniques.[12] The most common sequencing approach to analyze the microbiome, which has been used to compile most of the data collated by the Human Microbiome Project (HMP), is amplicon analysis of the 16S ribosomal RNA (rRNA) gene.[13, 14] In this method, a 16S rRNA region is amplified by PCR with primers that recognize highly conserved regions of the gene and sequenced.[15] An alternative approach to the 16S rRNA amplicon sequencing method is whole genome shotgun sequencing (WGS) which uses sequencing with random primers to sequence overlapping regions of a genome. The major advantages of the WGS method are that the taxa can be more accurately defined at the species level. Another important consideration is that the 16S and WGS methods commonly utilize different databases for classification of taxa. However, WGS is more expensive and requires more extensive data analysis.[16-18]

THE DEVELOPMENT OF GUT MICROBIOTA

Intestinal microbiota development is a complex process that begins in utero and continues for the first 2-3 years of life.[19] Chu *et al.* first introduced the concept that gut microbes should be considered as a mediating factor in the relationship between early life nutrition and later life disease, as a component of the Developmental Origins of Health and Disease (DOHaD) hypothesis.[20] Traditionally, it was thought that the intestinal tract is sterile at birth; new evidence indicates that the colonization of the infant's gut may begin in utero from the placenta.[21, 22]

Emerging evidence indicates that the fecal microbiota during infancy is shaped by several prenatal and postpartum environmental factors.[23-28] Previous studies found differences in the infant fecal microbiota on mode delivery, initiation and duration of breastfeeding and also infant exposure to antibiotics.[23, 29] Moreover, most recent research is focusing on how maternal diet and gestation weight gain affect the infant microbiome.[30-32] Chu *et al.*, indeed, showed that a maternal high-fat diet in gestation is associated with persistent and specific alterations in the offspring microbiome, with specific highlights on the relative depletion of *Bacteroides* species.[31] Another specific prospective study was identified four distinct microbiota profiles: *Bifidobacterium*-dominant, entero/*Veilonella*-dominant, *Bacteroides*-dominant, and *Escherichia*-dominant. Infants whose mothers had higher gestational weight gain were less likely to have *Bacteroides*-dominant profile and were also associated with lower bacterial community richness.[32]
However, it is accepted to date that several factors influence the microbiome over these critical early months and years of life and by 2 to 3 years of age, the child's microbiota has come to closely resemble that of an adult in terms of composition.[33] Thereafter, the microbiota is thought to remain relatively stable until old age when changes are seen, possibly related to alterations in digestive physiology and diet.[34]

FACTORS MODULATING MICROBIOTA HEALTH

Several factors can interfere with the precious homeostatic interaction between the micriobiota and the host, can influence the composition of the microbiota in health and

must be accounted for in the interpretation of findings in disease. Foremost among these is diet. Both short-term dietary interventions and long-term dietary patterns influence the composition and diversity of gut microbiota.[19, 35] General characteristics of the diet as well as the relative concentrations of specific components, such as carbohydrate, protein fat, fiber, and vitamins, have all been shown to influence the composition of the microbiota, even leading to different enterotypes, as cited above.[8] On the dietary components, non-digestible carbohydrates, or fiber, have received the most attention as it is the primary energy source for the most gut microbes and influences bacterial fermentation, total bacteria numbers and species composition.[19] As widely known, the human host does not contain digestive enzymes capable of breaking the molecular linkages contained in most complex carbohydrates and plant polysaccharides. Thus, these polysaccharides are metabolized by microbes in the colon. Holscher *et al.* summarized the current evidence linking dietary fiber and prebiotic consumption to human gastrointestinal microbiota composition and function.[36]

Dietary fibers, carbohydrate polymers which are neither digested nor absorbed, are subjected to bacterial fermentation in the gastrointestinal tract and thus impact the composition of bacterial communities as well as microbial metabolic activities, including the production of fermentative end products. Some dietary fibers can also be classified as prebiotic. Prebiotics are defined as "*selectively fermented ingredients that result in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.*" [37] A review recently published by Sharon *et al.*, discusses the impact of consumption of dietary fibers and prebiotics on the gastrointestinal microbiota, including the role of the ingredients' physiochemical properties and dose, as well as the phenotypic responses related to the composition of the resident microbiota. Briefly, clinical studies conducted in adolescents or adults free of gastrointestinal diseases that utilized molecular methods to assess ≥2 microbes and fermentative profiles and were published in the last 5 years (2011-2016) revealed that galactooligosaccharide (GOS), inulin, xylooligosaccharide, and arabinoxylan oligosaccharides induced blooms in Bifidobacterium spp. while, soluble corn fiber and polydextrose stimulated more diverse changes in microbes in the *Bacteroidetes* and *Firmicutes* phyla. Main results support the designation of GOS and inulin as prebiotic fibers. The differential effects of consumption of the fibers is driven by their chemical structures. GOS are generally composed of galactose polymers linked by b-1,6 bonds and b-1,4 linkage to the terminal glucose, and a DP between 2 and 10.75. Each fiber's distinct molecular structure provides a partial explanation for the differential effects of consumption of the human gastrointestinal microbiota.[37, 38]

Antibiotics are another crucial point affecting our microbiota composition, leading to "eubiotic" effect or a "dysbiotic" result.[39] Both drugs' characteristics (class, pharmacokinetics, pharmacodynamics, dosage, duration, etc.) and host-related factors, including age, lifestyle and microbiota composition, should be considered in order to prevent a dysbiotic impairment.[40, 41] It has been postulated with supporting evidence that antibiotic and other exposures during the early years of life, when the microbiome is in evolution, may be especially deleterious and could result in metabolic and inflammatory disorders later in life.[42]

To sum up, many variables, such as Host genetics, age, and environmental factors such as where and who you live with, use of pre-, pro-, and antibiotics, exercise and diet have been described to influence the short- and long-term composition of the microbiome.[19, 43, 44]

PHYSIOLOGICAL FUNCTIONS OF THE GUT MICROBIOTA

The intestinal microbiota is considered a signaling hub that integrates environmental inputs, such as diet, with genetic and immune signals to affect the host's metabolism, immunity, including autoimmunity and autoinflammation [45-47] and elaborate networks linked to several organs.

The metabolic functions of the gut microbiota

The metabolic functions of the microbiome continue to be revealed and molecular key pathways are consistently arising.
It is long time recognized the importance of gut micro-organisms as a whole essential metabolic organ in biotransformation of bile acids and breakdown of otherwise indigestible dietary components, such as plant polysaccharides and production of short-chain fatty acids, a major energy source for colonic epithelium, from fermentable carbohydrates. In this setting, microbiota promotes absorption of monosaccharides from the gut lumen, with consequent induction of hepatic lipogenesis; moreover, it suppresses fasting-induced adipocyte factor (Fiaf), a lipoprotein lipase inhibitor, allowing the deposition of triglycerides in the adipocytes.[48] Obesity is associated with an alteration of gut microbiota composition: obese mice show a 50% reduction in *Bacteroidetes* and a similar increase in *Firmicutes*; in addition, "obese" microbiota displays an increased capacity to harvest energy from the diet.[49] Related data come out also from humans: both gut microbiota composition and representation of metabolism-related microbial genes are different in lean versus obese twins.[50] Obesity and insulin resistance are characterized by a low-grade inflammation status.[51] Gut microbiota may interfere in host metabolism by the modulation of inflammatory pathways. Enrichment in Gram-negative species results in an increase of lipopolysaccharide (LPS) absorption, which leads to the so-called "metabolic endotoxemia." [52]
Most recent papers showed that farnesoid X receptor (FXR) regulates the synthesis, transport and enterohepatic circulation of bile acids (BA) by modulating the expression of related genes in the liver and small intestine.[53] The composition of the gut microbiota is linked to metabolic diseases, notably obesity and non-alcoholic fatty acid disease (NAFLD). Bacterial metabolism of bile acids can modulate FXR signaling in the intestine by altering the composition and concentrations of FXR agonist and antagonist. FXR agonist enhances while FXR antagonist suppresses obesity, NAFLD and insulin resistance.[54, 55]
Other activities promoted by our intestinal microbes include synthesis of biotin, folate, and vitamin K. Moreover, intestinal microbiota has been linked to enteric bacterial enzymes such as azoreductase to convert prodrugs such as sulfasalazine to active drug metabolites (*e.g.*, aminosalicylate). Other examples of bacterial action on drug bioavailability include the metabolism of l-dopa to dopamine and degradation of digoxin. Not all of the metabolic changes induced by the enteric microbiota are beneficial to the host, however, and although bacteria probably

degrade some carcinogens, they also might promote the production of carcinogens from dietary procarcinogens.

The microbiome and innate immunity

Gut microbiota is strictly involved in the development of the mucosa- or, gut-associated immune system (mucosa-associated lymphoid tissue or gut-associated lymphoid tissue), immunologic tolerance, epithelial and barrier function, motility, and vascularity.[56, 57] As suggested in the most recent literature, the interactions between the innate immune system and the intestinal microbiota contribute to orchestrate the whole-organism physiology. First players in this crosstalk are the intestinal epithelial cells, extensively equipped with innate immune receptors. Pattern recognition receptors (PRSs), Toll-like receptors (TLRs), and NOD-like receptors are only few examples of molecular elements involved in the host-microbial interface.[58-60] The microbiome influences the function of myeloid cells at all stages of their development.[61] Notably, microbiota seems to drive myeloid-cell differentiation and function through PRR signaling. This microbiota-driven modulation in the myeloid cell pool influence the susceptibility of the host to a variety of disorder, ranging from infections till therapies for cancer disease. Even the lymphoid arm of the innate immune system is strictly related to gut microbiota, mainly involving innate lymphoid cells (ILCs).[62] The mechanisms through which the microbiota controls the development of the innate immune are beginning to be understood although the principles and purpose of innate-immune control over temporal dynamics in microbiota function remain unknown.[63]

Dysbiosis and impaired immune response play a key role in some digestive diseases, with particular attention to liver and pancreatic disease. Indeed, the presence of imbalance in gut microbiota is associated with endotoxemia and this propagates liver injury due to nonalcoholic steatohepatitis and alcohol. The composition and functionality of the microbiome changes with the development of cirrhosis, decompensation, and with treatments for these conditions. Gut microbiota can be used to predict clinically relevant outcomes in cirrhosis.[64]

The role of the microbiome in the development of pancreatic disorders, as well, is increasingly recognized. The translocation of gut bacteria and endotoxins following gut barrier failure is a key event contributing to the severity of acute pancreatitis, while small intestine bacterial overgrowth is common in patients with chronic pancreatitis and further worsens their symptoms and malnutrition.[65] In addition, specific molecular mimicry link the microbiome and *Helicobacter pylori* with autoimmune pancreatitis. Changes in the oral microbiome typical of periodontitis seem associated with an increased risk of developing pancreatic cancer. The composition of the gut microbiota is also unbalanced in the presence of risk factors for pancreatic cancer, such as obesity, smoking and diabetes. *Helicobacter pylori* infection, atrophic body gastritis and related decreased gastric acid secretion also seem associated with the risk of pancreatic cancer, although this area needs further research.[66]

The gut-brain axis

Concurrently, a growing body of evidence points to the microbiota playing a significant role in modulating brain function and

behavior and that the "microbiota-gut-brain axis" poised as a bidirectional communication pathway enabling gut microbes to communicate with the brain, and the brain with the gut.[67] The microbiota-gut-brain axis is a dynamic matrix of tissues and organs including the brain, glands, gut, immune cells and gastrointestinal microbiota that communicate in a complex multidirectional manner to maintain homeostasis.[68] The mechanisms of communication are complex and are slowly being unraveled; they include immune, neural, endocrine and metabolic pathway.

In this perspective, metabolic products of the microbiome, including neurotransmitters and neuromodulators, impact not only on the enteric neuromodulatory apparatus but also seem capable of influencing the development and function of the central nervous system.[69] Furthermore, synthesis and release of neurotransmitters from bacteria has been reported; the inhibitory neurotransmitter γ-aminobutyric acid (GABA) can be produced by *Lactobacillus* and *Bifidobacterium* species, whereas *Escherichia*, *Bacillus* and *Saccharomyces* spp. can produce noradrenaline (norepinephrine). On the other hand, *Candida*, *Streptococcus*, *Escherichia*, and *Enterococcus* spp. have been reported to produce serotonin, and *Bacillus* can produce dopamine, whereas certain *Lactobacillus* spp. can produce acetylcholine.[70] These microbially-synthesized neurotransmitters can cross the mucosal layer of the intestines, and possibly mediate physiological events in the brain. Brain development has been studied in germ-free mice and several models of studies are always purposed to assess the evolution of the microbiota in ageing and neurodegenerative diseases.[70]

Even mechanisms underlying visceral pain have been recently linked to alterations in gut microbes. Thus, both central and peripheral pathways associated with pain manifestation and perception are altered as a consequence of the microbiota-gut-brain axis imbalance.[71] This assumed particularly importance in the subset of irritable bowel syndrome (IBS) patients. Indeed, in IBS patient cohorts, numerous independent research groups have shown distinct gut microbiota populations when compared to healthy controls. This was recently reviewed by Mayer *et al.*[72] Moreover, probiotic interventions appear to be beneficial to IBS patients. Manipulation of the gut microbiota through the use of probiotic and prebiotics treatments have shown that by augmenting so-called "good bacteria," such as *Bifidobacteria* and *Lactobacillus*, in the gut, visceral hypersensitivity can be reversed in preclinical models. A mixture of eight probiotic bacteria strains (VSL#3) was shown to have protective effects against development of visceral hypersensitivity in the neonatal maternal separation model. Moreover, TPH1, tryptophan hydroxylase 1, the gene for the enzyme responsible for synthesizing serotonin, a key neurotransmitter involved in IBS treatment, was markedly up-regulated by neonatal maternal separation and this effect was reversed by VSL#3 intervention.[73] *Bifidobacterium* species particularly, *Bifidobacterium infantis* 35624 has been shown to be particularly effective at ameliorating visceral hyperalgesia in both stress-induced visceral hypersensitivity and colitis.[74]

CONCLUSIONS

Several mechanisms of our health have been linked to the rich microbial heritage housed in our gastrointestinal tract and many others hypothesis will be formulated regard to the imbalance of each organ. The most ad-

vanced discover was to understand that gut microbiota is an essential signaling hub, involving pattern more complex than merely expected intestinal functions.

Most research available in this setting arose from studies on animal models, and only in the last years did researchers investigate how gut microbiota affects human physiology, leading to several diseases in case of impairment. From this point of view, intestinal microbes have become the target of therapies able to modulate the microbiota assessment and to date many molecules are suggested as possible treatment.[75-78, 83] Fecal transplantation has become a recognized treatment for *C. difficile* infection antibiotic-resistant,[79-85] but also it is arising, as well, as a promising practical in several diseases. Unexplored features of our microbiome are still extended and future trials on human patients need to be performed to reach a complete knowledge of this symbiotic treasure.

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