REVIEW

From the intestinal flora to the microbiome

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ABSTRACT

In this article, the history of the microbiota is reviewed and the related concepts of the microbiota, microbiome, metagenome, pathobiont, dysbiosis, holobiont, phylotype and enterotype are defined. The most precise and current knowledge about the microbiota is presented and the metabolic, nutritional and immunomodulatory functions are reviewed. Some gastrointestinal diseases whose pathogenesis is associated with the intestinal microbiota, including inflammatory bowel disease, irritable bowel syndrome and celiac disease, among others, are briefly discussed. Finally, some prominent and promising data with regard to the fecal microbiota transplantation in certain digestive illness are discussed.

Key words: Microbiota. Microbiome. Metagenome. Dysbiosis. Phylotype. Enterotype.

FROM THE INTESTINAL FLORA TO THE MICROBIOME

In 1683, Anton van Leeuwenhoek described some *"animalcules"* he had observed in the gastrointestinal tract under a microscope that he himself had made, unaware that he was making the first description of the appearance of a bacterium (1). Since then significant progress has been made. Almost two centuries later, in 1861, Louis Pasteur, the brilliant French bacteriologist, discovered anaerobic intestinal bacteria (2). Pasteur himself is credited with the thought *"The role of the infinitely small in nature is infinitely large"* (3).

Ilya Metchnikov, a Ukrainian scientist who won the Nobel Prize in 1908 and a professor at the Pasteur Institute in Paris, had previously proposed that the so-called lactic acid bacteria (LAB) provided health benefits and were in some way capable of promoting human longevity. He suggested that the so-called "intestinal autointoxication" and the resulting effect on aging could be suppressed by modifying the intestinal flora. By replacing proteolytic microbes, such as *Clostridium* (which produce toxic substances such as phenols, indoles and ammonia via the digestion of proteins) with useful microbes such as *Lactobacillus* (4).

The term *microbiome* was coined in 2001 by Joshua Lederberg, an American molecular biologist. He was one of the three researchers who won the Nobel Prize in Medicine in 1958, awarded for their work in genetic studies of bacteria. Lederberg stated that symbiotic microorganisms and humans form a great metabolic unit, a view which recognizes that the bacteria located within the human body are in fact protecting us (5). In recent years, biomedical research has led to advances in our knowledge of the gut microbiota (referred to as intestinal flora until 2014). However, there is still a great deal to learn, much more than what we have already learnt during the last three centuries.

Over the last few years, two major projects have been decoding the structure and functionality of the human microbiota, as well as its relation to disease. The MetaHIT (Metagenomics of the Human Intestinal Tract; www.metahit.eu), funded by the European Union, and the Human Microbiome Project (http://hmpdacc.org), funded by the National Institute of Health of the United States of America.

Building on the article *"Microbiota and the gastrointestinal system,"* by C. Barbés Miguel, which was published in 2001 in the *Point of View* section of this journal (6), this *review* provides an update with some of the knowledge acquired during the past 16 years about this exciting organism. Before going further, it is important to define the terms that will be used in this article, along with the already known nomenclature of prebiotic, probiotic and symbiotic: *microbiota, microbiome, metagenome, pathobiont, dysbiosis* and *holobiont* (7).

Microbiota refers to the community of living microorganisms residing in a particular ecological niche, such as the human gut (colon). The *microbiome* is the ensemble formed by microorganisms, their genes and their metabolites in a given ecological niche. About 9.9 million microbial genes have been identified in the human fecal microbiome (8). The *metagenome* refers to all the genetic material present in an environmental sample, which in this case is the

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entire human and bacterial (microbial, in general) genome. *Pathobiont* are benign endogenous microbes with the ability, under the conditions of an altered ecosystem (dysbiosis), to cause certain pathologies. *Dysbiosis* is the loss of balance between the cells of a human organism and the bacterial (microbial, in general) cells that inhabit it. Finally, the *holobiont,* also known as the *superorganism*, refers to the totality of organisms in a given ecosystem (in this case, humans and the shared microbial ecosystem). Humans are in fact superorganisms governed in part by the microorganisms we host (9).

The microbiota has become fashionable. It no longer only interests the medical profession but also readers of the general press, such as *El País.* In the Sunday supplement of *El País* on the 8th of January 2017 (http://elpaissemanal.elpais.com/documentos/viaje-nuestras-profundidades/), Juan José Millás published an article-interview entitled *"Viaje a nuestras profundidades: en el intestino está la clave".* Within this article, some of the current knowledge of the human gut and microbiota was reviewed with great accuracy and precision by professor Carlos López-Otín, the renowned Aragonese scientist and professor of Biochemistry and Molecular Biology in the Faculty of Medicine at the University of Oviedo.

The *Journal of the International Microbial Society* (JISM), dedicated to covering a variety of new strategies and innovations in the field as well as clinical applications derived from studies on gut microbiota, is another indicator of the wider interest in this subject. The Fifth World Congress of the International Society of Microbiota (https://www.microbiota-site.com/) was held in October 2017 in Berlin, under the banner *"Targeting Microbiota".*

The vast majority, more than 90%, of bacteria present in the human system reside in the colon. The quantity was previously estimated to be around 10^{14}, i.e., about 100 billion (10). Recent studies using much more precise techniques have concluded that the volume of bacteria in the colon is less, around 10^{11} per gram. Considering that the volume of the colon is around 0.4 l or kg (400 g), it can be concluded that there are around 3.8×10^{13} (38 billion) bacteria in the colon (11) of a "typical male". This is defined as a male between 20 and 30 years old, weighing around 70 kg and 170 cm tall (12). Reviews of the literature suggest that bacterial concentrations in the colon do not appear to modify significantly over time, from childhood to old age (13).

With regard to the number of human cells in a typical adult male, the quantity is estimated at 3.0×10^{13} or about 30 billion, of which 84% (25×10^{12}) are erythrocytes (11). One might think that man is little more than a transporter of bacteria. With these updated numbers, the ratio between bacteria and human cells has fallen to approximately 1.3:1 (in a 70 kg male). Almost a 1:1 ratio, which should replace the values of 10:1 (14) or 100:1 which were quoted in the literature before more accurate measurements became available (11).

It has also been estimated that the total mass/weight of the bacteria in the colon is approximately half the weight of the colon itself, which is about 200 g (50 to 100 g dry weight) as the colon weighs close to 400 g (15). The weight of the colon thus represents 0.3% of the total body weight, a figure significantly lower than the 1-3% (with 1 to 2 kg of the total body weight attributed to bacterial mass) that the colon was believed to comprise until recently (11).

The gut microbiome is immensely diverse. It hosts more than 1,000 different bacterial species (16), mainly anaerobic bacteria (17). According to some studies, the number and diversity of these increase with the age of the host. While other studies suggest the opposite, a reduction and loss of diversity of the microbiota over time, with deficient and dysbiotic functioning influenced by age, environmental factors and lifestyle (18).

The microbiome is defined mainly by two bacterial filotypes*, Firmicutes* and *Bacteroidetes* (the latter accounting for 90% of the gut microbiota), and to a lesser extent, *Actinobacteria.* The first of these includes a large number of genera, the most important are *Lactobacillus* and *Clostridium*. *Bacteroides* include bacteria belonging to the genus *Bacteroides* and *Prevotella.* The main genus belonging to the *Actinobacteria* phylum in the human intestine is *Bifidobacterium* (19). The *phylotype* is a taxonomic group defined by the degree of similarity between DNA sequences of the 16S gene and not by phenotypic characteristics.

An important advance in the knowledge of the gut microbiota occurred in 2011, when the *enterotypes* were defined in adults (20) as the different groups of gut microbiota in accordance with certain states of equilibrium. Each enterotype is differentiated by the variation of the presence of the three predominant bacterial genera: *Bacteroides* (type 1 enterotype), *Prevotella* (type 2 enterotype) and *Ruminococcus* (type 3 enterotype), which are probably related to long-standing dietary patterns. The type 1 enterotype is associated with a diet rich in protein and fat and type 2 with carbohydrate consumption (21). The category seems to be independent of gender, age, nationality or body mass index. The type 1 enterotype is the most prevalent in European subjects, appearing in 56% of subjects followed by type 2, at 31% (22).

Previously, bacterial diversity studies were mainly carried out via culture techniques, which provided a biased view of the bacterial composition of the fecal microbiota. The subsequent development of high-throughput sequencing techniques as well as the development of bioinformatic tools has led to a comprehensive description of the bacterial community that inhabits the gastrointestinal tract and has been a turning point with regard to the understanding of bacterial colonization of the human gut. Our colleague Francisco Guarner M.D., of the Vall d'Hebron Hospital in Barcelona (22), has played a prominent role in this field.

The functional aspects of the normal human gut microbiota comprise the following: metabolic and nutritional functions, antimicrobial protection, maintenance of the integrity of the intestinal mucosa and regulation of the immune response (23-25).

With regard to the metabolic functions, the following should be emphasized:

1. Anaerobic bacterial fermentation of dietary fiber carbohydrates leads to the formation of short chain fatty acids (SCFA), which are the preferred respiratory fuel

for the colonocites. These SCFA have an anti-inflammatory effect as they inhibit certain pro-inflammatory cytokines and, interestingly, are able to induce apoptosis of malignant colon cancer cells (26). The SCFA produced by carbohydrate fermentation include acetate, propionate and butyrate, which are absorbed by the colon (23). The majority of the propionate is metabolized by the liver, where it acts to reduce serum cholesterol and glucose levels (27). Butyrate is the major provider of energy to cells in the colonic epithelium. The SCFA promote the integrity of cell junctions in the colon, increase the rate of proliferation of epithelial cells, accelerate epithelial repair in response to injury and facilitate the differentiation of epithelial cells, with the consequential effects against colon cancer (28).

2. The gut microbiota has recently been identified as a new factor involved in the management of body weight. The microbiota is involved in energy metabolism via the energy obtained from the diet, specifically, in the regulation of the storage of body fat, the regulation of lipogenesis and the regulation of the oxidation of fatty acids (23,29). Current evidence suggests that certain changes in the gut microbiota, in particular an increase in *Firmicutes* and a decrease in *Bacteroidetes* (30), play an important role in the development and maintenance of obesity, probably interacting with genetic factors. In addition to many other mechanisms (31), one of the most important factors that contributes to obesity includes a higher energy intake from the colon via the fermentation of non-absorbable carbohydrates (23). In contrast, the gut microbiota may also play a decisive role in anorexia nervosa and the severe weight loss resulting from this condition, as well as the associated mental disorders, anxiety and depression (31).
3. The gut microbiota synthesizes vitamin K and several components of vitamin B, including vitamin B_{12} (24,32). However, the latter is unlikely to be available directly to the human host due to the physiology of its absorption, which requires binding to factor R in the stomach, transfer to the intrinsic factor in the small bowel and absorption of the complex in the terminal ileum.

With regard to *immunomodulation* or the regulation of the immune response, in healthy subjects, the microbiota is in homeostatic symbiosis with the host via a functional intestinal epithelial barrier that contains high concentrations of secretory IgA (IgA S). The latter is produced by the plasma cells located in the Peyer's plaques and forms complexes in the lamina propia with the commensal bacteria and the microbiota in the intestinal lumen and selectively present the bacterial components to the dendritic cells. These cells induce the production of anti-inflammatory interleukin 10 (IL-10), which contributes to a class change from IgA S to IgA. All of this ensures effective communication between the microbiota and the immune system, inducing a tolerogenic environment towards the microbiota and, at the same time, stimulating the activity of the immune system (33,34). The composition of the microbiota helps to maintain immunological homeostasis, which suggests that the microbiota could be an additional organ of the human organism (35).

The microbiota-gut-brain axis is a bidirectional system that should be added to this complex. In one direction, the brain may indirectly affect the gut microbiota via changes in secretion, motility and/or intestinal permeability. In addition, it may directly influence the microbiota via neuronal networks through the release of substances by the enterocromaffin and immune cells. In the other direction, the gut microbiota communicates with the brain by direct stimulation of certain receptors, via vagal afferents or via the recently described humoral pathway. All of this can alter brain morphology and neurochemistry and specifically GABA and serotonin levels. This microbiota-brain communication is involved in the perception of visceral pain (36) and in the modulation of the immune response and emotions (37,38).

Below is a brief review of some gastrointestinal diseases whose pathogenesis is associated with the gut microbiota. In some cases, such as inflammatory bowel disease and irritable bowel syndrome, there is strong evidence supporting the implication of the microbiota. However, in others, such as celiac disease, the body of evidence suggests a less important role. There are also other processes, such as colorectal cancer, gastric cancer and hepatocellular carcinoma, in which the microbiota seems to be involved.

Inflammatory bowel disease (IBD) is clearly associated with intestinal dysbiosis and the gut microbiota seems to play a clear role in its pathogenesis. However, it is still unclear whether such dysbiosis is causative, contributes to, or is a consequence of the disease. It is likely that all three possibilities occur (39,40). More advances are needed in bacterial culture and experimental models and a wider implementation of bioinformatics in order to improve our understanding of the role of the gut microbiota in IBD.

With regards to experimental models, the gnotobiotic mouse has a known microbiota obtained from animals free from microorganisms and seems to be a useful model to investigate the functional role of the IBD-associated dysbiotic microbiota. Using this model, it has been shown that intestinal dysbiosis may potentially contribute to the pathogenesis of IBD, increasing the pro-inflammatory immune response of the host (41). The data from this study suggests that dysbiosis in patients with IBD is a key factor in the onset and maintenance of intestinal inflammation and not merely a secondary outcome.

A recent meta-analysis (42) found that the mean level of *Bacteroides* was significantly lower in patients with Crohn's disease (CD) and active ulcerative colitis (UC) compared to patients in remission and normal controls. Thus, it appears that the inflammatory activity in IBD causes a significant reduction of *Bacteroides.*

With regard to *irritable bowel syndrome (IBS),* gut microbiota disturbances have been linked to its pathophysiology for many years. The Rome Working Group concluded (43) that there is good evidence to support the idea that the gut microbiota is altered in IBS. The microbiota participates in the different mechanisms involved in the pathophysiology of IBS. This includes intestinal and colonic motility, visceral sensitivity, intestinal mucosal barrier and neuro-immune signals, as well the gut-brain-microbiota axis (44), all of which are involved in the pathogenesis of this disorder.

A recent meta-analysis in China (45) confirmed that there were alterations in the microbiota in patients with IBS,

probably connected to the pathogenesis of IBS. These alterations were different among patients from China, who had a decreased number of *Bifidobacteria* and *Lactobacillus* and a greater number *of E. coli* and *Enterobacterium*, with no significant change in the quantity of *Bacteroide*s, whereas in patients from other regions, such as Europe, there was a decrease in the number of *Bifidobacteria* and a higher number of *Bacteroides*. This finding is in line with the differences in the enterotype population observed in different regions, as discussed at the beginning of this article.

With regard to *celiac disease* (CeD), both genetic determinants and environmental exposure to gluten are necessary for complete manifestation; neither of these alone is enough. Epidemiological and clinical data suggest that other environmental factors, including infections, alterations in the composition of the gut microbiota and type of early diet, may also play a role in the development of the disease. This interaction is a *sine qua non* condition for the development of CeD. The deterioration of the interaction between microbiota, innate immunity and genetic and dietary factors leads to the alteration of homeostasis and inflammation, causing intestinal tissue damage (46). Differences in the microbial composition have been observed between patients with CeD and healthy individuals. Different studies also indicate that the microbiota may be involved in the manifestation of the disease. Some epidemiological studies have indicated that several factors influence both the risk of developing CeD and the composition of the gut microbiota, strongly supporting the link between the microbiota and the onset of the disease (47-50).

Another entity in which the microbiota seems to play an important role via different mechanisms is non-alcoholic fatty liver disease (51). The microbiota is also implicated in colorectal cancer (CRC) (52,53), hepatocellular carcinoma (54), and gastric cancer (55). However, a thorough description of these conditions is beyond the scope of this article.

It is worth mentioning an excellent study by several Spanish researchers in the journal *Aging* (56), which focused on an animal model, the *Caenorhabditis elegans* worm. This study showed that worms with a diet rich in *Bacillus subtilis* (gram positive) lived longer (43 to 58% longer) than those with a standard diet rich in *Escherichia coli* (gram negative), even though the latter is more nutritious. The difference in longevity seemed to be due to the fact that *B. subtilis* does not synthesize CoQ, an antioxidant that is synthesized by *E. coli.* In this case, the antioxidant seems to be harmful. In summary, the study indicated that the microbiome may influence life expectancy, i.e., longevity, and that, undoubtedly, diet composition affects the health of the organism. Yet another reason to continue investigation into our microbiota.

To conclude, we would like to briefly mention the possibilities opened by the transplantation of fecal microbiota for the treatment of certain gastrointestinal diseases (57,58). The efficacy of this process has been clearly demonstrated in cases of *Clostridium difficile* infection (59,60) and it is also beginning to be used in cases of IBD (61). While the available evidence is still insufficient to recommend the procedure, it has shown good results for ulcerative colitis (62-64). However, there is still a long way to go.

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