Chapter 2

Gut-Brain Axis: Role of Microbiota in Parkinson's Disease and Multiple Sclerosis

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Additional information is available at the end of the chapter

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Abstract

It has recently been discovered that the digestive tract is lined with about 100 million nerve cells; the digestive tract has been baptized, metaphorically speaking, as "the second brain," which contains a multitude of neurotransmitters, viruses, and bacteria that help regulate our emotional state. This second brain, known as the enteric nervous system, is a unique anatomical unit that extends from the esophagus to the anus. Like the nervous system, it produces a whole series of psychoactive substances, such as serotonin, dopamine, and opioids for pain, and synthesizes benzodiazepines. In it, we find the microbiota: a set of microorganisms (viruses and bacteria). Together with the brain, the microbiota directly influences mood, character, or sleep. Knowledge about the possible relationship of the microbiota with frequent neurological diseases is still just beginning. Recently, possible changes in the microbiota have been linked to the onset of Parkinson's disease (PD). Also, today, we know that there are differences between the microbiota of healthy people and people with multiple sclerosis and that these differences have also been related to the disease and its evolution.

Keywords: brain, intestine, microbiota, neurodegeneration

1. Introduction

Currently, we know that the enteric nervous system, although it has a certain sovereignty or autonomy, will always need to communicate with other systems in order to carry out functions of its authorship or of other adjacent systems. Thus, in a classical way, the enteric nervous system interacts with the central nervous system but also exhibits a certain exchange of stimuli with other systems. Through this connection afferent and efferent responses are generated, with the consequent exchange of information. Remember that the afferent neurons send information of the intraluminal chemical content, mechanical state of the intestinal wall, and tissue situation (inflammation, pH, cold, and heat) to the central nervous system. The responses follow an extrinsic efferent pathway where the main neurotransmitter is norepinephrine. In addition, the efferent neurons come from the prevertebral nodes that control motility and secretion and the paravertebrals that control the flow of gastrointestinal blood vessels. Also, the relaxation of the gastric *fundus* and gastric and pancreatic secretion is mediated through vagal neurons. In contrast to what happens in the upper digestive tract, the distal colon and rectum are innervated by pelvic nerves. In general, vagal stimulation inhibits motor activity, gastrointestinal secretion, sphincter contraction, and blood flow, while, on the contrary, spinal stimulation stimulates them. The responses may have extrinsic afferent pathways where spinal and vagal reflexes are also activated. The primary afferent vagal neurons have their cell bodies in the nodosum and jugular ganglia and project medially to the brain, while the spinal neuronal bodies are found in the roots of the dorsal ganglia. The vagal pathways transmit information about the physiological state of the digestive organs and regulate inflammatory responses, while the spinal pathways transmit the painful impulses.

2. Gut microbiota

Since 2007, the genome of 500 bacterial species that normally reside in the human intestine, and which together are known as microbiome or microbiota, has been identified. Our intestinal tract contains over 100 billion microbes, the vast majority in the colon, exceeding the number of human cells by a factor [1]. This complex microbial community is known as the gastrointestinal microbiota, and it consists of bacteria, *archaea,* eukaryotes, fungi, and viruses. Therefore, it has been proposed that the human being is a "meta-organism" with 10–100 times more bacterial than human cells, which integrate metabolically and immunologically. The composition of the microbiota, in addition to location, is influenced by age, sex, race, and other factors like diet, medication (especially antibiotics), stress, smoking, or gastrointestinal infections, as well as from each individual [2]. Even within each person, there are great variations in their composition if measured at different times. Although it is impossible to define the concept of healthy microbiota today, we do know that the richness and diversity of the microbiota are indicators of its health and that its impoverishment is associated with obesity and metabolic markers [2]. As for the qualitative composition, numerous studies are emerging that try to relate certain classes of microorganisms with different physiological states. It is proposed that there are those that improve the metabolic state, resistance to infections, cancer, autoimmunity, inflammation, endocrine signaling, and brain functionality

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(gut-brain axis): *Bacteroides, Bifidobacterium, Clostridium* group XIVa and IVa (butyrate producing), *Eubacterium, Faecalibacterium, Lactobacillus, and Roseburia* are considered today [3]. The relationship between the microbiota and the human being has been redefined from commensal to a mutualist relationship, where the bacteria provide biological functions not coded genetically in our organism, which go from metabolic activity to immunological homeostasis, considering the microbiota as a fundamental, virtual organ in the pathophysiological and immunological responses. There are studies related to the role of the intestinal microbiota in human health, infections, and neurological diseases [4] (**Figure 1**).

It has rarely been thought that microorganisms and the brain interact except in instances where pathogens penetrate the blood-brain barrier, which is the cellular strength that protects the brain from infections and inflammation [5]. When they do, they can have strong effects. Bidirectional communication between the brain-nervous system and the microbiota is well known in specific cases. Pavlov's experiments showed how a sound processed by the brain can condition the physiology of the digestive system, stimulating, among other things, gastric secretion [6].

Clostridium botulinum can colonize the intestine and from there release its toxin that blocks the release of acetylcholine at the neuromuscular synapse. In the case of hepatic encephalopathy, brain dysfunction is a consequence of substances produced in the intestine, and its treatment includes the use of antibiotics and probiotics [7]. Certain autoimmune neurological diseases, such as Guillain-Barré syndrome, may have their origin in certain intestinal bacteria and *Campylobacter jejuni.* The virus that causes rabies generates aggression, agitation, and even a fear of water, but for decades the vast majority of the body's natural microbes had not

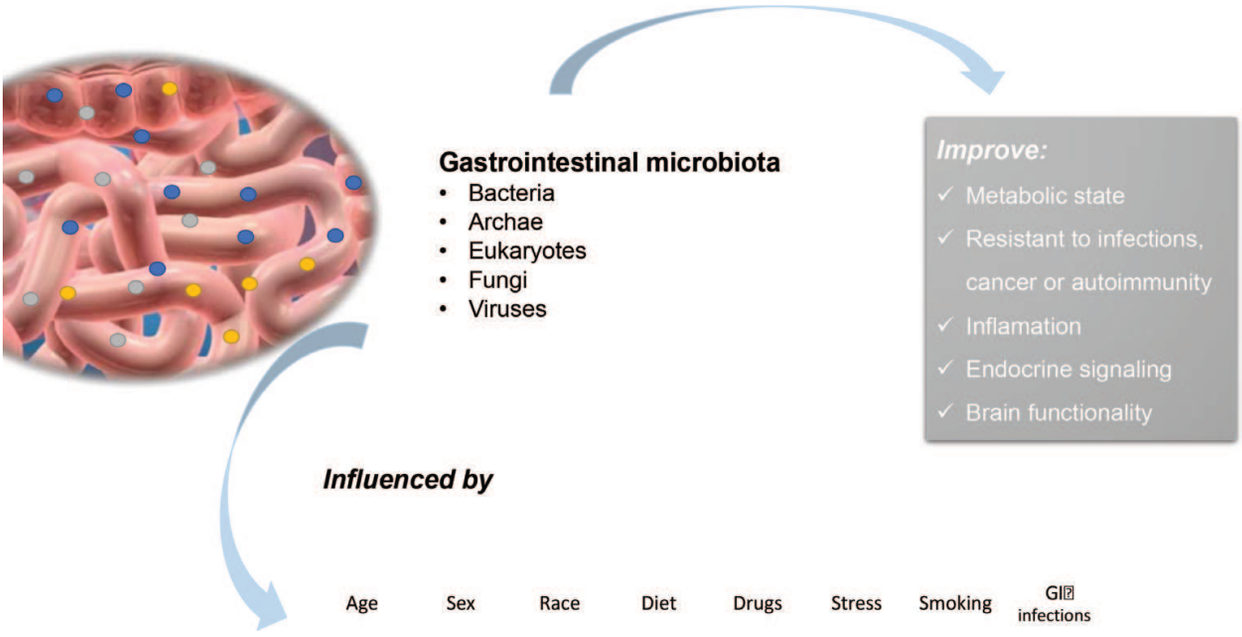

Figure 1. The composition of the intestinal microbiota is influenced by physicochemical conditions and also depends on the anatomical region along the GI. The type and number of microbial species that persist and colonize the GI tract are determined by a combination of factors including but not limited to the host genetics, medications, diet, environmental factors, and to the inflammatory state of the host. In elderly patients, we can observe changes in the composition of the microbiota in comparison to young people, which could lead to a dysbiosis.

been described, and while the idea that they could influence neurobiology hardly prevailed, that is slowly changing [8]. On the other hand, metabolic functions include the degradation of polysaccharides to short-chain fatty acids, such as butyrate, propionate, and acetate, with anti-inflammatory properties and the main energetic substrate of colonocytes, and thus are implicated in the barrier function of the gut mucosa [9]. Some bacteria, particularly *lactobacilli*, have been implicated in cholesterol metabolism and in the production of vitamins K and B and are also involved in the metabolism of xenobiotics, drugs, antibiotics, or bioactive products, conditioning pharmacokinetics, and the production of certain toxins involved in many diseases [9].

2.1. Microbiota and immune response

The microbiota contributes to various immunological functions. In the gut barrier, it prevents colonization and growth of pathogenic microorganisms, and it matures the immune barrier, both stimulating the innate response through Toll-like receptors (TLR) and NOD-like receptors (NLR) as the adaptive one, with an important role in the secretion of mucins, antimicrobial peptides, defensins, and IgA [10]. Regarding the development of the systemic immune response, in working with germ-free mice, the microbiota has been observed to intervene in the regulation and maturation of Peyer's plaques, mesenteric lymph nodes, and germinal centers [11]. It also regulates the number of plasma cells producing IgA, gut Tγδ cells, and CD4 + T lymphocytes in the lamina propria or intraepithelial and is involved in the gene expression of TLR and the major histocompatibility complex II [12]. The microbiota also conditions the development of effector T cells and the production of cytokines, highlighting the influence on Th and Treg lymphocytes involved in the autoimmune response and its regulation and, therefore, in autoimmune diseases in general and in multiple sclerosis in particular [13]. Germ-free mice (mice raised to lack intestinal microbiota) present a reduction in Th1 and Th lymphocytes, balancing the T-immune response to Th, which is reversed by reconstituting the normal gut microbiota in these animals. It has been proposed that the microbiota is involved in the passage of stationary T lymphocytes to pro-autoimmune T lymphocytes, so that mutualist microorganisms induce the production of Th at a steady state, which, in the presence of a proinflammatory microenvironment, promoted by certain cytokines such as IL-12, IL-23, IL-1β, or TGF-β3, would pass to pathogenic Th, a producer of IFN-γ, contributing to the progression of the inflammatory bowel environment [14]. It has been shown that the involvement of a single bacterium, the segmented filamentous bacteria, can contribute to this Th pro-autoimmune activity [14] (**Figure 2**).

As for the Treg, the microbiota is indispensable for its development and function. These lymphocytes regulate the inflammation that is generated against microbial stimuli through IL-10 [15]. Numerous microbial agents have been linked to the induction of Treg, notably the role of *Bacteroides fragilis* and specifically its polysaccharide A (PSA) with the development of IL-10 producing Foxp3+ regulatory T cells and with the prevention and cure of experimental colitis or shock in animal models, showing its key role in the regulation of immunological tolerance [16]. The aforementioned short-chain fatty acids, especially butyrate, balance the immune system to an "anti-inflammatory state" by increasing the production of IL-10 and IL-4, reducing

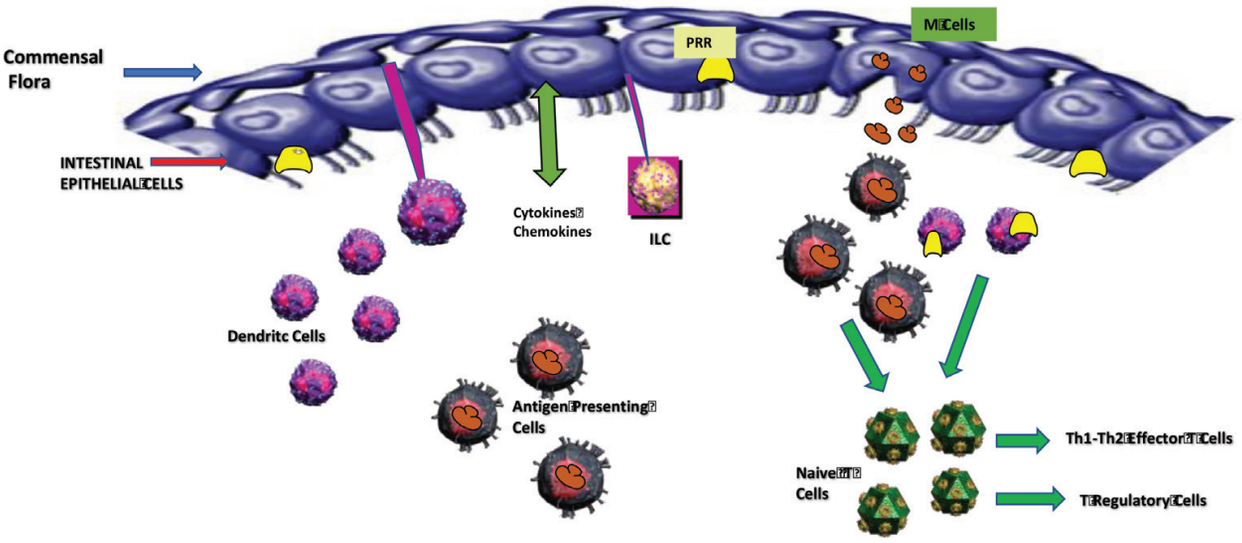

Figure 2. Microbiota stimulation leads to B cell switch to lga, regulatory T-cell induction, and T-cell differentiation to Th17. This image is a modification of QIAGEN's original at www.qiagen.com/mx/shop/genes-and-pathways/pathway-details/?pwid=468.

vascular adhesion of VCAM-mediated leukocytes, inhibiting function of IFN-γ and therefore its proinflammatory capacity, and regulating the Treg lymphocytes and the inflammatory function of leukocytes [17] (**Figure 3**).

2.2. Gut and nervous system

The surface of the gut mucosa is the most extensive of the organism and also houses the largest number of lymphoid structures in the human body. The innervations of the digestive tract are very abundant and are structured in three levels of plexus: functions of the enteric autonomic nervous system include bowel motility, vaso-regulation and permeability control, and secretion of certain gastroenteropancreatic hormones [18]. In addition, similar to what occurs in the blood-brain barrier, there are numerous astrocyte terminations at the border of the intestinal barrier that represent a potential pathway for communication with the rest of the nervous system. In the intestine we have about 100 million neurons, which are more than the spinal cord contains [19]. This multitude of neurons in the enteric nervous system allows us to feel the inner world of our gut and its contents. Much of this neuronal arsenal is evidenced in the elaborate daily routine of digestion, through decomposing food and absorbing nutrients. Expelling waste requires chemical, mechanical, and rhythmic muscle contractions that move everything to the end. Therefore, equipped with its own reflexes and senses, it can control the behavior of the gut independently of the brain. This nervous system of the intestine is connected to the brain in a bidirectional way. On one hand, the bowel receives information from the brain, and on the other hand, the bowel sends messages to the brain [20]. This communication of the intestine with the brain occurs both through the nervous system and the bloodstream and is called the gut-brain axis. Typical examples of this bidirectional circuit would be the increase in intestinal peristalsis (colicky pain and diarrhea) when our brain perceives a

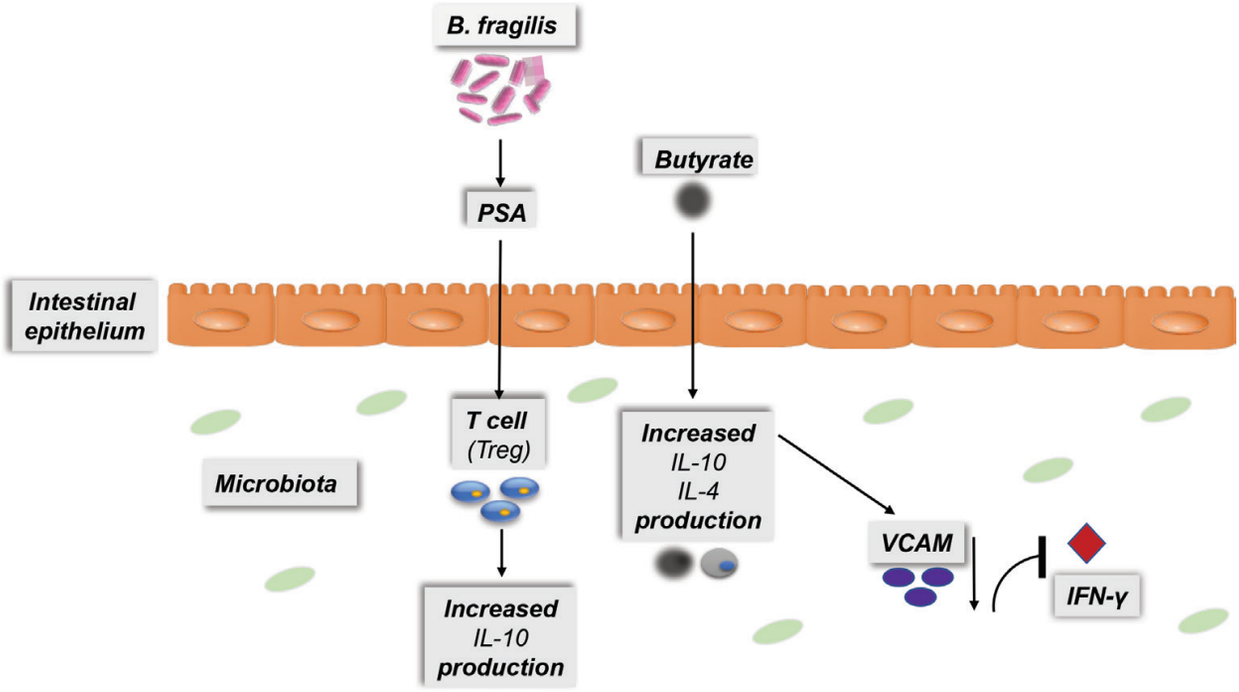

Figure 3. Regulation of inflammation by Treg, through IL-10 and IL-4 generated against microbial stimuli, *Bacteroides fragilis* specifically its polysaccharide a (PSA).

danger or, in the opposite direction, the sensation of satiety that our brain perceives when we have ingested a certain quantity of food [20] (**Figure 4**).

The intestine, besides having a nervous system of its own, is also an ecosystem. Colonization by the intestinal microbiota affects the brain development of mammals and their behavior during adulthood [21]. Through measurements of motor activity and behavior related to anxiety, it has been demonstrated in mice that the microbial colonization process triggers signaling mechanisms that affect these neural circuits, so that the gut microbiota can influence normal brain development and behavioral functions [22], and the microbiota is capable of modifying the expression of some risk genes or is part of the mechanisms that alter the cognitive functions observed in patients with gastrointestinal diseases [22]. The alteration of this microbiota and gut-brain axis could explain some of the mechanisms of the pathogenesis of diverse cerebral diseases like, for example, Parkinson's disease, multiple sclerosis, depression, Alzheimer's disease, etc., although today its etiology still remains unknown [23]. In studies in mice, it has been shown that alterations in the intestinal microbiota could be responsible for alterations in social behavior and that supplementation with probiotics such as *B. fragilis* administered in the early stages of adolescence in mice could reduce brain alterations [23]. But the influence of the microbiota on the brain is unknown in detail, beyond the simple examples previously uncovered, which do not reflect the full extent of this relationship. Much more articulate is the observation of what happens to mice whose digestive tract has remained sterile throughout their development as "germ-free" [23]. It has been found that in these animals the microglia does not mature properly and it is very difficult to provoke experimental allergic encephalitis and that these mice also have changes in their behavior, with increased responses to stress, and most amazingly, certain areas of their brain, like the amygdala and hippocampus, show structural differences.

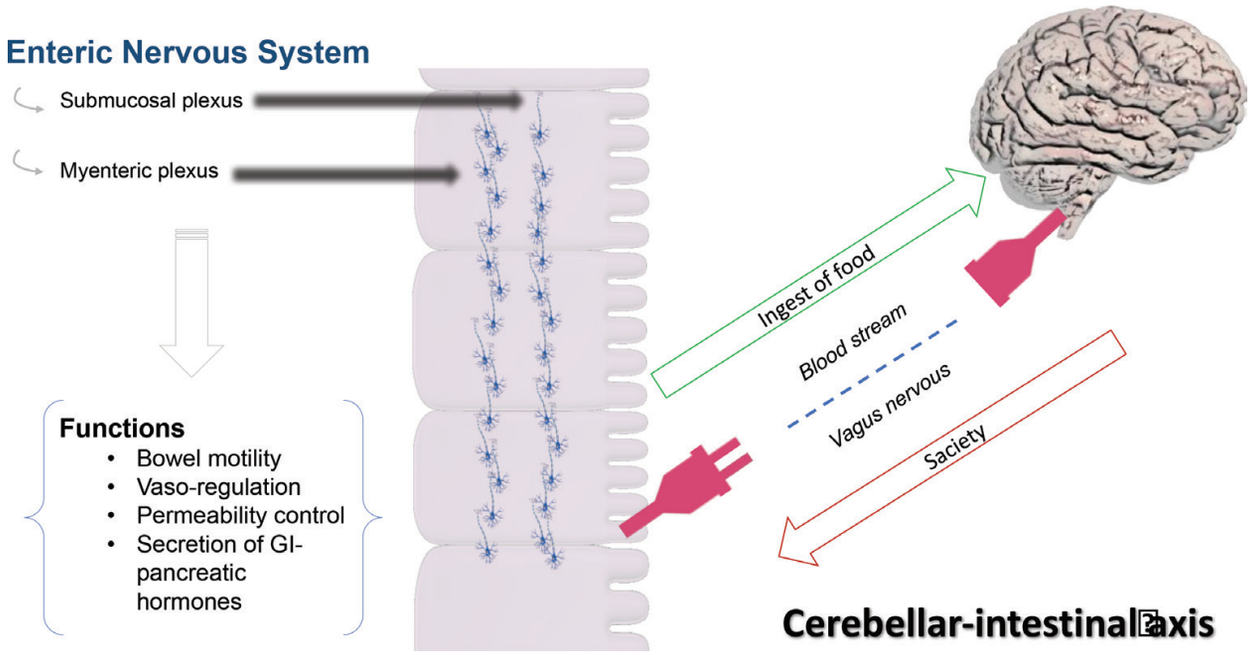

Figure 4. Key aspects of gastrointestinal physiology are controlled by the enteric nervous system, which is composed of neurons and glial cells. These cells of the enteric nervous system are connected to the central nervous system (in a bidirectional way). As an example, when we ingest food, through neural pathways and immune and endocrine mechanisms, we will perceive the sensation of satiety.

Given the role of the *vagus* nerve in the communication of signals between the gut and the brain, many investigations that seek to explore the connections between microorganisms and the CNS have examined the function of this nerve. Thus, it has been shown that both pathogenic and nonpathogenic bacteria appear to activate the *vagus* nerve. For example, subdiaphragmatic vagotomy attenuates the expression of c-fos in rats inoculated with *Salmonella typhimurium*; the combination of a *C. rodentium* infection and stress causes an increase in the activation of the vagal ganglion in mice; intraduodenal injection of *L. lactis* La1 activates the gastric vagal nerve in rats; subdiaphragmatic vagotomy blocks the anxiolytic and antidepressive effects of the chronic ingestion of *L. rhamnosus* in normal adult mice while avoiding the associated alterations in the expression of GABAAα2 mRNA in the amygdala; and vagotomy abolishes the ability of *B. longum* to attenuate anxiety induced by DSS colitis.

3. The second brain

The relationship between the brain, the emotions, and the digestive tract is intense. So much so that many scientists refer to the intestine as the "second brain" or "gut-brain," since the digestive tract contains a very complex neural network with a neuronal function very similar to the activity of the head.

The presence of receptors to various neurotransmitters in the intestine has been demonstrated: it is known that some intestinal molecules, such as serotonin 5-HT, can modulate the pathogenic potential of *Pseudomonas fluorescens* by affecting its motility and pyoverdine production but without affecting its growth. It has been reported that gut microbiota can control the

tryptophan metabolism of the host by enhancing the fraction of tryptophan available for the kynurenine route and decreasing the amount available for 5-HT synthesis [24]. Free fatty acid receptor 3 (FFAR3) receptors for short-chain fatty acids (SCFAs) have been detected in submucosal and myenteric ganglia, and the responsiveness of enteric neurons to glucose, amino acids, and fatty acids has been demonstrated [24]. For example, there are receptors to the stress hormones epinephrine and norepinephrine, and this increases by more than four orders of magnitude in the human gut in the presence of *Clostridium*/*Bacteroides*. A recent report has demonstrated that *Vibrio cholerae* can respond to epinephrine and norepinephrine (enhancing the growth rate, swimming motility, and production of virulence factors such as iron sequestering phenotypes) by means of specific sensor proteins [24]. Less information is available on prokaryotic Glu receptors; however, 100 prokaryotic channel proteins with putative Glu-binding domains have recently been identified through a bioinformatic study. Among them, 22 proteins have been found to be homologs of vertebrate ionotropic Glu receptors. Multiple Glu receptor types (including ionotropic, types 1 and 4 metabotropic receptors, and heterodimeric TAS1R1 + TAS1R3, L-Glu taste receptors) have been detected in gastrointestinal (GI) epithelial cells and/or enteric neurons in the stomach, small intestine, and colon. And mGlu4 receptors have been detected in the mucosa of both the gastric *antrum* and duodenum, while both mGlu4 and mGlu7 receptors have been identified in the colon epithelium. A role of mGluRs in the human colon in the control of colon peristalsis and electrolyte transport has been described. High levels of mGlu7 and mGlu8 have been detected in myenteric neurons, where they are possibly involved in the regulation of gut motility [24]. The $GABA_B$ receptors are abundantly expressed in the GI tract. GABA and its ionotropic and metabotropic receptors are widely distributed throughout the ENS, in both submucosal and myenteric neurons, from the stomach to the ileum. The release of 5-HT by endothelial cells in the small intestine of guinea pig is modulated by $GABA_A$ and $GABA_B$ receptors. Involvement of $GABA_B$ receptors in modulation of sensitivity of vagal and spinal afferents has been reported [24].

To think that the intestine acts as a second brain is not something new if we look at the more oriental cultures [25]. For them, the belly was and is the center of the vital energy of the organism where they integrate mind and body. The small brain that we have in the gut works in connection with the big brain in the skull and partly determines our mental state and plays a key role in certain diseases that affect other parts of the body. In addition to neurons, all types of neurotransmitters in the brain are present in the digestive system. The enteric nervous system secretes the same substances as those found in our central nervous system. There are nerve pathways that specifically connect the brain areas related to our emotions and thoughts, the immune system, the endocrine system, and the enteric nervous system to each other. When there is a disfunction between any of these conected systems, pathological symptoms may appear in any of the others even without direct damage to them [25, 26] (**Figure 5**).

Is well known that 90% of total body serotonin is synthesized in the intestine and it has a direct implication in gastrointestinal physiology. In this sense, our diet is important because this serotonin is formed from a tryptophan, an essential amino acid, which is only obtained through food. Here we begin to see the relationship between the brain, the intestine, and the diet [27].

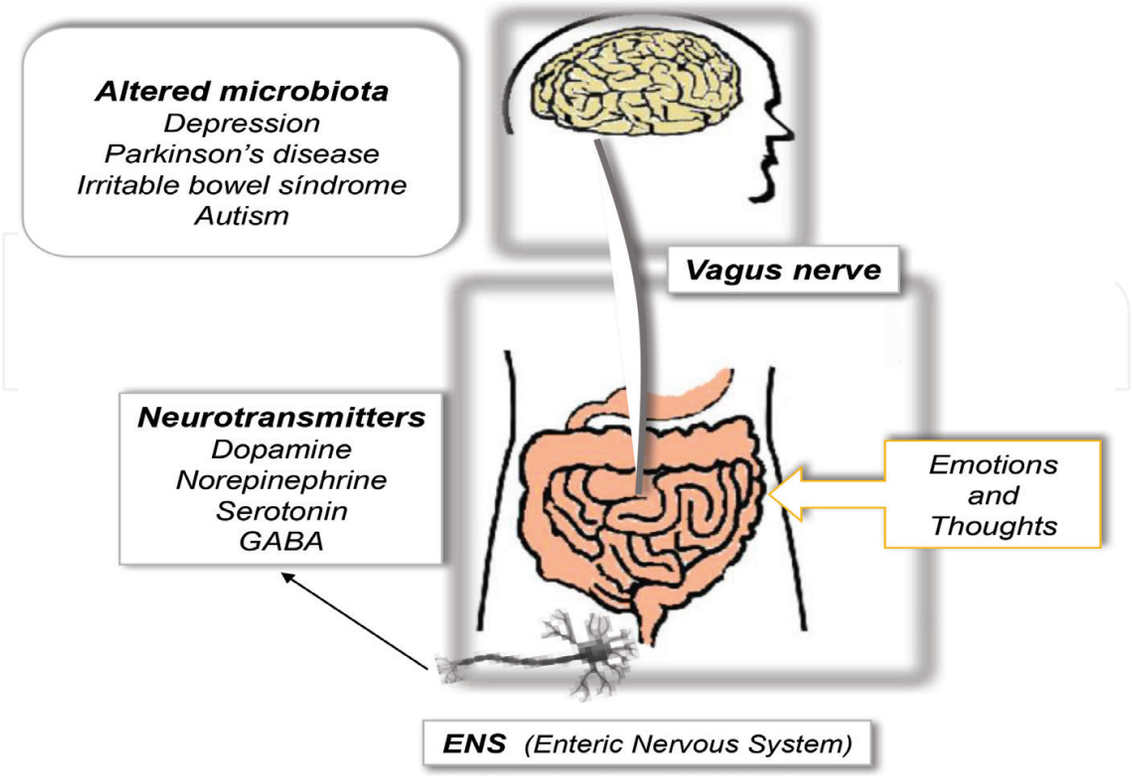

Figure 5. Second brain. In the intestine we can find neurotransmitters that are exported to the CNS.

3.1. Is the gut autonomous?

The gut has the ability to work in two ways: independently and in connection with the brain. This connection to the brain (gut-brain axis) is bidirectional in that it goes from the brain to the intestine and vice versa. We have long known that many emotional alterations, psychological issues, affect at the intestinal level, such as feelings, sadness, and loss of, or increase in, appetite. We mentioned above that serotonin in the intestine works as a neurotransmitter in the inhibition of anger, aggression, body temperature, mood, sleep, vomiting, and appetite and is responsible for keeping our state of equilibrium in balance (its different levels in our organism are related to depression). Here, more than 50% of the activity of dopamine also occurs: a neurotransmitter that among its functions regulates the levels of pleasure in our brain. Its secretion occurs during pleasant situations and encourages us to seek that activity or pleasing occupation. This means that food, sex, and various drugs are also stimulants for the secretion of dopamine in the brain in certain areas such as the nucleus accumbens and the prefrontal cortex [28].

3.2. Some other interactions in the gut-brain axis

Memory: the protein that burns the body fat is also responsible for memory, which is why obese people are more prone to dementia. **Well-being**: mood is lodged in the stomach since 90% of serotonin, the "happiness hormone," is produced and stored there. **Sleep**: when we

relax the gut, our stomach neurons produce benzodiazepines that relax and induce sleep. **Stress:** in an emergency the brain takes energy from the bowel, and the guts send signals like upset stomach. **Gluttony:** the trillions of bacteria that lodge in the gut choose their own nutrients to thrive, and sometimes they are greedier than you. **Fear:** panic causes the brain to frighten the large intestine. It no longer has time to absorb fluid, and the result is diarrhea. The relationship between the brain, the microbiota, and the emotions is little investigated. There are very preliminary studies. Knowing exactly, at the clinical level, how it can impact is difficult to pin down. There are studies that point to the idea of using probiotics as a complementary treatment to drugs that treat anxiety or depressive disorders, as they may help amplify the effects, but this is still quite preliminary. Probiotics or foods rich in healthy bacteria, such as yogurts and other fermented milks, exhibited a positive influence on our behavior: *Lactobacillus* and *Bifidobacterium* bacteria are capable of producing gamma-aminobutyric acid, a neurotransmitter of the brain that regulates many psychological processes and whose dysfunction is related to anxiety and depression [29].

4. Neurodegenerative diseases

Knowledge of the possible relationship of the microbiota with frequent neurological diseases is still new. Several studies have been carried out to analyze the type of microbiota and many neurological diseases. Recently, changes in the microbiota have been linked to the onset of Parkinson's disease (PD). A current theory proposes PD as a disease that progresses parallel to the propagation of insoluble protein accumulations in the nervous system [30]. The enteric autonomic nervous system could be one of the starting points of this pathological accumulation of proteins, and a change in the microbiota that increases local inflammation and oxidative stress could initiate the pathological cascade, similar to what happens in experimental models. In addition, digestive autonomic changes, such as precocious constipation, are almost a preclinical constant in PD, and all this would support this hypothesis [31]. In certain neurological diseases, immunomodulatory drugs that seek to reestablish a situation in which the anti-inflammatory cytokines predominate in the system are used. The important relationship of the intestinal microbiota with the immune system offers the possibility of acting on the intestinal bacteria to achieve this change. Experimentally, and through this mechanism, treatment with certain antibiotics has influenced the prognosis of cerebral infarctions in experimental animals [32]. It is possible that the lack of knowledge about the etiopathogenesis of many neurological diseases and the gut microbiota prevents us from seeing the magnitude of the relationship between them and the possibilities of intervention to protect health or prevent or ameliorate diseases. Beyond the knowledge of all agents of the microbiota, their genes, and their functions, it is even more important to identify the molecules they produce and their effects on metabolism. Advances in proteomics and metabolomics with practical applications in the daily clinic may be the key to establishing microbiota profiles and the different relationships with neuronal diseases [33] (**Figure 6**).

4.1. Parkinson's disease

Recently, possible changes in the microbiota have been linked to the onset of Parkinson's disease (PD). A current theory suggests that PD is a disease that progresses parallel to the

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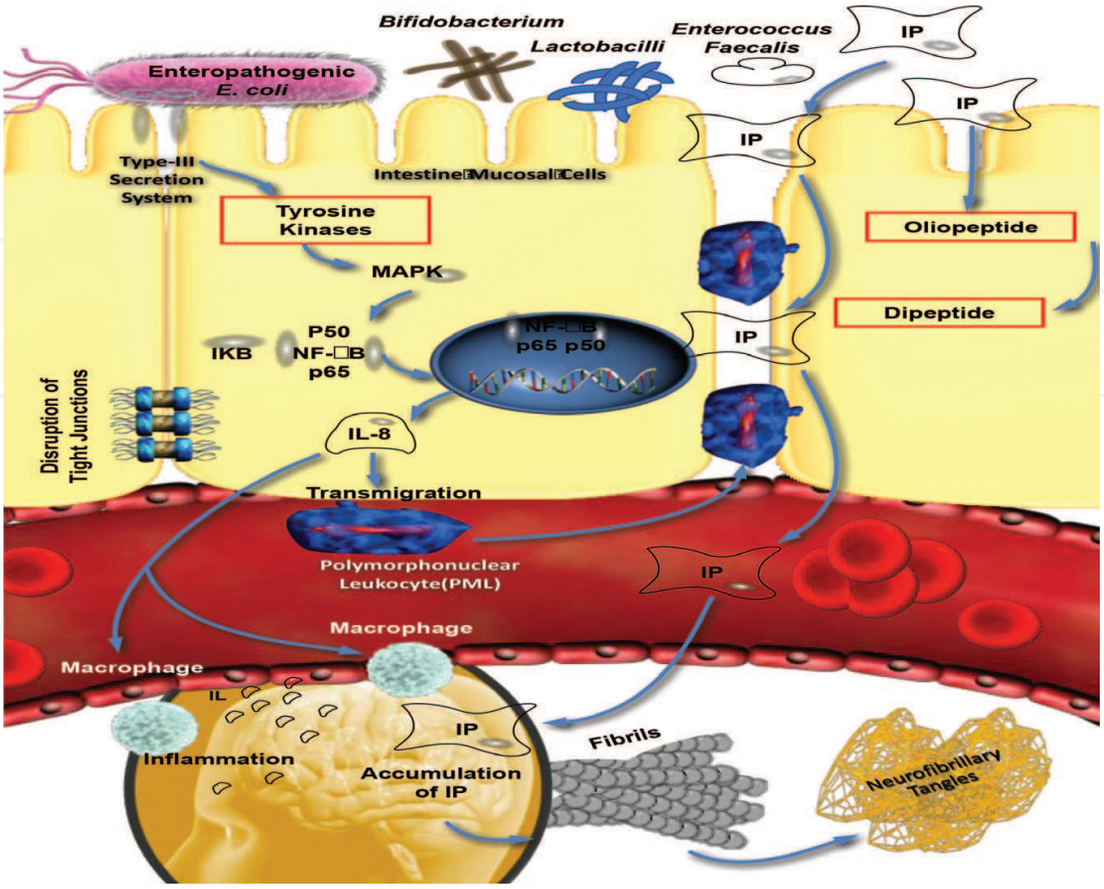

Figure 6. This figure shows the effect of the microbiota on the increased inflammation that results in Parkinson's disease. In addition, the accumulation of insoluble proteins may be an explanation for the pathological accumulation of proteins in the nervous system. IP, insoluble protein; NF-kB, nuclear factor kappa enhancer of activated B cells; MAPK, mitogen-activated protein kinase; P50, cytochromes P450; IKB, inhibitor of kB; IL-8, interleukin 8. This image is a modification of QIAGEN's original at www.QIAGEN.com/es/shop/genes-and-pathways/pathway-details/?pwid=29.

propagation of accumulations of insoluble proteins in the nervous system. The enteric autonomic nervous system could be one of the starting points of this protein accumulation. A change in the microbiota that increased local inflammation and oxidative stress could start the pathological cascade, similar to what happens in experimental models of PD. In addition, the autonomic changes to digestive disorders, such as precocious constipation, are almost a preclinical constant in PD, and all this would support this hypothesis [34]. In Parkinson's disease, a direct correlation between the amount of *Enterobacteriaceae* microbes in the gut of patients, and the degree of severity in the problems of balance and mobility was detected: Scheperjans explains that the abundance of *Enterobacteriaceae* was related to a high degree of postural instability and gait difficulty; therefore, there is a connection between the intestinal microbiota and the motor symptoms of our patients [35]. The question is, if the differences are permanent and whether the intestinal bacteria are linked to the progression of the disease and therefore to its prognosis. This fact implies that if we can establish the basis of the relationship between the intestinal microbiota and PD we will be in a much better position for developing new strategies for prevention of the disease and its progression [35, 36]. Microbial metabolites have been shown to influence the basic physiology of the blood-brain barrier. Intestinal microorganisms break down complex carbohydrates into short chains of fatty acids with a set of effects; for example, fatty butyric acid strengthens the blood-brain

barrier by adjusting the connections between cells. Also, there are recent studies of neurotransmitters that could enable them to communicate with neurons. For example, it has been studied how certain metabolites of the bacterial microbiota promote the production of serotonin in the cells lining the colon, an interesting finding given that some antidepressant drugs work promoting serotonin at the junctions between neurons [37]. Even though the association of gastrointestinal disorders and PD has been studied extensively, it does not occur in the same way with the study of the influence of the gut microbiota on PD. The first studies in this regard were limited to evaluating the association between *Helicobacter pylori* infestation and PD. These investigations were based on the causal role of *H. pylori* in a variety of human diseases including chronic gastritis, peptic ulcers, and stomach cancer and in the well-known association of PD with gastric ulcers [38–41]. Several studies have demonstrated the existence of an association between PD and levels of *H. pylori* infestation. In a small, case-control study, a fivefold increase in *H. pylori* antibody levels was observed among patients older than 80 years of age with Parkinsonian manifestations [42]. Similarly, in patients with PD, a threefold increase in *H. pylori* antibody levels compared to control subjects has been reported. More recently, in an extensive study conducted in Denmark that included a total of 4484 PD patients diagnosed between 2001 and 2008, and a total of 22,416 controls, it was shown that chronic infections with *H. pylori* or the presence of gastritis contributed to PD or that there are pathologies related to this disease that precede the occurrence of motor symptoms. Additionally, it has been shown that the eradication of *H. pylori* infections decreases PD symptoms [43]. Composition of the *fecal microbiome* has been compared between patients with PD and control subjects in the District Hospital of Helsinki and Uusimaa [44]. In this study, 72 patients with idiopathic PD and an equal number of control individuals matched by sex and age were included. The existence of alterations of the intestinal microbiome in patients with PD was demonstrated, and such alterations were associated to the motor phenotype. The average abundance of the *Prevotellaceae* was reduced in 77.6% in the patients with PD in comparison with the control subjects. The relative abundance of *Prevotellaceae* of 6.5% or less had a sensitivity of 86.1% and a specificity of 38.9%, while a classifier obtained by logistic regression based on the abundance of four bacterial families and the severity of constipation identified PD patients with a sensitivity of 66.7% and a specificity of 90.3%. On the other hand, the relative abundance of *Enterobacteriaceae* was positively associated with the severity of postural instability and difficulty walking. The enterotype of the intestinal microbiota represented by *Prevotella* has been associated with increased levels of short-chain fatty acids with a neuroactive health-promoting function and with a high capacity for the synthesis of thiamine and folate. From these observations, it has been proposed that the observed decrease in the abundance of *Prevotella* could be associated to the previously reported decrease in the levels of these vitamins in PD patients and that the supplementation of these vitamins and short-chain fatty acids may have potential therapeutic effects in patients with PD.

4.2. Demyelinating diseases

There is a broad line of research on the relationship between intestinal microbiota and optic neuromyelitis and mainly with experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), and an emerging line that is trying to extrapolate the results of the EAE to the MS. Patients with aquaporin-positive neuromyelitis optical have a

higher serum antibody response to gastrointestinal antigens than healthy controls, especially in extensive myelitis, which seems to be related to the control of the microbiota on autoimmune inflammation [45]. The peripheral existence of aquaporin-specific T cells, capable of developing Th responses, which showed cross-reactivity with a homologous sequence in an epitope present in *Clostridium perfringens*, representative of the commensal flora, has also been demonstrated, insinuating a mechanism of responsible cellular mimicry of activating said Th response [46]. Demyelination usually occurs as a phenomenon secondary to an infectious disorder or a toxin. In the primary demyelinating processes, its cause is unknown, but an autoimmune mechanism is thought to occur because its appearance sometimes follows a virosis, an antiviral vaccination, or an alteration of the microbiota. Demyelination tends to be segmental or patchy and often affects multiple zones at once or in succession. Often remyelination occurs, with repair, regeneration, and complete recovery of nerve functions. However, any extensive loss of myelin is often followed by axonal degeneration and, often, also of cellular soma, and both processes can be irreversible. Demyelination should be considered for any patient suffering from a neurological deficit without any other explanation. Primary demyelinating disorders are suggested by:

- Diffuse or multifocal deficits
- Sudden onset, especially in young adults
- Appearance within weeks after an infection or vaccination
- A course with ups and downs
- Symptoms indicative of a specific demyelinating process (e.g., unexplained optic neuritis or internuclear ophthalmoplegia that suggests multiple sclerosis).

4.3. Multiple sclerosis

MS is caused by a combination of genetic and environmental factors. Among the causal factors, it could be that a certain individual with certain bacterial microbiota could more easily develop the disease. However, if we want to identify what happens with the gut microbiota, we might also include this within the environmental causes of the individual and understand the conditioning of the disease. The first line of work is to identify if the bacterial flora plays a role in multiple sclerosis and the second, if it plays a role, is to determine which bacteria are protective and which are harmful [21, 47]. This surprising finding was made possible by the recent development of genetically modified mice. In the absence of exposure to any external influences, inflammatory reactions emerge in the brains of these animals that are similar to those associated with multiple sclerosis in humans, yet this only occurs when mice have intact gut microbiota [48]. Mice without microorganisms in their gut, which remained in a sterile environment, remained healthy. When vaccinated, animals bred under sterile conditions, with normal intestinal microorganisms, also became ill; however, the gut microbiota influences the immune systems in the digestive tract, and mice without intestinal flora have fewer T cells. On the other hand, the spleen of these animals produces fewer inflammatory substances, like cytokines, and, in addition, B cells produce few or no antibodies against myelin and restore the microbiota of mice, and their T cells and B cells increase their production of cytokines and antibodies [49].

Another group of habitual commensal bacteria related to the regulation of the immune response and studied in EAE is lactic acid bacteria. Within this group is *Pediococcus acidilactici,* which is administered orally and induces an IL-10 mediated response that decreases the severity of EAE both therapeutically and prophylactically, through the inhibition of IL-17 and IFN-γ and a decrease in cellular infiltrates in the CNS. In this case, the responsible, related lymphocytes, rather than being TCD4 + FoxP3+ (with a slight increase), were the type 1 regulatory T lymphocytes (Treg1) [50]. The potential use of probiotics in EAE has been investigated. *Bifidobacterium animalis* has been used during lactation of rodents that were later induced for EAE, with a reduction in the duration of clinical symptoms, curiously only in male mice. Using a combination of three strains of *Lactobacillus*, they demonstrated that the combination, but not each separately, reversed EAE in mice, associated with an increase in TregFoxP3 + lymphocytes and IL-4, IL-10 and TGFβ1 in the nodules of lymphatic vessels and the spleen [50]. Using other mixtures of probiotics (*Lactobacillus, Bifidobacterium, and Streptococcus*), similar results have been obtained, as well as the association of IL-10 and the development of Treg in the mechanism, which, as previously mentioned, leads to a lower polarization of lymphocytes T helper toward Th1/Th1 [51]. Scientists would now like to analyze the total microbial genome of patients with multiple sclerosis and thus identify differences in the intestinal flora of healthy individuals and patients with multiple sclerosis. Scientists are sure that the microbiota can also trigger an exaggerated reaction of the immune system against the myelin layer in people with a genetic predisposition for multiple sclerosis. Therefore, nutrition can play a central role in the disease since diet largely determines the bacteria that colonize the intestine. Changing eating habits could explain, for example, why the incidence of multiple sclerosis has increased in Asian countries in recent years. Apparently the immune system is activated in two stages. First, the T cells in the lymphatic vessels of the gut are activated and proliferate together with the proteins of the surface of the myelin layer, and these stimulate the B cells to form pathogenic antibodies. Both processes trigger inflammatory reactions in the brain that progressively destroy the myelin layer, a process that is very similar to the way multiple sclerosis develops in humans [52]. How does the intestinal flora influence the health of the brain? This is an area that arouses more and more interest in those who work with neurodegenerative diseases, and understanding this balance and how to control it can open the way to a new type of probiotic-based therapy (foods that contain live bacteria that may be beneficial), synbiotics which stimulate the growth of existing beneficial bacteria (**Figure 7**).

In another study, it has been shown that there is a link between intestinal commensal bacteria and autoimmune pathologies in murine models of MS. In one study, 34 pairs of monozygotic twins were selected, one ill and the other not. This choice permits eliminating the influence of genetic factors and reducing the environmental factors in the appearance of MS. In advance, they compared fecal microbiota without finding important differences, except for an excess of *Akkermansia* in untreated sick subjects. To test the functionality of these intestinal floras, they selected five pairs of twins. The intestinal microbiota of each individual was transferred to rodents predisposed to autoimmune encephalomyelitis, which is the animal model of MS. This transplant triggered the disease in more than 60% of the animals that received microbiota from subjects with MS, compared to 30% in those who received the microbiota of healthy subjects. The analysis of the intestinal microbiota of the transplanted animals revealed an increase of the *Sutterella* deficit in the animals that received the microbiota of subjects with MS. However, the

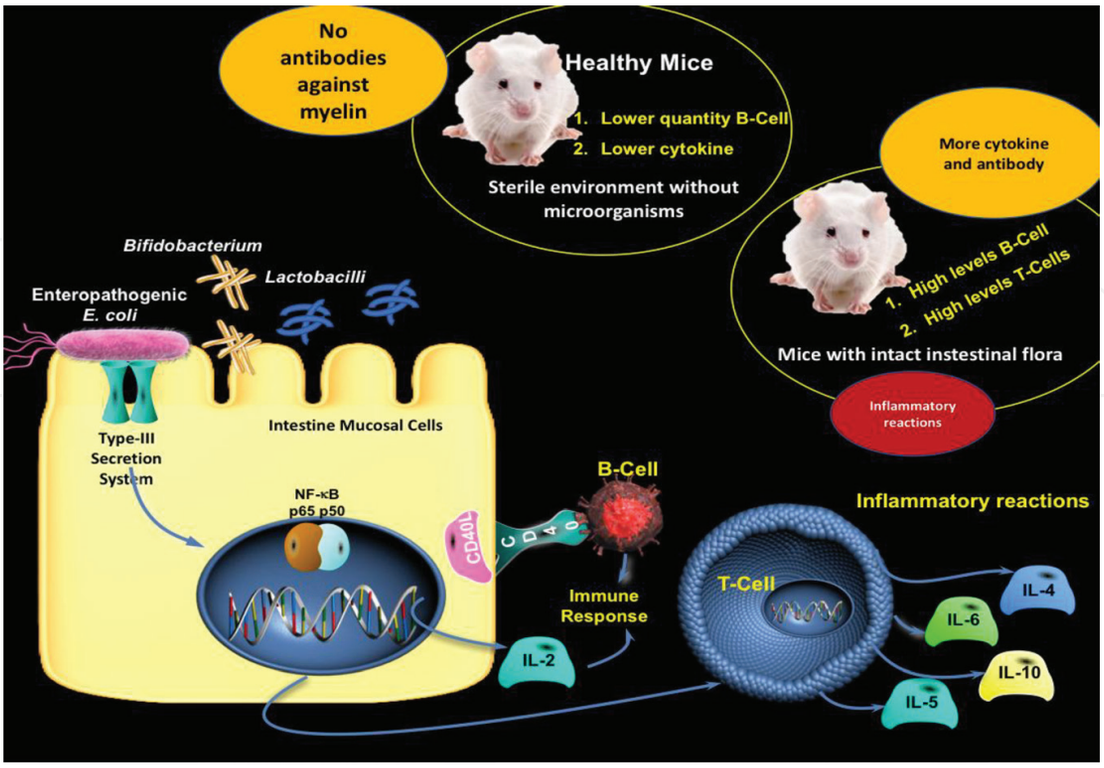

Figure 7. This figure shows an inflammatory pathway in mice very similar to that developed in MS patients. This inflammatory reaction is associated with modifications in the intestinal microbiota in mice. This image is a modification of QIAGEN's at www.QIAGEN.com/es/shop/genes-and-pathways/pathway-details/?pwid=29.

presence of this bacterium was associated with a better defense against inflammatory diseases. At the immune level, the study shows a deficit in the production of interleukin 10 in the animals that received the EM microbiota. In parallel, the blockade of this cytokine in rodents that received the healthy microbiota increased the incidence of autoimmune encephalomyelitis, which suggests that this molecule has a regulatory role in autoimmune diseases of the central nervous system [53].

5. Alteration in protein conformation: microbiota and nervous system

One of the problems that exist in common in several neurological diseases is alterations in the folding of proteins. It is the process by which the sequence of amino acids adopts a three-dimensional structure that constitutes its native form. In some proteins, in addition to the native and unfolding states, there are partially folded states known as intermediates. The concentration of proteins in the cytoplasm is high. Despite this, proteins in the native state are not normally added. On the other hand, the denatured state has a very short half-life. In this sense, various evidences strongly suggest that the aggregation is due to the specific association of non-native states. Several diseases that exhibit deposits of aggregated proteins have been associated with genetic factors, that is, point mutations in the protein that cause their aggregation. How do mutations facilitate aggregation? In physicochemical terms, mutations

can alter the stability or speed of interconversion between the native form and the fibrillar form; denaturing conditions have been found that favor the presence of non-native conformations, which act as precursors of the formation of the altered proteins. Another coincidence that exists in several neuropathologies is that we know what is happening (not always everything), but the root cause, what or who initiates, is unknown; we mention that this is multifactorial and, in it, we cover part of our ignorance. A particular case is the stabilization of the folding of α-synuclein, which is involved in Parkinson's disease, dementia associated with Lewy bodies, and the variant of Alzheimer's disease also associated with Lewy bodies.

As mentioned above, a large number of proteins without homology, or not associated with diseases, present conformational structural alterations. If so, why are not all proteins added? And at this point is when we have the obligation to analyze these neuropathologies in a systemic way. Our body has taken thousands of years to perfect itself, and we often forget that the set of systems that make up our body is a unit and that this is in constant interaction between its organs and systems but also with the environment and other organisms that are part of it, including the microbiota, which is currently telling us how there are close dialogs between our gut and our nervous system and other systems in a constant back and forth of information in both directions. In most diseases described above, their diagnosis is another challenge because only if we are strict enough we will only say probable Parkinson's disease, and this will only be corroborated with its *postmortem* study, the same happens with Alzheimer's. When classifying these diseases, we reach another coincidence in most of them—sporadic or genetic—and their percentages between them are similar 85–90% vs. 15–10% (respectively). Where the genetic and the environment are usually variations of the same symphony, at the end of the day, the relationship between the nervous system and the microbiota of the gut is a fact that invites us to reflect on the individuality of the systems and the need for research in translational medicine.

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