The Intestine – Anatomy, immunology, barrier function, microbiome and central regulations

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SUMMARY

The intestine, with its complex anatomy and intricate physiology, plays a fundamental role in maintaining overall health and well-being. Understanding the diverse functions of the intestine and the factors that can disrupt them is crucial for preventing and managing a wide range of diseases and pathological conditions. This review article explores the multifaceted role of the intestine in regulating human health across physiological and pathological states.

A systematic search for relevant literature was conducted using the following electronic databases: PubMed, Web of Science, Scopus, and Google Scholar. The search terms included a combination of keywords related to the intestine, its functions, and relevant conditions (e.g., "intestine", "mucosa", "gut barrier dysfunction", "microbiome", "gut-brain axis", "central regulations", "bacterial translocation"). For this review, we included only **peer-reviewed, full-text articles published in English** that directly addressed the intestine's role in regulating human health across physiological and pathological states. This review explores the intestine's unique structure and its critical neuroregulatory connection with the brain, the gut-brain axis (GBA). Subsequently, we focus on the pathological consequences of gut barrier dysfunction and the microbiome's contribution to both intestinal and extraintestinal health. Finally, we address the processes activated within the intestine during stress responses.

Key words: Intestine – Mucosa – Gut barrier dysfunction – Microbiome – Gut-brain axis – Central regulations – Bacterial translocation

LIST OF ABBREVIATIONS

GBA - gut-brain axis
GIT - gastrointestinal tract
GI - gastrointestinal
ANS - autonomic nervous system
ENS - enteric nervous system
IBD - inflammatory bowel diseases
MUC2 mucin – high-molecular weight glycoprotein
TLR2 – toll-like receptor 2
TLR4 – toll-like receptor 4
TLR7 – toll-like receptor 7
TLR9 – toll-like receptor 9
NOD1 – intracellular pattern-recognition receptor 1
NOD2 – intracellular pattern-recognition receptor 2

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Submitted: June 19, 2024. **Accepted:** September 2, 2024

https://doi.org/10.52083/SUQE1748

MyD88 - protein, myeloid differentiation primary response 88

IBS – irritable bowel syndrome

MALT – mucosa-associated lymphoid tissue

GALT – GIT-associated lymphoid tissue

FAE – follicle-associated epithelia

M cells – microfold cells

MNP – mononuclear phagocytes

NLRC4 - an adaptor that is activated in response to activation of a NAIP receptor

NAIP – cytosolic receptor

ICC – intestinal cells of Cajal

SIBO – small intestinal bacterial growth

TMA – trimethylamine

TMAO – trimethylamine N-oxide

LCA – lithocholic acid

ICs - immune checkpoints

ICIs – immune checkpoint inhibitors

TME – tumor microenvironment

ALS – amyotrophic lateral sclerosis

miRNA – microRNA

GDF5 – differentiation factor 5

ACVR – activation A receptor

OA – osteoarthritis

DDH – developmental dysplasia of the hip

ncRNAs – non-coding RNAs

MCs – mast cells

INTRODUCTION

The intestine, once viewed primarily as a digestive organ, has emerged as a critical regulator of human health (Kataoka, 2016; Valdes et al., 2018). Beyond its well-known role in nutrient absorption, this complex organ manages a multitude of functions. It acts as a selective barrier, filtering out harmful pathogens while allowing essential nutrients to pass (Ahluwalia et al., 2017). Additionally, the intestine maintains a fascinating bidirectional communication pathway with the brain, known as the gut-brain axis (GBA) (Mayer et al., 2022). This complex connection influences not only digestion and nutrient absorption but also mood and cognitive function (Quigley, 2017).

Furthermore, residing within the intestine is a diverse community of microorganisms known as the gut microbiome (Jovel et al., 2018). This microbial ecosystem plays a vital role in digestion, immune function, and even mental well-being (Generoso et al., 2020). Disruptions in the gut microbiome have been associated with various health issues (Di Vincenzo et al., 2024).

This review investigates the complicated workings of the intestine, exploring its structure, the gut-brain axis, and the microbiome.

ANATOMY AND PHYSIOLOGY OF THE INTESTINE

The digestive tract or gastrointestinal tract (GIT) is a continuous tube that extends from the oral cavity to the anus, around 10 meters in length. It is composed of multiple organs (subunits), each serving vital functions, the most prominent of which is to efficiently process food. Others include immune and regulatory roles, with an emphasis on the GIT-microbiome equilibrium (Peate, 2021; Régnier et al., 2021; Ramires et al., 2022).

The small intestine consists of the duodenum, jejunum, and ileum. The first two are anatomically divided at the duodenojejunal flexure, supported by the ligament of Trietz (Nassar et al., 2021). However, the demarcation between the jejunum and ileum is less distinct (Odze and Goldblum, 2014). Despite this, some distinct features become apparent along the intestine, progressing from proximal to distal. For example, the jejunum has a thicker wall in the proximal region and greater mesenteric adipose tissue in the distal region. The jejunum also has a smaller diameter and more prominent and palpable circular folds. These features are helpful for surgeons in identifying a specific bowel segment (Mills, 2019).

The large intestine, also known as the colon, can be roughly divided into two halves: the right and left colon. This division corresponds to the embryologic derivation of the midgut (mesenteron) on the right and the hindgut (proctodaeum) on the left, and is consistent with distinguishing blood supply, innervation, and venous drainage. The right colon includes the caecum, appendix, ascending and proximal transverse, while the left

is represented as the distal transverse, descending, sigmoid colon, and rectum (Mills, 2019).

On histology, despite functional differences, most of the organs of GIT have a similar wall structure, consisting of a mucosa, a submucosa, a muscular layer, and a serosa or adventitia on the surface (see Fig. 1).

The mucosa itself further divides into the surface epithelium, lamina propria mucosae, and muscularis mucosae. The latter is a thin double layer of inner circular and outer longitudinal muscle which constitutes the border between the mucosa and submucosa (Uchida and Kamikawa, 2007). This unique structure is of utmost importance for the histological diagnosis of tumor invasion, as it marks the clinical transition between T0 tumors, which are confined to the mucosa, and T1 tumors, invading the submucosa (Loughrey et al., 2018). The lamina propria, a layer of loose connective tissue, contains small blood vessels, lymphatics, and a resident population of hematopoietic cells (Odze and Goldblum, 2014). The density of these immune cells varies along the intestine, with a characteristic decrease towards the left colon (Mills, 2019). These immune cells will serve as a part of the intestinal immune system (Odze and Goldblum, 2014).

The submucosa consists of loose connective tissue that houses blood and lymph vessels in addition to the submucosal nervous plexus. Thus, it is referred to as "tela submucosa" rather than "tunica".

The muscularis propria is made up of two sublayers: an inner circular layer and an outer longitudinal layer (Loughrey et al., 2018). The thickness of these layers varies depending on the segment and physiological contraction (Mills, 2019). In between these layers, there is the myenteric nervous plexus, which coordinates their contraction (Wattchow et al., 2008).

The serosa is a smooth membrane composed of a thin layer of connective tissue and a thin layer of cells that produce serous fluid to lubricate internal structures. This lubricating fluid helps re-

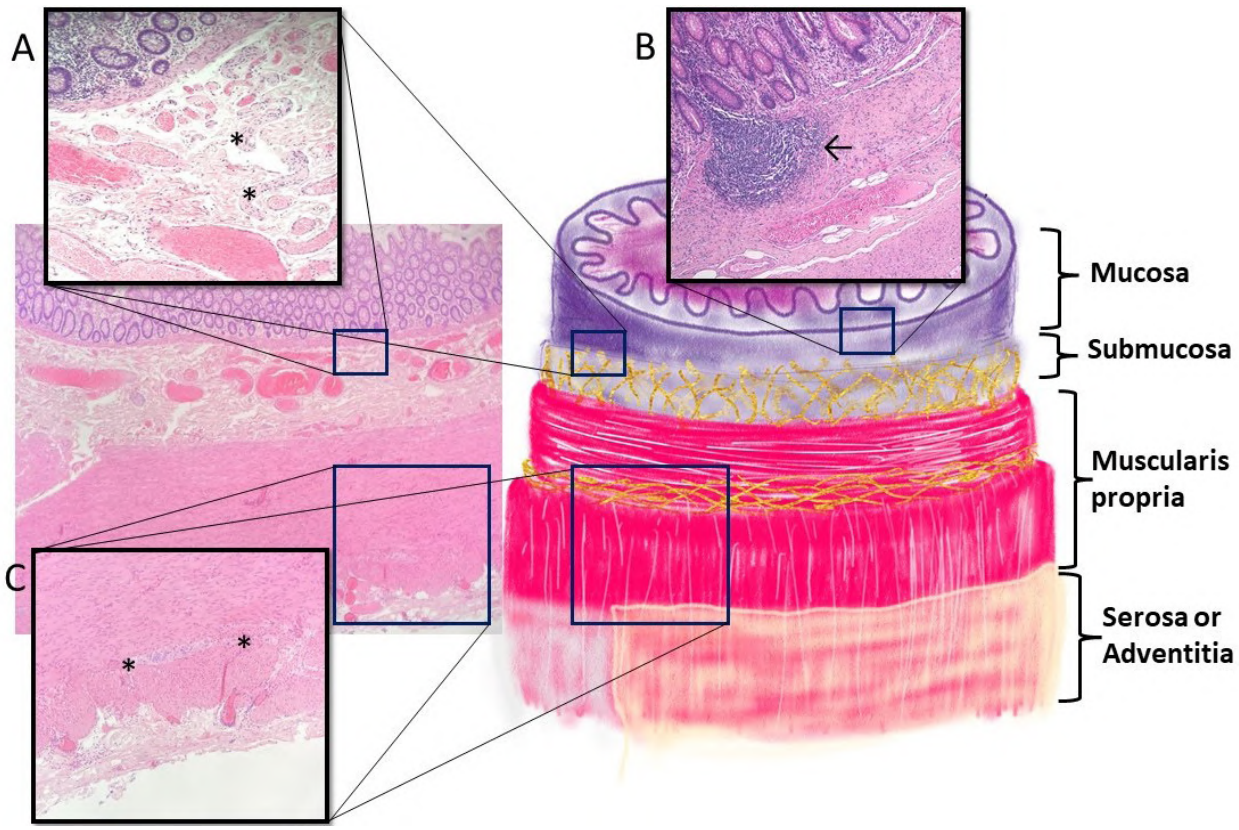

Fig. 1.- Histology and diagram structure of the intestinal wall: **A.** submucosa: lose connective tissue rich in the vasculature and nervous bundles of the submucosal plexus (asterisk). **B.** mucosal-submucosal junction. A lymphoid aggregate of the MALT is depicted (arrow), in this case protruding through muscularis mucosa into the submucosal space. **C.** Muscularis propria, subserosa, and serosa: ganglion of the myenteric plexus is appreciated between the inner circular and outer longitudinal muscle layers (asterisk).

duce friction during movement. The serosa covers intraperitoneal organs, while the adventitia covers retroperitoneal organs and serves to hold structures together rather than reducing friction between them (Ogobuiro et al., 2023).

Intestinal blood supply comes primarily from the superior and inferior mesenteric arteries. The lower two-thirds of the rectum receive blood from the internal iliac and internal pudendal arteries (Kahle et al., 1992). Intestinal veins drain into the superior and inferior mesenteric veins, the internal iliac, and internal pudendal veins. Lymph drains via the lymphatic vessels into the lymph nodes and the intestinal trunk. Lymphatic vessels in the loops of the small intestine drain to superior mesenteric nodes. Lymphatic vessels of the large intestine from the proximal to distal portion drain into the superior and inferior mesenteric nodes, the internal iliac nodes, the pararectal nodes, and the superficial inguinal nodes respectively (Fritsch and Kuehnel, 2014).

Like the rest of the alimentary canal, innervation of the intestine is provided by two independent roots: extrinsic - the autonomic nervous system (ANS), and intrinsic - the enteric nervous system (ENS).

The ANS is located outside of the GIT tube. Sympathetic and parasympathetic stimulation can greatly inhibit or enhance gastrointestinal functions. Autonomic nerves also carry visceral afferent fibers, through which the afferent impulses for supraregional reflexes flow. In the central nervous system, nuclei of the autonomic system lie in different regions. Sympathetic neurons occupy the lateral horn in the thoracic and upper lumbar segments of the spinal cord. Parasympathetic neurons form nuclei in the brain stem and sacral spinal cord. The highest integration organ of the ANS is the hypothalamus. The reticular formation of the brain stem also participates in this regulation. Sympathetic neuron fibers communicate with the sympathetic trunk, represented as ganglia within the nervous plexuses on both sides of the abdominal aorta. Then, it continues within splanchnic nerves to the visceral ganglia. From there, postganglionic branches extend to the viscera enter internal organs alongside blood vessels, and form a special network. The principal cranial nerve of the parasympathetic system is the vagus nerve, which divides into plexuses for viscera. Similarly, the plexuses divide from autonomic nerves and the sacral spinal cord. The vagus nerve provides parasympathetic innervation as far as a point between the middle and left thirds of the transverse colon (Cannon – Boehm point). Beyond this area, parasympathetic innervation is provided via the sacral spinal cord (S2-S4/5), giving rise to the sacral splanchnic nerves. Their fibers pass to autonomic plexuses along the blood vessels (add a similar chain for parasympathetic).

The ENS lies entirely in the wall of the GIT, from the esophagus to the anus, and is largely independent of external innervation. It is composed of two plexuses: the intramural plexus (submucous or Meissner's plexus), and the myenteric plexus (Auerbach's plexus), both composed of a network estimated at approximately 100 million neurons. This enormous number of neurons within the GIT tissue resembles an independent nervous organ (Guyton and Hall, 2006; Kahle and Frotscher, 2015). The submucous plexus forms an irregular three-dimensional network within the entire submucosa. It controls mainly secretion and local blood flow. The myenteric plexus fills a narrow space between the transverse and longitudinal muscle layers, and is responsible for regulating wall movement (Johnson, 2018).

Endocrine, paracrine hormones and neurotransmitters control gastrointestinal motility, secretion, perfusion, and growth. All endocrine hormones for GIT are produced in the endocrine cells of the mucosa (Guyton and Hall, 2006; Silbernagl and Despopoulos, 2009; Fritsch and Kuehnel, 2014). Endocrine peptide hormones include gastrin, cholecystokinin, secretin, gastric inhibitory peptide, and motilin. Paracrine transmitters in the GIT are histamine, somatostatin, and prostaglandin (Guyton and Hall, 2006; Silbernagl and Despopoulos, 2009).

Different types of enteric neurons release various neurotransmitters, such as acetylcholine, norepinephrine, adenosine triphosphate, serotonin, dopamine, cholecystokinin, substance P, vasoactive intestinal polypeptide, somatostatin, leu-enkephalin, metenkephalin, and bombesin. While the precise functions of some of these neurotransmitters are still being unraveled, they play a vital

role in coordinating various aspects of gut health.

The CNS affects the ENS by modulating the ANS to control gastrointestinal functions. Gut-brain axis impairment is one of the main important causes of gut disorders (functional gastrointestinal disorders and gastroesophageal reflux diseases). These are associated with impaired autonomic dysfunction, mainly due to suppressed vagal tonus and overactive sympathetic one. ANS regulates intestinal homeostasis, so gastrointestinal disorders could be treated by restoring autonomic dysfunction via neuromodulation (Guyton and Hall, 2006; Ali and Chen, 2023).

The disbalance of the ANS is observed in various pathologic conditions and the vagus nerve is a key component of the neuro-immune and brain-gut axis regulations. It has an anti-inflammatory effect and plays a key role in the pathophysiology and treatment of chronic inflammatory disorders. The vagus nerve stimulation represents an alternative treatment (non-drug therapy) in various gastrointestinal disorders (e.g. IBD, irritable bowel syndrome) (Bonaz et al., 2017).

IMMUNE DEFENSE OF THE INTESTINE

The GIT comprises a large internal surface area, which requires an effective immune defense system. Its contact surface area is about 100 m^2, which is 60 times larger than the surface area of the skin. The epithelial lining of the gut is in constant contact with the environment and with billions of bacteria that constantly challenge the intestinal immune system. Various mechanisms maintain a delicate balance between the gut wall and environmental factors: saliva contains mucin, IgA, and lysozyme, low pH gastric juice has a bactericidal effect, Peyer's patches have immunocompetent lymphatic tissue and hepatic Kupffer's cells are macrophages.

The intestine relies on various microstructures to regulate important physiological functions. These microstructures include the microbial flora, the mucus barrier of the intestinal wall, the structure of the epithelial wall, the mucosa-associated lymphoid tissue, and M cells, as illustrated in Fig. 2.

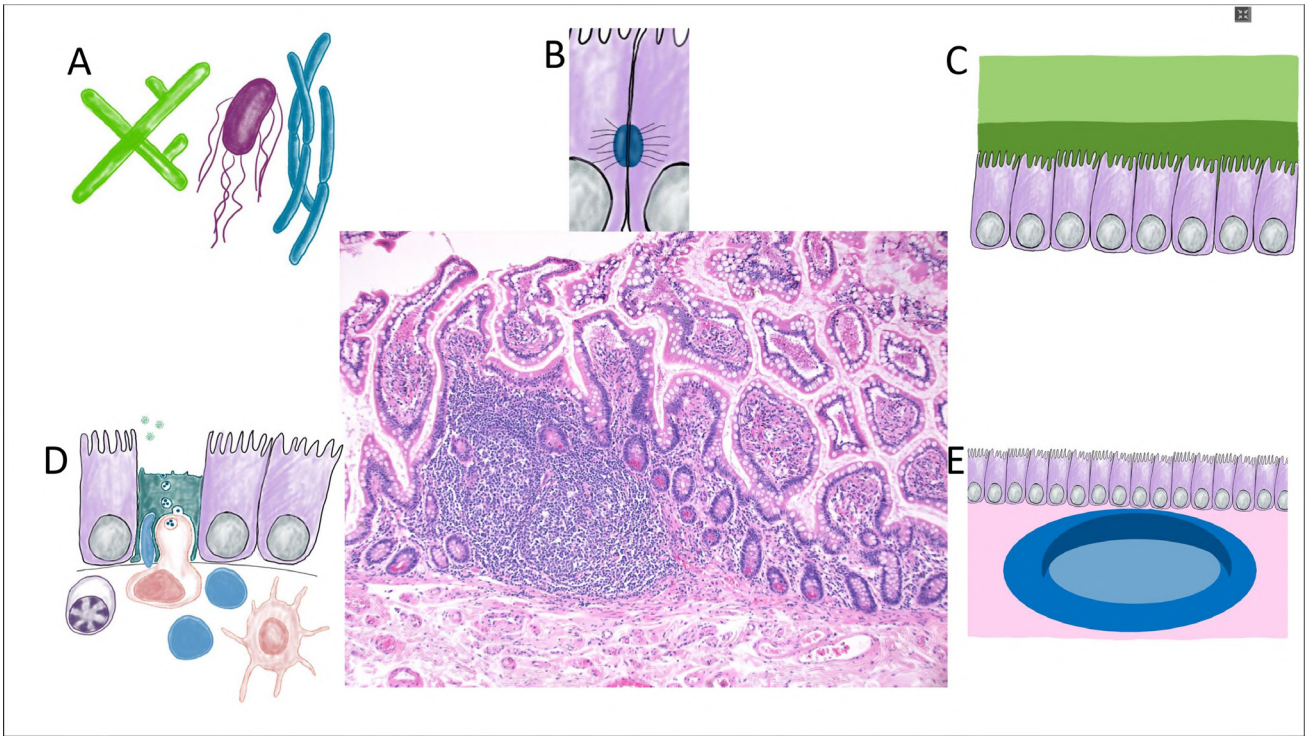

Fig. 2.- Gut defense system: **A**. bacterial microflora: a healthy gut microbiome is pivotal for developing food tolerance, reduction of toxic metabolic by products of digestion and induing physiological immune response **B.** Tight junction (Zonula occludens) provide a barrier between the lumen and the lamina propria, limiting paracellular permeability. **C**. double mucus layer: acts as a physical barrier shielding the epithelium from microorganisms and antigens, the outer layer is the habitat for the commensal flora. **D**. M cells: highly specialised cells between epithelial cells, responsible for transcytosis of luminal antigens and introducing them to subepithelial immune cells. **E.** Gut associated lymphoid tissue: complex lymphoid aggregates with formation of lymphoid follicles responsible for regulating specific immune response.

Microbial flora

The intestinal tract is initially sterile at birth and later becomes colonized with orally introduced anaerobic bacteria. In humans and other mammals, colonization of the infant's gut is thought to largely begin at birth, when delivery through the birth canal exposes the infant to its mothers´ vaginal microbiota, thereby initiating a critical maternal influence over the offspring´s lifelong microbial signature (Foster et al., 2017).

The bacterial content of physiological intestinal flora gradually increases within the intestine, the upper part of the small intestine of a healthy adult contains 0-10^4 bacteria per ml of intestinal content, the ileum contains up to 10^6, and finally, the large intestine contains 10^{11} up to 10^{12}/ml. These bacteria increase the activity of intestinal immune defense by way of "physiological inflammation", which prevents the spread of pathogens, their metabolic activity is also useful for the host (Silbernagl and Despopoulos, 2009).

Hasaniani et al. (2024) have described the important role of bacterial colonization of the digestive system hosting an extensive array of microorganisms. Among these, pathogenic microorganisms in the intestines have developed strategies to compete with beneficial microflora for essential nutrients. The process of mucin production plays a fundamental role in constructing the structure of mucus, which serves as a protective barrier against pathogens, enzymes and toxins. Research has unveiled that specific probiotic strains such as *Lactiplantibacillus plantarum 299v* and *Lacticaseibacillus rhamnosus LGG*, can induce the production of intestinal mucins. These mucins have a key role by hindering the attachment of harmful bacteria like *Escherichia colli* to the surface of intestinal epithelial cells.

Intestinal wall mucus barrier

Mucus is the first physical hurdle that bacteria encounter upon entering the digestive tract. It acts as a shield for the epithelium, protecting it from harmful microorganisms and antigens, but also as a lubricant for intestinal motility. It consists of two layers: an inner layer, firmly adherent to the epithelial cells and approximately 50 µm thick, and an outer layer, looser and less adherent, approximately 100 µm thick, according to measurements made in animal models.

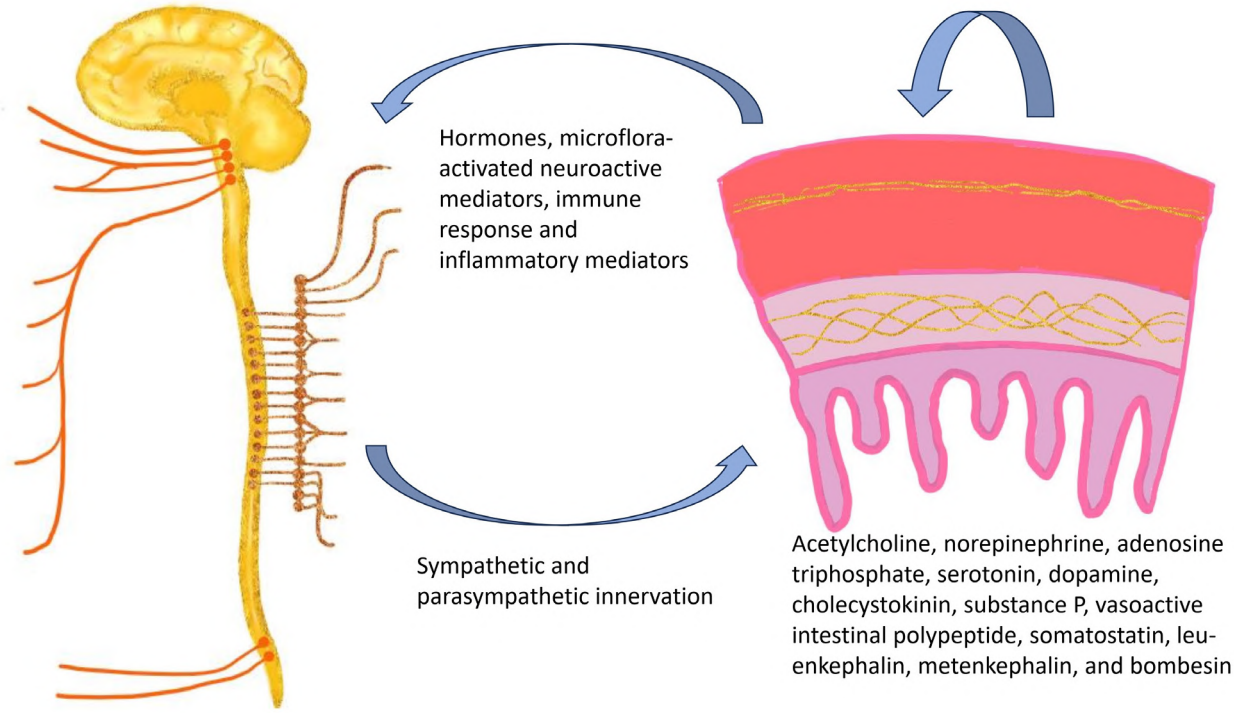

Fig. 3.- Gut-brain axis: Brain signaling to the gut is mediated through the autonomic nervous system, mainly through vagal innervation. Gut signaling to the brain occurs through multiple mechanisms, humoral factors, neuroactive substances, and systemic immune mediators. Meanwhile, the gut maintains autonomous function through the enteric nervous system, endocrine, paracrine hormones, and neurotransmitters.

The inner mucosal layer is dense and does not allow bacteria to penetrate, thus making the epithelial cell surface free from bacteria. As mucus secretion continues, the inner layer turns into the outer layer, which is the habitat of commensal flora. Both layers are organized around the highly glycosylated mucin MUC2, which forms an amorphous polymer-like cover and is secreted by goblet cells. MUC2 mucin is a molecule that has been preserved along the evolution since the first metazoans (Paone and Cani, 2020).

An important function of the chemical barrier is to maintain the abundance and composition of the gut microbiome. The chemical barrier included antimicrobial peptides, gastric acid, digestive enzymes, mucopolysaccharides, glycoproteins, glycolipids, and other compounds. It is believed that microbiome regulation in the small intestine is mainly carried out through pattern recognition receptors (Fawkner-Corbett et al., 2017). The microbiome population is maintained, either by preventing colonization or through direct killing mechanisms. The production of antimicrobials that lyse target cells is one of the main mechanisms for regulating the homeostasis of the gut microbiome. Bacterial contact with Toll (LTR2, TLR4, TLR7, and TLR9), NOD1, and NOD2 receptors activate adapter proteins, for example, MyD88, and genes responsible for the synthesis of cytokines and chemokines epithelial cells. This in turn triggers the synthesis of cytokines by immune cells, which activate genes responsible for the synthesis of antimicrobial peptides (Senchukova, 2023).

The composition of the microbiome is also influenced by various factors, such as hygiene, and dietary content, e.g., typical "Western diet" is low in fiber and high in sugar and fat, oxygen concentration, microbial adhesion, host stress, and other factors (Gomaa, 2020).

Hasaniani et al. (2024) in their review have described some important aspects modulating the function of the epithelial barrier integrity. The gastrointestinal epithelium refers to the layer of cells that lines the inner surface of digestive tract. This epithelium acts as a barrier between the body's internal environment and the external world, including microorganisms. It serves as the first point of contact between the host and the microorganisms present in the gut. It has been also suggested that probiotics can directly impact the environment of the intestine and its microbial composition, or indirectly affect it by interacting with the existing microbiota. One of the significant roles of probiotics is maintaining the integrity of the gastrointestinal epithelial barrier and preventing the passage of unwanted substances into the bloodstream.

Epithelial wall structure

The intestinal epithelium forms a relatively impermeable barrier between the lumen and the submucosa. This barrier function is maintained by a complex of proteins composing the tight junction that is located at the subapical aspect of the lateral membranes of cells. Tight junction consists of numerous proteins, in which zonula occludens and occludin are well described. These proteins are considered to be involved in the regulation of paracellular permeability. Disorganization of tight junctions causes disruption of the intestinal epithelial barrier and leads to an increase in paracellular permeability, as seen in inflammatory bowel diseases and intestinal infections (Mifkovic et al., 2009).

Increased intestinal permeability is considered an early event in irritable bowel syndrome (IBS) that leads to low-grade immune cell infiltration of the gut mucosa. Evidence for the remodeling in IBS has been provided by electron microscopy, which detected enlarged spaces between epithelial cells and cytoskeletal condensation in the gut biopsies of patients (Barbara et al., 2021).

Morphological and functional changes in intestinal permeability are also caused by abnormal gene and protein expression of tight junction proteins, including a reduction in the expression of occludin and zonula occludens protein 1. These findings have been corroborated by genetic and epigenetic findings in tight junction proteins claudin 1, claudin 2, and cingulin (Ding et al., 2013). Tight junction changes are probably the result of a combination of bacterial-mediated and proteasome-mediated degradation triggered by low-grade inflammation. Inflammatory mediators which increase intestinal permeability include

eicosanoids, histamine, and proteases (Grenham et al., 2011).

Disruptions in the gut barrier, bacterial overgrowth, increased permeability, and changes in the immune system can all contribute to bacterial translocation. This is defined by the passage of viable indigenous bacteria from the intestinal lumen to mesenteric lymph nodes and other territories. Its diagnostic criteria rely on the isolation of viable bacteria in mesenteric lymph nodes (Bellot et al., 2013).

Mucosa-associated lymphoid tissue

The intestine houses a complex immune system known as the Mucosa-associated lymphoid tissue (MALT), also referred to as Gut-associated lymphoid tissue (GALT). This network of lymphoid organs strategically positioned within the mucosal lining throughout the digestive tract (esophagus, stomach, small intestine, large intestine, and appendix) plays a vital role in the immune response. It consists of intraepithelial lymphocytes, immune cells of the lamina propria, solitary lymph nodules within the lamina propria, and aggregated lymphoid nodules, known as Peyer's patches within the lamina propria and submucosa (Fritsch and Kuehnel, 2014).

The arrangement of lymphatic vessels in the small intestine is similar to that of the colon, with the key difference being a more extensive submucosal network in the colon (Al-Kofahi et al., 2027). Regional lymph nodes, also known as mesenteric lymph nodes, are another component of the MALT system (Jaffe et al., 2017). Peyer's patches are specific to the mucosa and submucosa of the ileum and appendix. A typical feature of the appendix is the massive collection of lymphatic tissue, in the form of aggregated lymphoid nodules from the submucosa to the mucosa. It became apparent that the appendix fulfills a role in the gut immune system, and its shape is suitable for the retention of intestinal bacteria (Nigam, 2019).

The structure of Peyer's patches is roughly parallel to that of lymph nodes, apart from an expanded marginal zone extending all the way to the surface epithelium; they contain lymphoid follicles, composed predominantly of B lymphocytes and lymphoblasts, in addition to follicular center T helper cells and macrophages, surrounded by parafollicular T cell zones occupied by T cells and interdigitating dendritic cells. The mucosal lamina propria harbors mature plasma cells, macrophages, and occasional B and T lymphocytes. Intestinal plasma cells produce dimeric IgA, which is transported to the intestinal lumen by transcytosis. In neonates, GI mucosa is protected by the IgA in breast milk. Small populations of plasma cells also secrete IgM, IgG, and IgE mucosal intraepithelial lymphocytes constitute a heterogeneous population of T cells, including natural killer T cells and cytotoxic T cells (Jaffe et al., 2017).

Peyer's patches trap antigens by way of phagocytosis. In the T-cell-dependent germinal cell type response; T cells present the information to B cells in the lymphoid follicle. On exposure, B cells transform into blast cells (immunoblasts or centroblasts), which give rise to specific effector cells. B cells migrate into the lymphatic circulation, and general blood circulation, then home back to the intestinal mucosa. B cells presented with antigen develop into the IgA-secreting plasma cells, which is a method of reciprocal communication between lymphocytes and new enterocytes. Antigen contact within a Peyer's patch can lead to a generalized immune response throughout the entire small intestine, mediated by migrating activated B cells (Fritsch and Kuehnel, 2014; Silbernagl and Despopoulos, 2009).

M cells

The intestinal epithelium within the vicinity of lymphoid follicles of GALT has special features. Under steady-state conditions, approximately 10% of the epithelial cells within these follicle-associated epithelia (FAE) are microfold (M) cells. These cells have unique morphological features, including the presence of a reduced glycocalyx, irregular brush border, and reduced microvilli. In contrast to the neighboring enterocytes within the FAE, M cells are highly specialized for the transcytosis of gut lumen macromolecules, particulate antigens, and pathogenic or commensal microorganisms across the epithelium. Following their transcytosis across the FAE, antigens exit into the intraepithelial pocket beneath the M-cell basolateral membrane,

which contains a special microenvironment with various populations of lymphocytes and mononuclear phagocytes (MNP), a heterogenous population of macrophages and classical dendritic cells. Studies show that in the absence of M cells, or antigen sampling by M cells, antigen-specific T-cell response in the Peyer's patches of mice orally infected with *Salmonella Typhimurium* is reduced. Thus, efficient M-cell-mediated sampling of gut lumenal antigen is an important initial step in the induction of some mucosal immune responses (Al-Shboul, 2013).

Elements of immune protection can also include an increase in "tolerance" to a microbe, or a toxin of microbial origin and the death of infected cells. In particular, flagellins of pathogenic bacteria that have overcome the epithelial barrier can activate NAIP/NLRC4 in macrophages, which causes the death of epithelial cells and their expulsion into the intestinal lumen. The importance of the intestine as an immune organ also stems from the fact that it not only protects against external pathogens but participates in the formation of immune tolerance to food substrates and the normal gut microbiome. The main cytokines involved in the formation of immunological tolerance are IL-10 and TGF-beta, which are produced by CD4+ T cells, some populations of macrophages, and other cells. These substances have an anti-inflammatory effect, limiting the expansion of effector cells and inducing the proliferation of regulatory T cells (Senchukova, 2023).

Intestinal cells of Cajal

Intestinal cells of Cajal (ICC) are found throughout the entire length of the gastrointestinal tract and lie in close contact with nerve terminals. These cells provide junctions with each other and also with smooth muscle cells. Electrical coupling between smooth muscle cells and ICC has a key physiological role. Smooth muscle tissue contains a various interstitial cell with an important regulatory function during normal and pathophysiological responses. The main ICC function is the transduction of neural inputs from enteric motor neurons, mechanotransduction, and mechanosensitivity, pacemaker cells generating electrical slow waves – the basis for gastrointestinal motility, generating responses to specific neurotransmitters, expressing a variety of receptor neurotransmitters, hormones, paracrine substance, inflammatory mediators, mediation of postjunctional responses to neurotransmission. Also setting the membrane potential gradient of intestinal smooth muscles (Al-Shboul, 2013; Blair et al., 2014; Sanders et al., 2016).

Reduction of ICC, defective ICC function, and ICC abnormality is associated with a wide range of gastrointestinal motility disorders and diseases (Al-Shboul, 2013; Blair et al., 2014; Sanders et al., 2016).

INTESTINAL BARRIER DYSFUNCTION AND RELATED DISEASES

The passage of intestinal microorganisms or their by-products beyond the epithelial barrier is a proposed risk factor for numerous human diseases including inflammatory bowel diseases, obesity, cancers, and postoperative complications. The mesentery is the key intermediary tissue site that precipitates the physiological and immunological potential of these "escaped" microbial signals. By interrogating the environmental drivers of host-microbe signaling at the mesentery and beyond, mechanistic links between bacterial translocation and numerous pathophysiological processes in humans are emerging. Importantly, these represent potential intervention points to mitigate the negative consequences of bacterial translocation (Ha and Devkota, 2023).

The ability of the various intestinal barrier components to ensure physiological permeability even in the presence of pathogenic factors is essential for the maintenance of health. When barrier protection fails, immune cells come in direct contact with antigens in the intestinal lumen, which results in the impairment of normal physiological functions, such as immune response to pathogens (bacteria, viruses, fungi, and parasites), recognition of self-antigens, tolerance towards the commensal flora, and the desensitization to food antigens. Numerous evidences showed a significant association between gastrointestinal and extra-intestinal diseases and permeability alteration, including diabetes mellitus type I, multi-

ple sclerosis, other autoimmune diseases, acute pancreatitis, infectious gastroenteritis, and small intestinal bacterial growth (SIBO) (Bellocchi et al., 2022).

In the intestine, a specific group of immune cells (ILC3 – 3 innate lymphoid cells) produces a protein (IL-22 - Interleukin-22) that is crucial for maintaining a healthy gut lining. Jacquelot N. et al. [85] reported a new role for a protein called PD-1 (programmed cell death 1), typically linked to immunity against infections, in boosting IL-22 production by ILC3. The presence of gut bacteria and inflammatory signals influence PD-1 expression on these immune cells. The findings suggest PD-1 as a potential target to regulate gut health (Jacquelot et al., 2024).

Intestinal transplantation (IT) presents a life-saving therapy for intestinal failure, yet faces significant immunological hurdles. High graft immunogenicity, attributed to constant antigen exposure and abundant lymphoid tissue, leads to the highest rejection rates among solid organ transplants. Potent immunosuppression is utilized, but reliable non-invasive rejection monitoring remains elusive. Future directions should prioritize deciphering two-way allorecognition, fostering regulatory immune cell populations to reduce immunosuppressive burden, and developing assays for memory T-cell involvement. Additionally, exploring circulating donor DNA, microbiota alterations, and changes within the intestinal mucosa itself hold promise for identifying much-needed non-invasive biomarkers for improved IT outcomes (Rumbo and Oltean, 2023).

It is postulated that permeability alteration could be the primary pathogenic cause even in diseases not directly related to the mucosal barrier function, such as IBD and celiac disease. While in other diseases, such as liver cirrhosis, it would lead to the translocation of microbial antigens into the entero-hepatic circulation, with the consequent exacerbation of liver fibrosis and portal hypertension, and a further increase in permeability (Lopetuso et al., 2015). Intestinal abnormalities are also often present in relation to defects in connective tissue, neurodegenerative disorders, or larger-scale genetic aberrations, such as Down syndrome, Ehler-Danlos syndrome, and Huntington's disease (Stan et al., 2020). Some of these conditions are being studied for possible treatment with stem cells (Csobonyeiova et al., 2020; Harsányi et al., 2019, 2020).

Quantitative and qualitative changes in microbiota composition can lead to an increase in the production of potentially toxic metabolites, such as secondary bile acids, TMAO, and hydrogen sulfide, and an increase in the risk of developing intestinal, cardiovascular, neurological, oncological, and other diseases. TMAO is a molecule resulting from the oxidation in the liver of a microbial metabolism product trimethylamine (TMA). TMA is formed in the colon from choline, bataine, and carnitine. The main food precursors of TMA are meat, fish, poultry, and eggs. These changes may be related to diet, lifestyle, age, medications, and other factors. Plasma TMAO levels are determined by several factors, including diet, age, gut microbiota, drug intake, and liver flavin monooxygenase activity. The main TMA producers are *Clostridia, Shigella, Proteus, Aerobacter,* and *Eubacterium sp.* Taking antibacterial drugs can increase sensitivity to viral infections, increase the of developing malignant neoplasms, and contribute to the resistance to chemotherapy drugs and immune checkpoint inhibitors in cancer patients. Some authors attribute an increase in malignant neoplasms with the use of antibiotics to a decrease in the synthesis of intestinal metabolites with antitumor activity, for example, LCA (lithocholic acid) and cadaverin (Senchukova, 2023).

Bao H. et al. (2024) described the correlations among iron homeostasis, gut microbiota and the microenvironment, and intestinal immunity. It has been found that iron can not only maintain the balance of the internal environment to avoid diseases, such as anemia and hereditary hemochromatosis but also affect the composition and abundance of gut microbiota.

The protective layer of intestinal mucus is principally sensitive to ischemia and hypoxia. For example, in acute pancreatitis, a mass of cytokines and vasoactive agents, such as nitric oxide and thromboxane A2 are generated, which reduce intestinal tissue perfusion and thus cause intestinal mucous microcirculation disturbance, ischemia-reperfusion injury, and oxidative stress

injury. Furthermore, chronic ischemia can cause intestinal epithelial atrophy, increase mucous permeability, and finally damage the intestinal mucous barrier (Cen et al., 2018).

In the intestine, a specialized immune cell population termed intraepithelial lymphocytes (IELs) occupies a strategic position, balancing tolerance and defense at the critical interface between the luminal environment and underlying tissue. Remarkably, IEL subsets, despite arising from distinct developmental pathways, converge on a shared functional profile characterized by epithelial localization, innate-like properties, and limited T-cell receptor diversity. This restricted antigen recognition suggests a focused immune response by IELs, potentially influenced by epithelial cell interactions and dietary factors. Furthermore, IELs' capacity for both promoting gut health and contributing to pathology upon dysregulation highlights their potential as novel therapeutic targets in inflammatory bowel diseases and cancer (Lockhart et al., 2024).

In the intestinal crypts, Paneth cells serve as sentinel regulators of gut homeostasis. Their development is meticulously orchestrated by Wingless-related integration site (Wnt), Notch, and bone morphogenetic protein (BMP) signaling pathways, resulting in cells packed with potent antimicrobial peptides and nurturing growth factors. These peptides meticulously regulate the composition of gut microbiota, fending off both commensal and pathogenic bacterial threats to the epithelial barrier. Simultaneously, Paneth cells secrete growth factors that orchestrate the proper function of intestinal stem cells. Notably, Paneth cells exhibit remarkable plasticity, transforming into stem-like cells to repair epithelial integrity during injury (Cui et al., 2023).

Gamma-delta (γδ) T cells are a major cell population in the intestinal mucosa and are key mediators of mucosal tolerance and microbiota composition. The mechanisms by which intestinal γδ T cells interact with the gut microbiota to maintain tolerance are to be elucidated. Rezende et al. (2023) have found that antibiotic treatment impaired oral tolerance and depleted intestinal γδ T cells, suggesting that the gut microbiota is necessary to maintain γδ T cells. The authors have also found that mice deficient for γδ T cells ($\gamma\delta^{-/-}$) had an altered microbiota composition that led to intestinal immune dysregulation and impaired tolerance. Intestinal immune γδ T cells shaped the gut microbiota and regulated intestinal homeostasis by secreting the fecal micro-RNA let-7f. Oral administration of let-7f to $\gamma\delta^{-/-}$ mice rescued mucosal tolerance by promoting the growth of the $\gamma\delta^{-/-}$-microbiota-depleted microbe *Ruminococcus gnavus*. Rezende et al. (2023) concluded that γδ T cell-selected microbiota is necessary and sufficient to promote mucosal tolerance, is mediated in part by γδ T cell secretion of fecal micro-RNAs, and is mechanistically linked to restoration of mucosal immune responses.

γδT cells have gained popularity in the field of immunotherapy in recent years. They have extraordinary antitumor potential and prospects for clinical application. Immune checkpoint inhibitors (ICIs), which are efficacious in tumor patients, have become pioneer drugs in the field of tumor immunotherapy, since they were incorporated into clinical practice. γδT cells that have infiltrated into tumor tissues are found to be in a state of exhaustion or anergy with upregulation of many immune checkpoints (ICs) on their surface, suggesting that γδT cells have a similar ability to respond to ICIs as traditional effector T cells. Studies have shown that targeting ICs can reverse the dysfunctional state of γδT cells in the tumor microenvironment (TME) and exert antitumor effects by improving γδT-cell proliferation and activation and enhancing cytotoxicity. Clarification of the functional state of γδT cells in the TME and the mechanisms underlying their interaction with ICs will solidify ICIs combined with γδT cells as a good treatment option (Gao et al., 2023).

THE GUT-BRAIN AXIS

During embryogenesis, development of the gastrointestinal neuromuscular system occurs between the 4th and 7th weeks with neural crest cells migrating to the gut (Mills, 2019). First, the myenteric plexus is formed followed by the submucosal. Between the 6th to 9th weeks, the circular and longitudinal muscle layers form. The interstitial cells of Cajal appear around week 9 and become closely associated with the myenteric plex-

us (Odze and Goldblum, 2014).

The intestine is innervated by the enteric nervous system, a complex peripheral neural circuit embedded within the gut wall comprising sensory neurons, motor neurons, and interneurons. While the ENS is capable of independently regulating basic gastrointestinal functions (motility, mucous secretion, and perfusion), central control of gut functions is provided by vagal and spinal motor inputs that serve to coordinate gut functions with the general homeostatic state of the organism (Browning and Travagli, 2014). This central control over the ENS is important for adaptive gut responses during stressful events that signal a homeostatic threat to the organism. Modern society is characterized by the ubiquity of stressors that affect every individual to different extents. Experimental, clinical, and epidemiological data have shown that chronic activation of the stress response may participate in the development of various somatic as well as neuropsychiatric diseases (Mravec et al., 2018).

Loh et al. (2024) in their review have described the interaction between microglia and gut microbiota which begins early in life. A recent study demonstrated that early-life administration of a broad-spectrum antibiotic cocktail led to altered microglial morphology and myelin-related gene expression in adolescent mice, accompanied by anxiety-like and compulsive-like behaviors. Throughout the host lifespan, the gut microbiome provides essential signals to microglia during health and disease. Notably, among the neuronal and glial cells, microglia are the most vulnerable to alterations in the intestinal microbiome.

The complex communication network between the gut and the CNS comprises the ENS, sympathetic and parasympathetic branches of the ANS, neuroendocrine signaling pathways, and neuroimmune systems. Afferent spinal and vagal sensory neurons carry visceral feedback from the gut to the thoracic and upper lumbar spinal cord and the nucleus of the solitary tract within the caudal brainstem, engaging polysynaptic inputs to higher brain regions, including the hypothalamus and limbic forebrain. Bi-directional control is provided by descending autonomic neural projections from the cingulate and insular cortices, amygdala, bed nucleus of the stria terminalis, and hypothalamus, all of which are positioned to alter vagal and spinal autonomic outflow to the gut (Grenham et al., 2011).

Collectively, the microbiota-brain-gut axis is thought to communicate not only via these neural routes but also via humoral signaling molecules and hormonal components. Together, this intricate network exerts effects that alter both intestinal and brain function (Mayer et al., 2015; Bonaz et al., 2017).

The vagus nerve has been proposed to serve as the most important neural pathway for bidirectional communication between gut microbes and the brain (Forsythe et al., 2014). Recent investigations suggest that gut microbiota affects brain activity through the microbiota-gut-brain axis under both physiological and pathological disease conditions like Parkinson's disease. Further dopamine synthesis in the brain is induced by dopamine-producing enzymes that are controlled by gut microbiota via the microbiota-gut-brain axis. Also, alpha-synuclein deposition and the associated neurodegeneration in the enteric nervous system that increases intestinal permeability, oxidative stress, and local inflammation, account for constipation in Parkinson's disease patients. The trigger, which causes blood-brain barrier leakage, immune cell activation, inflammation, and ultimately neuroinflammation in the central nervous system is believed to be due to the chronic low-grade inflammation in the gut (Nair et al., 2018).

Neurological diseases, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis, are often associated with functional gastrointestinal disorders. These gastrointestinal disturbances may occur at all stages of neurodegenerative diseases, to such an extent that they are now considered an integral part of their clinical picture. Several lines of evidence support the contention that, in central neurodegenerative diseases, changes in gut microbiota and enteric neuro-immune system alterations could contribute to gastrointestinal dysfunctions as well as initiation and upward spreading of the neurologic disorder (Pellegrini et al., 2018).

Recent research on microRNA (miRNA) and autoimmune diseases showed that miR-16 is implicated in the etiology of various autoimmune diseases, including inflammatory bowel disease. In a mouse model, inhibition of miR-16 ameliorates inflammatory bowel symptoms (Chen et al., 2020). miR-16 and miR-125b have been found to play a role in the dysregulation of the intestinal epithelial barrier (Martínez et al., 2017). miR-16 was found to also regulate the expression of growth differentiation factor-5 (GDF5), activin A receptor (ACVR), and different genes. GDF5 is mainly associated with disorders of the connective tissue such as osteoarthritis (OA) or developmental dysplasia of the hip (DDH), a genetic anomaly often accompanied by various other abnormalities, e.g. intestinal fragility in Ehlers-Danslos syndrome (Harsanyi et al., 2020a, b; 2021a, b).

ACVR has been studied in Helicobacter pylori-induced gastric intestinal metaplasia (Chen et al., 2020). These findings show, that the roles of non-coding RNAs (ncRNAs) are only yet being revealed and these connections may be even more extensive than previously believed (Yan et al., 2019; Harsanyi et al., 2020a, b; 2021a, b). Some miRNA-affected pathways are associated with connective tissue defects, precancerosis, gut microbiome regulation, and intestinal immunity (Bi et al., 2020; Singh et al., 2021). A relationship between the intestine, gut microbiota, and brain functions has been reported many times, where dysregulation of the gut-brain axis ultimately lowers patients' health standards and causes anxiety or depression (Petra et. al., 2015; Lach et al., 2018; Kupcova et al., 2022).

Over the years, mast cells (MCs), have received a lot of attention as an important player in neuroimmune interactions in the gut. They are located primarily in perivascular spaces (2-3% of all cells in the lamina propria and about 1% of all cells in the submucosa) (Buhner et al., 2017).

Significant and growing data in the literature support the actions of GI luminal microorganisms to modulate gut-brain signaling via vagal afferents (the so-called microbiota-gut-brain axis). Although gut microbiota might normally be expected to activate vagal afferents directly only under conditions in which intestinal permeability is compromised (e.g., after inflammation or stress), luminal bacteria may activate vagal afferents indirectly, after stimulation and release of neuroactive mediators from enteroendocrine cells or gut-associated lymphoid tissue. In addition to their potential role in the regulation of gut functions, such as motility, secretion, and immune responses, gut microbiota is established to contribute to "higher" CNS functions, including mood, stress-related psychiatric conditions, and memory functions. For example, the administration of *Citrobacter rodentium* to mice increases anxiety-like behaviors in a vagally dependent manner, whereas *Bifidobacterium longum* NC3001 normalizes anxiety-like behavior after nematode-induced GI inflammation (Bellocchi et al., 2022).

Matsumoto et al. (2013) examined the role of microbiota in cerebral metabolism, and described that tested mice have an altered metabolic profile compared to their conventionally colonized counterparts, with 10 of these metabolites thought to be specifically involved in brain function. Clinical studies suggested that ingestion of probiotics can decrease anxiety and depression. Large clinical trials evaluating the efficacy of antibiotic and probiotic "psychobiotic" therapies, as well as in-depth sequencing of the microbiome, are required to further elaborate on the role of the microbiota-gut-brain axis in several pathophysiological situations (Browning et al., 2017).

The gastrointestinal microbiota is a diverse and numerous ecosystem that inhabits the entire gastrointestinal tract and has a systemic influence on our health. Owing to its enormous complexity and high interindividual variability, the microbiota is still in large part undefined regarding the scope of its contribution to human physiology and tolerable compositional variations under which normal functions are preserved. Diet changes affect the abundance of particular microbial groups. The macrobiotic signature is very stable, to observe a profound effect, the dietary change must be dramatic. For example, vegans switch to high-fat and high-protein diets (Nigam et al., 2019).

Individual differences in life-long stress responsiveness and susceptibility to stress-related disorders have been linked both to genetic and environmental factors, particularly early-life

exposures that can alter the developmental assembly and function of central neural circuits. Intriguingly, it has become increasingly clear that bacteria are required for normal brain development (Stilling et al., 2015; Foster et al., 2017).

Loh et al. (2024) in their review have described that the gut microbiota contributes to host physiology and brain health by generating a variety of metabolites through bacterial de novo metabolism and by modifying host-derived molecules. The authors discuss the mechanisms of the microbiota–gut–brain axis in neurodegenerative diseases using a metabolite-centric approach. The presence of a species possessing specific biosynthetic capabilities does not guarantee in vivo production of downstream metabolites in pharmacologically relevant quantities. Moreover, multiple gut microbes can produce the same metabolite. Thus, examining the intestinal microbiota through a functional metabolic lens (metabolite-centric), rather than focusing on taxonomic or phylogenetic aspects, is more valuable for understanding the intricate interactions between the microbiota and the host.

Some of the processes, structures and mediators described in this chapter are depicted in Fig. 3.

CONCLUSIONS

The intestine has several unique features. It is the largest immune-competent organ in our body and it is supported by an enormous number of nerves. Most of the nerve structures belong to the ENS, which regulates the intestinal function autonomously. The remaining extrinsic nerves connect the central nervous system with the gut and belong to either the afferent gut-brain or the efferent brain-gut axis. Both the sensory as well as motor pathways ramify extensively once entering the gut wall. The enteric immune system is an effective defense system maintaining a functional barrier and thus protecting the host from harmful invaders and noxious substances. Conceptually, it is important to realize that all three systems – ENS, extrinsic nerves, and enteric immune system – enter into an intimate anatomical and functional association which was reported in more recent investigations. The interplay between ANS and the immune system is clinically very important in organ inflammation and other diseases. The immune defense of the enteric nervous system is still not understood properly and further scientific studies could improve the diagnostic and therapy algorithms for intestinal diseases.

REFERENCES

AHLUWALIA B, MAGNUSSON MK, ÖHMAN L (2017) Mucosal immune system of the gastrointestinal tract: maintaining balance between the good and the bad. *Scand J Gastroenterol*, 52(11): 1185-1193.

ALI MK, CHEN JDZ (2023) Roles of heart rate variability in assessing autonomic nervous system in functional gastrointestinal disorders: a systematic review. *Diagnostics (Basel, Switzerland)*, 13(2): 293.

AL-KOFAHI M, YUN JW, MINAGAR A, ALEXANDER JS (2017) Anatomy and roles of lymphatics in inflammatory diseases. *Clin Exp Neuroimmunol*, 8(3): 199-214.

AL-SHBOUL OA (2013) The importance of interstitial cells of Cajal in the gastrointestinal tract. *Saudi J Gastroenterol*, 19(1): 3-15.

BAO H, WANG Y, XIONG H, XIA Y, CUI Z, LIU L (2024) Mechanism of iron ion homeostasis in intestinal immunity and gut microbiota remodeling. *Int J Mol Sci*, 25(2): 727.

BARBARA G, BARBARO MR, FUSCHI D, PALOMBO M, FALANGONE F, CREMON C, MARASCO G, STANGHELLINI V (2021) Inflammatory and microbiota-related regulation of the intestinal epithelial barrier. *Front Nutr*, 8: 718356.

BELLOCCHI C, CARANDINA A, MONTINARO B, TARGETTI E, FURLAN L, RODRIGUES GD, TOBALDINI E, MONTANO N (2022) The interplay between autonomic nervous system and inflammation across systemic autoimmune diseases. *Int J Mol Sci*, 23(5): 2449.

BELLOT P, FRANCÉS R, SUCH J (2013) Pathological bacterial translocation in cirrhosis: pathophysiology, diagnosis and clinical implications. *Liver Int*, 33(1): 31-39.

BI K, ZHANG X, CHEN W, DIAO H (2020) MicroRNAs regulate intestinal immunity and gut microbiota for gastrointestinal health: A comprehensive review. *Genes*, 11(9): 1075.

BLAIR PJ, RHEE PL, SANDERS KM, WARD SM (2014) The significance of interstitial cells in neurogastroenterology. *J Neurogastroenterol Motil*, 20(3): 294.

BONAZ B, SINNIGER V, PELLISSIER S (2017) The vagus nerve in the neuro-immune axis: implications in the pathology of the gastrointestinal tract. *Front Immunol*, 8: 293876.

BROWNING KN, TRAVAGLI RA (2014) Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions. *Compr Physiol*, 4(4): 1339-1368.

BROWNING KN, VERHEIJDEN S, BOECKXSTAENS GE (2017) The vagus nerve in appetite regulation, mood, and intestinal inflammation. *Gastroenterology*, 152(4): 730-744.

BUHNER S, BARKI N, GREITER W, GIESBERTZ P, DEMIR IE, CEYHAN GO, ZELLER F, DANIEL H, SCHEMANN M (2017) Calcium imaging of nerve-mast cell signaling in the human intestine. *Front Physiol*, 8: 971.

CEN ME, WANG F, SU Y, ZHANG WJ, SUN B, WANG G (2018) Gastrointestinal microecology: a crucial and potential target in acute pancreatitis. *Apoptosis*, 23: 377-387.

CHEN Y, SHAN T, QU H, CHEN Y, WANG N, XIA J (2020) Inhibition of miR-16 ameliorates inflammatory bowel disease by modulating bcl-2 in mouse models. *J Surg Res*, 253: 185-192.

CSOBONYEIOVA M, POLAK S, DANISOVIC L (2020) Recent overview of the use of iPSCs Huntington's disease modeling and therapy. *Int J Mol Sci*, 21(6): 2239.

CUI C, WANG F, ZHENG Y, WEI H, PENG J (2023) From birth to death: The hardworking life of Paneth cell in the small intestine. *Front Immunol*, 14: 1122258.

DI VINCENZO F, DEL GAUDIO A, PETITO V, LOPETUSO LR, SCALDAFERRI F (2024) Gut microbiota, intestinal permeability, and systemic inflammation: A narrative review. *Intern Emerg Med*, 19(2): 275-293.

DING L, LU Z, LU Q, CHEN YH (2013) The claudin family of proteins in human malignancy: a clinical perspective. *Cancer Manag Res*, 5: 367-375.

FAWKNER-CORBETT D, SIMMONS A, PARIKH K (2017) Microbiome, pattern recognition receptor function in health and inflammation. *Best Pract Res Clin Gastroenterol*, 31(6): 683-691.

FORSYTHE P, BIENENSTOCK J, KUNZE WA (2014) Vagal pathways for microbiome-brain-gut axis communication. *Adv Exp Med Biol*, 817: 115-133.

FOSTER JA, RINAMAN L, CRYAN JF (2017) Stress & the gut-brain axis: regulation by the microbiome. *Neurobiol Stress*, 7: 124-136.

FRITSCH H, KUEHNEL W (2014) Internal Organs, Color Atlas of Human Anatomy, Volume 2, 6th edition, Thieme.

GAO Z, BAI Y, LIN A, JIANG A, ZHOU C, CHENG Q, LIU Z, CHEN X, ZHANG J, LUO P (2023) Gamma delta T-cell-based immune checkpoint therapy: attractive candidate for antitumor treatment. *Mol Cancer*, 22(1): 31.

GENEROSO JS, GIRIDHARAN VV, LEE J, MACEDO D, BARICHELLO T (2020) The role of the microbiota-gut-brain axis in neuropsychiatric disorders. *Braz J Psychiatry*, 43(3): 293-305.

GOMAA EZ (2020) Human gut microbiota/microbiome in health and diseases: a review. *Antonie Van Leeuwenhoek*, 113(12): 2019-2040.

GRENHAM S, CLARKE G, CRYAN JF, DINAN TG (2011) Brain-gut-microbe communication in health and disease. *Front Physiol*, 2: 94.

GUYTON AC, HALL JE (2006) Textbook of Medical Physiology, 11th edition, Elsevier.

HA CWY, DEVKOTA S (2023) Bacterial translocation to the mesentery. In: *The Mesentery and Inflammation.* Cham: Springer International Publishing, pp 93-109.

HARSÁNYI S, ZAMBORSKÝ R, BÖHMER D, DANIŠOVIČ L (2019) Developmental dysplasia of the hip - etiopathogenesis, diagnostics and genetic aspects. *Lekarsky Obzor*, 7: 200-205.

HARSANYI S, ZAMBORSKY R, KOKAVEC M, DANISOVIC L (2020a) Genetics of developmental dysplasia of the hip. *Eur J Med Genet*, 63(9): 103990.

HARSANYI S, ZAMBORSKY R, KRAJCIOVA L, KOKAVEC M, DANISOVIC L (2020b) Developmental dysplasia of the hip: a review of etiopathogenesis, risk factors, and genetic aspects. *Medicina*, 56(4):153.

HARSANYI S, ZAMBORSKY R, KRAJCIOVA L, BOHMER D, KOKAVEC M, DANISOVIC L (2021a) Association analysis of GDF5 and contributing factors in developmental dysplasia of the hip in infants. *Ortop Traumatol Rehabil*, 23(5): 335-339.

HARSANYI S, ZAMBORSKY R, KRAJCIOVA L, KOKAVEC M, DANISOVIC L (2021b) Genetic study of IL6, GDF5 and PAPPA2 in association with developmental dysplasia of the hip. *Genes,* 12(7): 986.

HASANIANI N, RAHIMI SM, AKBARI M, SADATI F, POURNAJAF A, ROSTAMI-MANSOOR S (2024) The role of intestinal microbiota and probiotics supplementation in multiple sclerosis management. *Neuroscience,* 551: 31-42.

JACQUELOT N, XIONG L, CAO WH, HUANG Q, YU H, SAYAD A, ANTTILA CJ, BALDWIN TM, HICKEY PF, AMANN-ZALCENSTEIN D, OHASHI PS (2024) PD-1 regulates ILC3-driven intestinal immunity and homeostasis. *Mucosal Immunol*, 17(3): 371-86.

JAFFE ES, HARRIS NL, VARDIMAN J, ARBER DA, CAMPO E (2010) Hematopathology e-book. *Elsevier Health Sciences*.

JOHNSON LR (2018) Gastrointestinal Physiology E-Book. *Elsevier Health Sciences.*

JOVEL J, DIELEMAN LA, KAO D, MASON AL, WINE E (2018) The human gut microbiome in health and disease. In: Nagarajan M (editor). *Metagenomics: Perspectives, Methods and Applications.* Academic Press, London, pp 197-213.

KAHLE **À**, LEONHARDT H, PLATZER W (1992) Color Atlas & Textbook of Human Anatomy, Vol. 2. Internal organs. New York: George Thieme Verlag.

KAHLE W, FROTSCHER M (2015) Nervous System and Sensory Organs. Color Atlas of Human Anatomy, Volume 3, 7th edition. Thieme.

KATAOKA K (2016) The intestinal microbiota and its role in human health and disease. *J Med Invest*, 63(1.2): 27-37.

KUPCOVA I, DANISOVIC L, GRGAC I, HARSANYI S (2022) Anxiety and depression: what do we know of neuropeptides? *Behav Sci*, 12(8): 262.

LACH G, SCHELLEKENS H, DINAN TG, CRYAN JF (2018) Anxiety, depression, and the microbiome: a role for gut peptides. *Neurotherapeutics*, 15(1): 36-59.

LOCKHART A, MUCIDA D, BILATE AM (2024) Intraepithelial lymphocytes of the intestine. *Annu Rev Immunol*, 42(1): 289-316.

LOH JS, MAK WQ, TAN LKS, NG CX, CHAN HH, YEOW SH, FOO JB, ONG YS, HOW CW, KHAW KY (2024) Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct Target Ther*, 9(1): 37.

LOPETUSO LR, SCALDAFERRI F, BRUNO G, PETITO V, FRANCESCHI F, GASBARRINI A (2015) The therapeutic management of gut barrier leaking: the emerging role for mucosal barrier protectors. *Eur Rev Med Pharmacol Sci*, 9(6): 1068-1076.

LOUGHREY MB, QUIRKE P, SHEPHERD NA, WILLIAMSON L (2018) Standards and datasets for reporting cancers. Dataset for histopathological reporting of colorectal cancer. September 2018. *Royal College of pathologists*, document G049.

MARTÍNEZ C, RODIÑO-JANEIRO BK, LOBO B, STANIFER ML, KLAUS B, GRANZOW M, GONZÁLEZ-CASTRO AM, SALVO-ROMERO E, ALONSO-COTONER C, PIGRAU M, ROETH R (2017) miR-16 and miR-125b are involved in barrier function dysregulation through the modulation of claudin-2 and cingulin expression in the jejunum in IBS with diarrhea. *Gut*, 66(9): 1537-1538.

MATSUMOTO M, KIBE R, OOGA T, AIBA Y, SAWAKI E, KOGA Y, BENNO Y (2013) Cerebral low-molecular metabolites influenced by intestinal microbiota: a pilot study. *Front Syst Neurosci*, *7*: 9.

MAYER EA, TILLISCH K, GUPTA A (2015) Gut/brain axis and the microbiota. *J Clin Invest*, 125(3): 926-938.

MAYER EA, NANCE K, CHEN S (2022) The gut–brain axis. *Annu Rev Med*, 73: 439-453.

MIFKOVIC A, SKULTETY J, PINDAK D, PECHAN J (2009) Specific aspects of acute pancreatitis. *Bratisl Lek Listy*, 110(9): 544-552.

MILLS S (2019) Histology for pathologists. Lippincott Williams & Wilkins.

MRAVEC B, HORVATHOVA L, PADOVA A (2018) Brain under stress and Alzheimer's disease. *Cell Mol Neurobiol*, 38: 73-84.

NAIR AT, RAMACHANDRAN V, JOGHEE NM, ANTONY S, RAMALINGAM G (2018) Gut microbiota dysfunction as reliable non-invasive early diagnostic biomarkers in the pathophysiology of Parkinson's disease: a critical review. *J Neurogastroenterol Motil*, 24(1): 30.

NASSAR S, MENIAS CO, PALMQUIST S, NADA A, PICKHARDT PJ, SHAABAN AM, GABALLAH AH, ELSAYES KM (2021) Ligament of Treitz: anatomy, relevance of radiologic findings, and radiologic-pathologic correlation. *Am J Roentgenol*, 216(4): 927-934.

NIGAM Y, KNIGHT J, WILLIAMS N (2019) Gastrointestinal tract 5: the anatomy and functions of the large intestine. *Nursing Times*, 115(10): 50-53.

ODZE RD, GOLDBLUM JR (2014) Odze and Goldblum Surgical Pathology of the GI Tract, Liver, Biliary Tract and Pancreas. Elsevier Health Sciences.

OGOBUIRO I, GONZALES J, SHUMWAY KR, TUMA F (2023) Physiology, Gastrointestinal. [Updated 2023 Apr 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, Available from: https://www.ncbi.nlm.nih.gov/books/NBK537103/

PAONE P, CANI PD (2020) Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut*, 69(12): 2232-2243.

PEATE I (2021) The gastrointestinal system. *Br J Healthc Assist*, 15(3): 132-137.

PELLEGRINI C, ANTONIOLI L, COLUCCI R, BLANDIZZI C, FORNAI M (2018) Interplay among gut microbiota, intestinal mucosal barrier and enteric neuro-immune system: a common path to neurodegenerative diseases? *Acta Neuropathol*, 136: 345-361.

PETRA AI, PANAGIOTIDOU S, HATZIAGELAKI E, STEWART JM, CONTI P, THEOHARIDES TC (2015) Gut-microbiota-brain axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. *Clin Ther*, 37(5): 984-995.

QUIGLEY EM (2017) Microbiota-brain-gut axis and neurodegenerative diseases. *Curr Neurol Neurosci Rep*, 17(12): 1-9.

RAMIRES LC, SANTOS GS, RAMIRES RP, DA FONSECA LF, JEYARAMAN M, MUTHU S, LANA AV, AZZINI G, SMITH CS, LANA JF (2022) The association between gut microbiota and osteoarthritis: does the disease begin in the gut? *Int J Mol Sci*, 23(3): 1494.

RÉGNIER M, VAN HUL M, KNAUF C, CANI PD (2021) Gut microbiome, endocrine control of gut barrier function and metabolic diseases. *J Endocrinol*, 248(2): R67-R82.

REZENDE RM, COX LM, MOREIRA TG, LIU S, BOULENOUAR S, DHANG F, LESERVE DS, NAKAGAKI BN, LOPES JR, TATEMATSU BK, LEMOS L (2023) Gamma-delta T cells modulate the microbiota and fecal micro-RNAs to maintain mucosal tolerance. *Microbiome*, 11(1): 32.

RUMBO M, OLTEAN M (2023) Intestinal transplant immunology and intestinal graft rejection: from basic mechanisms to potential biomarkers. *Int J Mol Sci*, 24(5):4541.

SANDERS KM, KITO Y, HWANG SJ, WARD SM (2016). Regulation of gastrointestinal smooth muscle function by interstitial cells. *Physiology*, 31(5): 316-326.

SENCHUKOVA MA (2023) Microbiota of the gastrointestinal tract: Friend or foe? *World J Gastroenterol*, 29(1): 19.

SILBERNAGL S, DESPOPOULOS A (2009) Color Atlas of Physiology. 6th ed. Thieme, New York.

SINGH R, ZOGG H, RO S (2021) Role of microRNAs in disorders of gut–brain interactions: clinical insights and therapeutic alternatives. *J Pers Med*, 11(10): 1021.

STAN TL, SOYLU-KUCHARZ R, BURLEIGH S, PRYKHODKO O, CAO L, FRANKE N, SJÖGREN M, HAIKAL C, HÅLLENIUS F, BJÖRKQVIST M (2020) Increased intestinal permeability and gut dysbiosis in the R6/2 mouse model of Huntington's disease. *Sci Rep*, 10(1): 18270.

STILLING RM, RYAN FJ, HOBAN AE, SHANAHAN F, CLARKE G, CLAESSON MJ, DINAN TG, CRYAN JF (2015) Microbes & neurodevelopment–Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. *Brain Behav Immun*, 50: 209-220.

UCHIDA K, KAMIKAWA Y (2007) Muscularis mucosae-the forgotten sibling. *J Smooth Muscle Res*, 43(5): 157-177.

VALDES AM, WALTER J, SEGAL E, SPECTOR TD (2018) Role of the gut microbiota in nutrition and health. *BMJ*, 361: k2179.

WATTCHOW D, BROOKES S, MURPHY E, CARBONE S, DE FONTGALLAND D, COSTA M (2008) Regional variation in the neurochemical coding of the myenteric plexus of the human colon and changes in patients with slow transit constipation. *Neurogastroenterol Motil*, 20(12): 1298-1305.

YAN L, LIANG M, HOU X, ZHANG Y, ZHANG H, GUO Z, JINYU J, FENG Z, MEI Z (2019) The role of microRNA-16 in the pathogenesis of autoimmune diseases: A comprehensive review. *Biomed Pharmacother*, 112: 108583.