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# Enteric Nervous System in Inflammatory Bowel Disease Genesis: Review

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**Published Date:** December 29, 2015

## ABSTRACT

The Enteric Nervous System (ENS) is a collection of neurons in the gastrointestinal tract that constitutes the "brain of the gut" and can function independently of the central nervous system. The enteric nervous system encompasses the intrinsic neural circuits of the gastrointestinal tract, which are organized into a vast network of interconnected ganglia distributed into two concentric layers within the gut wall, the outer myenteric or Auerbach's and the inner submucosal or Meissner's plexus. Patients with chronic inflammatory bowel diseases have several structural and functional abnormalities of the ENS. Although it has been hypothesized that these abnormalities are secondary to the inflammatory process, i.e. to tissue injury or via the effects of soluble mediators of the inflammatory process itself, the occurrence of lesions in the ENS in macroscopically uninvolved areas may actually suggest that they precede mucosal inflammation. Recent advances have highlighted the ENS as playing a key role in the control of gut immune homeostasis, and that alterations of the ENS could be directly associated in the development of inflammatory bowel disease. When intestinal homeostasis is disrupted by severe inflammation, pathologic changes of ENS neurons and glial cells occur, compromising gut motility and secretion.

## DEVELOPMENT, ANATOMY AND PHYSIOLOGY

The enteric nervous system is a collection of neurons in the gastrointestinal tract that constitutes the "brain of the gut" and can function independently of the central nervous system [1,2]. Notwithstanding this, there are multiple afferent and efferent connections with the central nervous system. Extrinsic afferent neurons convey information to the CNS, while parasympathetic and sympathetic motor pathways interact with the Enteric Nervous System (ENS) to modify gut activity [3-5]. The enteric nervous system encompasses the intrinsic neural circuits of the gastrointestinal tract, which are organized into a vast network of interconnected ganglia distributed into two concentric layers within the gut wall, the outer myenteric or auerbach's and the inner submucosal or meissner's plexus [2]. The myenteric plexus lies between the longitudinal and circular layers of muscle and extends the entire length of the gut. It primarily provides motor innervation to the two muscle layers and secretomotor innervation to the mucosa, but there are numerous projections from the myenteric plexus to the submucosal ganglia and to enteric ganglia of the gallbladder and pancreas [6]. The submucosal plexus of humans (Figure 1) exists in two connected but separate layers, whereas smaller mammals, including rodents, have only one [7]. Enteric ganglia contain the cell bodies and neurites of intrinsic primary afferent neurons, interneurons, and motor neurons, which together form microcircuits involved in the reflex regulation of motility, blood flow, and secretion [8-10]. The intrinsic primary afferent neurons are located in both plexuses and respond to changes in the chemical and contractile state of the organ by the discharge of action potentials and the release of neurotransmitters [11]. Interestingly, axons from myenteric and submucosal neurons innervate the lamina propria and are found in close proximity to lymphoid cells and follicles in mice [12,13], which suggests a potential role for enteric neurons in immunomodulation. However, the physiological and pathophysiological significance of the proximity of enteric varicosities to immune cells has not yet been fully elucidated.

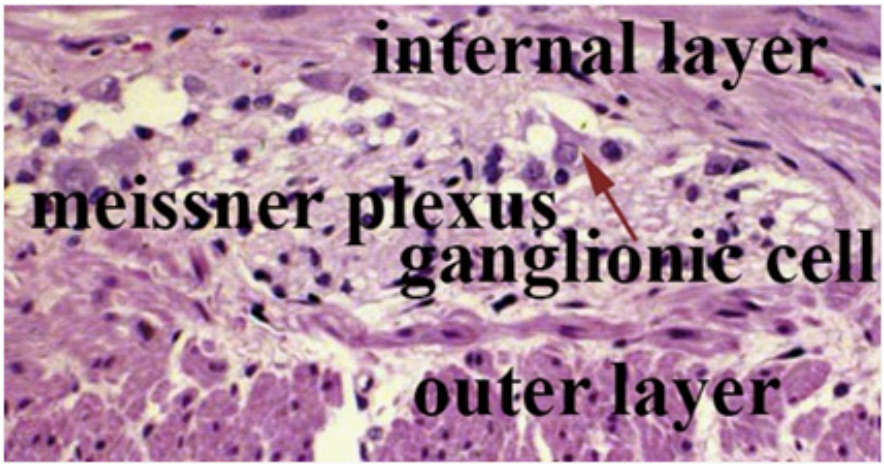


Figure 1: Hematoxylin-eosin stained of Meissner's plexus. Arrow indicates the ganglionic cell. Magnification 250 xs.

The enteric nervous system develops from Neural Crest-Derived Cells (NCCs), the majority of which migrate from the vagal region of the neural tube. During ENS development, there is a critical balance among migration, survival, proliferation, and differentiation of neural crest cells [14]. A fine control of these processes guarantees that a sufficient number of NCCs enter the foregut at the correct time while proliferation and differentiation are maintained at a balanced level at migration wave front. It is known that the microenvironment plays a pivotal role to regulate the extent of ENS formation influencing on neural crest cells number and gut colonization.

During their migration, a sub-population of Enteric Neural Glial Cells (ENCCs) starts to express neuronal markers [15-17]. Furthermore, many neurons begin to extend caudally-projecting neurites that grow in close association with migrating ENCCs [18-21].

Many transcription factors, receptors, extracellular ligands and adhesion molecules that regulate ENCC migration and differentiation have been identified [14,21-25]. One of these receptors, Ret, is involved in the development of enteric ganglia derived from vagal-neural-crest cells. RET is a transmembrane tyrosine kinase receptor that is expressed in ENCDCs as they migrate through the bowel. It is the signaling receptor for four ligands: Glial Cell Line-Derived Neurotrophic Factor (GDNF), neurturin, artemin, and persephin that activate RET by binding to the glycosylphosphatidylinositol-linked GDNF family of receptors (GFRα1, GFRα2, GFRα3, and GFRα4, respectively). A gene on chromosome 10q11.2 that encodes a receptor for tyrosine-protein kinase is involved in various cellular processes, RET, signalize supports ENS precursor survival, proliferation, migration, differentiation, and neurite growth [26-28].

Endothelin-3 and endothelin-B receptors also have a role in the migration and development of the enteric nervous system, as well as in the development of melanocytes from the neural crest. Both targeted disruption of the endothelin-3 gene in mice and naturally occurring mutations of this gene (in lethal spotting mice) cause aganglionic megacolon and coat-color spotting required finely tuned control over muscular activity and fluid secretion to efficiently break down macroscopic food particles, efficiently extract nutrients, and maintain a healthy luminal microbiome. An important arbiter of these processes is the ENS, a network of neurons and glia within the wall of the bowel that controls most aspects of intestinal function. In humans, the ENS contains approximately 5×108 neurons of >15 functional classes comprising a wide range of neurotransmitters, projection patterns, and electrical properties [24].

An SRY-related HMG-box transcription factor, also familiar as SOX 10, is expressed in the neural tube prior to neural crest delamination, in migratory ENCDCs, and in mature enteric glia. Using genetic fate mapping of SOX10, a neural crest cell marker [29], it has been demonstrated that in postnatal gut, SOX10 positive cells can act as neuroprogenitor cells and regenerate neurons after damage. PHOX2B is a home domain transcription factor expressed in the NC-derived autonomic nervous system, including the developing ENS and adult enteric neurons [24].

Molecules controlling ENS morphogenesis are relatively poorly understood, but several classic morphogens are known to have important roles in ENS development.

The hedgehog pathway is involved indirectly and directly in the developing ENS. Hedgehog proteins have important roles as morphogens. For example, localized Sonic Hedgehog (SHH) expression is critical for defining anterior-posterior patterning of digits in the limb [30] and dorsoventral patterning in the spinal cord [31]. Similarly, in the bowel, localized expression of hedgehog proteins in the epithelium is essential for concentric patterning of the bowel wall. The hedgehog ligands SHH and Indian Hedgehog (IHH) are expressed by the gut epithelium during bowel development [32].

Enteric glia is important components of the ENS and also forms an extensive network in the mucosa of the gastrointestinal tract. Initially regarded as passive support cells, it is now clear that they are actively involved as cellular integrators in the control of motility and epithelial barrier function. Enteric glia forms a cellular and molecular bridge between enteric nerves, enteroendocrine cells, immune cells, and epithelial cells, depending on their location [10]. Enteric Glial Cells (EGCs) outnumber enteric neurons by 4:1 and are located within ganglia and extraganglionic sites, including the smooth muscle layers and the intestinal mucosa. Based on morphological features and location, EGCs are subdivided into distinct subtypes that share molecular and functional characteristics. Despite the realization that the different subpopulations of EGCs make critical and unique contributions to intestinal homeostasis, the dynamic relationship between spatially segregated EGCs, the physiological signals that regulate their steady-state equilibrium, and their response to trauma or disease remain unknown [33,34]. Motility is regulated by the innervation of the smooth muscle layers by myenteric neurons, superimposed upon the pacemaker activity of interstitial cells of Cajal [35,36]. Enteric neurons display a wide variety of neurotransmitters and neuropeptides determining the chemical coding of the ENS. Enteric neurotransmitters and neuropeptides are able to bind and influence a variety of immune cells. The motor neurons that innervate the circular and longitudinal muscle layers release a number of excitatory and inhibitory neurotransmitters. Acetylcholine (ACh) and substance P primarily mediate contraction, whereas Vasoactive Intestinal Peptide (VIP), Nitric Oxide (NO), and purines elicit relaxation. Cholinergic enteric neurons are targeted by both branches of the autonomic nervous system. Evidence of direct interactions between “basket-like” cholinergic endings and cholinergic myenteric neurons was recently reported [37]. This vagal innervation of cholinergic enteric neurons stimulates the production of acetylcholine and may represent the neuronal circuitry by which the vagus nerve mediates its anti-inflammatory signal. ChAT+ fibers, most likely originating from the enteric neurons, are found in close proximity to resident intestinal macrophages [38].

Motor neurons from the submucosal plexus, along with a small minority of myenteric neurons, innervate the mucosal epithelium release transmitters that cause chloride ion and water secretion [7]. Morphological and functional abnormalities of the ENS, the complex neuronal network that autonomously regulates most gastrointestinal functions, have been consistently reported in several inflammatory bowel disorders such as ulcerative colitis and Crohn, s disease [39,40].

## ENTERIC MICROBIOTA, CENTRAL AND ENTERIC NERVOUS SYSTEMS

Microbiota can influence ENS activity by producing molecules that can act as local neurotransmitters, such as GABA, serotonin, melatonin, histamine and acetylcholine [41] and by generating a biologically active form of catecholamines in the lumen of the gut [42]. Lactobacilli also utilize nitrate and nitrite to generate nitric oxide [43] and to produce hydrogen sulfide that modulates gut motility by interacting with the vanilloid receptor on capsaicin-sensitive nerve fibers [44].

The ENS represents also the target of bacterial metabolites. One of the main product of bacterial metabolism are Short-Chain Fatty Acid (SCFAs), such as butyric acid, propionic acid and acetic acid, that are able to stimulate sympathetic nervous system [45], mucosal serotonin release [46] and to influence memory and learning process [47 - 49].

As the ENS (and enteric glial cells, in particular) is known to express microbial pattern recognition receptors (such as TLR2 and TLR4) it will be of interest to determine whether microbiota control the dynamics of enteric glia directly or via an intermediary cell type. Irrespective of the mechanisms, recent experiments identify EGCs as a major target of gut microbiota and suggest that microbes and their products regulate the development and maturation of other glial cell networks of the nervous system. [50,51]. The basic principals of brain – gut -microbiota axis mechanism [49] have been presented in (Table 1).

Table 1: Brain gut microbiota axis in IBD genesis.

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|---|
| From gut microbiota to brain: |
| Production, expression and turnover of neurotransmitters (i.e. serotonin, GABA) and neurotrophic factor (BDNF) |
| Protection of intestinal barrier and tight junction integrity |
| Modulation of enteric sensory afferents |
| Bacterial metabolites |
| Mucosal immune regulation |
| From brain to gut microbiota: |
| Alteration in mucus and biofilm production |
| Alteration in motility |
| Alteration of intestinal permeability |
| Alteration in immune function |

Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. Previous studies have been demonstrated transcriptome changes of differentiated astrocytes and reprogramming of Schwann cells exposed to lipopolysaccharides and leprosy bacilli, respectively [52,53]. Changes in the organization and function of enteric glia, an essential component of a key role station along the gut-brain axis, or potential deficits of central nervous system glia, could contribute to neuroendocrine and behavior abnormalities associated with changes in gut microbiota [54]. It will be interesting to determine whether microbiota regulates the homeostasis of mEGCs in all segments of the mammalian intestine and identify additional factors that contribute to the generation and supply of enteric glial cells to the intestinal mucosa. Understanding further the interplay between microflora and EGCs will provide

an excellent model system to examine the effects of commensal and pathogenic micro-organisms on the host nervous system and help elucidate the pathogenesis and ultimately develop novel therapeutic strategies for GI disorders [55,56].

## INFLAMMATORY BOWEL DISEASE (IBD) AND ENS

The role of the enteric nervous system in the regulation of the intestinal epithelial barrier and the gut immune response has fuelled an increased interest in the last few years. Recent advances, summarized in this review, have highlighted the ENS as playing a key role in the control of IEB functions and gut immune homeostasis, and that alterations of the ENS could be directly associated in the development of Inflammatory Bowel Disease (IBD) and its associated symptoms. When intestinal homeostasis is disrupted by severe inflammation, pathologic changes of ENS neurons and glial cells occur, compromising gut motility and secretion [39,57,58]. Patients with chronic inflammatory bowel diseases (Crohn, s disease and ulcerative colitis), have several structural and functional abnormalities of the ENS. Although it has been hypothesized that these abnormalities are secondary to the inflammatory process, i.e. to tissue injury or via the effects of soluble mediators of the inflammatory process itself (cytokines, arachidonic acid metabolites, oxygen-derived free radicals), the occurrence of lesions in the ENS in macroscopically uninvolved areas may actually suggest that they precede mucosal inflammation. A common finding from studies of the inflamed colon is a reduction in the number of enteric neurons that does not appear to be restricted to specific neural populations [59-61]. Enteric neuron density remains diminished following the resolution of inflammation [60]. The loss of enteric neurons appears to occur downstream of the activation of caspase-dependent apoptosis. Enhanced activation of the P 2X7 receptor, pannexin-1-signaling complex [34], and neutrophil infiltration of enteric ganglia [59] have each been proposed as causes of neuronal apoptosis in the inflamed bowel. Despite a loss of enteric neurons, several studies have been demonstrated that there is enhanced neurite outgrowth following colitis. The neurochemical content of enteric neurons also changes during inflammation. Immunoreactivity for VIP decreases and substance P increases in tissues from colon of IBD patients compared to controls [40,62]. Additionally, immunoreactivity for VIP, NO synthase, and neuropeptide Y increases in tissues from the ileum of Crohn's disease patients compared to controls [7]. Numerous studies have identified substantially altered electrophysiological properties of enteric neurons from the inflamed and post-inflamed gut. At rest, IPANs are relatively inexcitable because of their prolonged after hyperpolarizing potential, which limits AP firing [63]. Hyperexcitability of intrinsic primary afferent neurons from inflamed regions of the gastrointestinal tract has been well documented in guinea pig with trinitrobenzene sulfonic acid induced colitis during and following the resolution of inflammation [64-67]. Another important effect of colitis is that it alters enteric neurotransmitter release as well as effector tissue responsiveness to this neurotransmitter. Vasoactive Intestinal Peptide (VIP), a well-established immunomodulator, is expressed by enteric neurons. Sympathetic and vagal efferent fibers make contact with VIP positive enteric neurons providing another neuronal mediator by which

the brain can affect the gut immune response [68]. Treatment with VIP in a TNBS-colitis model decreased the severity of the inflammation suggesting a beneficial role of this neuropeptide on the colonic inflammation [69]. In experimental colitis and CD patients, an increase in the number of VIP+ neurons was measured and correlated with an elevated colonic VIP content. However, contradictory results were found on VIP expression in ulcerative colitis and Crohn's Disease (CD) patients leaving under debate the role of VIP in the colonic inflammation [60].

Acetilcholin released from submucosal vasomotor neurons binds to endothelial muscarinic receptors, which stimulates endothelial NO synthase. NO produced from endothelial cells diffuses into the adjacent vascular smooth muscle cells where it activates soluble guanylyl cyclase and causes smooth muscle relaxation and vasodilation [67, 70-72].

At present, there is no definite cure for these diseases and although the etiology of IBD remains unknown there are some new insights into its pathophysiology. Inflammatory bowel disease, Crohn, s disease and ulcerative colitis, are characterized by a significant increase of proinflammatory cytokines in the gut, which trigger and support the inflammatory processes. Several lines of evidence implicate Glial Fibrillary Acidic Protein (GFAP)-positive enteric glia cells in regulating the inflammatory response in the gut as well as the integrity of the gut epithelium [40,73,74].

Genetic ablation of enteric glia cells in mice induced fatal hemorrhagic jejuno-ileitis with histopathological changes closely resembling changes described in human Crohn, s disease [75]. Destruction of EGC by auto-immune mechanisms was shown to induce gut inflammation [76]. However, the number and distribution of GFAP-positive EGCs in the inflamed and non-inflamed gut of IBD patients is controversially discussed and only few data exist on EGCs in the intestines of IBD patients and during gut inflammation [76,77]. It is speculated that the enteric nervous system is disturbed in chronic inflammation of the gut [40,73-76]. Previous data was showed an increase of GDNF and GFAP in sections of patients with CD [77].

Von Boyen and co-authors [78] were demonstrated that in the inflamed tissue of patients with ulcerative colitis, both, GFAP-positive EGCs and Glial-Derived Neurotrophic Factor (GDNF) is also highly increased. Some investigators were outlined that GDNF secretion is increased in the inflamed mucosa of CD and that GDNF acts anti-apoptotic on intestinal epithelial cells [77,79]. Enteric glial cells serve as the main source of mucosal GDNF and a loss of enteric glial cells, lead to a severe inflammation of the gut by a disruption of the mucosal barrier [80].

A defect EGC network is postulated for CD. Although GDNF - anti-apoptic agent for epithelial cells- and GFAP is increased in the inflamed gut of CD, it is significantly less than in the inflamed intestines of UC patients or patients with infectious colitis. The diminished GFAP expression in the non-inflamed and in the inflamed tissue of CD patients might be an indicator for a chronic dysfunction of EGCs. As loss of EGCs lead to gut inflammation and several animal models demonstrate EGCs and their secreting factors (GDNF, b TGF- b, GSNO) as essential tools for maintaining the integrity of the gut [76,80,81], it might be speculated that the reduced EGC network of CD patients is part of the pathophysiological puzzle in this disease. Some previous studies were described ultrastructural abnormalities of intersticial cells cajal such as swelling

of mitochondria, decreased electron density, autophagosomes and partial depletion of the cytoplasm in patients with IBD [58,82]. Villanaci and co-authors [58] have been found decreased number of EGC in the submucosal plexus in the individuals with Crohns disease compared with healthy controls. The few studies on animal models have showed that loss of glial cell function may increase gut permeability and alter the barrier function of the intestine [80] and this abnormality might contribute to the pathogenesis of CD [83]. As EGC cells can also act as antigen presenting cells, the loss of these cells can be a link with the current hypothesis suggesting that CD may be due to an abnormal response to the intestinal flora [84]. Villanaci and associates [58] have revealed myenteric plexitis in approximately 75% of patients with Crohn, s disease and about 56% of ulcerative colitis. The significance of plexitis remains unclear. It may be related to the development of the disease however. The presence of myenteric plexitis in proximal resection margins of ileocolonic resection specimens from patients with CD indeed been shown to be predictive of early endoscopic CD [85]. In fact, many of the above abnormalities we found in IBD patients were more evident in tissue samples macroscopically not involved by the inflammatory process, suggesting that ENS abnormalities may represent an early pathogenetic factor, and that the subsequent inflammation may mask some findings, due to an architectural distortion of the tissue with rearrangement and packing together of the enteric structures. On the other hand, experimental animal models have demonstrated that inflammation in the gut alters the cellular components of enteric reflex circuits in non-inflamed regions in ways similar to those at sites of active inflammation. These changes underlie altered function in non-involved regions during episodes of intestinal inflammation [86].

Furthermore, the intestines of Crohn,s disease patients are characterized by a reduced number of EGCs with a diminished secretion of GDNF during inflammation and might be a part of the pathophysiological processes in Crohn,s disease. EGC processes are in close proximity to gut epithelial cells and these cells secrete several mediators implicated in mucosa barrier function. EGCs are producers of transforming growth factor b (TGF- b) that promote intestinal barrier function [81]. Recently it was shown that nitric oxide metabolite S-nitrosogluthatione, a novel potent inducer of intestinal barrier function in human colon, is secreted by EGCs [87].

In the last two decades, enormous progress has been made in the characterization of soluble factors that regulate gut functionality at physiological and inflamed conditions. Among signaling molecules, Wnt family proteins (derived from Wingless, the Drosophila melanogaster segment-polarity gene, and Integrase-1, the vertebrate homologue – Wnts) are reported to play a pivotal role in the development of gut [88,89] balancing the homeostasis of intestine epithelium [90]. Besides Wnt proteins, Glial Cell-Derived Neurotrophic Factor (GDNF), Nerve Growth Factor (NGF), Fibroblast Growth Factors (FGF), Epithelial Growth Factor (EGF), Leukemia Inhibitory Factor (LIF), interleukin 6 (IL6), and Lipopolysaccharide (LPS) from gram-negative enteric bacteria [91,92] modulate strongly the ENS or gut function. In case congenital or acquired defects reduce the bioavailability of enteric neurotrophic factors, a compromised ENS development and a dysfunction of the immune system [93] are observed to promote the inhibition of GSK3β activity [94,95] and enterocolitis [96], suggesting that a possible cross talk between Wnt/β-catenin pathway and immune response could be involved.

The results of the experimental study by Liddo and coauthors [97] were suggested the existence of neuronal surveillance through FZD9 and Wnt3a in enteric myenteric plexus. Although the total number of FZD9 positive cells cultured under standard and basal conditions did not significantly change, the expression level of FZD9 was upregulated in response to GDNF, bFGF, and NGF, suggesting a possible involvement of Wnt signaling in neuronal and glial differentiation. Interestingly, under in vitro-simulated inflammation, Wnt signaling was demonstrated to exert an anti-inflammatory activity to negatively control NF-kB pathway.

However, it is unknown whether these ENS defects, ranging from subtle changes to severe structural modifications, are secondary to inflammatory processes or rather have a role in the pathogenesis of gut disorders. Alterations in the composition of the gut microbiota, derangement in signaling of innate immune receptors such as Toll-Like Receptors (TLRs), and modifications in the neurochemical coding of the ENS have been associated with a variety of gastrointestinal disorders [98,99]. The activation of TLR2 by microbial products controls the ENS structure and regulates intestinal neuromuscular function [100]. Because TLRs are expressed in the central nervous system and their signaling is involved in nervous system development, authors hypothesized that TLR2 signaling is critical for ENS homeostasis. Brun and co –authors [100] have shown that TLR2 is expressed in the ENS and intestinal smooth muscle layers. Its absence induces architectural and neurochemical coding changes in the ENS, leading to gut dysmotility and to higher IBD susceptibility. The finding that ENS anomalies resulting from inadequate TLR2- driven Glial Cell Line Derived Neurotrophic Factor (GDNF) availability were completely corrected by GDNF treatment reveals the prominence of the TLR2-GDNF axis ensuring ENS integrity and in the resolution of IBD outcomes [101]. Some clinical study [102] was showed that expression of glial cell-derived neurotrophic factor, the critical neurotrophin for the post-natal ENS is upregulated in intestinal smooth muscle cells by inflammatory cytokines, leading us to explore the relationship between intestinal smooth muscle cells growth and GDNF expression. Also, these authors were concluding that in the inflamed intestine, smooth muscle proliferation supports the ENS, and thus its own re-innervation, by expression of GDNF.

## CONCLUSION

Enteric nervous system is undeniably involved in the pathogenesis of the inflammatory bowel disease, interfered in several mechanisms: genetic, immunological and direct microbiota affects consequences. Level and the type of affection of the ENS might be useful in differentiation two major subtypes of IBD, ulcerative colitis and Crohn's disease. Considering all the factors that influence the status of ENS, a novel strategy for IBD therapy is advisable to be discussed, and this strategy ought to take care about the beneficiary effect of probiotics to the impact of the brain gut microbiota axis in IBD pathogenesis.

## ACKNOWLEDGEMENT

Papers is partially financed by the grant III41020 Ministry of Education, Science and Technological Development, Republic Serbia.

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