Gut-Brain Axis in IBD

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The gut–brain axis is a bidirectional communication system driven by neural, hormonal, metabolic, immunological, and microbial signals. Signaling events from the gut can modulate brain function and recent evidence suggests that the gut–brain axis may play a pivotal role in linking gastrointestinal and neurological diseases. Accordingly, accumulating evidence has suggested a link between inflammatory bowel diseases (IBDs) and neurodegenerative, as well as neuroinflammatory diseases.

Keywords: gut-brain axis ; IBD ; MS ; PD ; ex vivo organ models

1. Introduction

Inflammatory Bowel Diseases (IBDs) are prototypic immune-mediated inflammatory diseases (IMIDs) that affect the gastrointestinal (GI) tract and have a globally increasing prevalence and multifactorial etiology. The two main entities include Crohn's Disease (CD) and ulcerative colitis (UC), which differ in extent and localization of inflammation. Previous clinical and preclinical studies have highlighted important components in the pathophysiology of both diseases. These include (a) local immune-cell populations and their associated mediators [1][2][3], (b) genetic predisposition [4][5][6], (c) the epithelial barrier [7][8][9] as a shield of the bowel wall against (d) altered intestinal microbiota, as well as other environmental factors that can be altered, for example, via diet [10][11][12][13]. Emerging evidence further indicates that the enteric nervous system (ENS) strongly influences mucosal immunity and thus might represent an important contributor to IBD development and progression [14][15]. Disruption of ENS function may have bidirectional consequences for the gut and the central nervous system (CNS) [16]. In this context, visceral hypersensitivity and chronic pain are common debilitating symptoms of IBD suggesting an interaction between the gut, the ENS and CNS. It is currently believed that pain, including neuro-immune interactions in the inflamed gut, mediate an increase in intestinal permeability that ultimately leads to an aggravation of disease activity and that resolution of inflammation is associated with reduced abdominal pain [15][17]. Interestingly, UC was originally considered a psychosomatic disorder until the latter half of the 20th century [18]. With the discovery in 1954 of the therapeutic effect of steroids for UC [19][20], it became obvious that it originates in the digestive tract and is a primary severe chronic inflammatory disease. In contrast, irritable bowel syndrome (IBS) is a prevalent functional gastrointestinal disorder characterized by chronic abdominal pain or discomfort and altered bowel habits due to visceral hypersensitivity. Chronic, subtle and low-grade subclinical inflammation of the intestinal mucosa may also be involved in the pathology of this disease condition (reviewed in Ng et al., J Inflamm Res) [21]. Moreover, intestinal malabsorption syndromes (e.g., Vit B12 deficiency) can cause severe neurologic and neuropsychiatric symptoms culminating, e.g., in funicular myelosis or psychosis [22].

The close association between the gut and the brain was uncovered in the 21st century and is now known as the gut–brain axis. Microbial–neuro–immune–endocrine modulation of this bidirectional communication system likely plays a pivotal role in the pathogenesis of gastrointestinal and neurologic diseases (**Figure 1**).

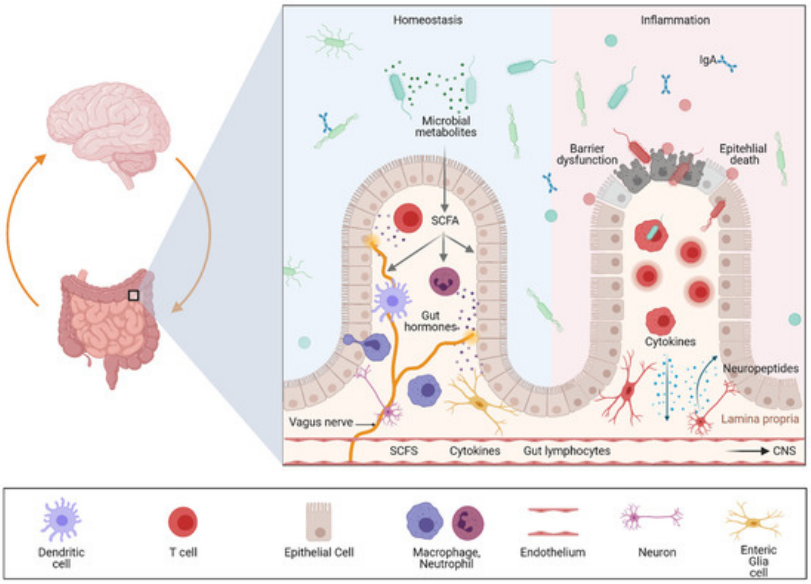

Figure 1. Impact of intestinal inflammation on gut–brain communication. The figure was generated with biorender.com (8 July 2021). Multiple pathways exist through which gut–brain communication can be modulated including neural, hormonal, metabolic, immunological, and microbial signals. Signaling events from the gut can modulate brain function, and recent evidence suggests that a dysregulated gut–brain axis plays a pivotal role in linking gastrointestinal and neurological diseases involving neuroinflammation as well as neurodegeneration. Cytokines released by the mucosal immune system in response to local inflammation or infection can be released in the periphery reaching the CNS via the bloodstream. Similarly, circulating gut-primed immune cells can cross the blood–brain barrier (BBB) and modulate immune responses in the CNS. In addition, bacterial metabolites, such as short-chain fatty acids (SCFAs) are neuroactive metabolites of dietary fibers that can further influence gut–brain communication and neuroinflammation. In addition to immune signals and microbial metabolites, signaling from the vagus and enteric nervous system affect gut mucosal and anatomically distant tissues such as the CNS. These peripheral signals promote neuroinflammation and neurodegeneration.

Accordingly, enteric dysbiosis and translocation of bacterial products as well as inflammatory soluble factors derived from the inflamed intestinal mucosa across the gut–epithelial barrier and blood–brain barrier (BBB) have been widely recognized as major factors for structural and functional alterations in the CNS. Thus, it is not surprising that the impact of mucosal immune dysfunction and the contribution of the gut microbiota to peripheral inflammation is also in the focus of neurodegenerative diseases.

Similarly, there is a bidirectional association between MS and IBD. In general, data derived from this systematic review of published patient data suggest that both IBD and MS patients seem to have a 50% increased risk of MS or IBD comorbidity, with no difference between CD or UC [23].

Importantly, depending on the study population, the risk for developing CNS pathologies among IBD is highly variable and data regarding the individual risk for neurodegeneration among CD und UC patients largely vary.

2. Genetic Evidence for an Association between Gut and Brain in the Context of Inflammation

The majority of PD cases are of sporadic origin; however, about 10% are familial and the most common monogenic forms of PD are pathogenic variants on the *LRRK2* gene that encode leucine-rich repeat kinase 2 (LRRK2) [24], a multidomain protein with a catalytic core that can fulfill kinase and GTPase activity. It also has a scaffold function allowing LRRK2 to interact and recruit several other signaling molecules. The coding variants associated with PD cluster within the enzymatic core of LRRK2 and are thought to disrupt the enzymatic functions of this protein. Accordingly, preclinical studies have indicated that targeting the activity or expression of LRRK2 is neuroprotective.

Recent genome-wide association studies (GWAS) have shown that the association between the *LRRK2* locus and IBD includes several *LRRK2* genetic variants [25]. Although the interaction of alterations in LRRK2 function and CD mechanisms is still unknown, increasing evidence supports the fact that LRRK2 plays a role in mediating autophagy in Paneth cells, which would explain its strong association with CD because defects in Paneth cell autophagy have been described as hallmarks of this IBD prototype [26]. In line with this notion, preclinical studies using mice with a LRRK2 deficiency showed a specific impairment in the expression of antimicrobial peptides by Paneth cells [27].

The genetic risk factors associated with MS and IBD are not well described in the literature. Genetic studies of MS cohorts suggest that this autoimmune disease is provoked following exposure to environmental factors, which might be responsible for loss of tolerance and peripheral activation of myelin-specific T cells. GWAS have supported the complexity of MS pathology and uncovered immune-related gene variants linking MS to other autoimmune diseases such as IBD [28]. Further systematic studies are needed to better delineate the genetics between IBD and MS.

3. Evidence from Gut–Brain Communication in Preclinical Mouse Models

Beside the epidemiological and genetic evidence, several preclinical studies support the importance of gut–brain communication in intestinal inflammation. In the dextran sulfate sodium (DSS)-induced colitis, a rodent model of IBD, it was demonstrated that, parallel to local inflammatory responses in the gut mucosa, increased expression of *IL6* and *iNOS* (Nitric oxide synthase, inducible; *NOS2*) was found in the cerebral cortex. The authors further described microglial activation by increased immunoreactivity for the pan-myeloid cell marker ionized calcium-binding adapter molecule 1 (Iba1) and elevated cytokine levels [29]. Another study analyzed the impact of intestinal inflammation by DSS administration in a model of dopaminergic neurodegeneration by LPS injection in the substantia nigra [30]. The authors demonstrated that inflammatory responses in the gut reinforced the inflammatory and deleterious effects of LPS induced neuroinflammation as indicated by increased levels of TNF-α, GFAP, and IL-6 in serum and the substantia nigra of the animals.

Interestingly, there are several recent animal studies suggesting that α-synuclein, a key protein involved in PD pathology, accumulates not only in the brain but also in the gut. Surprisingly, this could not only be observed in α-synuclein transgenic mice but also in mice subjected to DSS (experimental colitis drives enteric α-synuclein accumulation and Parkinson-like brain pathology). In line with previous examinations, this study observed that experimentally induced colitis in transgenic mice exacerbated α-synuclein pathologies in the CNS. While highly interesting, the effect of α-synuclein aggregates on ENS homeostasis has not been studied. So far, only one study demonstrated that increased α-synuclein expression following colitis was associated with phosphorylation in the myenteric plexus of common marmosets [31].

In summary, available studies suggest that inflammation is associated with peripheral alterations affecting CNS homeostasis through factors accumulating in the gut or systemically. In line with this hypothesis, another recent study demonstrated activation of microglial cells and reduction in occludin and claudin-5 expression in the brain suggesting an impaired BBB following experimental colitis [32]. These data further suggest that DSS-induced colitis increases systemic inflammation which then results in cortical inflammation via up-regulation of serum cytokines. Interestingly, the same group further observed that a decrease in dopaminergic function was associated with an increase in gastrointestinal inflammation, suggesting a bidirectional gut–brain interaction. Accordingly, mice studies showed that Experimental Autoimmune Encephalomyelitis (EAE), a model for MS in rodents, is accompanied by loss of mucosal immune homeostasis [33].

4. The Different Levels of Gut–Brain Communication

4.1. Neuronal Communication

The GI tract is the only internal organ that has its own independent nervous system, the enteric nervous system (ENS) [34][35]. This digestive system can be innervated by intrinsic enteric neurons and by extrinsic efferent and afferent nerves.

It has been shown that neuropeptide-containing (peptidergic) neurons within the colonic wall are key players in neurogenic inflammation as they release neuropeptides into the adjacent tissue. These peptides can induce vasodilation, plasma extravasation and leukocyte migration. Moreover, these neuropeptides have been shown to not only regulate intestinal homeostasis but also inflammation [36]. Accordingly, experimental studies could demonstrate that peptidergic neurons release neuropeptides that orchestrate colonic inflammation in a complex way. Calcitonin gene-related peptide (CGRP) and substance P (SP) seem to be the link between neuronal activation and the consecutive mucosal immune response. In murine colitis models, mice deficient in neutral endopeptidase (an enzyme responsible for the extracellular degradation of SP) displayed and aggravated colitis, while mice deficient in substance P showed a strong attenuation of colitis severity [15][37][38]. In sharp contrast, CGRP-deficient mice showed increased susceptibility to experimental colitis. Neuropeptide release is controlled by transient receptor potential (TRP) channels; therefore, neuropeptides released locally in the gut may function as mediators at the interface between the nervous system, the mucosal immune system and other cell compartments such as the epithelium or endothelium. Interestingly, recent single cell analyses revealed a significant expression of risk genes for diseases that feature intestinal and CNS involvement in the ENS, suggesting that it is involved in gut–brain disease communication [16].

4.2. Microbial Communication

While the importance of the gut microbiome was described some time ago for IBD and a variety of other immune and metabolically driven diseases, the essential role of the gut microbiota in CNS inflammation was discovered only a few years ago [39].

Several clinical studies highlighted a reduced diversity and altered composition of the gut microbiota (dysbiosis) not only in mouse models of neuroinflammation and neurodegeneration, but also as a common feature of patients with PD [40][41][42][43][44] and MS [45][46][47][48][49][50][51][52]. While dysbiosis has been shown in many clinical and preclinical studies, a disease-relevant microbiota for neuroinflammation or neurodegeneration is debatable. In addition, it still remains unclear whether dysbiosis can modulate inflammatory processes in the CNS or if it is merely the consequence of neuroinflammation/neurodegeneration. In support of a rather causative function of gut microbes, germ-free mice were resistant to spontaneous EAE, a striking notion that was explained by a lack of local activation of T cells in the gut and the subsequent deficient triggering of pathogenic antibody production by activated B cells. A translational study could demonstrate that transplanting faecal microbiota from PD patients exacerbated motor dysfunction in an α-synuclein transgenic mouse model [53].

4.3. Immunological Cross Talk

In summary, there is growing evidence suggesting that the gut is strongly involved in various neurological diseases via direct and indirect mechanisms. The key components are intestinal microbes and their products (e.g., metabolites) and immune education in the mucosal immune system, including immune cells releasing proinflammatory cytokines. Key to the regulation of these processes is the intestinal epithelium, which is capable of translating microbial and inflammatory signals to the immune system and secreting peptides as well as hormones, which are involved in the metabolic processing of dietary nutrients. Although this network is of strong clinical relevance for both intestinal and neurological diseases, we are just beginning to understand the underlying molecular mechanism and how organ crosstalk is regulated during health and disease. Accordingly, we need novel model systems to better understand microbiota–gut–brain communication on a cellular level. In the last chapter we therefore focus on novel human-specific preclinical model systems that will help to uncover disease mechanisms, which might allow us to better understand and modulate the function of this complex system.

5. Ex-Vivo Organ Models

5.1. Brain Organoids

The complex process of human brain development is achieved through the spatially and temporally regulated release of key patterning factors. Initially, the goal of in vitro modeling of brain cells was achieved by PSC derived two-dimensional neural cultures. Compared to the brain, the complexity of these cultures is low, and from the beginning the driving motive was for more advanced in vitro models concerning cell composition, maturation, and tissue architecture. The ability of PSC cells to form rosette-like structures, delineating the self-organization potential of neural progenitors to form neural tube-like structures was found two decades ago [54] and then refined, resulting in structures reminiscent of early stages of brain development [55][56][57]. A major breakthrough came with a series of publications from the Sasai and Knoblich labs [58][59]. Inspired by the cell's intrinsic development program and self-patterning ability, they provided a pea-size brain model, termed "brain organoid". Specifically, this work demonstrated the generation of broad brain regional identities with a minimal set of chemical guidance. The Lancaster study implemented two new crucial experimental steps: (I) embedding of pre differentiated cellular aggregates in matrigel, followed by (II) spinning in a three-dimensional device over the course of months. These experimental changes led to the reorganization and expansion of the neuroepithelium. Brain organoids contained cortical regions but also generated a large variety of brain regions including the midbrain, hindbrain and retina.

The ultimate comparison of the complexity of this model is the human brain. Here, OMICS techniques like single-cell RNA-Sequencing (scRNA-seq) were instrumental. Analyses of brain organoids at different times of differentiation showed that the developmental steps and fates of cell populations mimicked processes in the human fetal brain [60]. The latest updates on the generation of brain organoids provided 3D structures with brain-specific regional identities, including the forebrain [61], midbrain [62] and hippocampus [63]. As further outlined below, the ability to introduce epithelial barriers into organoid models is highly relevant when considering organoids for studying inter-organ communication. In a recent study, such a selective barrier was successfully modeled for the first time, namely, through choroid plexus organoids, including self-contained compartments [64].

Moreover, the fusion of regionalized brain organoids indicated that brain circuits can be modeled in these assembloids [65][66]. Another important aspect of using brain organoids for modeling concerns the reconstruction of circuits of interest. A brilliant example is the reconstruction of the motor circuit using 3D cortical motor assembloids [67].

5.2. Enteric Nervous System (ENS)

Human stem cell-based modeling of the enteric nervous system (ENS) is less frequently found than differentiations of CNS neural cells. The ENS, a vast network of neuronal and glial cells, is derived from neural crest (NC) progenitor cells. Studer and Fattahi implemented human PSC-based protocols for NC induction and regional specification [68][69], which led to the development of a robust method for directing the fate of hPSCs towards the enteric NC and further to the vagal ENS. The application of hPSC-derived enteric neural lineages provided a powerful platform not only for ENS-related disease modeling of neurodevelopmental processes like Hirschsprung disease [69] but also for studies of cell–cell interaction with CNS-derived neural cells and with the intestinal system to better understand inflammatory diseases.

5.3. Intestinal Organoids

The human GI tract can be separated into the foregut, midgut, and hindgut [70][71]. Each of these regions gives rise to defined tissues and organs. The foregut endoderm is the embryonic progenitor for the oral cavity, pharynx, esophagus, stomach, proximal duodenum and parts of the hepatobiliary system such as the liver parenchyma and pancreas. Other parts of the small intestine (distal duodenum, jejunum, ileum) as well as colon, rectum, anal canal, and also the epithelium of the bladder and urethra develop from the midgut and hindgut endoderm. Organoids recapitulating the GI tract or hepatobiliary–pancreatic (HBP) system can be derived from PSC and adult stem cells. Following the direction of PSC in an endodermal direction, individual protocols mimic the respective developmental cues (reviewed by [72][73][74]). Intestinal organoid protocols are based on modulation by *WNT3A*, *Notch*, *FGF4*, *EGF*, and *BMP/Nodal* signaling [75][76][77]. A detailed protocol for generating 3D human intestinal tissues (organoids) in vitro from human PSC was provided by the Spence lab [78]. Recent work by the Helmrath and Wells groups introduced a tissue-engineering approach with PSCs to generate human intestinal tissue containing a functional ENS [79]. ENS-containing intestinal organoids grown in vivo formed neuroglial structures similar to a myenteric and submucosal plexus, contained functional interstitial cells of Cajal, and showed electromechanical coupling that regulated waves of propagating contraction. The authors further showed an example of how this system can be used to study motility disorders of the human gastrointestinal tract. Interestingly a recent study demonstrated that patient-derived (UC) organoids recapitulated colitic reactivity offering opportunities to tailor interventions to the individual patient [80]. In this context, a recent study demonstrated that PSC-derived intestinal organoids can be used for compound testing [81].

Beside PSC-derived organoids, intestinal as well as biliary and pancreatic organoids can easily be derived from the tissue of the adult stem-cell niche or adult somatic tissue. When these stem cells are grown in a three-dimensional environment, they self-organize into organoids that replicate key structural and functional features of the corresponding part of the GI or HBP tract they are derived from. Interestingly, even they share a common developmental origin, the physical turnover is highly variable between the different organs, which results in the fact that the efficiency of generating the organoids and maintaining the cultures varies among the organs [82][83][84]. Accordingly, these adult stem cell-derived organoids can be differentiated from organoids derived from high-turnover stem cells (e.g., intestinal epithelium) and slow-turnover organs such as the liver and pancreas [85][86]. The gastrointestinal tract has a further advantage as its maintenance and repair relies on a small population of actively cycling tissue-resident stem cells found in a very specific location at the bottom of the invaginations of the mucosa known as “crypts” [82]. In sharp contrast, the liver and pancreas do not contain a defined stem-cell niche, making it difficult to obtain the corresponding progenitor cells. Of note, small intestinal organoids derived from adult stem cells of the gut were the first 3D miniorgans described more than 10 years ago by the group of Hans Clevers [87]. He showed that two factors were important for maintaining these cells in a 3D structure: (I) an extracellular matrix (mostly with matrigel, a solubilized basement membrane preparation extracted from the Engelbreth–Holm–Swarm mouse sarcoma) and (II) a culture medium supplemented with growth factors that resemble the in vivo stem-cell niche environment. This medium mainly includes factors that stimulate the WNT signaling pathway and alters BMP signaling. Intestinal organoids can be used to study the pathophysiology of a variety of human inflammatory diseases, such as IBD and celiac disease, and infectious disorders (*Helicobacter*, *Salmonella*) as well as metabolic, and neoplastic diseases [7][88][89][90][91][92][93][94][95][96][97]. Patient-derived organoids further allow drug screening and personalized approaches for treating diseases. Importantly, in sharp contrast to those of the brain, intestinal organoids, including epithelial and ENS organoids, can be derived from PSC and ASC. This allows the comparison of organoids with identical genetic background, but only one population (ASC) was exposed to an inflammatory environment.

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