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Gut-brain axis: does intestinal inflammation affect hippocampal neurogenesis and medulloblastoma development?

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Gut-brain axis: does intestinal inflammation affect hippocampal neurogenesis and medulloblastoma development? Inflammatory bowel diseases (IBD) are a group of disorders that cause chronic inflammation in the intestines, associated with altered intestinal permeability, which in turn causes an immune response to enteric antigens in a genetically susceptible host. IBD incidence is high in industrialized countries and continuously rises in emerging economies.

Patients with IBD also often have increased costs associated with co-morbidities, including mental disorders. Compared to healthy controls, double the rate of depression has recently been reported in IBD patients, with this prevalence being heightened during active IBD (Masanetz et al., 2022). IBD also increases the risk of intestinal cancer that represent the second most common cause of death after cardiovascular diseases in these patients (Chang et al. 2018). Augmented risk for a variety of extraintestinal malignancies has also been reported in IBD patients, with inflammation and/or immunosuppression as a result of IBD treatment, as the main driving factors for IBD-related carcinogenesis (Chang et al., 2018). A gut-immune-brain axis has been very recently described as an important route for neuropsychiatric morbidity in IBD (Masanetz et al., 2022) and a higher risk of Alzheimer's and Parkinson's diseases has also been found in IBD patients (Szandruk-Bender et al., 2022). However, little is known about gut-brain communications and the molecular interplay between intestinal and neuronal molecules that may play a role in IBD and the mechanism(s) of these central nervous system pathologies is still largely unknown.

An emerging area of challenge in IBD is the mechanistic understanding of related brain manifestations in humans, and although its elucidation has been hampered by the scarce accessibility to human brain samples, well-established models of colitis are successfully being used to explore the pathogenesis of brain-related IBD manifestations. In particular, fully characterized mouse models of experimental colitis, represents a valuable tool to investigate the crosstalk between the intestine and the brain and to evaluate the IBD consequence on cancer and non-cancer brain pathologies. Different IBD mouse models exist, ranging from chemically induced colitis models to genetic models, to models which are induced by manipulation of the immune response, or addition of specific bacteria, each able to replicate many, but not all, aspects of human disease. The dextran sodium sulfate (DSS) model of chemically-induced colitis via drinking water, is a robust rodent model of either acute or chronic colonic inflammation, depending on administration strategies, with similar disease features seen in ulcerative colitis patients, including diarrhea, body weight loss, mucosal ulcers, shortened colon, and dysbiosis, as well behavioral alteration (Gampierakis et al., 2021; Vitali et al., 2022). Consequently, the vast majority of IBD studies in rodents are conducted using chemically-induced intestinal inflammation. Notably, several studies have recently addressed the influence of DSS-induced experimental colitis on adult neurogenesis (Zonis et al., 2015; Nakagawasai et al., 2020; Salvo et al., 2020; Gampierakis et al., 2021, Vitali et al., 2022) and overall, either acute or chronic colitis was shown to affect neurogenesis in the hippocampal dentate gyrus. In particular, both acute and chronic DSS-induced colitis was shown to inhibit the production of adult-born neurons in the dentate gyrus and to increase the microglia expression in the hippocampus (Zonis et al., 2015). DSS-treated mice were shown to exhibit reduced adult hippocampal neurogenesis and increased activation of microglia and astrocytes (Nakagawasai et al., 2020). More recently, acute colitis by DSS was reported to increase neurogenesis; though animals with chronic colitis had normal level of neurogenesis but the newborn neurons showed deficits in the integration into the functional circuitry. Notably, mice with DSS-induced chronic colitis also showed behavioral alterations consisting of impaired ability to respond to a novel spatial environment and exhibited dysregulated expression of the immediate early gene Arc, a marker of neuronal activity (Gampierakis et al., 2021).

In this framework, we have investigated the crosstalk between the intestine and the brain, by evaluating the short and long-term effects of intestinal inflammatory processes, such as those occurring in the context of IBD, on neurogenesis and neuroinflammation in the hippocampus using a DSS-induced experimental colitis mouse model (**Figure 1**; Vitali et al., 2022). Using different DSS administration strategies (1 DSS cycle of 7 days or 3 cycles of 7 days each followed by a recovery of 14 days with tap water), we induced acute or chronic colitis with clinical features of IBD patients (weight loss, diarrhea, shortening of the colon and histological changes at the level of the intestinal mucosa). We also showed activation of an inflammatory process in the colon as indicated by macrophage infiltration and increased expression level of pro-inflammatory cytokines and oxidative stress markers [interleukin (IL)-6, and inducible nitric oxide synthase]. In the hippocampus of mice with DSS-induced acute colitis, intestinal inflammation resulted in increased expression of inflammatory-related genes (IL-6, IL-1β, S-100, Tgf-β, and Smad-3), as well as microglia activation, a marker of neuroinflammation. Chronic DSS treatment also resulted in neuroinflammation in the hippocampus, as indicated by astrocyte activation. These findings demonstrated that IBD-driven immune reactions propagate to the central nervous system and influence brain-resident immune and glial cells. Inflammatory cytokines released in the hippocampus may be amplified by microglia activation and our data support this hypothesis as in the hippocampus of acutely DSS-treated mice we detected significant IL-1β upregulation, which was not observed in the colon.

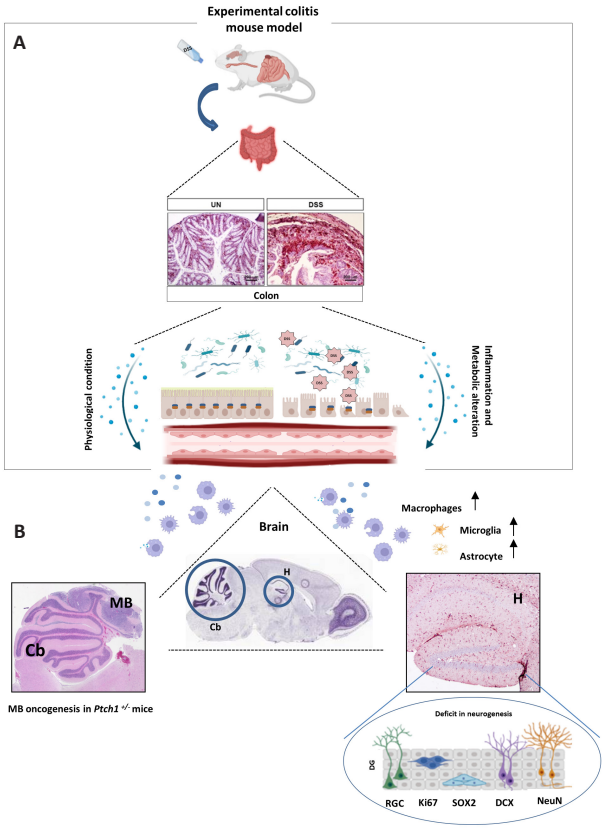

Figure 1 | **Crosstalk between the intestine and the brain in a mouse model of DSS-induced colitis.**
(A) DSS administration via drinking water in mice triggers intestinal inflammatory processes with cytokines release and colon macrophage infiltration. (B) Inflammation also propagates to the brain although we report regional differences in the peripheral inflammatory response caused by DSS experimental colitis in the brain. In particular, chronic colitis enhanced neuro-inflammatory response in the hippocampus but not in the cerebellum. Consequently, mice with colitis displayed alterations in adult hippocampal neurogenesis but not changes in the rate of MB tumorigenesis, suggesting the existence of regional-specific mechanisms by which chronic intestinal inflammation affects the brain. Cb: Cerebellum; Dcx: doublecortin; DG: dentate gyrus; DSS: dextran sulfate sodium; H: hippocampus; MB: medulloblastoma; NeuN: neuronal nuclear protein; RGL: radial glia-like; Sox2: SRY (sex determining region Y)-box 2; UN: untreated. Created with BioRender.com and adapted from BioRender templates (2020) https://app.biorender.com/biorender-templates.

Neuroinflammation is known to negatively regulate hippocampal neurogenesis and failing or altered neurogenesis has been associated with a number of neuropsychiatric diseases including anxiety and depression. Adult hippocampal neurogenesis is a multistep process that involves the proliferation, survival, differentiation and integration of newborn neurons into pre-existing neuronal networks. In our study, by evaluating the cellular composition of the subgranular and granular zone of dentate gyrus, we showed that the presence of neuroinflammation in the hippocampus of mice with colitis affects hippocampal neurogenesis, inducing deficits in different cell populations. Both acute and chronic colitis caused alterations in cell populations of adult dentate gyrus with staminal/progenitor features such as glial fibrillary acidic protein, Sox2 (SRY (sex determining region Y)-box 2), and Ki67 that were in fact more responsive to intestinal inflammation compared to Dcx^{+} (Doublecortin) newborn neuronal populations. Noteworthy, neural precursor cells constitutively express receptors for pro-inflammatory cytokines (Green et al., 2012) and the pro-inflammatory cytokines IL-1β and IL-6, overexpressed in the hippocampus of our mice with acute colitis, are shown to be potent suppressors of neurogenesis (Vallières et al., 2002).

Furthermore, in our investigations (Vitali et al., 2022), in order to unravel the metabolite role in IBD pathogenesis, we have compared the fecal metabolomic profiles of acute and chronic colitis, identifying marked metabolomics alterations in DSS-treated compared to untreated mice. However, consistently with the accumulation of metabolic changes along with the progression of acute to chronic colitis, these metabolic changes were more pronounced in chronic colitis animals (Vitali et al., 2022). The identified differentially accumulated metabolites and corresponding pathways analysis in mice with chronic colitis highlighted a set of common alterations in (i) alanine, aspartate and glutamate metabolism, (ii) thiamine metabolism, (iii) lipid and (iv) phenylalanine metabolism, providing the groundwork for understanding these processes during disease progression. Interestingly, these differentially accumulated metabolites are related to four disease signatures, including irritable bowel syndrome, Crohn's disease, ulcerative colitis, colorectal cancer as expected and to autism, strengthening the knowledge that other kinds of mental disorders could be associated with intestinal inflammation and metabolic imbalance.

Finally, as IBD patients are at increased risk of developing intestinal and extraintestinal cancers, including brain tumors (Chang et al., 2018; Mehrian-Shai et al., 2019), to our knowledge there are no experimental studies investigating the relationship between intestinal inflammation and the onset of brain tumors. To address a possible causal relationship between gut-related inflammation and brain cancer susceptibility, we induced colitis through DSS administration in *Ptch1*$^{+/-}$/C57BL/6 mice, a well-established mouse model of medulloblastoma recapitulating many of the histological and molecular features of the human tumor counterpart (Hahn et al., 2000). Medulloblastoma, the most common malignant nervous system tumor in childhood, is thought to arise from disruptions in cerebellar development. Our data showed the absence of neuroinflammation in the cerebellum of DSS-treated mice and no differences in medulloblastoma development between untreated and DSS-treated mice. Therefore, in our experimental model, the intestinal inflammation associated with colitis markedly influences brain homeostasis impairing hippocampal neurogenesis but not oncogenesis of the cerebellum, offering support to the hypothesis that the observed increased cancer risk in IBD patients may result from the immunosuppressive medications often used in IBD. Further experimental studies with mouse models of oncogenesis, in which IBD can be induced, are recommended as they might help to discern whether the increased risk of extraintestinal malignancy is related to immunoinflammation or immunosuppression.

Conclusions and future perspectives: Although the link between gut inflammation and neuropsychiatric morbidity in IBD patients is well-recognized, most of the studies are observational and the mechanistic understanding of brain dysfunction during disease progression remain rather limited. Robust mouse models of colitis are of great help in establishing functional connections between colon inflammation, metabolic alterations, neuroinflammation and alteration of hippocampal neurogenesis and, indeed, the body of mechanistic knowledge on IBD-related central nervous system comorbidity is largely based on findings produced in chemically-induced colitis in mouse models. Using a DSS-induced experimental colitis mouse model, we have reported short and long-term effects of intestinal inflammatory processes, such as those occurring in the context of IBD, on metabolic imbalance, on neurogenesis and neuroinflammation in the hippocampus, collectively suggesting a systemic immune reaction propagating to the brain. However, in our settings, neuroinflammation was not a generalized phenomenon in the brain, as it did apparently not involve the cerebellum, and in fact, the tumor rate in a well-characterized mouse model of cerebellar tumorigenesis was not influenced by the presence of DSS-driven gut inflammation.

There is a growing need for studies investigating the driving factors in the pathogenesis of neuropsychiatric comorbidities in IBD for a more comprehensive understanding of the epidemiological observations of this multifaceted disease. Moreover, the crosstalk between the intestine and the brain is bidirectional and, evidences accumulating over the past decade, have suggested that this pathological link may also represent a novel therapeutic target for treating neurodegenerative diseases (Nandwana et al., 2022). Moreover, targeting the patients' specific gut microbiota may alleviate neurological symptoms in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease (Nandwana et al., 2022). Therefore, investigating whether induction of intestinal inflammation may exacerbate the severity of neural manifestation in neurodegenerative mouse models might help to gain a better understanding of the gut-brain-axes in the contest of neurodegenerative pathophysiology.

Overall, well-characterized IBD animal models are a valuable tool to extend the molecular understanding of IBD-related neurobiological alterations and have the potential to address many knowledge gaps, also helping to identify targets for better therapeutic options for the treatment of IBD-associated comorbidities such as depression or neurodegenerative diseases.

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References

Chang M, Chang L, Chang HM, Chang F (2018) Intestinal and extraintestinal cancers associated with inflammatory bowel disease. Clin Colorectal Cancer 17:e29-37.

Gampierakis IA, Koutmani Y, Semitekolou M, Morianos I, Polissidis A, Katsouda A, Charalampopoulos I, Xanthou G, Gravanis A, Karalis KP (2021) Hippocampal neural stem cells and microglia response to experimental inflammatory bowel disease (IBD). Mol Psychiatry 26:1248-1263.

Green HF, Treacy E, Keohane AK, Sullivan AM, O'Keeffe GW, Nolan YM (2012) A role for interleukin-1β in determining the lineage fate of embryonic rat hippocampal neural precursor cells. Mol Cell Neurosci 49:311-321.

Hahn H, Wojnowski L, Specht K, Kappler R, Calzada-Wack J, Potter D, Zimmer A, Müller U, Samson E, Quintanilla-Martinez L, Zimmer A (2000) Patched target Igf2 is indispensable for the formation of medulloblastoma and rhabdomyosarcoma. J Biol Chem 275:28341-28344.

Masanetz RK, Winkler J, Winner B, Günther C, Süß P (2022) The gut-immune-brain axis: an important route for neuropsychiatric morbidity in inflammatory bowel disease. Int J Mol Sci 23:11111.

Mehrian-Shai R, Reichardt JKV, Harris CC, Toren A (2019) The gut-brain axis, paving the way to brain cancer. Trends Cancer 5:200-207.

Nakagawasai O, Yamada K, Takahashi K, Odaira T, Sakuma W, Ishizawa D, Takahashi N, Onuma K, Hozumi C, Nemoto W, Tan-No K (2020) Liver hydrolysate prevents depressive-like behavior in an animal model of colitis: Involvement of hippocampal neurogenesis via the AMPK/BDNF pathway. Behav Brain Res 390:112640.

Nandwana V, Nandwana NK, Das Y, Saito M, Panda T, Das S, Almaguel F, Hosmane NS, Das BC (2022) The role of microbiome in brain development and neurodegenerative diseases. Molecules 27:3402.

Salvo E, Stokes P, Keogh CE, Brust-Mascher I, Hennessey C, Knotts TA, Sladek JA, Rude KM, Swedek M, Rabasa G, Gareau MG (2020) A murine model of pediatric inflammatory bowel disease causes microbiota-gut-brain axis deficits in adulthood. Am J Physiol Gastrointest Liver Physiol 319:G361-374.

Szandruk-Bender M, Wiatrak B, Szeląg A (2022) The risk of developing Alzheimer's disease and Parkinson's disease in patients with inflammatory bowel disease: a meta-analysis. J Clin Med 11:3704.

Vallières L, Campbell IL, Gage FH, Sawchenko PE (2002) Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. J Neurosci 22:486-492.

Vitali R, Prioreschi C, Lorenzo Rebenaque L, Colantoni E, Giovannini D, Frusciante S, Diretto G, Marco-Jiménez F, Mancuso M, Casciati A, Pazzaglia S (2022) Gut-brain axis: insights from hippocampal neurogenesis and brain tumor development in a mouse model of experimental colitis induced by dextran sodium sulfate. Int J Mol Sci 23:11495.

Zonis S, Pechnick RN, Ljubimov VA, Mahgerefteh M, Wawrowsky K, Michelsen KS, Chesnokova V (2015) Chronic intestinal inflammation alters hippocampal neurogenesis. J Neuroinflammation 12:65.

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