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NEUROIMMUNE MECHANISMS IN THE MICROBIOTA-GUT-BRAIN AXIS

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INTRODUCTION

Gut microbiota has been increasingly recognized as a key factor in human health and disease, shaping and educating our immune system, participating in nutrient digestion and absorption, affecting host metabolism, playing key roles in gastrointestinal tract function, and even affecting distant organs, such as the brain (*Collins* et al., 2012; *Macpherson* et al., 2023). There are many pathways through which the gut microbiome can communicate with the brain, including the immune system, neuroendocrine system as well as using direct communication with the neural system (*Collins* et al., 2012).

GUT-BRAIN COMMUNICATION

Bacteria can signal through immune cells, as neurons express receptors for many cytokines and chemokines, which help to shape the function and development of the neural system (*Deverman* and *Patterson*, 2009). Multiple studies demonstrated that bacteria influence the activation of peripheral immune cells, thus regulating responses in neuroinflammation, brain injury, autoimmunity and neurogenesis (*Fung* et al., 2017).

Serotonin is one of the most abundant neurotransmitters in the gut and the brain, with effects on mood, cognition, and memory. Most of the human serotonin is being produced within the gut by the enteroendocrine cells (*O'Mahony* et al., 2015), and the intestinal microbiome can affect this production. It has been shown that indigenous spore-forming bacteria promote serotonin biosynthesis from colonic enterochromaffin cells, which supply 5-HT to the mucosa, lumen and circulating platelets, significantly impacting host physiology (*Yano* et al., 2015).

Gut bacteria produce many bioactive molecules that can affect the neural system as byproducts of bacterial fermentation, including short chain fatty acids (SCFAs), but they can also produce neurotransmitters found in mammals, including acetylcholine and GABA (*Lyte*, 2014). *Yunes* and colleagues showed that among 135 strains of human-derived Lactobacilli and Bifidobacteria strains, 43% had ability to produce GABA from its precursor monosodium glutamate (*Yunes* et al., 2016). Furthermore, the genes underlying GABA production, gadB and gadC, were also identified in the phyla Bacteroidetes, Proteobacterium and Firmicutes, indicating that the ability to produce GABA are widely distributed among many gut-derived bacterial species.

Gut bacteria can signal directly to the brain through neural pathways, such as the vagus nerve, which likely serves as an early danger signal during infection (*Goehler* et al., 2005), but can also mediate beneficial effects of probiotics

on anxiety-like behaviour (*Bercik* et al., 2011a; *Bravo* et al., 2011). The communication between the gut and the brain is bi-directional, as neural mediators, as well as stress can affect the composition and function of the gut microbiome. For example, adherence of *Escherichia coli* O157:H7 to murine caecal mucosa can be modulated by norepinephrine and dopamine, suggesting that stress exposure may influence host susceptibility to enteric infections (*Chen* et al., 2003). Furthermore, chronic social stress alters the composition of the gut microbiome and exacerbates *Citrobacter rodentium*-induced inflammation (*Galley* et al., 2017), which can be improved by treatment with probiotics.

The gut microbiome affects the function as well as the structure of the brain. Compared to conventionally raised mice, germ-free mice display more exploratory (anxiolytic) and less depressive-like behaviour associated with altered expression of multiple genes regulating neurotransmitter and neurotrophin production (*Bercik* et al., 2011b; *Diaz Heijtz* et al., 2011). Germ-free mice also have altered blood-brain barriers, changes in morphology of the amygdala and hippocampus, altered myelination profiles and plasticity, and global defects in microglia (*Braniste* et al., 2014; *Hoban* et al., 2016; *Luczynski* et al., 2016; *Erny* et al., 2017). Interestingly, most of these abnormalities, including behaviour, normalize after bacterial colonization. However, it is unknown what specific neuroimmune mechanisms mediate and initiate these changes.

THE GUT MICROBIOME AND BEHAVIOUR

We studied mouse behaviour, in germ-free conditions and at several time points after colonization with either complex or simple microbiota. We confirmed that germ-free mice display more exploratory and less depressive-like behaviour, as assessed by the light preference and the tail suspension tests, respectively, compared to mice born and raised conventionally (in the presence of microbiota). Colonization of adult germ-free mice with either complex (Specific Pathogen Free) or simple (Altered Schaedler Flora) microbiota normalized their behaviour and altered expression of brain neurotrophins; these changes were observed already at 2 weeks post-colonization. Interestingly, monocolonization of mice with a laboratory strain of *E. coli* was sufficient to normalize their behaviour and brain chemistry.

We found that this behavioural normalization was dependent on the activation of the innate, but not the adaptive, arm of the immune system through TLR and NOD signalling, and that intestinal dendritic cells but not macrophages played a key role in this process. Finally, we discovered that the normalization of behaviour was associated with many changes in the neuroimmune system, both in the gut and the brain, which included altered expression of multiple brain proteins involved in neural plasticity (*Philip* and *Kraimi*, unpublished data).

Although these results were obtained in mice, the observed impact of the bacterial colonization on the neuroimmune system can be likely extrapolated to humans, bearing on psychiatric conditions, in which altered innate immune signalling has been implicated (*Ratajczak* et al., 2018). Indeed, our results raise the possibility that perturbation of initial (postnatal) microbiota-host cross-talk could affect normal brain development and function. In that respect, a large population-based study

found that bacterial infections and use of antibiotics at very early age increase risk of developing psychiatric diseases later in life (*Köhler-Forsberg* et al., 2019).

THE GUT MICROBIOME AND PAIN

Apart from mood and behaviour, the gut microbiome has been shown to affect perception of somatic and visceral pain (*Verdú* et al., 2006; *Amaral* et al., 2008; *Yang* and *Chiu*, 2017), although the mechanisms are not fully understood. We have recently shown in patients with irritable bowel syndrome (IBS), a disorder where microbiome plays a key pathophysiological role, that altering intake of poorly digestible dietary fibre improves gut symptoms, mainly abdominal pain (*McIntosh* et al., 2017). This was associated with decrease in urinary histamine, which correlated with severity and frequency of pain (*Keshteliu* et al., 2019), as well as changes in the gut microbiota profiles (*McIntosh* et al., 2017). Histamine is a neuroimmune mediator, produced by mast cells and basophils, known to be involved in the control of intestinal permeability and visceral sensitivity (*Boeckxstaens* and *Wouters*, 2017). However, histamine can be produced by gut bacteria as many microbes possess the histidine decarboxylase (*hdc*) gene encoding the enzyme capable of converting histidine into histamine (*Takahashi* et al., 2003; *Fiorani* et al., 2023).

To investigate the putative role of gut microbiome in the abdominal pain in IBS patients we employed our previously developed microbiota-humanized mouse model, that mimics many features of IBS, including altered gastrointestinal transit, low grade inflammation, increased intestinal permeability and anxiety-like behaviour (*De Palma* et al., 2017). We chose faecal microbiota samples from IBS patients with either high or low urinary histamine, and used samples from healthy volunteers as control. Mice were then placed on human-like diet with high content fermentable fibre, and their visceral sensitivity was assessed three weeks later.

Although all mice colonized with IBS microbiota displayed greater bowel distension compared to mice with healthy control microbiota, only mice colonized with microbiota from patients with high urinary histamine displayed higher pain responses to colorectal distension (*De Palma* et al., 2022). When incubating caecal contents of these mice with excess histidine, we found 40 times higher production of histamine, compared to caecal contents of mice colonized with microbiota from healthy volunteer or patients with low urinary histamine. We identified a specific bacterium, *Klebsiella aerogenes* MQ, which generated up to 100 times more histamine than any other strain investigated.

When examining host responses to bacterial histamine, we found a greater density of colonic mast cells, which were often co-localized with neural cells. Interestingly, mast cell hyperplasia and co-localization with neural fibres were previously shown in colonic biopsies from patients with IBS, and this finding correlated with severity of abdominal pain (*Barbara* et al., 2004). Using immunohistochemistry, we found that the H4 receptor (H4R), but not H1, H2 or H3 receptor, expression was upregulated in mice with high bacterial histamine and was present in multiple cell types, including goblet cells, mast cells, lymphocytes, macrophages and enteroendocrine cells. Treatment with a

H4R antagonist normalized both pain responses and mast cell levels in mice colonized with histamine producing microbiota.

Placing mice with histamine producing microbiota on a low fermentable diet decreased pain signalling and mast cell levels, which was associated with decrease in colonic lactic acid producing bacteria and lactic acid, a main determinant of colonic pH. We found that *in vitro* histamine production by *K. aerogenes MQ* was pH dependent, in agreement with previous reports (*Landete* and *De las Rivas*, 2008), with optimal pH of 6.5 We thus incubated *K. aerogenes MQ* with lactobacilli and colonized germ-free mice with mixture of lactobacilli and *K. aerogenes MQ*. In both instances, histamine production dramatically decreased suggesting that fermentable fibre creates an ideal environment for histamine production, thus explaining results from our clinical trial (*McIntosh* et al., 2017).

Altogether, our data suggest that bacterial histamine can trigger chronic abdominal pain, at least in a subset of patients with IBS. Based on our own data and a publicly available dataset (*Mars* et al., 2020), we estimate that between 15 and 25% of IBS patients have high histamine producing microbiota. Identifying this subgroup will be crucial as these patients may truly benefit from a low fermentable diet, and presence of *K. aerogenes* or bacteria with similar *hdc* activity in the gut could guide dietary recommendations, microbiota-directed therapies, or possible use of H4 receptor antagonists.

CONCLUSIONS

In summary, accumulating evidence suggest that gut bacteria can interact with both the peripheral and the central neural system, either directly or through immune or endocrine systems. Better understanding of these mechanisms and their implications are likely to help in management of patients with chronic gastrointestinal diseases, as well as neuropsychiatric disorders, in which microbiota has an important pathophysiological role.

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