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THE EFFECT OF INFECTION AND ANTIBIOTICS ON GUT AND BRAIN

PREMYSL BERCIK

Farncombe Family Digestive Health Research Institute, Department of Medicine, Division of Gastroenterology, McMaster University, Hamilton, Canada

A growing body of evidence now supports the view that the gut and the brain can communicate and influence each other's function. There are multiple pathways involved in this bi-directional communication, including neural and humoral mechanisms (*Bercik* et al., 2012). The afferent neural pathways include the vagus nerve, spinal afferents and the enteric nervous system (ENS). The gut can influence brain's function by the release of multiple regulatory peptides and pro-inflammatory cytokines, while the brain modulates gastrointestinal function through the autonomic nervous system, hormonal and immune pathways.

Gastrointestinal infection and subsequent inflammation can markedly affect behaviour and central nervous system (CNS) function. Acute inflammation induces a complex of symptoms, termed cytokine-induced sickness behaviour, which include both a neurovegetative and a psychological component (*Dantzer* and *Kelley*, 2007). Neurovegetative symptoms include loss of appetite, sleepiness, fatigue and fever, while psychological symptoms comprise depression, anxiety and cognitive dysfunction. Chronic exposure to pro-inflammatory cytokines results in changes in behaviour and CNS function, as evidenced during experimental cytokine cancer therapy (*Denicoff* et al., 1987). Psychiatric symptoms are also a frequent side effect of interferon treatment for chronic hepatitis C (*Renault* et al., 1987; *Keefe*, 2007).

Most of the evidence that gastrointestinal infections affect brain function comes from experimental animal studies. *Lyte* et al. (1998) demonstrated that mice infected with *C. jejuni* display anxiety-like behaviour, even during the early stage of the infection, when a systemic immune response was undetectable. This suggests that non-immune factors are also involved in gut-brain communication. The same group has shown that the abnormal behaviour in infected mice was present as early as 4 hours post-infection, and this was associated with increased neural activation pattern in vagal and central autonomic pathways, suggesting that the vagus plays a crucial role in the early detection of pathogens (*Gaykema* et al., 2004; *Goehler* et al., 2005). We have shown that chronic infection with *H. pylori* induces changes in the gastric cholinergic nerve function, as well as up-regulation of substance P and CGRP within spinal afferents (*Bercik* et al., 2002). This was accompanied by increased sensitivity to gastric distension and altered gastric emptying (*Bercik* et al., 2009). The chronically infected mice also displayed an abnormal feeding pattern, eating more frequently but smaller amounts of food per feeding bout, which is similar to eating habits in patients with functional dyspepsia, whose principle complaint is early satiety and abdominal fullness. When examining the brain chemistry of these mice, we found that chronic infection altered expression of pro-opiomelanocortin (POMC) in the arcuate nucleus, one of the brain centres

involved in food control, as well increased expression of tumour necrosis factor alpha (TNF-α) in the median eminence, one of the circumventricular organs (*Bercik* et al., 2009). Interestingly, some of these abnormalities did not fully normalize even 2 months after successful *H. pylori* eradication, suggesting that chronic infection may alter permanently the neural and gut function. The infected mice also displayed a trend for anxiety-like behaviour, when assessed by the light preference test, and this abnormal behaviour became more prominent during repeated testing. On closer examination it became clear that the control mice learned and adapted their behaviour in time becoming less anxious, while the infected mice maintained the abnormal behavioural pattern. The results suggest that chronic gastrointestinal infection may affect cognition and memory.

To further explore the effect of chronic infection and inflammation on behaviour, we have used model of chronic non-invasive parasite *Trichuris muris,* which in susceptible mice leads to mild-to-moderate colitis. We have found that mice chronically infected with *T. muris* have anxiety-like phenotype compared to control healthy mice (*Bercik* et al., 2010). This abnormal behaviour was accompanied by decreased expression of brain-derived neurotrophic factor (BDNF) in the hippocampus, which is one of the main brain centres involved in the regulation of mood and memory (*Deng* et al., 2010). BDNF is an important neurotrophin that regulates neural plasticity, and its lower levels were associated with anxiety and depression (*Duman* and *Monteggia*, 2006; *Martinowich* et al., 2007). The infected mice had modestly, but statistically significantly increased serum concentration of the pro-inflammatory cytokines TNF-α and IFN-γ. Both of these cytokines have been shown to alter tryptophan/serotonin metabolism with increasing production of kynurenine, which is able to induce anxiety/depression-like behaviour in a dose dependent fashion (*O'Connor* et al., 2009a,b). Indeed, we found that mice with *T. muris* infection had increased levels of kynurenine, which normalized together with mouse behaviour, after treatment with anti TNF-α agent etanercept (*Bercik* et al., 2010). These data suggest that chronic gut inflammation triggers alterations in the function and biochemistry of the CNS through immune mediated pathways.

It is possible that changes in gut microbiota induced by administration of antibiotics are associated with immune activation in the gut and subsequently with changes in behaviour. We have shown in a murine model, that a one-week oral treatment with non-absorbable antimicrobials induced significant alteration in gut microbiota composition, as well as mild acute inflammation (*Verdú* et al., 2006). This was associated with increased sensitivity to colorectal distension and up-regulation of substance P in the intestine. Interestingly, treatment with probiotic *L. paracasei* ameliorated visceral sensitivity and normalized expression of substance P.

In a recent study using a different mouse strain and lower concentration of the same antimicrobials, we were able to induce a significant intestinal dysbiosis, but which was not associated with over inflammation or changes in neurotransmitter content in the gut (*Bercik* et al., 2011). Interestingly, the mice treated with antimicrobials increased their exploratory behaviour and became more active. This was associated with an increase in BDNF content in the hippocampus and decreased BDNF in the amygdala. This is consistent with the role of hippocampus and amygdala in mood modulation, as

over-activation of the amygdala has been implicated in depression and anxiety (*Drevets*, 2000). Since intraperitoneal administration of antimicrobials did not alter the mouse behaviour and germ-free mice given the same antimicrobials orally did not exhibit changes in behaviour, we can conclude that the increased exploratory behaviour in orally treated mice was due to changes in gut microbiota composition. Interestingly, germ-free mice displayed marked anxiety behaviour after colonization with commensal bacteria (*Bercik* et al., 2011). Overall these data suggest that intestinal microbiota has the capacity to modulate behaviour of the host.

To investigate this concept further, we have compared the behaviour and microbiota composition of NIH Swiss and BALB/c mice. While NIH Swiss are adventurous, with high exploratory drive, BALB/c mice are shy and cautious. Interestingly, these two mouse strains also had different microbiota composition. We thus derived both mouse strains under germ-free conditions and then colonized them with their own microbiota or with microbiota from the other mouse strain. While the mice colonized with the homologous microbiota displayed identical behaviour as their conventional counterparts, germ-free NIH Swiss mice colonized with BALB/c microbiota became more shy and hesitant, and germ-free BALB/c mice colonized with NIH Swiss microbiota became more daring and active (*Bercik* et al., 2011). The changes in mouse behaviour were accompanied by alterations in hippocampal BDNF levels, but no change in immune markers or gut neurotransmitter content was observed. Thus the intestinal microbiota can affect CNS function through immune-independent pathways.

In summary, accumulating data from animal studies suggest that gut inflammation and intestinal microbiota have a profound effect on gut function, behaviour and brain biochemistry of the host. Microbiota-gut-brain communication is complex and likely involves multiple pathways, including neural, immune and metabolic mechanisms. Up to date, human data on microbiota-gut-brain axis is limited and clinical trials are needed to extend our understanding on the role of bacteria in both health and disease.

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