ROLES OF GUT MICROBIOTA IN ENERGY METABOLISM DISORDERS, INFLAMMATION, ANXIETY AND DEPRESSION

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1. ABSTRACT

Gut microbiota has recently been shown to have various effects on human epigenetics and physiology. Its composition is established at postnatal colonization and later affected by e.g. dietary choices and antibiotic treatments, which may modulate this constantly present source of neural, endocrine and immune signals to either protect one from or predispose one to a large variety of disorders throughout life. This work focuses on the emerging field of gut microbiome-brain axis (MBA) research which studies the effect of intestinal microbes on brain development and (dys)function. It is shown that the gut microbiota are needed for normal endocrine and behavioral responses to stressors and that their pro-inflammatory activity may cause e.g. anxiety and depression. Dysbiotic state of the microbiota often co-occurs with common diet-dependent metabolic and immune disorders such as obesity and metabolic syndrome which all own a chronic systemic inflammatory component. Such synergic pro-inflammatory activity increases the risk for sickness behavior akin to symptoms of depression as well as plethora of other inflammation-dependent disorders. The role of gut microbiota in psychiatric diseases is now generally regarded as a paradigm shift for how they are perceived. Quali- and quantitative assessment of MBA activity along with dietary habits and co-morbid disorders provides a holistic view for the connections between the gut microbiota, metabolic, immune and psychiatric disorders that will help us to prevent and treat both physical and mental disorders more efficiently and effortlessly than before.

2. INTRODUCTION

Gut microbiota and its health effects is one of the most understudied field of medicine that has been gaining momentum only recently. PubMed search for “human gut microbiota” returns 4174 hits and shows that the number of these publications have been substantially increasing only after the year 2005. In this work the term ‘microbiota’ is defined as the whole of $10^{14}$ gut microbes ($10x$ the number of human cells) found at and between the duodenum and the distal colon, consisting of symbiotic, neutral (commensal) and pathobiotic anaerobic bacteria, archaeabacteria, protozoa and fungi of individual compositions. Pathobiotics such as *Escherichia coli* may cause an infection that activates the innate immune system. Commensal bacteria seem to form the most of the microbiome. In line with the notion made first by Paracelsus, the quantity of a substance determines its qualitative effect also in the form of gut microbiota. Here this refers to commensal bacteria such as some members of the clade of *Lactobacillales* (e.g. certain species of *Lactobacilli* and *Bifidobacterium*) which are generally thought to be beneficial for our health mainly by competing with pathobiotics for space, but which in overt quantities could convey negative health effects. Symbiotic or probiotic microbes (e.g. *Lactobacillus rhamnosus*, *Bifidobacterium infantis*, related *B. longum* NCC3001 and *Akkermansia muciniphila*) on the other hand convey positive effects either via bacteriostatic and/or bacteriocidic metabolites, and/or substances that provide building blocks, beneficial modulatory signals, antioxidants and/or anti-inflammatory protection for the body.
2.1. Stress and inflammatory reactions modulating the gut microbiota composition

Both physical and mental stressors may activate responses that are linked to inflammation via immune reactions (Slavich and Irwin 2014). These responses are mediated by two interconnected systems: the (limbic-)hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). Sympathetic effects are caused by adrenergic messengers noradrenaline (NA) and adrenaline which are mainly released by the pontine locus coeruleus (LC, responsible for e.g. stress- and panic reactions) in the central nervous system (CNS) and by the adrenal medulla in the periphery. LC may activate the limbic system which in turn may activate the HPA axis that releases stress hormones (glucocorticoids) such as cortisol from the adrenal cortex in to the peripheral circulation.

The fast-acting and often brief behavioral responses caused by the SNS activity are collectively called as “the five f’s”: fright, freeze, fight, flight, and making love. Persistent activation of the SNS may also modulate behavior via pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor alpha and chemokine CCL2 that are released by the cells of the innate immune system (Dantzer et al 2008, Slavich and Irwin 2014). This release is dependent on NAergic stimulation of the beta-adrenergic receptors which activates e.g. the pro-inflammatory NF-kB transcription factor leading to upregulation of several hundreds of gene-products that participate in wide array of defense mechanisms. NF-kB activity is also upregulated with social adversity, the transcriptional profile however varies according to their qualitative nature (Slavich and Irwin 2014). Autonomic effects of the SNS are counteracted by the parasympathetic nervous system that uses acetylcholine (ACh) as its neurotransmitter which also functions as a neuromodulator in the CNS.

[Glucocorticoids] elevated by the HPA-activity produce enhanced functionality in stressful situations, support adaptive SNS responses and act as anti-inflammatory signals in intermittent situations. Chronic elevations caused e.g. by persisting social threat is instead detrimental to normal physiology of the periphery and the CNS due to catabolic effects and the developing glucocorticoid insensitivity that turns HPA activity as pro-inflammatory. Patients with anxiety and/or depression (A/D) have persistent flattened cortisol response, heightened overall [cortisol] and altered cortisol sensitivity (Dantzer et al 2008, Slavich and Irwin 2014). Glukocorticoids can also change gut microbiota composition and alter intestinal permeability and barrier function, which in case of A/D leads to leaky gut and increased circulating levels of e.g. pro- and/or anti-inflammatory (depending on the individual’s microbiome) bacterial cell wall components such as (lipo)polysaccharides that can be counteracted with probiotics such as L. farciminis leading to decreased gut permeability and HPA activity (Cryan and Dinan 2012).
2.2. Vagus nerve, the anti-inflammatory reflex and obesity

Many neural, endocrine (glucocorticoid) and immune (cytokine) pathways that mediate the bidirectional communication between the microbiota, enteric nervous system and the CNS require the tenth cranial nerve (n. vagus) as a mediator (Mayer et al 2014). According to a review by Pavlov and Tracey (2012), the vagus is a paired parasympathetic nerve that “regulates metabolic homeostasis by controlling heart rate, gastrointestinal motility and secretion, pancreatic endocrine and exocrine secretion, hepatic glucose production, and other visceral functions. In addition, the vagus nerve is a major constituent of a neural reflex mechanism—the inflammatory reflex—that controls innate immune responses and inflammation during pathogen invasion and tissue injury.” It extends from and to the medulla oblongata and from there to all visceral organs in the periphery. In the CNS it synapses e.g. bidirectionally with medullar nucleus tractus solitarii region (NTS), which in turn synapses e.g. with LC. Functionally, its peripheral parts are divided to afferent fibers (comprising over 80% of all of its fibers), acting as sensors for various visceral signals including inflammatory molecules, and efferent fibers, which mediate the brain-to-gut and brain-to-immune communication. It affects the innate immune cells via anti-inflammatory cholinergic pathway that suppress the NF-κB activity and the release of pro-inflammatory cytokines in type 7 nicotinic ACh receptor activation-dependent manner (Pavlov and Tracey 2012). Prevention of excessive inflammation requires both fibers (Slavich and Irwin 2014).

Dysfunctional vagal anti-inflammatory response (dVAR) is often seen in obesity which is characterized by chronic low-grade peripheral inflammation as well as metabolic and immune dysfunction that in turn aggravates peripheral inflammation caused by e.g. dysbiosis. This is evident in e.g. obese individuals who are developing insulin resistance and metabolic syndrome which can be reversed by selective cholinergic stimulation of the efferent vagus-mediated anti-inflammatory response (Pavlov and Tracey 2012). Vagal stimulation activates the NTS which in turn stimulates NAergic LC that improves mood, promotes neurogenesis and suppress inflammation by decreasing excitatory signaling and stimulating serotonergic dorsal raphe neurons as well as central and vagal cholinergic neurons (Cheyuo et al 2011).

2.3. Depression, anxiety and cytokine-induced sickness behavior

A/D are inflammation-related in humans (Dantzer et al 2008, Slavich and Irwin 2014). Stressors such as social rejection and major disappointments can initiate the “conserved transcriptional response to adversity” that activates the SNS and HPA-axis which at least in chronic situations overstimulates NF-κB activity and elevates [pro-inflammatory cytokines] in
the CNS and periphery. These cytokines can affect the brain and behavior by causing “cytokine-induced sickness behavior” (CISB) akin to clinical depression. Symptoms of CISB include lethargy, amotivation, depression, failure to concentrate, anorexia, sleep disturbances, reduction in personal hygiene or social withdrawal (Dantzer et al 2008). Reason for this effect is hypothesized to be a mental feedforward loop which activates pain-related neural systems that anticipates physical (and abstract) traumas and initiates reaction to them in advance. This purely mind-induced form of brain inflammation manifesting as a mood disorder can also be treated with anti-inflammatory medication such as paracetamol in the case of social rejection, which is one of the most potent inducer of systemic inflammation both in animal models and humans (Slavich and Irwin 2014).

Emotional and behavioral signs of A/D may be explained by aberrant stress and inflammatory responses but depression also exhibits cognitive dysfunction which is related to dysfunctional neural networks. Prolonged elevations of [glucocorticoids] and inflammatory processes cause brain atrophy especially at hippocampi which leads to deficits in learning and memory. Opposing signals such as those created by brain-derived neurotrophic factor (BDNF) leads to decreased anxiety, heightened neuroplasticity and regeneration of neuronal connections that may eventually normalize dysfunctional neuronal networks and thus mood and cognitive abilities (Dantzer et al 2008, Cheyuo et al 2011, Slavich and Irwin 2014).

3. MICROBIOMERIC SIGNALS, CNS AND ENERGY METABOLISM

In addition to protecting against opportunistic pathogens, modulating immune functions, metabolizing dietary nutrients/drugs and influencing their absorption, gut microbiota also produce neurotransmitters and other neuromodulators that can affect several aspects of (patho)physiology and mood (Cryan and Dinan 2012, Mayer et al 2014). Microbes that produce neurotransmitters include e.g. *Lactobacillus* spp. and *Bifidobacterium* spp. that produce inhibitory γ-amino butyric acid (GABA) with e.g. anxiolytic effects; *Escherichia* spp., *Bacillus* spp. and *Saccharomyces* spp. produce NA; *Candida* spp., *Escherichia* spp., *Enterococcus* spp. and *Streptococcus* spp. produce serotonin which has roles e.g. in mood and immune regulation; *Bacillus* spp. produce dopamine that modulates e.g. in mood and motivation; *Lactobacillus* spp. produce ACh and probiotics such as *Akkermansia* spp. increase endocannabinoid levels as well as opioid and cannabinoid receptor concentrations in the gut epithelium, producing anti-inflammatory and antinociceptive effects that may affect the cortical pain areas and thus CISB. CNS and immune effects of these neurometabolites are often (e.g. in case of most probiotics) but not always vagally mediated (Cryan and Dinan 2012, Mayer et al 2014).
Gut microbiota also produce other metabolites that affect the CNS, energy metabolism and epigenetics. Short-chain fatty acids (SCFAs) such as acetate (C\textsubscript{2}), n-butyrate (C\textsubscript{4}) and propionate (C\textsubscript{3}) are produced mainly in the colon by bacterial fermentation of complex carbohydrates (resistant starch and digested dietary fibers). Apart from being important energy sources, affecting the development of the immune system and modulating endocrine functions (e.g. leptin and neuropeptide YY secretion) by stimulating free fatty acid receptors, they are also inhibitors of histone deacetylase (HDAC) enzymes that de-acetylate other proteins and compactify chromatin making it transcriptionally inactive (Vinolo et al 2011, Lin et al 2012). SCFAs thus increase gene transcription by inhibiting HDAC-dependent epigenetic changes and affect intracellular signal transduction e.g. by inhibiting NF-κB (Vinolo et al 2011). C\textsubscript{2} and C\textsubscript{4} have been shown to protect from diet-induced obesity without hypophagia whereas C\textsubscript{3} has an anorectogenic effect (Lin et al 2012). There’s a causal and perhaps bidirectional link between microbiome composition and obesity (Cryan and Dinan 2012). SCFAs are also anti-inflammatory as they decrease [pro-inflammatory cytokines] and increase [anti-inflammatory lipid mediators] including lipoxins, maresins, protectins and resolvins (Vinolo et al 2011).

### 3.1. Effects of diet and gut microbiota on anxiety and depression

Lack of “microbimergic” (vagal) communication leads to hyperreactive anxiety evident as HPA-axis hyperactivity and decreased [BDNF]\textsubscript{CNS} in germ-free mice. Antibiotic treatment of ordinary lab mice also produces dysbiosis that leads to similar decreases in [BDNF] and enhanced stress responses. This behavioral phenotype is reversible with early \textit{B.infantis} colonization (as happens also naturally in human infants) and is aggravated with enteropathogenic \textit{E. coli} (Mayer et al 2014). If this true, probiotic microbes should stimulate the vagus nerve, attenuate inflammation and relieve A/D in at least in a subgroup of depressed patients and research animals. This has been shown to occur with mice treated with \textit{L. rhamnosus} which modulated their CNS GABAergic signaling and decreased A/D-related behavior that was preventable with vagotomy (Bravo et al 2011).

Dietary lipids that interact with gut microbiota may cause metabolic dysfunction and inflammation that manifests as depression-like symptoms and other inflammation-related pathologies. In a study by Caesar and co-workers (2015), mice fed with saturated lipids (lard) developed increased innate immune system-related Toll-like receptor activity and white adipose tissue inflammation together with insulin insensitivity, whereas this was not seen with germ-free mice or those that had been fed with fish oil. Feeding with lard increased intestinal load of \textit{Lactobacilli} which was correlated with the increased adiposity and inflammatory
signals, while fish oil diet was correlated with increase in intestinal \textit{A. muciniphila}. Transplantion of this probiote in to lard-fed mice provided partial protection against the inflammatory and metabolic disturbances.

4. CONCLUSIONS

Stress and unhealthy food with disproportionately large amount of saturated fats may affect intestinal microbiome composition and cause obesity and inflammation that may manifest itself as A/D due to CISB. Obesity is often seen together with dVAR that aggravates and prolongs the peripheral inflammation as well as starves the CNS of vagal anti-inflammatory and neurotrophic stimulation. Rebalancing the microbiome with probiotes and healthy diet provides partial protection against metabolic and immune dysfunction-related pathologies including e.g. metabolic syndrome, diabetes and A/D. More research is needed to determine how gut microbial signals are transmitted, what their exact effects are and how they cause or protect us from pathologies throughout the body.

5. REFERENCES


