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Mini review

The microbiome and disorders of the central nervous system☆

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ABSTRACT

Alterations of the gut microbiota have been associated with stress-related disorders including depression and anxiety and irritable bowel syndrome (IBS). More recently, researchers have started investigating the implication of perturbation of the microbiota composition in neurodevelopmental disorders including autism spectrum disorders and Attention-Deficit Hypersensitivity Disorder (ADHD). In this review we will discuss how the microbiota is established and its functions in maintaining health. We also summarize both pre and post-natal factors that shape the developing neonatal microbiota and how they may impact on health outcomes with relevance to disorders of the central nervous system. Finally, we discuss potential therapeutic approaches based on the manipulation of the gut bacterial composition.

1. The human gut microbiota

1.1. What is the microbiota

The human gastrointestinal (GI) tract is colonized by bacteria, archaea and eukaryotes and approximates the same number as the number of human cells in the body (Sender et al., 2016). The term used to refer to the different organisms within this microbial community is microbiota while microbiome refers to the collective microbial genes. The bacteria composition differs depending on the section of the GI tract considered (Hooper and Gordon, 2001). Approximately 700 species have been detected in the mouth, while 95 have been detected in the oesophagus (Pei et al., 2004; Aas et al., 2005). The stomach comprises between 10^2 and 10^4 cells per gram of content due to the low pH and peristaltic waves (Delgado et al., 2013). In the small intestine, bacterial population increases from 10^4, near the stomach, up to 10^7 cells per gram of content, near the colon due to the slower peristalsis (O'Hara and Shanahan, 2006; Delgado et al., 2013). Bacterial density increases in the large intestine from 10^8 in the cecum up to 10^{12} cells per gram of content in the stool (Dethlefsen et al., 2006). At this level 800 species have been suggested to be present, with *Firmicutes* and *Bacteroidetes* being the most abundant phyla (Turnbaugh et al., 2007).

Alteration of the microbial diversity, especially during early-life has been associated with a negative health outcome later in life. An overgrowing number of publications have provided novel insights on the links between the microbiota and disorders of the central nervous system including; depression and anxiety, irritable bowel syndrome (IBS), autism spectrum disorders (ASD) and Attention-Deficit Hypersensitivity Disorder (ADHD).

In this review we aim to summarize and discuss the current knowledge of the implications of the gut microbiota in the gut-brain communication with relevance to these disorders. Moreover, we discuss how modulation of the intestinal microbiota may improve certain core symptoms of disorders of the central nervous system.

1.2. The microbiota across the life span

It has been thought for quite a while that during fetal life, the GI tract is sterile. However, microbial presence has been recently described in the placenta, the organ through which nutrients and oxygen are transferred form the mother's blood to the fetus, suggesting that exposure to bacteria may occur before birth. Interestingly, the placenta bacterial composition ranging from *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria* phyla was most similar to that present in the mouth than gut or vagina (Aagaard et al., 2014). The origin of these bacterial species is still not fully elucidated.

Upon delivery the neonate is exposed to vaginal, fecal and skin bacteria from the mother which are responsible for first substantial GI colonisation (Dominguez-Bello et al., 2010). The first bacteria colonising the infant gut are represented by the facultative species *Enterobacteriaceae*, *Staphylococcus* and *Streptococcus* which remain predominant during the first two weeks of life (Bezirtzoglou, 1997). The

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consumption of oxygen by these bacteria prepares the environment for the colonisation of strictly anaerobes such as *Bacteroides* and *Bifidobacterium* species. Bacterial composition during the first year of life is also affected by mode of feeding (Roger et al., 2010). With the introduction of solid food, by the end of the first year of life, the composition of the gut micorbiota begins to converge towards an adult-like profile (Palmer et al., 2007). Although changes in lifestyle, dietary habits and illness have been described to have an impact on the individual gut microbial composition, during adult life the gut microbiota is relatively stable and characterised by two dominant phyla: *Firmicutes* and *Bacteroidetes* (90%) (Costello et al., 2009). However, age-related changes occur in the gut microbial population composition. Although the total anaerobic bacteria are stable in elderly populations, shifts in genera composition have been described (for review see (Woodmansey, 2007)).

1.3. Functions of the microbiota in health

The microbiota maintains a symbiotic relationship with the host contributing to essential functions such as food digestion, energy harvest and storage, the development of the immune system, maintenance of intestinal barrier function and integrity and protection against pathogenic organisms.

The microbiota and in particular members of the genus *Bacteroides*, is responsible for the digestion of otherwise indigestible carbohydrates derives from the diet leading to the production of important nutrients such as short chain fatty acids (SCFA) including acetate, propionate and butyrate which represents a rich source of energy for the host (Macfarlane and Macfarlane, 2003). Moreover, the gut microbiota has also been shown to play a role in lipid metabolism. Indeed, *Bacteroides thetaiotaomicron* has been shown to up-regulate the expression of colipase which plays a critical role in lipid metabolism by stimulating the activity of pancreatic lipases (Hooper et al., 2001). Vitamins including vitamin K and B12 are also produced by the metabolic function of the gut microbiota (Macfarlane and Macfarlane, 2003).

Bacteria within the gut are associated with the development of the systemic and intestinal immune systems including the innate and the adaptive immune system. Several studies have demonstrated that the intestinal microbiota is essential for the normal development and function of effector and regulatory T cells, shaping a normal gut associated lymphoid tissues (GALT), IgA secretion, modulation of Group 3 innate lymphoid cells as well regulation of the production of IL-8 and IL-10 by resident macrophages in the lamina propria (for review see (Jandhyala et al., 2015)).

It is also suggested that the gut microbiota plays a central role in maintaining the integrity and function of the intestinal barrier that regulate nutrients, electrolytes and water absorption and prevents the entry of pathogenic microorganisms (Farhadi et al., 2003). Key features of the intestinal barrier are the mucus layer containing antibacterial proteins and IgA and a monolayer of epithelial cells connected by tight junctions (Groschwitz and Hogan, 2009; Johansson et al., 2011). Interestingly, a recent preclinical study has shown that differences in microbiota composition influence the mucus phenotype (Jakobsson et al., 2015). The importance of the gut bacteria in regulating tight junction expression has been widely demonstrated both *in vivo* and *in vitro* through the use of different probiotics (Ulluwishewa et al., 2011; Bergmann et al., 2013; Yang et al., 2014).

There is a bidirectional communication network between the CNS and the GI tract which involves neural and metabolic pathways, immune and endocrine mechanisms (Cryan and O'Mahony, 2011; Bercik et al., 2012; De Palma et al., 2014; Dinan et al., 2015) (see Fig. 1). The microbiota plays a key role in this communication. Under normal physiological conditions this axis is responsible for the modulation of digestive processes (i.e. motility, secretion), immune function, perception and emotional response to visceral stimuli (Mayer et al., 2006). The high co-morbidity between stress-related psychiatric symptoms such as anxiety with GI disorders including IBS and inflammatory bowel disease (Bonaz and Bernstein, 2013; Dinan and Cryan, 2013) are further evidence of the impact of this axis (Fig. 2).

For the purpose of this review we have mentioned the major systems that are influenced by the gut microbiota relevant to the disorders highlighted below. This is not an exhaustive list and we urge the reader to refer to (Jandhyala et al., 2015) for a broader overview.

1.4. Methods to analyse the microbiota

Originally, the identification and enumeration of the microorganisms colonising our gut was carried out through culture based techniques which can be both qualitative and quantitative and relatively cheap although labour intensive. However, this method only allowed isolating less than 30% of the microorganisms present in our gut due to the fact that most are strictly anaerobes. Dominant genera including *Bacteroides*, *Clostridium*, *Bifidobacterium* were identified with the improvement of anaerobic culturing techniques. Another limitation of this technique is that some microorganisms have symbiotic relationships and thus would not survive in pure cultures.

The development of high throughput gene sequencing technologies has provided most of the information (diversity, structure, stability and dynamics) that we have to date in relation to the composition of the gut microbiota. The target of these analyses is the 16S ribosomal RNA (rRNA) gene sequence which is highly conserved in these microorganisms but also presents with variable regions that are used as target for phylogenetic identification.

These techniques include quantitative polymerase chain reaction (qPCR) which uses fluorescent primers to amplify and quantify the DNA present in the samples. Temperature or denaturing gradient gel electrophoresis (TGGE or DGGE), which separates complex mix of 16S rRNA gene amplicons based on their different DNA sequences using respectively linear temperature gradient or denaturing gradient gel. Terminal-restriction fragment length polymorphism (T-RFLP) which is a semi-quantitative analysis, rapid and cheap based on the fragmentation of the 16S rRNA gene amplicons by restriction endonucleases with band visualization. Fluorescent in situ hybridisation (FISH) based on the use of a fluorescently labelled oligonucleotide probes targeting the complementary 16S rRNA sequences.

The more recent development of high throughput techniques such as DNA microarray allows in-depth phylogenetic identification of the microbiota. Direct sequencing of 16S rRNA gene amplicons including 454 Pyrosequencing, Illumina and SOLiD allows the sequencing of a high number of DNA templates and the detection of bacteria that are in low abundance. Microbiome shotgun sequencing involves the random fragmentation of DNA, sequencing of these fragments and reconstruction of overlapping sequences into a consecutive sequence (for a detailed review see (Fraher et al., 2012)).

2. Prenatal factors affecting gut colonisation

Prenatal perturbations such as stress, infection, preterm birth and diet have been shown to have long-term effects on health and microbiota composition.

2.1. Stress

Maternal stress during pregnancy has been shown to alter the composition of the vaginal microbiota in mice and in particular the abundance of *Lactobacillus* bacteria. Interestingly, this effect was correlated with lower abundance of *Lactobacillus* in the gut microbiota of their offspring (Jašarević et al., 2015). Moreover, male pups presented an increase in anaerobic bacteria like *Clostridium* and *Bacteroides* (Jašarević et al., 2015). This study also highlighted changes in metabolite profiles involved in energy balance as well as region and sex specific disturbances of amino acid profiles in the central nervous