Targeting the endocannabinoid system with microbial interventions to improve gut integrity

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ABSTRACT

The endocannabinoid system is a metabolic pathway involved in the communication between the gut microbiota and the host. In the gut, the endocannabinoid system regulates the integrity of the intestinal barrier. A compromised integrity of the intestinal barrier is associated with several disorders such as inflammatory bowel disorder, obesity and major depressive disorder. Decreasing the integrity of the intestinal barrier results in an increased translocation of bacterial metabolites, including lipopolysaccharides, across the epithelial layer of the gut, causing the subsequent inflammation. Targeting the endocannabinoid system in the gut can improve the integrity of the intestinal barrier. Currently, microbial interventions in the form of probiotics are under investigation for the treatment of diseases related to a compromised integrity of the intestinal barrier. However, the role of the endocannabinoid system in the gut is ambiguous since activity of the endocannabinoid system is increased in obesity and decreased in inflammatory bowel disease, emphasizing the need for development of personalized microbial interventions. This review discusses the role of the endocannabinoid system in regulating the gut barrier integrity and highlights current efforts to develop new endocannabinoid-targeted microbial interventions.

List of abbreviations

IBS irritable bowel syndrome
MDD major depressive disorder
LPS lipopolysaccharides
ECS endocannabinoid system
2-AG 2-arachidonoylglycerol
AEA N-arachidonylethanolamine
AA arachidonic acid
NPE-PLD N-acylphosphatidylethanolamine-lyase phospholipase D
FAAH fatty acid amide hydrolase
DAGL diacylglycerol lipase
MAGL monoacylglycerol lipase
2-LG 2-linoleoylglycerol
2-PG 2-palmitoylglycerol
CB1 ESC-G-protein coupled receptors 1
CB2 ESC-G-protein coupled receptors 2
SEA N-stearoylthanolamine
PEA N-palmitoylthanolamine
OEA N-oleoylthanolamine
LEA N-linoleoylthanolamine

(continued on next column)

DHEA N-docosahexaenoyl ethanolamine
2-OG 2-oleoyl glycerol
TRPV-1 transient receptor potential vanilloid-1
PPAR-γ peroxisome proliferator-activated receptor
GF germ-free
DIO diet induced obesity
HFHS high fat high sucrose
MGBA microbiota gut brain axis
TNBS trinitrobenzene sulfonic acid
IBS-C irritable bowel syndrome constipation
IBS-D irritable bowel syndrome diarrhea
HP high-fat
NAPE N-acyl-phosphatidylethanolamines
TEER transepithelial electrical resistance
ETA ethanolamine

1. Introduction

An enormous number of microorganisms, including bacteria reside...
in the human gut. Under normal circumstances, the gut microbiota is in a balanced and symbiotic relationship with the host (Li et al., 2008). Disruption of the balance between the host and its microbiota, which is called dysbiosis, can be detrimental for the host health. For example, dysbiosis is associated with gut related diseases such as, irritable bowel syndrome (IBS) (Wang et al., 2020), metabolic disorders such as obesity (Nagal et al., 2018), as well as brain related diseases including major depressive disorder (MDD) (Cheung et al., 2019). A possible factor that links dysbiosis to host malfunction is the integrity of the intestinal barrier.

The intestinal barrier is comprised of the lamina propria, epithelial layer and the mucus layer (Vancamelbeke and Vermeire, 2017). When the integrity of the intestinal barrier is compromised, its permeability is increased, and in turn, translocation of bacteria and their metabolites across the intestinal barrier becomes more prevalent (Bischoff et al., 2014). Bacteria and their metabolites can translocate across the intestinal barrier either transcellularly or paracellularly. Paracellular translocation is the movement of molecules in the space between adjacent epithelial cells. This space is not large enough to allow the translocation of a whole bacterium or large protein complexes, but translocation of ions and small bacterial metabolites, including lipopolysaccharides (LPS), does occur (Guerville and Boudry, 2016). Transcellular translocation is the crossing of apical and basolateral membranes of an epithelial cell by bacteria or their metabolites, which is often mediated by receptors (Karasov, 2017). A compromised integrity of the intestinal barrier results in activation of the immune system, and consequently increased production of pro-inflammatory compounds, which results in systemic inflammation (Camilleri, 2019)(Fig. 1).

A metabolic pathway at the crosslink of microbial dysbiosis and intestinal barrier integrity is the endocannabinoid system (ECS) (Can i et al., 2016). The ECS is associated with several diseases including inflammatory bowel disorders (Ahmad et al., 2017), neurological disorders (Cristino et al., 2020), and MDD (Ollson et al., 2019). Thus, microbial dysbiosis might interfere with the normal balance of the ECS, which alters the integrity of the intestinal barrier, and may lead to disease development (Muccioli et al., 2010). However, the precise molecular mechanisms involved are not yet fully elucidated. This review summarizes the state-of-the-art knowledge on the role of the ECS in regulating the gut barrier integrity and highlights current efforts to develop new ECS-targeted microbial interventions.

2. The endocannabinoid system

The ECS is comprised of endocannabinoids, enzymes, and receptors. The endocannabinoids present in the ECS are 2-arachidonoylgllycerol (2-AG), and N-arachidonylethanolamine (also known as anandamide (AEA)) (Fig. 2). In human plasma, the concentration of 2-AG and AEA is in a nanomolar range, where AEA ranges between 0.6 and 18.7 nM (Balvers et al., 2009; Matias et al., 2006) and 2-AG between 0.4 and 4.6 nM (Di Marzo et al., 2009; Fernández-Rodríguez et al., 2004). Endocannabinoids are synthesized from arachidonic acid (AA) and membrane precursors; phosphatidylinositol for 2-AG and phosphatidylethanolamine for AEA, respectively (Piomelli, 2003). The levels of endocannabinoids are tightly controlled in the human body. For example, AEA can be synthesized via several metabolic pathways (Leung Donnemene et al., 2006; Liu Jie et al., 2008) including N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) enzyme (Maccarrone Mauro, 2017) and degraded by fatty acid amid hydrolase (FAAH) enzyme. 2-AG is synthesized by diacylglycerol lipase (DAGL) and degraded by monoacylglycerol lipase (MAGL) enzyme (Maccarrone Mauro, 2017). Notably, MAGL is inhibited by 2-linoleoyl glycerol (2-LG) and 2-palmitoyl glycerol (2-PG), which could, in turn, enhance the effect of 2-AG (Ben-Shabat et al., 1998).

AEA and 2-AG function through the activation of the ESC-G-protein coupled receptors 1 and 2 (CB1 and CB2) (Can i et al., 2016; Chirichiri et al., 2018). CB1 is expressed in the brain and on the terminus of peripheral nerves, where it controls memory, motor activity and sensory perception (Can i et al., 2016). CB2 is expressed on myeloid cells, lymphoid tissue and peripheral organs (Leimuranta et al., 2018).

The ECS can be expanded with other metabolites, receptors and enzymes. The expanded ECS is called the endocannabinoidomide (Di Marzo, 2018). Besides CB1 and CB2, AEA can also activate the transient receptor potential vanilloid-1 (TRPV-1), peroxisome proliferator-activated receptor (PPAR-γ), and inhibit T-type Ca2+ channels. 2-AG can also activate TRPV1 (Di Marzo and Silvestrini, 2019). TRPV1 is located at the nerve terminals of extrinsic primary afferents (Ohl et al., 2017), where it is involved in peripheral inflammation, the integration of pain stimuli and modulation of noiceptive inputs to the brainstem and spinal cord (Cortright et al., 2007). The activation of TRPV1 by 2-AG and AEA has been shown to play a role in reducing pain (Storozhuk and Zhilos, 2017). PPAR-γ is involved in a variety of physiological processes, such as lipid and glucose homeostasis, fat cell differentiation, inflammation and aging (Han et al., 2017; Letterova et al., 2014; Wang et al., 2016). Additionally, next to AEA and 2-AG the endocannabinoidomide consists of other fatty acid derived metabolites such as N-stearoyl ethanolamine (SEA), N-palmitoyethanolamine (PEA), N-oleoyl ethanolamine (OEA), N-linoleoyl ethanolamine (LEA), N-docosahexaenoylethanolamine (DHEA), 2-oleoyl glycerol (2-OG) and 2-LG (Di Marzo, 2018). These endocannabinoidomide ligands, also have different targets. For example, PEA can directly activate PPAR-α, GPR55 and indirectly activate TRPV1 through activation of PPAR-α or via enhancing the effect of AEA (De Petrocellis et al., 2001; Paolo et al., 2013), DHEA activates GPR110, LEA and OEA activate TRPV1 and GPR119, OEA activates PPAR-α, and 2-OG and 2-LG activate GPR119 and TRPV1 (Di Marzo, 2018).

3. The endocannabinoid system in the gut

In the gut, both CB1 and CB2 are expressed on enteric nerves, enterocytes, and immune cells. In addition, CB1 is highly expressed on the enterococcal cells of the intestinal mucosa (Izzo and Sharkey, 2010). Activation of CB1 induces mesenteric vasodilation, suppresses secretion of fluid and acid, and stimulates motility of the GI tract (Izzo and Sharkey, 2010). Under normal conditions, the actions of the ECS in the gut are mostly mediated through CB1. However, during inflammation, activation of both CB1 and CB2 occurs resulting in the production of anti-inflammatory cytokines, which reduces intestinal inflammation and gastric damage (Fichna et al., 2013; Kinsey et al., 2011; Toczek and Malinowska, 2018).

Furthermore, Sykaras et al. showed that CB1 mRNA is highly expressed on I-cells, which produce cholecystokinin (Sykaras et al., 2012), and DiPatrizio et al. examined the role of the ECS in dietary fat intake. The authors showed increased levels of AEA and 2-AG in the small intestine when rats were sham fed. Administering a CB1 inhibitor to the duodenum reduced the amount of fat intake (DiPatrizio et al., 2011). Together, these results show that the ECS influences the energy balance through feeding behavior (Di Marzo, 2011; Piomelli, 2003). The ECS has also been described to be involved in regulation of the intestinal barrier integrity (Muladi et al., 2018). In obese mice, CB1 activation resulted in reduced levels of tight junction proteins. Importantly, germ-free (GF)-mice showed higher transcription of CB1 in the colon compared to conventionally raised mice, implying a key role for the microbiota in regulating the induces transcription of CB1 (Muccioli et al., 2010). Manca et al. confirmed the results of Muccioli et al. and showed that a fecal microbiota transplant (FMT) could reverse CB1 transcription in the colon. Furthermore, the authors showed decreased transcription of GPR55 in the whole GI tract of GF-mice compared to conventionally raised mice, which was upregulated in response to FMT (Manca et al., 2020), further confirming the significance of the microbiota in regulating the intestinal ECS.
Fig. 1. Intestinal barrier integrity in health and disease. The intestinal barrier is comprised of the mucus layer, epithelial cells and the lamina propria. During disease, expression of tight junction proteins is decreased. In turn, the space between epithelial cells is larger, resulting in increased paracellular translocation of metabolites produced by the microbiota in the lumen, which, in turn causes activation of the immune system and inflammation.
administration of the CB1 receptor antagonist SR141716A via oral gavage to mice with diet-induced obesity (DIO) showed an increase in *Akkermansia muciniphila* and a decrease in *Lachnospiraceae* and *Erysipelotrichaceae* in the colon compared to DIO mice treated with a vehicle and these changes were associated with an increase in mucus layer thickness (Mehrpouya-Bahrami et al., 2017). Moreover, Guida et al. showed a reduction in the levels of N-acyl-serotonins, which are lipids included in the endocannabinoidome, in homogenized tissue of the duodenum and jejunum of antibiotic treated mice, which was accompanied by a reduction in levels of AEA. In addition, the authors showed an elevation of the expression levels of TRPV1 in the hippocampus. Subsequently, the antibiotic treated mice showed more depressive-like symptoms compared to the controls. Interestingly, administration of *Lactobacillus casei* improved the depressive-like symptoms and, normalized the levels of N-acyl-serotonins and, in turn, the levels of AEA in the GI tract (Guida et al., 2018). This effect is possibly mediated by altering the microbial composition in the gut. In another study, mice were fed a high fat high sucrose (HFHS) diet and showed a significantly increased level of AEA in the ileum compared to mice fed a low fat low sucrose diet. Furthermore, levels of PEA and OEA were decreased in the HFHS-mice. However, after 56 days of the HFHS diet, the levels of PEA were restored. Additionally, the levels of 2-AG, 2-OG and 2-LG after 56 days showed a decreasing trend in the ileum. The altered levels of these metabolites can be explained by altered expression levels of FAAH mRNA and MAGL in the ileum. Using Spearman correlation analysis, the authors showed an association of AEA with the relative abundance of *Barnesiella, Adlercreutzia, Parasutterella, Porphyromonas, Enterococcus* and *Methylobacterium* species in the ileum (Lacroix et al., 2019).

The ECS in the gut is linked to the ECS in the brain via the microbiota-gut-brain axis (MGBA). The MGBA is a bidirectional communication network that includes the peripheral nerves, the enteric and the central nervous system, gut hormones, the gut microbiota and microbiota derived molecules (Farzi et al., 2018; Russo et al., 2017). One of the mechanisms involved in the MGBA is the regulation of the intestinal barrier via expression of tight junction proteins and mucus metabolism (Carabotti et al., 2015; Julio-Pieper et al., 2014). Therefore, a compromised integrity of the intestinal barrier is associated with brain-related disorders. For example, dysfunction of tight junction proteins is associated with Parkinson’s disease (Santos et al., 2019). Higher concentrations of immunoglobulin A and M are detected in the serum of depressed patients compared to healthy controls, indicating higher translocation of bacteria across the epithelial barrier (Maes et al., 2012). Furthermore, Ohlsson et al. found decreased levels of zonulin, a protein involved in assembling of tight junctions, in plasma of depressed patients compared to healthy controls (Ohlsson et al., 2019). Since microbial dysbiosis is associated with MDD and Parkinson’s disease and the ECS plays a central role in the integrity of the intestinal barrier, altering the ECS in the gut could be a mechanism by which microbial dysbiosis induces disease. For example, stress reduces the thickness of the mucus layer in the colon of rats compared to non-stressed rats, which was accompanied by microbial dysbiosis. Interestingly, these changes were also associated with upregulation of CB2 mRNA expression in the colon (Aguilera et al., 2019). Other endocannabinoidome ligands, in particular, PEA and OEA, have been reported as regulators of the intestinal barrier integrity. Karwad et al. investigated the effect of OEA and PEA on the permeability of a Caco-2 monolayer. The authors showed an increase in permeability when OEA was applied to the apical membrane, and a decrease in permeability when OEA was applied to the basolateral membrane. This effect was mediated via TRPV1. Moreover, PEA was shown to decrease permeability in a PPAR-α dependent manner when applied to both the apical and basolateral membrane (Karwad Mustafa et al., 2017; Karwad et al., 2019). Together, these results suggest that altered ECS metabolism induced by members of the gut microbiota could impair the health status.

### 4. The endocannabinoid system in health and disease

Alterations in the ECS are also associated with metabolic disorders. For example, in obese mice, the expression levels of the enzyme NAPE-PLD and CB1 receptor are elevated, whereas FAAH enzyme is reduced in adipose tissue compared to lean mice. Moreover, the concentration of AEA in obese mice is increased compared to lean mice, showing that an altered ECS balance is associated with obesity (Forte et al., 2020). The

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**Fig. 2.** Overview of the compounds and enzymes in the endocannabinoid system. The two compounds of the ECS are arachidonylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG). Membrane lipid precursors are the substrate for the synthesis of AEA by NAPE-phospholipase D (NAPE-PLD) and 2-AG by diacylglycerol lipase (DAGL). AEA is degraded into arachidonic acid (AA) and ethanolamine (ETA) by fatty acid amidehydrolase (FAAH), while 2-AG is degraded into AA and glycerol via monoacylglycerol lipase (MAGL) (Simon and Cota, 2017).
relationship between obesity and the ECS is mediated by lower expression levels of tight junction proteins in the colon of obese mice and an elevated concentration of LPS in their plasma compared to their lean counterparts, highlighting the role of the intestinal barrier in obesity (Muccioli et al., 2010). Similarly, intraduodenal infusion of a CB1 receptor antagonist to rats reduced food intake and reduced body weight (DiPatrizio et al., 2011). Elevated plasma levels of 2-AG were accompanied by an increase in the consumption of favorite food compared to normal food in humans (Monteleone et al., 2012), showing another link between the ECS and obesity. Moreover, when NAPE-PLD enzyme was selectively knocked out in hepatocytes in mice, the knockout mice developed an obese-like phenotype (Lefort et al., 2020). When NAPE-PLD was selectively knocked-out in adipose tissue of mice, the knockout mice developed an obese-like phenotype too (Geurts et al., 2015), highlighting the importance of NAPE-PLD in the development of obesity. When the microbiota of the mice with the adipose tissue-NAPE-PLD knockout was transferred to GF-mice the mice developed more fat mass, gained more weight and had a higher adipose index compared to GF-mice treated with WT microbiota (Geurts et al., 2015). When NAPE-PLD was selectively knocked-out in intestinal epithelial cells of mice, the mice accumulated more fat, showed higher liver mass, as well as altered microbiota composition when fed a high fat (HF) diet compared to wild type mice fed a HF-diet. However, treatment with A. muciniphila reduced body weight and fat mass of the NAPE-PLD knockout mice fed a HF-diet, indicating that intestinal epithelial NAPE-PLD is not required for the protective effects of A. muciniphila (Everard et al., 2019).

Similar to the development of obesity in combination with the ECS, more diseases are related to a malfunctioning ECS. For example, the stress hormone corticosterone compared to mice colonized with a mock community. While, the levels of AEA in the brain were reduced, the levels of LEA were increased in the cecum of mice colonized with C. albicans (Markey et al., 2020). Additionally, Manca et al. showed decreased levels of 2-AG, 2-OG, and 2-LG in the whole brain of GF-mice compared to conventionally raised mice. However, these low levels of 2-AG were not significantly reversed after FMT (Manca et al., 2020). In the brain, alteration of the ECS can result in, among others, mood, anxiety and depressive disorders (Parolaro Daniela et al., 2010; Stamparoni Bassi et al., 2018). Indeed, Hill et al., showed that stimulation of the CB1 receptor improved behavior of rats during the forced swimming test, similar to administration of conventional anti-depressants (Hill and Gorzalka, 2008). In addition, antagonizing the CB1 receptor may induce anxiety and depression (Moreira and Crippa, 2009). The extensive research regarding the role of the ECS in the brain and in mental disorders is reviewed elsewhere (Ashton and Moore, 2011; Meyer et al., 2018). Nevertheless, the studies mentioned above emphasize the potential effect of the gut microbiota on the ECS in the brain. However, the precise mechanisms underlying this effect is not completely clear and needs to be investigated.

5. Improving gut permeability with microbial interventions

Since the microbiota can influence the integrity of the intestinal barrier via the ECS, the alteration in the microbiota might reduce disease symptoms caused by a compromised integrity of the intestinal barrier. Probiotic intervention can be used as an ECS-targeted intervention in the microbiota, which is already under investigation. For example, administration of Lactobacillus acidophilus NCFM was found to induce CB2 expression in the colon of mice and rat, which showed analgesic effects against visceral pain (Rouesseau et al., 2007). Everard et al. showed an increase in 2-AG, 2-OG and 2-PG in the ileum after A. muciniphila administration to mice fed a HF-diet. Furthermore, A. muciniphila increased the inner mucus layer of the colon in HF-diet mice, whereby heat killed bacteria did not alter the mucus layer in HF-diet mice, indicating that the bacteria need to be alive to elicit an effect (Everard et al., 2013). The probiotic mixture VSL#3, containing a variety of Bifidobacteria, Lactobacilli spp. and Streptococcus thermophilus, is recognized to be potentially beneficial in chronic IBD (Chapman et al., 2006). Moreover, VSL#3 has been found to increase colonic CB1 expression in mice (Distrutti et al., 2014). In zebra fish, VSL#3 upregulates CB1 and CB2 expression and downregulates FAAH gene expression. Moreover, VSL#3 showed anti-inflammatory and immune stimulant capacities, which could be related to the upregulation of CB2 (Giacchetti et al., 2017). In search for probiotic treatment against obesity, Chen et al., introduced N-acyl-phosphatidylethanolamines (NAPE), the precursor for N-acylatedolamolamines such as AEA, in Escherichia coli Nissle 1917. After treatment with the genetically modified E. coli, plasma NAE levels were not affected in mice, which is likely due to the rapid conversion of NAE by FAAH. Although the NAE levels were not affected, changes were observed. Mice that were fed a HF-diet and treated with genetically modified E. coli gained less weight, and showed a reduction in food intake and meal size compared to control mice. These effects lasted for 12 weeks after bacterial administration. NAE levels were also elevated in the liver, suggesting a systemic effect of treatment with the genetically modified E. coli Nissle 1917 (Chen et al., 2014). Developing treatments targeting the ECS can help relieve symptoms associated with obesity (Fig. 3) and can lower the inflammatory response associated with a reduced integrity of the intestinal barrier (Fig. 4). For example, Alhouayeck et al. showed that inhibition of MAGL enzymes reduced tri-nitrobenzene sulfonic acid (TNBS)-induced colitis in mice by lowering the inflammatory response in a CB1 and CB2 mediated manner (Alhouayeck et al., 2011).

6. Conclusion and future perspectives

The ECS plays an ambiguous role in health and disease. CB1 inhibition reduces fat intake and obesity is associated with higher CB1 activation and a lower integrity of the intestinal barrier, indicating that a higher ECS activity is detrimental for host health (Muccioli et al., 2010) (Fig. 3). In contrast, lower ECS activity is associated with higher inflammation and lower integrity of the intestinal barrier in IBD. Activation of the ECS in IBD mice reduces inflammation via production of anti-inflammatory compounds (Gyires and Zádori, 2016; Leinwand et al., 2017) (Fig. 4). Since the gut microbiota is associated with alterations in the ECS and intestinal barrier integrity, a specific microbial dysbiosis can be the cause of the ambiguous role of the ECS in health in disease. To distinguish between cause and effect, more research is needed to elucidate the effect of endocannabinoids on bacteria and the metabolism of endocannabinoids by bacteria. Cohen et al. showed a wide distribution of a bacterial N-acyl synthase gene in stool samples. With liquid chromatography and mass spectrometry the authors showed production of several N-acyl amides in pure bacterial cultures and showed that these compounds could activate human GPR119 (Cohen et al., 2017). Friedman et al. incubated Vibrio harveyi with AEA and showed no effect on growth of the bacteria. However, incubation with 100 μg/mL AEA had an inhibitory effect on quorum sensing and motility (Friedman et al., 2019). Although, the physiological concentration of AEA ranges between 0.6 and 18.7 nM and V. harveyi is a marine bacterium it suggests unexplored effects of the ECS on the gut microbiota.
To better understand the role of the ECS in intestinal barrier functioning, a more detailed understanding of the integrity of the intestinal barrier is needed. Currently, the intestinal barrier integrity is often measured indirectly using biomarkers. Biomarkers can be measured in plasma, urine or fecal samples and include claudine, calprotectin and endotoxins (Vancamelbeke and Vermeire, 2017). However, biomarkers cannot always distinguish between paracellular and transcellular translocation, which region of the intestine is affected and, between...
bacterial origin of endotoxins. However, understanding the mechanism of translocation is essential for development of interventions. To answer some of these questions, transcript epileptial electrical resistance (TEER) has been used. TEER is an in vitro method which uses two compartments separated by a layer of cells or tissue and measures changes in ion current, whereby more intestinal permeability results in less electrical resistance due to higher ion flow between the compartments (Srinivasan et al., 2015). Impedance spectrophotometry is a TEER method whereby para-and transcellular movement can be distinguished, but can be technically challenging (Günzel et al., 2012). Furthermore, choosing the correct type of cells or tissue can be challenging and does not capture the full complexity of the intestine. Nevertheless, in vitro methods are necessary to better understand the molecular mechanisms of intestinal barrier integrity and can be used to investigate the role the ECS plays. Altogether, accumulating evidence shows that intestinal barrier integrity, microbiota and host health are intertwined. At the crossroads, the ECS integrity and can be used to investigate the role the ECS plays. Altogether, accumulating evidence shows that intestinal barrier integrity, microbiota and host health are intertwined. At the crossroads, the ECS integrity and can be used to investigate the role the ECS plays.