The endocannabinoid system in inflammatory bowel diseases: from pathophysiology to therapeutic opportunity

Mireille Alhouayek and Giulio G. Muccioli

Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Av. E. Mounier, 72, B1.72.01, 1200 Bruxelles, Belgium

Crohn’s disease and ulcerative colitis are two major forms of inflammatory bowel diseases (IBD), which are chronic inflammatory disorders of the gastrointestinal tract. These pathologies are currently under investigation to both unravel their etiology and find novel treatments. Anandamide and 2-arachidonoylglycerol are endogenous bioactive lipids that bind to and activate the cannabinoid receptors, and together with the enzymes responsible for their biosynthesis and degradation [fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)] constitute the endocannabinoid system (ECS). The ECS is implicated in gut homeostasis, modulating gastrointestinal motility, visceral sensation, and inflammation, as well as being recently implicated in IBD pathogenesis. Numerous subsequent studies investigating the effects of cannabinoid agonists and endocannabinoid degradation inhibitors in rodent models of IBD have identified a potential therapeutic role for the ECS.

The ECS in a nutshell

Endocannabinoids are lipid mediators that exert most of their functions by binding and activating two G protein-coupled receptors, cannabinoid receptor 1 (CB1) and 2 (CB2), that are both expressed throughout the gastrointestinal tract at various levels depending on the tissue and conditions. The two most thoroughly studied endocannabinoids are arachidonic acid derivatives, the N-acyl ethanolamine (NAE) N-arachidonoyl ethanolamine (anandamide) and the monoacylglycerol 2-arachidonoylglycerol. They are found at various levels throughout the gastrointestinal tract, with 2-arachidonoylglycerol being more abundant than anandamide. Their production from cell membrane lipid precursors is activity-dependent, and their actions are terminated via hydrolysis by specific lipases (Figure 1) [1]. Anandamide signaling is terminated by two amides, FAAH and N-acyl ethanolamine-hydrolyzing acid amidase (NAAA), whereas MAGL and α/β-hydrolase domain 6 (ABHD6) are two esterases involved in regulating 2-arachidonoylglycerol signaling [2]. Although FAAH and MAGL are considered the primary enzymes controlling anandamide and 2-arachidonoylglycerol levels, respectively, it is important to consider that under certain conditions (e.g., inflammation) or in specific cell types (e.g., macrophages as compared to endothelial cells), NAAA or ABHD6 may have

Glossary

b.i.d.: abbreviation for bis in die, the administration of a drug twice a day.

Bioactive lipids: lipids for which changes in their levels result in functional consequences. Usually requires the trilogy: lipid, enzymes producing and degrading the lipid, and downstream targets for the bioactive lipid.

Ethylenediaminetetraacetate (EDTA): EDTA is a metal chelator with high affinity for calcium. When an epithelial (or endothelial) cell monolayer is incubated in the presence of EDTA, the calcium depletion results in disruption of tight junctions and opening of the paracellular route.

Fluorescein isothiocyanate–dextran: a high molecular weight polymer of D-glucose conjugated to the fluorophore fluorescein. Its molecular weight prevents translocation of luminal LPS to the bloodstream, but during obesity, increased epithelial permeability leads to increased plasma LPS levels (significantly lower levels than during an infection or sepsis).

Haptens: small molecule that becomes immunogenic only once bound to a carrier (a bigger molecule such as a protein).

Lipopolysaccharide: a component of Gram-negative bacteria made of a hydrophobic domain known as lipid A (or endotoxin), an oligosaccharic core, and a distal polysaccharide (or O-antigen). Its binding to toll-like receptor 4 (TLR4) induces potent inflammatory responses. A normal epithelial barrier prevents translocation of luminal LPS to the bloodstream, but during obesity, increased epithelial permeability leads to increased plasma LPS levels.

Myeloperoxidase: a lysosomal peroxidase most abundantly expressed in neutrophils and involved in the respiratory burst. Increased myeloperoxidase activity in tissues mirrors increased infiltration of these tissues by neutrophils.

Oil of mustard (OM): is a small nerve fiber stimulant and a potent inflammatory irritant, usually administered intra rectally to provoke aloodynia (pain response to a usually painless stimulus) and visceral hyperalgesia (an exaggerated pain response). It has also recently been used for colitis induction.

Tight junctions: complexes of proteins that mediate epithelial cell-to-cell adhesion. By closing the space between two adjacent epithelial cells, tight junctions prevent molecules from crossing an epithelial by the paracellular route. Several of these proteins are transmembrane proteins (claudins, occludins, and junctional adhesion molecules or JAM), whereas others are cytoplasmic (zonula occludens proteins).

Trans-epithelial electric resistance (TEER): a measure of the resistance that an epithelial (or endothelial) confluent cell monolayer opposes to the passage of a current applied between two electrodes separated by the cell monolayer. It is used to determine the presence of functional tight junctions and thus the integrity of a confluent monolayer of epithelial or endothelial cells.

q.d.: abbreviation for quaque die, the administration of a drug once a day.

q.a.d.: abbreviation for quaque alternis die, the administration of a drug every other day.
a more predominant role. This is also true when considering the biosynthetic pathways of these mediators.

In addition to generating anandamide and 2-arachidonoylglycerol, the enzymes responsible for their biosynthesis produce other bioactive lipids (see Glossary). These other lipids do not always bind to cannabinoid receptors but they do share their metabolic pathways and some molecular targets with the endocannabinoids. For instance, N-palmitoyl ethanolamine and N-oleylethanolamine, differing from anandamide by their acyl chain, do not bind to cannabinoid receptors but to the nuclear receptors PPAR and to G protein-coupled receptors such as GPR119, which is highly expressed in the gut [3]. 2-Oleoylglycerol, a 2-arachidonoylglycerol analogue, also lacks significant affinity for the cannabinoid receptors but binds and activates GPR119 [4]. Finally, GPR55 and the receptor channel TRPV1 (activated, for instance, by anandamide) are additional targets related to the ECS.

Endocannabinoids and related molecules such as N-palmitoyl ethanolamine have pleiotropic effects in the organism, including in the gastrointestinal tract [5]. In this review, we focus on the role of the ECS in gut homeostasis and its potential benefits in inflammatory bowel diseases (IBD) (Box 1). The receptors mediating the effects of endocannabinoids and the enzymes controlling their levels represent prime pharmacological targets to interfere with the intestinal endocannabinoid signaling and study its physiopathological relevance. Over the years, selective pharmacological tools have been developed to selectively probe each element of the ECS, and some of these are listed in Box 2.

**Role of the ECS in gut homeostasis**

Cannabinoids have numerous effects on the gastrointestinal tract ranging from modulating food intake and feeding behavior [6] to gastroprotection and inhibition of nausea or vomiting [5]. Several ECS effects could play a role in IBD, including modulation of gastrointestinal motility, visceral sensation, inflammation, and intestinal permeability.

The inhibition of intestinal transit and colonic propulsion is of particular interest when considering inflammatory states of the bowel, where dysmotility is a clinically significant component of the disease. Cannabinoids and endocannabinoid degradation inhibitors decrease intestinal motility, peristalsis, and colonic propulsion in rodents, primarily in a CB1-dependent manner [7–12]. Accordingly,
pharmacological blockade of CB1, or its genetic deletion, result in prokinetic effects in the gastrointestinal tract (enhanced motility) [8,9,13,14]. It is likely that cannabinoids inhibit contractility by reducing acetylcholine release from enteric nerves because they do not inhibit contractions produced by exogenous administration of acetylecholine but reduce electrically-evoked acetylcholine release from myenteric nerves [7,15,16]. Additionally, cannabinoids have an inhibitory effect on the non-adrenergic, non-cholinergic excitatory transmission in the intestine [7,17,18]. Under normal conditions, CB1 mediates the effects of cannabinoids on motility; however, in inflammatory states both CB1 and CB2 receptor activation may reduce inflammation-induced hypermotility. For instance, in lipopolysaccharide (LPS)-induced intestinal inflammation, hypermotility was normalized following CB2 (but not CB1) activation [19,20], whereas in croton oil-induced hypermotility CB2 antagonism did not reverse the effect of the cannabinoid agonist CP55,940 [8]. In another study of LPS-induced inflammation, the FAAH inhibitor AM3506 normalized hypermotility in a CB1- and CB2-dependent manner, whereas colonic propulsion was CB1-dependent [21]. Interestingly, the effect of cannabinoids in cases of inflammatory hypermotility occurred at lower doses than in control states [8,22]. This could be due to the increased receptor expression during inflammation, to increased coupling of the receptor to effectors proteins, or both. Therefore, in an acute inflammatory setting, the cannabinoid-induced normalization of hypermotility should be beneficial. It is nevertheless important to consider the whole picture in chronic IBD where dysmotility is predominantly altered towards diarrhea, mainly due to secretory colon disturbances (increase in secretion and/or decrease in reabsorption) accompanied by reduced colon contractility. It is noteworthy, however, that small intestinal contractility appears to be enhanced, leading, in fine, to decreased oroecal transit time [23]. Cannabinoids can decrease intestinal transit time and hypersecretion. They inhibit choleria toxin-induced hypersecretion in the mouse small intestine and delay the onset of castor oil-induced diarrhea in the rat, via CB2 receptor activation [24,25]. However, cannabinoids have mostly been evaluated in acute inflammation and should therefore be evaluated for transit time and diarrhea reduction during colitis. Moreover, some patients might experience gastroparesis and a delay in small intestinal motility, in which cases, cannabinoids (if considered a potential treatment) should be used at doses that do not affect gastric emptying or intestinal motility. Feelings of discomfort, nausea, and pain are commonly associated with inflammatory disorders of the gastrointestinal tract. Cannabinoid receptors have been implicated in pain processing in various neuropathic and inflammatory states, mainly in somatic pain [26]. Putative actions of cannabinoids on visceral sensation have been proposed, and the effects of centrally and peripherally restricted cannabinoids have been recently investigated, implicating primarily CB1 [27–29] but also CB2 [27,30]. In one interesting study, the CB1/CB2 agonist WIN55,212-2 and the CB2 agonist JWH-015 (both at doses of 10 mg/kg) attenuated the degree of visceral sensitivity under baseline conditions. When assessed versus the heightened sensitivity due to colonic inflammation of trinitrobenzene sulfonic acid (TNBS)-induced colitis, both agonists were effective at a much lower dose (0.1 mg/kg) that had no effect under baseline conditions [27].
Box 2. The ECS toolbox

Potent and selective pharmacological tools are needed to dissect the role of the ECS in IBD. The cannabinoid receptor ligands as well as the inhibitors of endocannabinoid degradation used in the studies described in this review are listed in Table I [3,59].

Table I. Pharmacological modulators of the ECS

<table>
<thead>
<tr>
<th>CANNABINOID RECEPTORS LIGANDS</th>
<th>Drug</th>
<th>CB1 Kᵢ (nM)</th>
<th>CB2 Kᵢ (nM)</th>
<th>Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB₁/CB₂ receptors agonists</td>
<td>HU210</td>
<td>0.06–0.7</td>
<td>0.1–0.5</td>
<td>Full agonist at both receptors</td>
</tr>
<tr>
<td></td>
<td>CP55,940</td>
<td>0.5–5</td>
<td>0.69–2.8</td>
<td>Full agonist at both receptors</td>
</tr>
<tr>
<td></td>
<td>WIN55,212-2</td>
<td>1.9–123</td>
<td>0.3–16.2</td>
<td>When assayed in the same study, slightly more affinity for CB₂ receptors. Full agonist at both receptors</td>
</tr>
<tr>
<td></td>
<td>Δ²-THC</td>
<td>5–80</td>
<td>3–75</td>
<td>Δ²-Tetrahydrocannabinol is a nonselective partial agonist</td>
</tr>
<tr>
<td></td>
<td>JWH-133</td>
<td>677</td>
<td>3.4</td>
<td>Selective agonist at CB₂ receptors</td>
</tr>
<tr>
<td></td>
<td>AM1241</td>
<td>280</td>
<td>3.4</td>
<td>Selective agonist at CB₂ receptors</td>
</tr>
<tr>
<td></td>
<td>JWH-015</td>
<td>383</td>
<td>13.8</td>
<td>Selective agonist at CB₂ receptors</td>
</tr>
<tr>
<td>CB₁ receptors selective agonists</td>
<td>ACEA</td>
<td>1.4</td>
<td>&gt;2000</td>
<td>Arachidonyl-2'-chloroethylamide is a CB₁ receptor agonist</td>
</tr>
<tr>
<td>CB₂ receptors selective agonists</td>
<td>JWH-133</td>
<td>677</td>
<td>3.4</td>
<td>Full agonist at CB₂ receptors</td>
</tr>
<tr>
<td></td>
<td>AM1241</td>
<td>280</td>
<td>3.4</td>
<td>Protean agonist at CB₂ receptors</td>
</tr>
<tr>
<td></td>
<td>JWH-015</td>
<td>383</td>
<td>13.8</td>
<td>Full agonist at CB₂ receptors</td>
</tr>
<tr>
<td>CB₁ receptors selective antagonists</td>
<td>SR141716A</td>
<td>1.8–12.3</td>
<td>&gt;1000</td>
<td>Rimonabant behaves as an inverse agonist at CB₁ receptors</td>
</tr>
<tr>
<td></td>
<td>AM251</td>
<td>7.5</td>
<td>2300</td>
<td>Inverse agonist at CB₁ receptors</td>
</tr>
<tr>
<td>CB₂ receptors selective antagonists</td>
<td>AM630</td>
<td>5152</td>
<td>31.2</td>
<td>Inverse agonist at CB₂ receptors</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INHIBITORS OF ENDOCANNABINOID DEGRADATION</th>
<th>Drug</th>
<th>FAAH IC₅₀ (nM)</th>
<th>MAGL IC₅₀ (nM)</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid amide hydrolase inhibitors</td>
<td>UR8597</td>
<td>4.8</td>
<td>&gt;30 000</td>
<td>Irreversible carbamate-type inhibitor</td>
</tr>
<tr>
<td></td>
<td>AM3506</td>
<td>48</td>
<td>11 000</td>
<td>Sulfonylfluoride-type covalent inhibitor</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>88</td>
<td>&gt;30 000</td>
<td>Putatively reversible FAAH inhibitor</td>
</tr>
<tr>
<td></td>
<td>AA-SHT</td>
<td>5600</td>
<td>/</td>
<td>Arachidonoylserotonin is a FAAH inhibitor also able to antagonize TRPV1 receptor</td>
</tr>
<tr>
<td></td>
<td>VDM11</td>
<td>2000–11000</td>
<td>/</td>
<td>Also inhibits the putative anandamide transporter (IC₅₀=9000–25 000 nM)</td>
</tr>
<tr>
<td>Monoacylglycerol lipase inhibitors</td>
<td>JZL184</td>
<td>4690</td>
<td>6</td>
<td>Irreversible carbamate-type inhibitor</td>
</tr>
</tbody>
</table>

An intriguing observation is the modulation of cannabinoid receptors expression by gut microflora [31,32]. For instance, administration of *Lactobacillus acidophilus* increases CB₂ expression, resulting in analgesic functions in the gut, a response reversed by the CB₂ receptor antagonist AM630 [32]. It is well accepted that the microflora plays a role in IBD by initiating or maintaining the immune response, however, the microflora also seems to influence visceral pain sensation through cannabinoid receptor modulation. A point to consider, however, is that cannabinoid agonists differentially modulated afferent intestinal nerve sensitivity depending on the stimuli used [29]. Thus, the understanding of the underlying mechanisms of visceral sensitivity is important to effectively target visceral pain with cannabinoids.

Cannabinoids exert pharmacological actions on epithelial cells as well as immune cells, resulting in protective and immunomodulatory effects [33]. Indeed, cannabinoids such as anandamide, noladin ether, and arachidonoylcyclopropylamide (ACPA) induce wound-closure in human colonic epithelial cell lines, suggesting that delayed wound healing, a feature of IBD lesions, might be reversed by cannabinoids [34]. Additionally, *in vitro* studies suggest roles for both cannabinoid receptors in modulating inflammatory processes, including suppression of the activation of macrophages and mast cells, secretion of proinflammatory cytokines, and modulation of T helper lymphocytes by reducing the activated T cell population, inducing their apoptosis, and inhibiting their proliferation [33,35–40].

The intestinal epithelium is a selective barrier that plays an important role in gut homeostasis. It is permeable to nutrients and essential macromolecules but constitutes an obstacle for luminal antigens, intestinal bacteria, and harmful macromolecules. Increased epithelial permeability is implicated in IBD pathogenesis. Indeed, a leaky intestinal barrier allows access to the underlying mucosal tissue that becomes increasingly and permanently exposed to antigens of the microflora. This results in a change from immune tolerance to immune activation [41–43]. Pro-inflammatory cytokines are known to alter the barrier function [39,40], thus forming a vicious circle where increased inflammation leads to a further increase in intestinal permeability. In monolayers of Caco-2 cells, a model of the intestinal epithelial barrier, application of EDTA, pro-inflammatory cytokines, or LPS increases permeability as measured by a decrease in trans-epithelial
electric resistance (TEER) [31,44,45]. The endocannabinoids anandamide and 2-arachidonoylglycerol increased permeability when applied on the apical surface of Caco-2 monolayers [44], and they enhanced the EDTA- or cytokine-induced increase in permeability [44,45]. These effects were blocked by antagonism of CB1, but not CB2, implicating CB1 receptors in the control of intestinal permeability [31,44,45]. Actually, the CB2 antagonist AM251 decreased EDTA- and cytokines-induced alterations of epithelial permeability [44,45]. The in vivo relevance of CB1 receptors in controlling gut permeability was demonstrated in a study looking at the disrupted epithelial barrier found in obese mice [31]. Indeed, obesity is characterized by altered gut permeability leading to increased plasma LPS levels, that is, metabolic endotoxemia [46]. Treatment with the antagonist SR141716A reduced plasma LPS levels in obese mice, mirroring an improvement of the gut barrier function [31]. In another experiment, administration of the CB2 agonist HU210 to lean mice increased plasma LPS levels. HU210 administration also increased plasma levels of fluorescein isothiocyanate-dextran administered by oral gavage, confirming in vivo the cannabinoids-induced increase in gut permeability [31]. However, the anti-inflammatory effects of cannabinoids could have a beneficial impact on inflammation-induced alterations of the intestinal epithelial barrier (see below). Indeed, available treatments for IBD, such as anti-TNF-α antibodies or corticosteroids, indirectly restore barrier function by reducing inflammation [42,47–49].

In summary, the ECS seems well positioned to be a regulatory system under inflammatory conditions because the effects of cannabinoids were more evident in situations where increased gastrointestinal motility and visceral sensation were induced by an inflammatory stimulus. Moreover, the CB2 receptor, although not always relevant in a normal setting, seems to play an important role during gut inflammation, as is also the case in other organs or systems [e.g., the central nervous system (CNS)].

The ECS is off-balance in IBD, evidence from human and animal studies
Due to its implication in the homeostasis and functionality of the gastrointestinal tract, the ECS seems positioned to exert a protective role in many of the points where homeostasis breaks in IBD. The first evidence for this was provided by studies in knockout mice that revealed an increased susceptibility to dinitrobenzene sulfonic acid (DNBS)-induced and dextran sulfate sodium (DSS)-induced colitis in CB1−/− mice when compared with their wild type counterparts [50] (see Box 3 for an overview of the rodent models of IBD discussed in this review), indicating a protective role for the CB1 receptor in IBD. More recently, increased susceptibility to colitis was confirmed not only for CB1−/− mice but also for CB2−/− mice [51], suggesting that both cannabinoid receptors play complementary roles in intestinal homeostasis because the absence of one or the other cannabinoid receptor results in an exaggerated inflammatory response. Interestingly, the genetic disruption of both the CB1 and CB2 receptors did not result in further worsening of colitis, when compared with either CB1−/− or CB2−/− alone [51]. It is possible that in double knockout mice compensatory mechanisms, or the action of endocannabinoids on other receptors, account for the lack of an even more exaggerated inflammatory response. Together, these studies suggest that cannabinoid receptors play a predominant role in suppressing gut inflammation.

The initial studies in knockout mice prompted investigations of the endocannabinoid signaling pathways in human IBD and revealed an unbalanced ECS. Indeed, anandamide and N-palmitoylethanolamine levels are altered in colon mucosal biopsies of patients with ulcerative colitis (UC) or Crohn’s disease (CD), especially during acute flares, with no change in 2-arachidonoylglycerol levels [35,52,53] (changes in the ECS in human IBD are summarized in Table 1). NAE levels were reported to be either increased or decreased depending on the study. However, a closer look at anandamide-metabolizing enzymes in acute colitis seems to support the notion of

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**Box 3. Overview of mouse models of acute IBD**

Numerous rodent models of mucosal inflammation (chemically-induced, genetically-manipulated, and immune-mediated) are available to unravel the mechanisms implicated in IBD pathogenesis and investigate novel therapeutic strategies [77]. Although these models differ from one another, primarily in the method or mechanisms of colitis induction, the resulting inflammation is generally channeled into a final common pathway, mediated by an excessive Th1 T cell response (associated with secretion of IL-12, IFN-γ, and TNF-α) as is the case with most models, or an excessive Th2 response (associated with IL-4 and IL-5). Inflammation is driven by antigens of the normal mucosal microflora, such as LPS, and one factor tipping the balance between a Th1- or Th2-mediated response could be the nature of the antigen driving the inflammation or the specificity of the T cell receptor on reactive cells [78,79].

The majority of genetic models of IBD are gene knockout, such as IL-10−/− mice, where colitis develops spontaneously around 12 weeks of age in mice kept in a conventional environment. Although these models allow researchers to study the role of specific cytokines, their utility is limited for understanding the underlying causative factors in IBD [77,78,80].

To characterize the role of ECS in IBD, mostly chemically-induced models are used where colitis induction requires administration of a chemical agent such as DSS, DNBS, TNBS, or oxazolone. These models mimic the acute phase of the disease, such as epithelial response to injury or neutrophil infiltration, and thus are not relevant to address changes occurring in the chronic phases of colitis. They are nevertheless useful for studying the pathogenic or protective roles of specific systems and for proposing potential therapeutic strategies [77,81].

DSS, administered in drinking water for 5 days, disrupts the epithelial barrier and promotes increased cellular exposure to gut microflora. This leads to activation of mucosal phagocytes by luminal flora and, consequently, a nonspecific release of proinflammatory cytokines. In an intact immune system, a T cell-mediated response is superimposed on the initial macrophage-mediated inflammation leading to lymphocyte activation and a Th1 response. Colitis is assessed on day 7 [78,81].

TNBS and oxazolone are haptenating agents, where inflammation results from an immune response to a specific antigen (a hapten). Both agents are administered intrarectally with ethanol (to disrupt the mucosal barrier), however, they lead to different immune responses: whereas TNBS leads to a Th1-mediated response, oxazolone leads to Th2-mediated inflammation. These models are more aggressive than DSS; colon alterations develop in less than 24 h and colitis is assessed 4 days later [78,81].
decreased anandamide levels in IBD. Indeed, one study reported decreased expression of N-acylphosphatidylethanolamine selective phospholipase D (NAPE-PLD) with no change in FAAH expression [54], which was confirmed in a second study showing a decrease in NAPE-PLD activity and an increase in FAAH activity during acute flares of UC or CD [35]; one could infer that this leads to decreased anandamide levels. Thus, although there appears to be a deregulated anandamide tone, the whole picture of anandamide metabolism in the human colon during IBD remains to be developed.

Data concerning the levels of endocannabinoids in rodent models of IBD is also scarce. Anandamide levels have been shown to be increased in the inflamed colons of DNBS-mice and the submucosa of TNBS-rats, with no change in 2-arachidonoylglycerol levels [52]. Interestingly, anandamide levels in the mucosa of the same TNBS-rats remained unchanged [52]. This points to a differential regulation of anandamide levels in the various colon structures and might explain the differences in anandamide levels observed between human studies. In several rodent models of colitis, decreased or unchanged FAAH mRNA expression was reported at different time points during the course of the disease in the inflamed distal colon [55–57]. However, this decrease in mRNA expression was not accompanied by a decrease in FAAH activity [57], raising the question of whether the observed increase in anandamide levels is actually due to a decrease of its catabolism by FAAH or to increased synthesis. FAAH−/− mice challenged with DSS or DNBS exhibit less colonic inflammation, thus strengthening the potential role of anandamide in IBD [50]. We must keep in mind, however, that along with anandamide, FAAH hydrolyzes other NAEs, such as N-palmitoylethanolamine, as well as other bioactive lipids, the levels of which are also increased in FAAH−/− mice [58,59] and could be implicated in the observed reduction of inflammation.

Interestingly, in the case of 2-arachidonoylglycerol, expression of both diacylglycerol lipase (DAGL)-α and MAGL was increased in colon mucosal biopsies of patients with UC, which implies an increased 2-arachidonoylglycerol turnover during inflammation [54]. Note that maintenance of the DAGL-α/MAGL ratio [54] could explain the unchanged 2-arachidonoylglycerol levels observed both in humans and mice [35,52,57]. The question remains, however, whether the differences in expression of MAGL and DAGL-α translate into differences in enzymatic activity. Indeed, in TNBS-induced colitis decreased MAGL mRNA in the distal colon was not accompanied by decreased MAGL activity [57].

So far, data from human studies seem insufficient to provide a conclusion concerning the ECS tone in IBD, mainly due to the complexity in obtaining large enough cohorts of subjects and to a lack of sub-classification of these diseases in published studies. In general, one of the cannabinoid receptors appears to be increased in CD or UC, with the other increased or unchanged (Table 1) [35,54,60]. A sub-classification of acute UC according to clinical score (mild, moderate, or severe) identified various changes in cannabinoid receptors and enzymes expression according to the clinical score [54]. For instance, whereas
expression of DAGL-α is unchanged during mild colitis, it is significantly increased during moderate colitis. Interestingly, fewer differences were observed in severe colitis when compared with healthy controls, suggesting a diminished ECS response to the inflammatory insult at that stage \[54\]. A second interesting fact to consider is the modified ECS tone in quiescent phases of the diseases \[54\] reflecting either a role for cannabinoid receptors in acute inflammation or a diminished colonic functionality in chronic stages, given that cannabinoid receptors, aside from modulating inflammation, have other functional roles in the colon.

The discrepancies, between human studies, in the inflammation-induced variation of expression of cannabinoid receptors (Table 1) could be explained by varying degrees of infiltration and mucosal remodeling or alteration as well as an analysis of different zones of the inflamed colon (ulcerations or at a distance from ulcerations). Indeed, in normal colonic tissue, CB₁ immunoreactivity is most evident on the microvillus border of the apical surface of the colonic epithelium, as well as the smooth muscle and the submucosal nerve bundles \[34,54,60\]. By contrast, CB₂ expression was more evident in subepithelial macrophages and plasmocytes characteristically present within the lamina propria of normal bowel, with no evident staining in the colonic epithelial border \[34,54,60\]. In acute phase IBD, CB₁ immunostaining was evident in the same structures as normal tissue, in contrast to CB₂ immunoreactivity which was also evident in the microvillus border. This shift towards an upregulation of CB₂ expression in epithelial cells in IBD was consistent in all the studies, even when total CB₂ protein or mRNA was unchanged \[54,60\].

Unfortunately, animal studies do not shed light on these discrepancies in expression. Indeed, CB₁ expression was increased in the myenteric plexuses of DNSS-mice, owing to an increase in cells expressing CB₁, with no change in the overall neuronal population \[50\], whereas no difference was observed in CB₁ mRNA expression in the colon of TNBS-mice \[57\]. CB₂ expression, by contrast, was increased in one study of DSS-induced colitis and TNBS mice \[61\] but unchanged in a second study of TNBS mice \[57\].

**The ECS as a therapeutic target in IBD**

Overall, data from animal and human studies suggest an up-regulation of the ECS during bowel inflammation, either by increased receptor expression and/or increased endocannabinoids levels. Moreover, data from knockout mice underline a protective role of the ECS in the pathogenesis of colitis. Thus, its pharmacological modulation could be a therapeutic strategy for IBD.

Regardless of the model, colitis in rodents generally translates into colon shrinkage and thickening, inflammatory lesions such as tissue damage to epithelial and smooth muscle architecture and invasion by monocytic and granulocytic cells, as well as bowel dysmotility and diarrhea. Colon shrinkage is the result of neuromuscular-induced contractions of the colonic smooth muscle, which in a clinical context is the correlate of acute abdominal pain. Colon thickening is indicative of glandular hypersecretion, muscular hypertrophy, and edematous swelling of the colonic wall. Usual ways to assess colitis and the effect of treatments include evaluation of macroscopic and microscopic (histological) signs of colon inflammation, measurement of colon weight and length, and additional measures of colon inflammation such as inflammatory cytokines expression or myeloperoxidase activity. Treatment schemes for the TNBS- or DNSS-induced models generally consist of drug administration once prior to colitis induction and then once or twice daily for 3 days. In the DSS model, drugs are administered concomitantly with the start of DSS or 4 days later.

Various cannabinoid agonists have been used to evaluate the impact of ECS activation on colitis. Nonselective agonists such as Δ⁹-THC (10 mg/kg or 20 mg/kg, q.d.) \[62\], HU210 (0.05 mg/kg, q.d.) \[50\], WIN55,212-2 (2 mg/kg, b.i.d.) \[12\], or anandamide (5 mg/kg, q.d.) \[63\] were beneficial in murine models of colitis and reduced colon inflammation. ACEA, a CB₁-selective agonist, was also effective in alleviating inflammation in oil of mustard (OM)-induced colitis (2.5 mg/kg, q.d.) and DSS-induced colitis (10 mg/kg, b.i.d.) \[64\].

Because the use of CB₁ receptor agonists comes with central side-effects owing to activation of CB₁ receptors in the CNS, targeting solely CB₂ receptors might be a sound strategy for treating colitis, seeing as CB₂ activation is not associated with such side-effects. JWH-133, a CB₂-selective agonist, effectively reduces inflammation at the stated doses in DSS-induced colitis (10 mg/kg or 20 mg/kg, b.i.d.) \[36,64\], TNBS-induced colitis (20 mg/kg, q.d. or b.i.d.) \[61\], OM-induced colitis (2.5 mg/kg, b.i.d.) \[64\], and spontaneous colitis in IL-10⁻/⁻ mice (2.5 mg/kg, q.a.d.) \[36\]. In all of these models, CB₂ activation led to improved macroscopic as well as histological colon alterations and reduced cellular infiltrates. In the IL-10⁻/⁻ model of colitis, inflammation is primarily mediated by Th1 cells, and in this model, JWH-133 not only inhibited neutrophil infiltration in the lamina propria (LP) and mesenteric lymph nodes (MLN) but also reduced the number of activated T cells in the colon, probably due to increased apoptosis. The reduction in cellular infiltrates in JWH-133-treated mice was further confirmed with isolated cells from the LP and MLN of DSS-mice. CD11b⁺ cells were increased in the LP and MLN of untreated mice – consistent with the macrophage-induced inflammation characteristic of DSS – and reduced following JWH-133 treatment \[36\]. A similar effect was observed for INF-γ⁺ cells \[36\], consistent with the fact that INF-γ plays a critical role in colitis induction and progression.

Interestingly, JWH-133 was more effective in OM-induced colitis when administered therapeutically (24 h after colitis induction) versus prophylactically (24 h prior to colitis induction) \[64\]. This observation might offer insight into the mechanism of action of CB₂ agonists in colitis; CB₂ signaling might be related to a time-dependent recruitment of CB₂-expressing cells or to their activation state. Indeed, CB₂ is not highly expressed in the normal colon, whereas under inflammatory conditions its expression is increased. In this setting, CB₂ agonists might act primarily to inhibit inflammation by acting on CB₂-expressing cells, instead of preventing their recruitment. Additionally, this observation implies that cannabinoid signaling can be effective in correcting colitis even after its initiation and
not only at induction of disease. However, seeing as most studies to date used a pre-treatment scheme, further studies in a therapeutic setting are warranted to confirm, or disprove, this effect.

AM1241 (10 mg/kg or 20 mg/kg, b.i.d.), a CB2 agonist, was used in the context of TNBS-induced colitis with the same outcome as JWH-133 [61]. The effects of both agonists were confirmed to be CB2-mediated because they were reversed in the presence of AM630 (10 mg/kg or 20 mg/kg, q.d.) and absent in CB2−/− mice [36,61]. In one study, AM630 seemed to exacerbate TNBS-induced colitis [61], but this was not consistent over all measured parameters and was not confirmed by two other studies using AM630 in studies of TNBS-induced and DSS-induced colitis [57,65].

A potential way to avoid the central side-effects of CB1 receptor activation is to use peripherally-restricted cannabinoi receptor agonists. SAB378, a CB4/CB2 peripherally restricted agonist has been studied for treatment of DSS-induced colitis (0.1 mg/kg or 1 mg/kg, b.i.d.) and TNBS-induced colitis (0.1 mg/kg or 1 mg/kg, 1 h pre-TNBS and then 8 h and 24 h later), and its effect was compared to that of the brain-penetrating CB4/CB2 agonist WIN55,212-2 [12]. SAB378 treatment reduced inflammation in TNBS-induced colitis by an amount that was not statistically significant, and was ineffective in DSS-colitis [12]. This study suggests that central cannabinoid receptor activation might be required to achieve the anti-inflammatory effect of cannabinoid agonists on colitis; however, the doses used were low compared with other cannabinoid agonists used in the same models or to the doses of WIN55,212-2 used in the same study. Higher doses of SAB378 were suggested (but not tested) to be potentially efficient in reducing colitis, with the concern that they might exert inhibitory actions on gastrointestinal transit [12]. However, inhibition of gastrointestinal transit could prove beneficial in acute colitis where bowel dysmotility and diarrhea are often observed.

Several studies established a role for CB2 receptors in alleviating colitis, shying away from CB1 because of its potential central side-effects. Nevertheless, targeting both receptors could have added benefits versus a receptor-specific approach. Along this line, another strategy to explore is inhibiting endocannabinoid degradation to benefit from the natural turnover of endocannabinoids in inflamed tissues.

Anandamide was the first studied endocannabinoid based on its altered levels in human IBD and rodent models and the availability of several selective FAAH inhibitors. VDM11 increases anandamide tone by decreasing its cellular uptake (the first step in endocannabinoid degradation) and by inhibiting FAAH. VDM11 administration resulted in a significant improvement of colon inflammation in TNBS-induced colitis (5 mg/kg, b.i.d.) [55] and DNBS-mice (5 mg/kg, q.d.) [52] and increased anandamide levels in the colon of DNBS-mice [52]. Two inhibitors of FAAH were also assayed. AA-5-HT (10 mg/kg, q.d.) decreased inflammation in DNBS-mice, although to a lesser extent than VDM11 in the same study [52], possibly due to the absence of a significant increase in colon anandamide levels with AA-5-HT treatment [52]. Another FAAH inhibitor, URB597 (5 mg/kg, b.i.d.) was used in TNBS-mice at a dose known to increase anandamide levels in vivo [66] and resulted in a significant attenuation of inflammation [55]. Interestingly, combining VDM11 and URB597 was not more effective at reducing colitis than either drug given alone [55] suggesting that the individual doses used allowed for a maximal increase of anandamide levels. The beneficial effects of increased anandamide levels are CB1- and CB2-dependent because URB597 and VDM11 had no effect in CB1−/− or CB2−/− mice [55]. In addition, a newly described FAAH inhibitor, compound (39) in [67] (10 mg/kg, q.d.), also reduced inflammation in TNBS-colitis, although its effects on anandamide levels or FAAH activity in vivo were not reported [67].

The potentially increased turnover of 2-arachidonoylglycerol under conditions of inflammation is also an interesting fact to consider. Administration of JZL184 (16 mg/kg, b.i.d.) in TNBS-induced colitis effectively inhibited MAGL activity, led to increased 2-arachidonoylglycerol levels, and subsequently to a decrease in inflammatory markers [57], confirming the therapeutic potential of 2-arachidonoylglycerol. An interesting observation is that colitis-induced alterations of the intestinal barrier led to increased intestinal permeability, as is the case in IBD, and subsequently to endotoxemia and systemic as well as central inflammation. Decreasing colon inflammation improved epithelial barrier function and resulted in a decrease of colitis-derived systemic and central inflammation. The use of antagonists selective for CB1 (SR141716A; 3 mg/kg, q.d.) and CB2 (AM630; 10 mg/kg, q.d.) established that the effects of 2-arachidonoylglycerol were mediated by both receptors. In fact, blocking each receptor completely abrogated the beneficial effects of 2-arachidonoylglycerol on macroscopic and microscopic colon inflammation markers, suggesting that both cannabinoid receptors are needed to maintain colon integrity [57]. This is consistent with previously reported data from knockout mice, where in each knockout strain the remaining cannabinoid receptor was not sufficient to suppress knockout colitis or a complete abrogation of the beneficial effects of inhibitors of endocannabinoid degradation [51,55].

Concluding remarks
Since the first evidence implicating the ECS in IBD, much additional work has been done. The effects in animal models of IBD of modulating the four main targets of the ECS – the CB1 and CB2 receptors, FAAH, and MAGL – are now well described. Collectively, these studies show that increasing the ECS tone results in decreased inflammation and improved colon integrity. However, there are still numerous questions to be answered. One pertinent question would be determining which, if not both, cannabinoid receptor to target because both seem to play a role in colon homeostasis. However, there are no reports of a study comparing potent and selective CB1 and CB2 agonists, alone or in combination, in the same experimental setting. One characteristic of the ECS is the large number of enzymes and molecular targets that regulate the levels of endocannabinoids and mediate their effects, respectively. Thus, another direction for future research
Box 4. Non-psychotropic cannabinoids in IBD

Although anecdotal reports of Cannabis sativa preparations being used to treat IBD offer a potential therapeutic pathway for investigation, the psychoactive side-effects of Δ⁹-THC constitute a major obstacle to widespread use. Thus, components of the cannabis plant that do not bind and activate classical cannabinoid receptors, or synthetic analogues, have been tested as treatments for IBD. Cannabidiol (CBD), present in Cannabis sativa, has antioxidant, anti-inflammatory, and immunomodulatory effects and is devoid of psychoactive properties [82]. It has very low affinity for CB₁ and CB₂ receptors and could exert part of its actions through a PPARγ pathway [3,83]. CBD effectively reduces colon alterations in TNBS- and DNBS-induced colitis [56,84]. Moreover, in DNBS-induced colitis, CBD counteracted colitis-induced alterations in cytokine and endocannabinoid levels in the colon; CBD reduced IL-1β and increased IL-10, thus regulating the aberrant immune response, and diminished the colitis-induced increase in endocannabinoid levels [56]. Beneficial effects of CBD in IBD could extend further than its immunomodulatory properties. Indeed, CBD was shown not to affect colonic motility in vivo in normal mice, while inhibiting inflammation-induced hypermotility [62,85]. Furthermore, on Caco-2 cells, a model of the intestinal epithelial barrier, CBD reduced the inflammation-induced increase in epithelial permeability [45]. Moreover, CBD reduced nitrite production in vivo and oxidative stress in vitro in Caco-2 cells and in human colonic cultures derived from UC patients. This could be beneficial in this setting as oxidative stress is a tissue-destructive factor playing a role in IBD pathogenesis [56,83]. In another experiment, CBD potentiated the beneficial effects of Δ⁹-THC on colitis, significantly improving the effect of the same dose of Δ⁹-THC alone [62]. This could prove interesting if it holds true with other cannabinoids. Thus, further studies are warranted to determine if CBD could potentiate the effects of low doses of CB₁/CB₂ agonists (i.e., devoid of CNS effects) in order to have beneficial effects in IBD. Finally, O-1602, a synthetic atypical cannabinoid that binds to and activates GPR55 and lacks significant affinity for CB₁ and CB₂ [3] also showed some beneficial effects in DDS- and TNBS-induced colitis [86]. Indeed, O-1602 reduced colon alterations and myeloperoxidase activity, suggesting an influence on neutrophil recruitment, confirmed with chemotactic assays [86]. O-1602 retained its efficacy in CB₁⁻/⁻/CB₂⁻/⁻ mice as well as in GPR55⁻/⁻ mice [86]. In summary, this ‘class’ of non-CB₁/non-CB₂ cannabinoid-like drugs with anti-inflammatory actions in the colon deserves further investigation to make use of their potential as well as identify additional targets involved in IBD pathogenesis.

Box 5. Outstanding questions

- Which of the two cannabinoid receptors is the most effective target for countering colitis?
- Are CB₁ agonists the best method to avoid central side effects and decrease motility only when hypermotility is induced by inflammation?
- Is the protection conferred by FAAH blockade the result of elevated levels of one bioactive lipid (e.g., anandamide) or of several of the bioactive lipids regulated by FAAH?
- Beyond CB₁ and CB₂, what is the relevance of other targets of endocannabinoids and related compounds?
- Regardless of the target, would a treatment initiated post-colitis induction (i.e., more closely mimicking a therapeutic setting) work in reducing colitis?
- What is the exact state of the ECS during the development of IBD in humans?
- What are the underlying mechanisms of the ECS effects in IBD?
- How relevant are the translational studies in humans? To date, they are mostly performed with Δ⁹-THC, a partial agonist; are they always analogous to what we have learned from animal models?
- Where does modulation of the ECS stand in comparison to available therapies (from efficacy and safety standpoints)?
- Would targeting the ECS be a valuable therapeutic strategy in all forms of IBD?

would be determining the role in IBD of the less well-studied components of the ECS, such as GPR119 or GPR55 (Box 4). There is also the need for improved characterization of the ECS in human IBD and translational studies to assess its therapeutic potential in humans. For instance, how do cannabinoid therapies compare with available therapies; only two studies compared the effect of increasing anandamide levels to 5-aminosalicylic acid derivatives and found them to be equivalent [36,52]. If confirmed, this would suggest the potential use of ECS modulators for inducing remission in mild to moderate cases and maintenance of remission in the early stages of IBD. Whether targeting the ECS would be effective in later stages, where high doses of corticosteroids or immunosuppressors are needed, remains speculative. Highly valuable studies have been conducted, but the variations from one study to the other in output terms, and the intrinsic variability of the pathology, clearly require additional studies (Box 5).

The ability of the ECS to decrease inflammation in a number of inflammatory diseases is well recognized. The studies briefly outlined in this review clearly support the notion of the ECS as a potentially valuable therapeutic target also in IBD.

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