Manipulation of the endocannabinoid system in colitis: A comprehensive review

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Abstract

Background—Inflammatory bowel disease is a lifelong disease of the gastrointestinal tract whose annual incidence and prevalence is on the rise. Current immunosuppressive therapies available for treatment of inflammatory bowel disease offer limited benefits and lose effectiveness, exposing a significant need for the development of novel therapies. In the clinical setting, cannabis has been shown to provide patients with inflammatory bowel disease symptomatic relief, although the underlying mechanisms of their anti-inflammatory effects remains unclear.

Methods—This review reflects our current understanding of how targeting the endocannabinoid system, including cannabinoid receptors 1 and 2, endogenous cannabinoids anandamide and 2-arachidonoylglycerol, atypical cannabinoids, and degrading enzymes including fatty acid amide hydrolase and monoacylglycerol lipase, impacts murine colitis. In addition, the impact of cannabinoids on the human immune system is summarized.

Results—Cannabinoid receptors 1 and 2, endogenous cannabinoids, and atypical cannabinoids are upregulated in inflammation, and their presence and stimulation attenuates murine colitis, while cannabinoid receptor antagonism and cannabinoid receptor deficient models reverse these anti-inflammatory effects. In addition, inhibition of endocannabinoid degradation via monoacylglycerol lipase and fatty acid amide hydrolase blockade can also attenuate colitis development, and is closely linked to cannabinoid receptor expression.

Conclusions—While manipulation of the endocannabinoid system in murine colitis has proven to be largely beneficial in attenuating inflammation, there is a paucity of human study data. Further research is essential to clearly elucidate the specific mechanisms driving this anti-inflammatory effect for the development of therapeutics to target inflammatory disease such as inflammatory bowel disease.

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Introduction

Inflammatory bowel disease (IBD) is a complex, multifactorial, life-long disease of the gastrointestinal (GI) tract that is divided into 2 predominant phenotypes: Crohn's disease (CD) and Ulcerative colitis (UC). IBD is characterized by chronic inflammation of the GI tract and causes symptoms of abdominal pain, bloody diarrhea, and weight loss, as well as symptoms specific to pediatric-onset IBD including growth failure, impaired bone mineralization and pubertal delay. With an estimated annual disease-attributable direct cost in the United States in excess of $6.3 billion[1] and an estimated 1.6 million affected people in the United States[2], IBD is one of the most prevalent and costly GI disorders whose annual incidence and prevalence is on the rise. IBD places a significant burden on affected individuals because of the significant effects on quality of life, growth and development, and risk of malignancy later in life[3]. The cause of IBD and its flare-ups is thought to involve dysregulation of the immune response, and the conventional treatment includes immunosuppression to induce and maintain remission. Current immunosuppressive therapies available for the treatment of IBD offer limited benefits, lose effectiveness rapidly, and carry multiple long-term risks including malignancy, infection, and low bone density[4]. These faults of the available therapies as well as their known complications expose a significant need for the development of highly novel therapies for the treatment of IBD.

One pathway that may offer a novel approach to enhance the impaired host regulatory system in IBD patients is the endogenous cannabinoid (endocannabinoid) pathway. While cannabinoids are traditionally characterized as being synonymous with plant-derived *Cannabis* or marijuana (MJ), there is an innate, mammalian endocannabinoid system that includes endogenous ligands termed endocannabinoids, their cannabinoid receptors, and the proteins involved in endocannabinoid biosynthesis and degradation. Several physiological effects and pathophysiological roles have been proposed for the endocannabinoid system in the GI tract, including effects on epithelial growth and regeneration, immune function, motor function, appetite control, and secretion[5]. Anecdotal and limited scientific evidence suggests that MJ use may have a positive impact on IBD patients due to its analgesic and anti-inflammatory effects[6]. Cannabinomimetics can provide IBD patients symptomatic relief by improving appetite, stimulating weight gain, reducing abdominal pain and decreasing intestinal motility but their anti-inflammatory function remains unclear[7]. Due to legalization of medical MJ and its increased use in the setting of inflammatory diseases, this uncertainty has sparked a focus on basic science research to further elucidate the biologic effects of manipulation of the endocannabinoid system. In this review, we will focus on defining the endocannabinoid system, and highlight the current data from targeting of the cannabinoid receptors 1 and 2, endogenous cannabinoids, atypical cannabinoids, and the enzymes of degradation in murine colitis.

The Endocannabinoid System (ECS)

The endocannabinoid system is comprised of endogenous ligands termed endocannabinoids, their cannabinoid receptors, and the proteins involved in endocannabinoid biosynthesis and degradation. The primary endocannabinoids are arachidonoyl ethanolamines, also known as anandamide (AEA), and 2-arachidonoylglycerol (2-AG). These endogenous ligands are lipid mediators that, in contrast to classic neurotransmitters which are stored in vesicles, are synthesized on-demand from membrane precursors and phospholipids[8] including N-acyl-phosphatidylethanolamine-selective phospholipase D (NAPE-PLD), then released from cells immediately after production[5]. They activate receptors to elicit a biologic response, then become inactivated through reuptake by carrier proteins in the cell membrane named the endocannabinoid membrane transporters (EMT), followed by enzymatic degradation. AEA is degraded by fatty acid amide hydrolase (FAAH) in to arachidonic acid and ethanolamine and 2-AG is degraded by monoacylglycerol lipase (MAGL) in to arachidonic acid and glycerol[5].

The endocannabinoids AEA and 2-AG act primarily on two heterotrimeric G-protein coupled receptors, the cannabinoid 1 (CB_1) receptor and cannabinoid 2 (CB_2) receptor. CB_1 receptors are located primarily on central and peripheral neurons, and more specifically in the enteric nervous system, in the intrinsic neurons, extrinsic neurons such as the cell bodies of sensory neurons in the dorsal root ganglia and nodose ganglion, and vagal efferent nerves, as well as on epithelial cells[8]. In neurons, endocannabinoids act mainly presynaptically, modulating the transmission of other neurotransmitters including γ-aminobutyric acid, glutamate and acetylcholine[8]. CB_2 receptors are mainly expressed on immune cells, specifically, neutrophils, activated macrophages, and subsets of T and B cells, as well as on epithelial cells[9]. CB_1 receptors modulate neurotransmitter release, while CB_2 receptors are mainly associated with immune functions[5]. In addition to signaling through CB_1 and CB_2 receptors, endocannabinoids also activate the transient receptor potential vanilloid type 1 (TRPV1), which is mainly expressed by primary afferent neurons, as well as the orphan G protein-coupled receptor GPR55[5] (Figure 1).

Other endogenous molecules exhibit "cannabinoid-like" effects and may be termed atypical endocannabinoids, including 2-arachidonoylglycerol ether (noladin ether), N-arachidonoyl dopamine (NADA), virodhamine, N-homo-γ-linolenoyl-ethanolamine (HEA) and N-docosatetraenoyl-ethanolamine (DEA), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA). These additional molecules do not seem to bind to cannabinoid receptors but rather to non-CB_1 or CB_2 receptors or to a specific isozyme belonging to a class of nuclear receptors/transcription factors with three subsets, known as the alpha, beta (delta), or gamma peroxisome proliferator-activated receptors (PPARs). However, these atypical endocannabinoids may augment the effect of AEA by competitive inhibition of FAAH, and/or through allosteric effects on other receptors such as ion channel TRPV1, and thus play an important, although less defined role in the endocannabinoid system[10].

Cannabinoid 1 Receptor

CB_1 receptor expression is significantly increased in models of inflammation, with increased expression demonstrated by Western Blot analysis in croton oil-induced inflammation of the jejunum[11], by increased mRNA expression in dinitrobenzene sulfonic acid (DNBS)-induced colitis[12], dextran sulphate sodium (DSS) induced murine colitis[13] and in lipopolysaccharide (LPS) stimulated peritoneal macrophages[14], and was shown by immunostaining of colonic sections to be increased in myenteric ganglia in both oil of mustard (OM) and DSS-induced colitis, as well as in the endothelium of OM-induced colitis[15]. In addition, mRNA expression of CB_1 receptor is upregulated in both DNBS-induced colitis and control mice when treated with the atypical cannabinoid PEA[16]. Based on this link of upregulated expression of the CB_1 receptor in inflammation, many studies have sought to demonstrate the key role of the CB_1 receptor in attenuation of murine colitis. CB_1 receptor agonist ACEA attenuated inflammation in OM and DSS-induced colitis models[15]. Massa *et al* showed that treatment with CB_1 receptor agonist R(-)-7-hydroxy- 6-tetra-hydrocannabinol-dimethylheptyl (HU210) in DNBS-induced colitis consistently resulted in protection against colitis[12]. Furthermore, WIN55, 212-2, a weakly selective CB_1 receptor agonist, protects against TNBS-induced colitis[17] in one particular study; however, this model does not reflect the inflammatory cascade found in human disease. WIN 55, 212-2 was also found to be protective against DSS-induced colitis in WT mice[17,18], and this protective effect is further enhanced in C57BL $Mk2^{-/-}$ mice, demonstrating an amplified benefit with simultaneous activation of the CB_1 pathway while blocking p38/Mk2 pro-inflammatory pathway[18].

Genetically engineered knockout mouse models have been instrumental in elucidating the CB_1 receptor pathways of the endocannabinoid system. Both 2,4,6-trinitrobenzene sulfonic acid (TNBS) and DSS treatment induced more severe colitis in $CB_1^{-/-}$ mice than in wild type (WT) littermates, and treatment with the specific CB_1 antagonist N-(piperidino-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-pyrazole-3- carboxamide (SR141716A) mimicked the phenotype of $CB_1^{-/-}$ mice, showing an acute requirement of CB_1 receptors for protection from inflammation[12]. Engel *et al* reaffirmed this finding several years later by showing that TNBS-induced colitis in $CB_1^{-/-}$ mice had a higher stool score, increased colon weight, a shorter colon, higher macroscopic and histologic scores, and increased mRNA expression of IL-1β and TNF-α, indicating a more severe colitis in the absence of the CB_1 receptor[19].

Many compounds act via the endocannabinoid system in a broader sense and only recently have the pathways via CB_1 receptor been more clearly explained. One such study demonstrated that oral treatment with α,β-amyrin, a pentacyclic triterpene, reduced leukocyte infiltration and pro-inflammatory cytokine production, and reduced inhibition of adhesion molecule expression[13]. These changes indicated that α,β-amyrin attenuated DSS-induced colitis, and this protective and therapeutic effect was partially reversed with the use of CB_1 receptor antagonist AM251, while CB_2 antagonist aminoalkylindole 6-iodo-pravadoline (AM630) failed to reduce the anti-inflammatory benefit of α,β-amyrin[13]. In addition, the enzymatic degradation pathway of the compound PF-3845, a potent and selective FAAH inhibitor, is mediated by the CB_1 receptor pathway, as administration of CB_1 receptor antagonist AM251 blocked the anti-inflammatory effect of PF-3845 in TNBS-

induced colitis while CB_2 blockade with CB_2 selective antagonist AM630 had no effect on inflammation[20].

Overall, while these described studies provided no strong consensus of the mechanism of CB_1-mediated attenuation of intestinal inflammation, *in vitro* studies describing a CB_1-selective improvement in intestinal epithelial barrier restitution following inflammatory insult suggest that this may represent one mechanism through which CB_1 receptors are protective[21].

Cannabinoid 2 Receptor

Mirroring the upregulation of CB_1 receptors in inflammation, CB_2 receptor mRNA expression is increased in chemically-induced colitis[22], and CB_2 receptors are highly expressed on infiltrated immune cells in colonic sections in OM-induced colitis and to a lesser extent, in DSS-colitis as demonstrated by immunostaining[15]. However, *in vitro* LPS stimulation of peritoneal macrophages led to decreased mRNA expression of CB_2 receptors in one study[14].

Furthermore, CB_2 receptors have also been shown to play a critical role in the attenuation of murine colitis. Both plant derived 9-tetrahydrocannabinol (THC) and endogenous cannabinoid AEA demonstrated an anti-inflammatory effect in *in vitro* cell assays of antibody formation, and these effects were blocked by CB_2 receptor antagonist SR144528 but not by CB_1 receptor antagonist SR141716[23]. Moreover, CB_2 agonist JWH-133 has been shown to inhibit LPS/IFN-γ induced pro-inflammatory cytokine IL-12p40 *in vitro*[24], and induce apoptosis in activated T cells both *in vivo* and *in vitro*[25]. Additionally, CB_2 activation with JWH-133 also ameliorates inflammation in $IL10^{-/-}$ colitic mice[25], as well as in chemically-induced colitis[15 25]. These protective effects of JWH-133 in DSS-colitis were reversed in the presence of CB_2 receptor antagonist AM630[25]. Similar findings were demonstrated in TNBS-induced colitis; CB_2 agonists JWH-133 and AM1241 significantly reduced murine colitis, and CB antagonist AM630 exacerbated colitis and abolished the anti-inflammatory effect of JWH-133 when given as a pre-treatment in the colitis model[22]. Genetically engineered CB_2 receptor knockout mice further confirmed the importance of the CB_2 pathway in attenuation of murine colitis. In the previously described study, neither JWH-133 nor AM1241 maintained their anti-inflammatory effects on TNBS-induced colitis in $CB_2^{-/-}$ mice[22]. Engel *et al's* 2010 study reiterated the importance of the CB_2 receptor pathway by showing that TNBS-induced colitis in $CB_2^{-/-}$ mice had increased mRNA expression of IL-1β and TNF-α and a more severe colitis in the absence of the CB_2 receptor[19].

The CB_2 receptor pathway also modulates the effects of several atypical cannabinoids. In the DNBS-induced colitis model, the beneficial effects of treatment with atypical cannabinoid PEA were abolished by CB_2 antagonist AM630 (as well as by GPR55 and PPAR antagonists), but the CB_1 antagonist rimonabant did not have any effect[16]. The CB_2 receptor pathway was also found to modulate the favorable effects of cannabigerol (CBG), a non-psychotropic cannabinoid capable of reducing nitric oxide production in macrophages and attenuating murine DNBS-induced colitis in both a preventive (pretreatment) model and

therapeutic (established colitis) model[26]. In these models, treatment with CB_2 antagonist SR144528 further increased the inhibitory effect of CBG on nitric oxide production[26]. Finally, the plant metabolite β-caryophyllene (BCP) ameliorates DSS- and oxazolone-induced colitis in a dose dependent manner, and this effect was reversed in the presence of CB_2 antagonist AM630 and in the presence of PPAR antagonist GW9662, suggesting the anti-inflammatory effect of BCP not only involves CB_2 receptor pathway, but also the PPAR pathway[27].

Based on evidence that CB_2 receptor agonism demonstrates promising therapeutic benefits in murine colitis, several novel, highly selective CB_2 receptor agonists were developed, of which two of these compounds had promising *in vivo* anti-inflammatory effects in the DSS-induced colitis model[28]. Furthermore, El Bakali *et al* developed constrained analogues from 4-oxo-1,4-dihydroquinoline-3-carboxamides with improved affinity for the CB_2 receptor and increased selectivity over the CB_1 receptor and tested their effects on prevention and treatment of TNBS-induced colitis; the highly CB_2 receptor selective agonistic compound 26 (ALICB459) exerted a strong protective effect when given orally to mice with TNBS-induced colitis[29] (Table 1).

Endogenous Cannabinoids

Endogenous cannabinoids AEA and 2-AG signal via cannabinoid receptors and are also fundamental in the pathway to attenuate murine colitis. AEA is upregulated in LPS stimulated peritoneal macrophages[14], and levels of endogenous cannabinoids AEA and 2-AG are elevated via investigation by isotope dilution liquid chromatography-mass spectrometry in DNBS-induced colitis, while treatment with plant-derived, anti-inflammatory cannabinoid cannabidiol (CBD) significantly counteracted these changes[30]. Additionally, intraperitoneal administration of AEA ameliorates macroscopic and microscopic colonic inflammation and suppresses the expression of pro-inflammatory cytokines in AKR mice with TNBS-induced colitis[31]. Furthermore, selective MAGL inhibitor JZL184 prevents degradation of 2-AG and thus allows 2-AG to activate cannabinoid receptors, leading to decreased levels of pro-inflammatory cytokines and attenuation of inflammation in TNBS-induced murine colitis[32].

Atypical Cannabinoids

Little is known about the mechanistic pathways of atypical cannabinoids in murine colitis, but they appear to play a dynamic role in attenuation of inflammation. Atypical cannabinoid and GPR55 agonist O-1602 attenuates inflammation in both DSS-induced and TNBS-induced colitis in wild type mice, and this effect is preserved when tested in $CB_1^{-/-}$ and $CB_2^{-/-}$ mice and in $GPR55^{-/-}$ mice, indicating that this therapeutic effect may have been achieved by off-target effect or by alternate pathways[33]. Conversely, GPR55 antagonist CID16020046 attenuated inflammation in DSS-induced colitis, and $GPR55^{-/-}$ mice showed reduced inflammation scores as compared to wild type mice in the DSS model, both suggesting that GPR55 activates a pro-inflammatory signal in intestinal inflammation[34].

Atypical cannabinoid PEA, which has been shown to act via several targets, including CB_1 receptors, CB_2 receptors, TRPV1 channels, PPARs, and GPR55, was found to be increased 2.5 fold in DNBS-treated colonic tissue, and led to attenuation of inflammation in this murine model of colitis[16]. In addition, PEA counteracted DNBS-induced downregulation of GPR55 and TRPV1, however, GPR55 antagonist ML-191 and PPARα antagonist GW6471 reduced the anti-inflammatory effects of PEA while TRPV1 channel antagonist capsazepine had a synergistic anti-inflammatory effect[16].

Treatment of LPS activated peritoneal macrophages with non-psychotropic cannabinoid cannabichromene (CBC), a transient receptor potential ankyrin-type 1 (TRPA1) agonist, weak MAGL inhibitor, and inhibitor of endocannabinoid activation, significantly increased OEA levels[14,26]. CBC treatment also reduced pro-inflammatory cytokines and nitric oxide production, and this anti-inflammatory effect was mimicked by cannabinoid receptor and TRPA1 agonists carvacrol and cinnamaldehyde, further enhanced by CB_1 receptor antagonists rimonabant and AM251, with no change on CBC effect by CB_2 antagonist SR144528[14,26]. Finally, CBC treatment attenuated DNBS-induced colitis in ICR mice, although the exact mechanistic pathway of these effects is somewhat unknown[14,26].

MAGL and FAAH

As the enzymes responsible for degradation of endocannabinoids 2-AG and AEA, respectively, MAGL and FAAH are vital components in the ECS during the inflammatory cascade. A significant increase in FAAH activity was observed in croton oil treated mice[11], but in contrast, FAAH mRNA expression is significantly reduced in early stages of inflammation and returned to normal levels over time in multiple colitis models[30,35]. In addition to the previously reviewed effects of α,β-amyrin as an anti-inflammatory agent via the CB_1 receptor pathway in DSS-induced colitis, preventive treatment with α,β-amyrin in DSS-induced colitis caused a marked decrease in both monoglyceride lipase 1 (MGL1) and FAAH mRNA expression, suggesting that the α,β-amyrin beneficial effects could be related to the modulation of endocannabinoid hydrolase expression[13].

In addition, MAGL and FAAH blockade are important to attenuation of colitis, and are closely linked to CB receptors in the ECS. While FAAH inhibition was shown to ameliorate DSS-induced colitis in one study[36], it failed to treat colitis using the same model in another study[37]. The FAAH inhibitor PF-3845 successfully attenuated TNBS-induced colitis[37]. Potent MAGL inhibitor JZL184 attenuated TNBS-induced murine colitis while preserving the expression of MAGL and altering function only[32]. The MAGL inhibitory pathway also involves the CB_1 and CB_2 receptors, as administration of the MAGL inhibitor JZL184 in the presence of CB_1 receptor antagonist SR141716A and CB_2 receptor antagonist AM630 completely abolished the protective effect of MAGL inhibition[32]. Furthermore, FAAH inhibitor URB597 attenuated TNBS-induced colitis, and this anti-inflammatory effect was abolished in both $CB_1^{-/-}$ and $CB_2^{-/-}$ mice[35]. Lastly, $FAAH^{-/-}$ mice are impaired in their ability to degrade AEA and display protection from DNBS-induced colitis as compared to the more severe inflammation seen in their $FAAH^{+/+}$ littermates[12] (Table 2).

Impact of Cannabinoids in Human Disease

Several studies have looked at the endocannabinoid system and its effects on human immune cell function. Anandamide has been shown to suppress proliferation and cytokine release from human T-lymphocytes via the CB_2 receptor[38]. Also, human neutrophil transmigration *in vitro* is impaired by treatment with synthetic cannabinoid receptor agonist WIN 55,212-2, though the mechanism does not appear to be mediated via the CB_1 or CB_2 receptor[39], suggesting an off-target effect may be responsible.

In addition, two plant cannabinoids, THC and CBD, have received greatest interest for their therapeutic potential in humans. THC, the partial $CB_{1/2}$ receptor agonist which was the first identified active ingredient in cannabis with potent psychotropic effects[40], can suppress production of IFNγ by human CD4+ T cells *in vitro* albeit at relatively high concentrations[41]. THC has also been shown to enhance TGFβ production[42] in unfractionated human peripheral blood mononuclear cells and reduces both cyclic AMP[43] and IL-10 production[44] by human lymphocytes via the CB_2 receptor.

CBD first gained interest when it was shown to provide significant improvement in a phase II placebo-controlled clinical trial for the treatment of epilepsy[45]. CBD has been reported to suppress TNFα, IL-1β and IFNγ production by human peripheral blood mononuclear cells[46]. In contrast to THC, CBD appears to have little or no CB_1 receptor or CB_2 receptor affinity[47] and a number of possible alternative methods have been postulated for its mechanism of action, including prolonging of the effect of anandamide. In a study looking at CBD in schizophrenia, CBD enhanced AEA signaling[48] potentially by acting as a false substrate for FAAH. CBD can also enhance adenosine signaling by inhibiting its uptake via equilibrative nucleoside transporter 1[49]. CBD, however, has also been reported to strongly inhibit IL-10 production by a human HUT-78 T cell line[44]. Interestingly, CBD increases TGFβ production[50] at low doses, which is important for expansion of both Treg and Th17 cells, but does the reverse at high CBD concentrations suggesting that there may be a significant dose effect in patients.

Three observational studies focused on MJ use in adults with IBD report subjective improvement in symptoms with MJ use[51,52,53], but the study by Storr *et al* in 2014 also demonstrated increased surgical procedures in those with use of MJ for greater than 6 months[53]. A prospective trial using THC versus placebo for the treatment of Crohn's disease in 21 patients found statistically significant reductions in Crohn's Disease Activity Index scores and increased quality of life in the THC treatment group; however, there was no change in serum markers of inflammation in either the THC treatment or the placebo group[6]. Lastly, a clinical trial sponsored by Bial and performed by Biotrial studied the effect of a fatty acid amide hydrolase (FAAH) inhibitor as an analgesic in human subjects, but the study was aborted after 5 of 6 subjects developed significant neurologic side effects, including one death[54]. Subsequent investigations coupled with studies on other FAAH inhibitors, strongly suggest that this is highly unlikely to represent a class effect.

Discussion

The ECS and its components, including CB_1 and CB_2 receptors, endocannabinoids AEA and 2-AG, atypical cannabinoids, and endocannabinoid hydrolases FAAH and MAGL, are vastly interrelated but incompletely understood pathways that highly contribute to attenuation of murine colitis. Plant cannabinoids THC and CBD proved beneficial in DNBS-induced colitis in a bell-shaped dose-related response, but more importantly, the effects of the phytocannabinoids were additive, as CBD increased an ineffective THC dose to the level of an effective one[55]. As previously discussed, TNBS induced a more severe colitis in $CB_1^{-/-}$ mice, $CB_2^{-/-}$ mice, as well as in $CB_{1/2}$ double knockout mice as compared to WT littermates, however, there was no difference in the severity of colitis between $CB_1^{-/-}$, $CB_2^{-/-}$ or the $CB_{1/2}$ double knockout mice, indicating the importance of the presence of both cannabinoid receptor pathways in the attenuation of inflammation[19]. Moreover, covalent cannabinoid agonist AM841 attenuated both DSS and TNBS-induced colitis when administered both before induction of colitis as well as a treatment for existing inflammation, and this anti-inflammatory effect was abolished in the setting of $CB_1^{-/-}$ mice, $CB_2^{-/-}$ mice, as well as in $CB_{1/2}$ double knockout mice, which provides further evidence to support the vital role of both cannabinoid pathways in attenuation of murine colitis[56]. Finally, MAGL and FAAH blockade, as well as blockade of atypical cannabinoid receptor TRPV1[57], are important to attenuation of colitis, and studies demonstrate a close link of these endocannabinoid hydrolases to CB receptors in the ECS (Figure 2).

Investigators have produced similar results from manipulation of the endocannabinoid system in other disease models of inflammation. In the Mixed Lymphocyte Reaction (MLR), an in vitro correlate of organ graft rejection, selective CB_2 agonists JWH-015 and O-1966, as well as THC, significantly suppressed the MLR (thus inflammation) in a dose dependent fashion, and this effect was reversed in the setting of CB_2 antagonist SR144528 but not in the presence of CB_1 antagonist SR141716A[58], indicating that the anti-inflammatory effect was CB_2 receptor mediated. In the same MLR model, selective CB_2 agonist O-1966 increased percentage of T regulatory cells (Treg), decreased active markers of inflammation including transcription factors on T cells and decreased pro-inflammatory cytokines, but these anti-inflammatory effects were abolished in $CB_2^{-/-}$ mice, and the increased Treg percentage was reversed with administration of anti-IL10[59]. In Theiler's virus (TMEV)-activated macrophages, a viral model of multiple sclerosis, CB_2 agonist JWH-133 inhibited inflammation and this effect was reversed in the presence of CB_2 antagonist SR144528[24]. Thus, the mammalian ECS likely plays an important role in a multitude of inflammatory diseases, and with time, novel drug therapies targeting the ECS may prove critical for the advancement of therapeutics in inflammatory disease, including IBD.

Multiple studies have struggled to elucidate specific mechanistic pathways in attenuation of inflammation, but nonetheless, their findings highlight the importance of manipulation of the ECS to produce biologic effects. Two groups investigated intraperitoneal administration of peripherally-restricted CB agonists and found that they had no effect on murine colitis, suggesting an inclusive role of CNS receptors[56,17]. One group went a step further and demonstrated that intracerebroventricular (i.c.v.) administration of peripherally-restricted CB agonists attenuated colitis, imparting credibility to the concept that both central and

peripheral CB receptors are responsible for the protective and therapeutic effects of cannabinoids[56], and that the lack of efficacy in peripheral restriction could be overcome by systemic administration.

Furthermore, upregulation in expression of the components of the ECS in the setting of murine colitis may be location and timing dependent. In one study, there was marked upregulation of CB_2 expression in the proximal colon within one day of TNBS instillation which progressed to the distal colon by day three, consistent with the aboral direction of disease progression; these findings were mirrored in the DSS-induced colitis model as well[22]. An additional study notes that method of administration may be critical in attenuating inflammation, as intraperitoneal (i.p.) and rectal administration of CBD attenuated TNBS-induced colitis but oral administration made no effect on inflammation[60]. These studies highlight the complicated nature of the ECS, and suggest that further research to clearly elucidate the specific mechanistic pathways of the ECS will be crucial to the development of therapeutics to target inflammation.

Conclusion

There is a significant need for the development of highly novel therapies for the treatment of IBD, as IBD is one of the most prevalent and costly GI disorders whose annual incidence and prevalence is on the rise. Manipulation of multiple pathways of the ECS in murine colitis has proven to be beneficial in attenuating inflammation, and the ECS pathway may offer an innovative approach to enhance the impaired host regulatory system in IBD patients. However, studies on the impact of manipulation the endocannabinoid system in intestinal inflammation are lacking, as the few human trials on MJ use in IBD have evaluated subjective, although positive, results only and failed to evaluate objective histologic data. This review highlights the complicated nature of the ECS, its critical role in a multitude of inflammatory diseases, and emphasizes that further research to clearly elucidate the specific mechanistic pathways of the ECS is essential to the development of therapeutics to target inflammation.

Acknowledgments

Nonstandard Abbreviations

IBD	Inflammatory bowel disease
FAAH	Fatty acid amide hydrolase

MAGL	Monoacylglycerol lipase
GI	Gastrointestinal
UC	Ulcerative Colitis
CD	Crohn's disease
MJ	marijuana
ECS	Endocannabinoid System
AEA	anandamide
2-AG	2-arachidonoylglycerol
NAPE-PLD	N-acyl-phosphatidylethanolamine-selective phospholipase D
EMT	endocannabinoid membrane transporters
CB$_1$	Cannabinoid 1
CB$_2$	Cannabinoid 2
TRPV1	transient receptor potential vanilloid type 1
NADA	N-arachidonoyl dopamine
HEA	N-homo-γ-linolenoyl-ethanolamine
DEA	N-docosatetraenoyl-ethanolamine
PEA	palmitoylethanolamide
OEA	oleoylethanolamide
PPARs	peroxisome proliferator-activated receptors
DNBS	dinitrobenzene sulfonic acid
DSS	dextran sulphate sodium
LPS	lipopolysaccharide
OM	oil of mustard
TNBS	2,4,6-trinitrobenzene sulfonic acid
WT	wild type
THC	9-tetrahydrocannabinol
CBG	cannabigerol
BCP	b-caryophyllene
CBD	cannabidiol

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CBC	cannabichromene
TRPA1	transient receptor potential ankyrin-type 1
MGL1	monoglyceride lipase 1
MLR	Mixed Lymphocyte Reaction
Treg	Regulatory T cell
TMEV	Theiler's virus
i.c.v.	intracerebrovascular
i.p.	intraperitoneal
CB$_1$R	CB$_1$ receptor
CB$_2$R	CB$_2$ receptor

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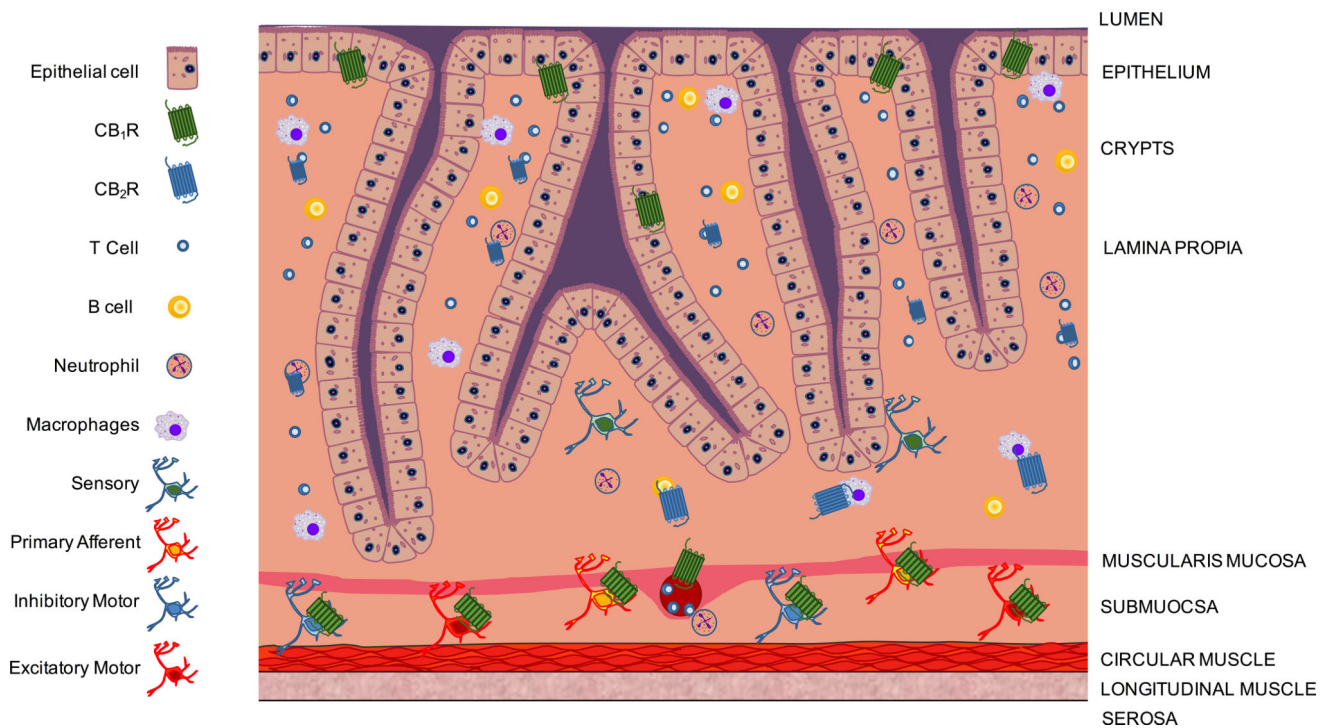
Figure 1. Colonic distribution of classical cannabinoid receptors CB$_1$ and CB$_2$

CB$_1$ receptors (CB$_1$R) are located in the intrinsic neurons, extrinsic neurons such as the cell bodies of sensory neurons in the dorsal root ganglia and nodose ganglion, and vagal efferent nerves within the enteric nervous system, as well as on epithelial cells. CB$_2$ receptors (CB$_2$R) are expressed on epithelial cells and immune cells including neutrophils, activated macrophages, and subsets of T and B cells.
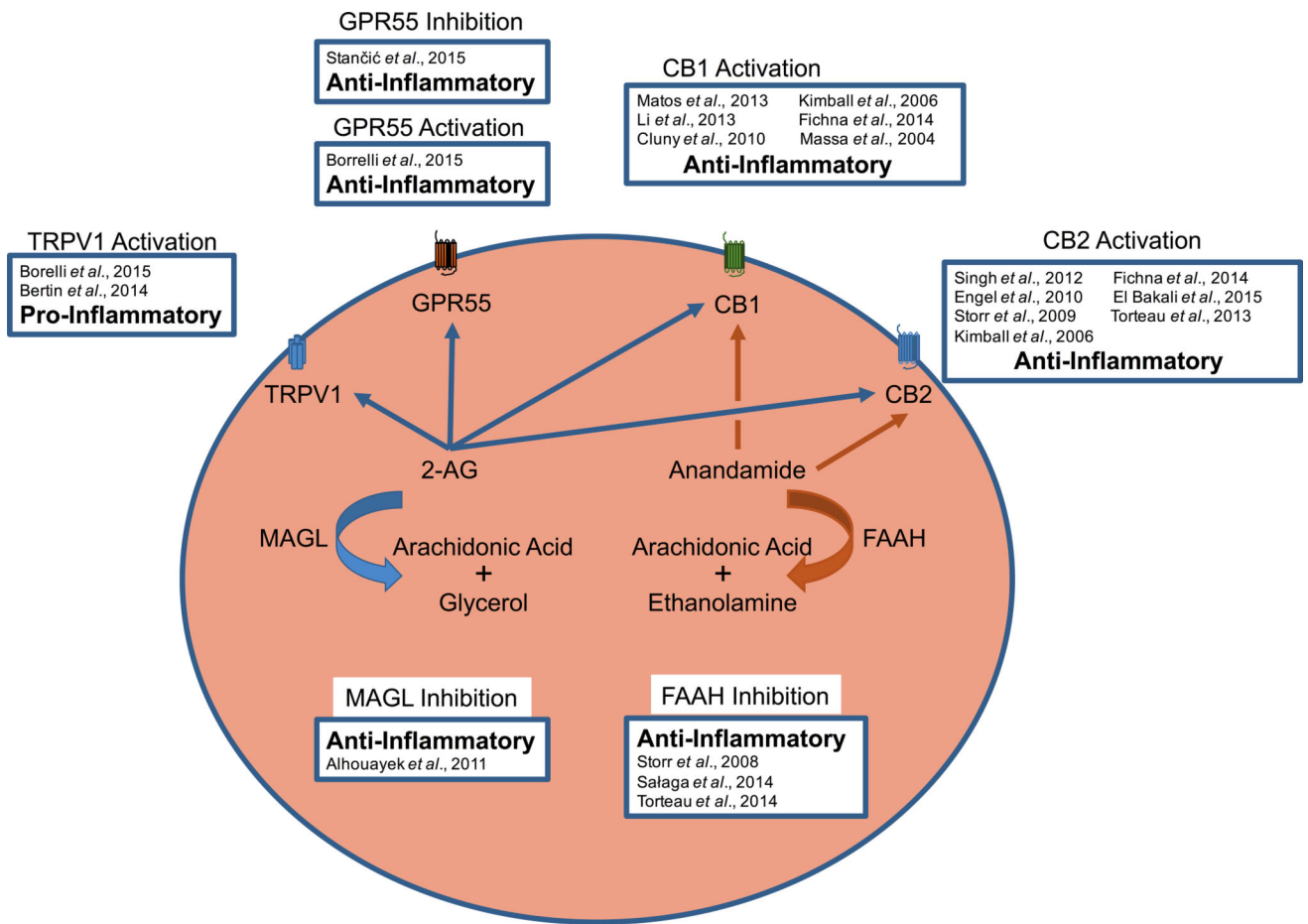
Figure 2. Therapeutic targeting of the endocannabinoid system in murine colitis
A schematic overview of cannabinoid receptors, the endogenous cannabinoids that act on them, pathway of enzymatic degradation of endocannabinoids, and how manipulation of this pathway impacts colitis.

Table 1

CB$_1$ and CB$_2$ targeted compounds have varying effects on colitis

Multiple compounds specifically target CB$_1$ or CB$_2$ receptors. CB$_1$ and CB$_2$ receptor agonism is largely anti-inflammatory while CB$_1$ and CB$_2$ receptor antagonism is pro-inflammatory.

Compound	Cannabinoid Receptor & Mechanism	Effect
ACEA	CB$_1$ agonist	Anti-inflammatory
HU210	CB$_1$ agonist	Anti-inflammatory
WIN55, 212-2	Weak CB$_1$ agonist	Anti-inflammatory
SR141716A	CB$_1$ antagonist	Pro-inflammatory
α,β-amyrin	CB$_1$ agonist	Anti-inflammatory
AM251	CB$_1$ antagonist	Pro-inflammatory
SR144528	CB$_2$ antagonist	Pro-inflammatory
JWH-133	CB$_2$ agonist	Anti-inflammatory
AM630	CB$_{1,2}$ antagonist	Pro-inflammatory
AM1241	CB$_2$ agonist	Anti-inflammatory
β-caryophyllene	CB$_2$ agonist	Anti-inflammatory
ALICB459	CB$_2$ agonist	Anti-inflammatory
AM841	CB$_{1,2}$ agonist	Anti-inflammatory
JWH-015	CB$_2$ agonist	Anti-inflammatory
O-1966	CB$_2$ agonist	Anti-inflammatory

Table 2

Atypical cannabinoid pathway and FAAH and MAGL targeted compounds have varying effects on colitis

Multiple compounds specifically act on atypical cannabinoid pathways TRPA1 or GPR55 and on enzymatic degradation pathways FAAH and MAGL. GPR55 pathway is pro-inflammatory, as antagonism has an anti-inflammatory effect. TRPA1 agonism, as well as FAAH and MAGL inhibition have anti-inflammatory effects on experimental colitis.

Compound	Receptor & Mechanism	Effect
CID16020046	GPR55 antagonist	Anti-inflammatory
cannabichromene	TRPA1 agonist & weak MAGL antagonist	Anti-inflammatory
carvacrol	TRPA1 agonist	Anti-inflammatory
cinnamaldehyde	TRPA1 agonist	Anti-inflammatory
PF-3845	FAAH antagonist	Anti-inflammatory
JZL184	MAGL antagonist	Anti-inflammatory
URB597	FAAH antagonist	Anti-inflammatory