Review Article

Cannabis, Cannabinoids, and the Endocannabinoid System—Is there Therapeutic Potential for Inflammatory Bowel Disease?

Tim Ambrose,a,b,*, Alison Simmonsa,b

aTranslational Gastroenterology Unit, John Radcliffe Hospital, Oxford, UK bMRC Human Immunology Unit, John Radcliffe Hospital, Oxford, UK

Corresponding author: Dr Tim Ambrose, BSc (Hons), MBChB, MRCP (UK) (Gastroenterology), c/o Prof. Alison Simmons, MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headley Way, Oxford OX3 9DS, UK. Tel.: 01865 222628; email: Timothy.ambrose@nhs.net

Abstract

Cannabis sativa and its extracts have been used for centuries, both medicinally and recreationally. There is accumulating evidence that exogenous cannabis and related cannabinoids improve symptoms associated with inflammatory bowel disease (IBD), such as pain, loss of appetite, and diarrhoea. In vivo, exocannabinoids have been demonstrated to improve colitis, mainly in chemical models. Exocannabinoids signal through the endocannabinoid system, an increasingly understood network of endogenous lipid ligands and their receptors, together with a number of synthetic and degradative enzymes and the resulting products. Modulating the endocannabinoid system using pharmacological receptor agonists, genetic knockout models, or inhibition of degradative enzymes have largely shown improvements in colitis in vivo. Despite these promising experimental results, this has not translated into meaningful benefits for human IBD in the few clinical trials which have been conducted to date, the largest study being limited by poor medication tolerance due to the Δ9-tetrahydrocannabinol component. This review article synthesises the current literature surrounding the modulation of the endocannabinoid system and administration of exocannabinoids in experimental and human IBD. Findings of clinical surveys and studies of cannabis use in IBD are summarised. Discrepancies in the literature are highlighted together with identifying novel areas of interest.

Key Words: Inflammatory bowel disease; cannabis; cannabinoids

1. Introduction

The use of cannabis, whether for medicinal or recreational purposes, dates back to ancient civilisation, featuring in Chinese medicine almost 5000 years ago and also described in Egyptian, Greek, Indian, and Middle Eastern cultures.1 Within Western medicine, the earliest work was performed by William O’Shaughnessy in the mid 1800s.2 He defined effects of Indian hemp on healthy animals and in human cases of rheumatism, hydrophobia, cholera, tetanus, and infantile convulsions.3 Despite medicinal use for millennia, work continues to try to understand the mechanistic role of cannabinoids in gastrointestinal disease, including human inflammatory bowel disease and animal models of intestinal inflammation.

2. The endocannabinoid system

The major active ingredient of Cannabis sativa, and that which causes the psychotropic effects, is Δ9-tetrahydrocannabinol [THC], and was only isolated from the plant in 19644 when technology advanced to allow isolation and characterisation of individual components of mixtures. However, the plant contains up to 100 cannabinoid constituents including cannabidiol [CBD].5 For some years...
after the identification of THC, the potential target[s] of action of cannabinoids remained elusive.

The discovery of cannabinoid receptor 1 [CB1] in the 1980s as both an abundant G-protein coupled receptor [GPR] in the human brain,4 and also at lower levels in immune cells,5,6 was followed by cannabinoid receptor 2 [CB2]. This was identified in the human HL60 promye locytic leukaemia cell line and has 44% homology to CB1.1 CB2 has been described in human immune cell subsets3,11,12 particularly those of a myeloid lineage.14 Since the identification of CB1/2, other putative cannabinoid receptors have been identified, including GPR18,15 GPR55,16 GPR119,17 transient receptor potential channels,6,13,15 and peroxisomal proliferator-activated receptors.29

Subsequently, endogenous ligands for these receptors, through which THC exerts its actions, were identified. Anandamide [AEA], a partial agonist of CB1 and CB2, with 2-arachidonoylglycerol [2AG], a monoacylglycerol, acting as a full agonist at both these receptors although with greater potency for CB1 than CB2.18,19,20 Other bioactive lipids related to these classical endocannabinoids have been described, including N-acyl-ethanolamines (palmitoylethanolamine [PEA], oleoylethanolamine [OEA]) and other monoacylglycerols (2-oleoylglycerol [2OG], 2-palmitoyleglycerol [2PG]). They may also bind to other cannabinoid-related receptors21 to exert actions independent of CB1/2. They may act to modulate 2AG signalling through an ‘entourage effect’,22,23 although whether this is to potentiate or inhibit is dependent on the experimental model used. Interestingly, gut microbes have been shown to produce lipid ligands with similarities to the wider endocannabinoids to signal through GPRs, including GPR119, to modulate host physiology particularly with glucose handling.34

2AG is synthesised from the action of diacylglycerol lipases [DAGL] α and β on diacylglycerols [DAGs] containing the arachidonate moiety. Metabolism of 2AG may proceed through hydrolysis, oxidation by COX2 or LOX enzymes, or epoxidation by components of the cytochrome P450 system,25,26 to produce arachidonic acid and eicosanoids [prostaglandins and leukotrienes]. In the murine brain, monoacylglycerol lipase [MGLL] accounts for 85% of 2AG hydrolytic activity, with further contributions by α/-hydroxylase domain 6 [ABHD6] and ABHD12.27 Fatty acid amide hydrolase [FAAH], the chief hydrolytic enzyme for AEA,28 also contributes slightly to 2AG hydrolysis. The contribution of MGLL to prostaglandin production downstream of 2AG appears to vary by tissue type—MGLL regulates prostaglandin production in murine liver and lung, and PLA2G4A in gut and spleen, with contributions from both enzymes in brain tissue.29 MGLL has also been demonstrated to hydrolyse prostaglandin glycerol esters [produced from 2AG by COX2 oxidation] and so may have more substrates than just monoacylglycerols.30

The cannabinoid receptors, together with their endogenous ligands and synthetic/degradative enzymes, form the endocannabinoid system [ECS] through which THC and other exocannabinoids act.

3. Inflammatory bowel disease

IBD can be classified into Crohn’s disease [CD] and ulcerative colitis [UC], based on characteristic clinical, radiological, endoscopic, and histological features. Although incompletely understood, the aetiology is thought to represent a complex interaction between genetic background, intestinal microbiota, environmental factors, and host immune response, resulting in persistent and dysregulated inflammation.

The incidence of IBD has increased in Western populations31,32 although it may be plateauing.33 However, this is not the case in the Asia-Pacific region where both incidence and prevalence are increasing.34 The increased incidence cannot be accounted for by substantial changes in host genetics, with environmental factors likely to be of critical importance [reviewed in 35 and briefly summarised in Table 1]. Although there has been an explosion in therapies for IBD, there remains an unmet clinical need for novel therapies and improved understanding of disease biology.

Genetic association studies have identified approximately 200 loci associated with IBD, with many shared between CD and UC.36,37 Differences in susceptibility alleles exist between ethnic backgrounds with, for instance, NOD2 and IL23R variants over-represented in European populations compared with East Asian populations.34,37 More recently, using high-density genotyping, 45 variants have been fine-mapped as potentially causal for IBD,38 and this approach may aid better understanding of disease mechanisms. Grouping of susceptibility loci using ontology pathway analysis has highlighted broad mechanisms of relevance in the immunological response in IBD, and this has informed subsequent study. This includes autophagy [ATG16L1, IRGM, LRKK2], endoplasmic reticulum stress [XBPI, innate immune cell sensing [NOD2], T cell tolerance [IL10, IL10R], IL23-pathways [IL23R, IL12B], lymphocyte trafficking [CCL7, IL8], and epithelial barrier function [MUC1, MUC3].39–42 Another development in the field of IBD genetics is evidence that genetic associations may be related to disease prognosis in addition to, or instead of, disease susceptibility.43 This might provide a novel avenue to stratify patients and target therapies accordingly.

4. Single nucleotide polymorphisms in ECS components in IBD

Single nucleotide polymorphisms [SNPs] in some ECS genes have been investigated in human IBD [Table 2]. The Q63R mutation in the CNR2 gene, encoding CB2, impairs endocannabinoid-induced inhibition of T cell proliferation.44 In an Italian paediatric IBD cohort, this mutation was associated with a more severe disease phenotype and shorter time to relapse for UC,45 but these were not replicated in a Turkish cohort of adult patients.46 This may be due to either age or ethnic differences between patients. The G1359A mutation in CNR1, encoding CB1, has shown to have a lower prevalence in patients with UC than in controls, and to be associated with lower body mass index.

| Table 1. Effect of environmental factors on rates of IBD. |
|-----------------------------------------------------------|
| Factor | General effect on rates of IBD |
|--------|--------------------------------|
| South to North migration | Increase |
| East to West migration | Increase |
| Smoking | Reduction [UC]; increase [CD] |
| Appendectomy | Reduction [UC]; increase [CD] |
| Antibiotics in childhood | Increase [Western populations]; reduction [Asia] |
| Improved hygiene/sanitation | Increase |
| COX inhibition | Increase [NSAIDs, aspirin]; reduction [COX2-inhibitors] |
| Reduced dietary fibre | Increase [CD] |
| High ω-3 PUFA | Increase |
| Vitamin D deficiency | Increase |
| Stress | Increase |
| Increased physical activity | Reduction |

COX, cyclo-oxygenase; PUFA, polyunsaturated fatty acids; NSAID, non steroidal anti-inflammatory drug; CD, Crohn’s disease; UC, ulcerative colitis.

Data extracted from reference 35.
5. ECS tone in IBD

There is disagreement on the tone of the ECS between studies of human IBD [summarised in Figure 1]. CB1 has been identified in the colonic epithelium [particularly crypts], some plasma cells of the lamina propria, smooth muscle, and the submucosal myenteric plexus. Conversely, CB2 localises in the absorptive and goblet cells of the epithelium, Paneth cells, and some subepithelial macrophages and plasma cells. CB2 was expressed at slightly higher levels than CB1 in one study. The effect of inflammation on expression of CB1 and CB2 is less clear. Increases in both CB1 and 2 in both CD and ‘acute phase IBD’ [a combination of UC and IBD-unclassified] have been observed. The authors of this study suggest that the changes in CB1 may be an effect of goblet cell depletion in epithelial architecture rather than a true increase in protein abundance. In other work, increases in either CB1 or alternatively in CB2 alone have been observed. A recent study of CB1 and CB2 gene expression in Crohn’s disease, the largest to date, demonstrated consistent detection of expression albeit at low levels in inflamed, non-inflamed, and healthy samples. A difference in expression patterns was seen between disease affecting the ileum, where both CB1 and 2 were reduced in inflamed/non-inflamed samples, and colon, where an increase was seen in CB1 and 2 in the non-inflamed but not inflamed samples.

Similar controversy exists for levels of endocannabinoids themselves. AEA has been shown to be increased in inflamed samples from UC patients yet reduced in another study. It is more plausible that AEA levels are indeed decreased in inflammation as it has been shown that there is reduced expression of the synthetic enzyme NAPE-PLD and increased expression of the hydrolytic enzyme FAAH in colonic inflammation. PEA is increased in inflammation in one study but does not change in another, and the two studies assessing 2AG levels both demonstrate no change in inflammation.

To date, only one study has attempted to define levels of MGLL and the DAGL enzymes in human IBD. MGLL was localised to the central portion of epithelial cells, polymorphonuclear cells of the lamina propria, and the myenteric plexus, and was shown to be increased in inflammation. Previously MGLL has been shown to be widely distributed in the rat intestine, increasing in expression from duodenum to distal colon. DAGLα was similarly increased in inflammation, but no difference was seen in DAGLβ.

There are a multitude of potential explanations for the differences between these studies. In particular, the patients studied were not always well phenotyped for disease severity and extent, and were frequently grouped together. Furthermore, the potential role of medications such as systemic immunosuppression in altering expression levels was not explored. In many studies, comparisons were made between inflamed mucosa in an IBD patient and healthy mucosa from a different person. ECS tone has been shown to be affected by diurnal variation and ethnicity, not to mention the likely confounding of any comorbidities such as irritable bowel syndrome, suggesting effects on motility, secretion or pain perception.

| Genotype | FAAH C385A | CNR1 G1359A | CNR2 Q63R |
|----------|------------|-------------|-----------|
|          | CC         | GG          | QQ        |
| Storr 2008 CD [n = 202] Controls [n = 206] | 67.3% | 53.3% | 10.9% |
| Storr 2009 CD [n = 435] UC [n = 167] Controls [n = 406] | 65.8% | 58.4% | 6.9% |
| CNR1 G1359A CD [n = 216] UC [n = 166] Controls [n = 197] | 53.3% | 58.4% | 11.9% |
| Yonal 2014 CD [n = 101] UC [n = 101] Controls [n = 101] | 10.9% | 6.9% | 11.9% |
| Striscuglui 2016 CD [n = 112] UC [n = 105] Controls [n = 600] | 2.7% | 18.1% | 16.0% |

Prevalence of genotypes are displayed alongside associations with disease phenotype. Data extracted from references 45–49.

IBD, inflammatory bowel disease; CD, Crohn’s disease; UC, ulcerative colitis; ECS, endocannabinoid system; EIM, extra-intestinal manifestations.
IL-6/8 in *ex vivo* biopsies, with the greatest benefit seen with Δ⁹-tetrahydrocannabinolic acid [THCA] which does not have psychoactive effects.⁶⁶ THCA has not yet been used in clinical trials.

### 6. In vivo studies

#### 6.1. Cannabinoid receptors

One of the earliest studies of the effect of cannabinoid receptor modulation identified that CB1⁺ mice exhibited more severe colitis following either dinitrobenzene sulphonic acid [DNBS] or dextran sulphate sodium [DSS] administration.⁶⁷ Subsequently this finding was confirmed in trinitrobenzene sulphonic acid [TNBS] colitis and extended to demonstrate that CB2⁻ and CB1⁻/CB2⁻ double knockout mice also display worsened colitis.⁶⁸ Although not reaching statistical significance, there was an additive effect of double knockout on macroscopic colonic inflammation but not histological inflammation. The effects of CB2⁻ have also been confirmed in DSS colitis. The authors also demonstrated that murine peritoneal macrophages stimulated *ex vivo* with lipopolysaccharide [LPS]/DSS upregulated components of the NLRP3 inflammasome, that this was exacerbated in CB2⁻ cells, and was improved by the CB2 agonist HU308.⁶⁹

The findings of genetic knockout studies have largely been confirmed by use of CB agonists/antagonists. DNBS colitis is worsened by prophylactic administration of the CB1 agonist SR141716A,⁷⁰ with DSS and oil-of-mustard colitis improved by prophylactic ACEA, a CB1 agonist.⁷¹ Similarly, the CB2 agonists JWH133,⁷² HU308,⁷³ or ALICB459⁷⁴ are beneficial in ameliorating chemical colitis models when administered prophylactically. ALICB459 is particularly appealing from a translational medicine perspective, as it was effective with oral rather than intraperitoneal administration. CB2 agonists improve, and CB2 antagonists worsen, chemical colitis when administered therapeutically, and that this is CB2-dependent.⁷²⁻⁷⁴

Although most studies have used chemical colitis models, JWH113 ameliorates colitis in the IL10⁻ model where mice develop spontaneous colitis by 12 weeks of age.⁷⁵ GP-1a, purported to be a CB2 agonist, improves ileitis when administered retro-orbitally in the TNFΔ⁻ model of Crohn’s-like ileitis. However, recent evidence would suggest that this compound may in fact be an inverse agonist of both CB1 and CB2 *in vitro*,⁷⁶ and so it remains to be confirmed whether CB2 agonism or inverse agonism is effective here.

Complementing the results from double knockout studies, use of less selective agonists also display benefits in colitis. WIN55,212 is an agonist of both CB1 and CB2 and ameliorates colitis whether used prophylactically or therapeutically in TNBS and DSS models.⁷⁷⁻⁷⁹ AM841 [CB1 agonist] improves colitis in a cannabinoid receptor-dependent manner, with the effect lost in CB1⁺ and CB2⁺ mice.⁷⁹ The same is true for the CB1 agonist, HU210,⁸⁰ which also has effects on sustaining intestinal barrier function in a TLR4-independent manner.⁸¹ Interestingly the effect of non-selective cannabinoid receptor activation may be through central rather than peripheral mechanisms. CB13 is an agonist of both CB1 and CB2, with poor penetration of the central nervous system.⁸² However, this compound was not effective in murine TNBS or DSS colitis when administered intraperitoneally, but was effective when injected intracerebroventricularly.⁸³ Equally ineffective was the peripherally restricted, non-selective agonist, SAB378.⁸⁴

#### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| T. Ambrose and A. Simmons |  |
| CB1/2 | Cannabinoid receptor type 1/2 |
| DSS | Dextran sulphate sodium |
| DNBS | Dinitrobenzene sulphonic acid |
| DGL | Diacylglycerol lipase |
| EC | Endocannabinoid |
| ECS | Endocannabinoid system |
| IBD | Inflammatory bowel disease |
| IHC | Immunohistochemistry |
| IB | Immunoblot |
| IB | Polymerase chain reaction |
| RC | Radiochromatography |
| HPLC | High performance liquid chromatography/mass spectrometry |
| ELISA | Enzyme-linked immunosorbent assay |

**Figure 1.** A summary of existing studies of the endocannabinoid tone in human ileum and colon in health and IBD. Studies of the ECS in human IBD have yielded conflicting results dependent on the technique used, the site of sampling [ileum vs colon], and the comparison used [inflamed vs non-inflamed vs healthy]. Only one study has assessed the synthetic [DAGL] and key hydrolytic [MGLL] enzyme involved in 2AG metabolism. No studies have examined the presence of ABHD6. Studies of the ECS in human IBD have yielded conflicting results dependent on the technique used, the site of sampling [ileum vs colon], and the comparison used [inflamed vs non-inflamed vs healthy]. Only one study has assessed the synthetic [DAGL] and key hydrolytic [MGLL] enzyme involved in 2AG metabolism. No studies have examined the presence of ABHD6.
Two studies have suggested interplay between cannabinoid signalling and p38 MAPK in the modulation of colitis severity. In the first, Mk2−/− mice, who lack a downstream substrate of p38, exhibit a less severe colitis in response to DSS. This study also demonstrated that WIN55,212 impairs phosphorylation of p38 in response to DSS in both wild-type and Mk2−/− mice. Subsequently a similar result has been obtained by using the p38 inhibitor SB203580. One mechanism therefore, by which colitis is ameliorated by cannabinoids, may be through effects on MAPK pathways.

6.2. Endocannabinoid lipid ligands
A recent study investigated rectal 2AG administration in chemical colitis. Here the authors used carbon nanotubes linked to 2AG, with the aim of reducing hydrolysis and improving the overall pharmacological profile. A single dose administered rectally 2 days before the instillation of TNBS in rats, and then a second dose 8 days after instillation, resulted in improvement of colitis. No effect was seen of free 2AG or of the carbon nanotubes alone.

In addition to the effect of 2AG on colitis, intraperitoneal AEA improves TNBS colitis. More evidence exists for the role of PEA in colitis, possible acting by inhibiting the induction of angiogenesis that is usually seen in chemical colitis. Intraperitoneal injection of PEA is beneficial in both established chemical colitis and when administered prophylactically. The effects are dose-dependent and require PPARα but not PPARγ. This study demonstrated a reduction in TLR4 and $100B expression on enteric glial cells and a reduction in MAPK signalling, as potential mechanisms of action. The effect of PEA is also dependent upon CB2 and GPR55. Adelmidrol is a PEA analogue which is effective orally in established colitis and when administered prophylactically. The effects are dose-dependent and require PPARα but not PPARγ. This study demonstrated reduced permeability of unstimulated Caco-2 [colon cancer] cells and affected expression of micro-RNAs in mesenteric lymph nodes and Peyers patches of colitic mice as a potential mechanism of action. One study has suggested that therapeutic FAAH inhibition may have additional benefits over prophylactic administration, although this remains to be replicated. Despite several studies demonstrating a benefit of FAAH inhibition, findings are not unanimous. One study demonstrated that PF3845 is effective at ameliorating colitis in the TNBS model but not the DSS model. The beneficial effects on TNBS were not replicated by a second group.

Inhibition of N-acylethanolamine-hydrlysing acid amide [NAAA] results in increased levels of PEA and not AEA, and improvement in colitis. This corroborates the studies performed using PEA.

6.3. Hydrolytic enzymes
6.3.1. MGLL
To date, only one study has assessed the role of MGLL inhibition in colitis. Rectal administration of TNBS was used to induce colitis, and the small molecule MGLL inhibitor JZL184 was administered prophylactically. Both macroscopic and microscopic colitis were ameliorated. This was associated with a reduction in mucosal and systemic pro-inflammatory cytokines such as IL-6, TNFα, and IL-12. MGLL inhibition also reduced LPS-induced endotoxaemia, which may suggest effects on mucosal barrier function. Certainly, THC and CBD have beneficial effects on intestinal permeability induced by EDTA in unstimulated Caco-2 [colon cancer] cells. The effects of 2AG and AEA in this model were dependent on apical [worsened permeability] versus basolateral [improved permeability] administration. The same is true for JZL184 administration in unstimulated Caco-2 cells. When cytokines were administered to Caco-2 cells to mimic inflammation, JZL184 worsened permeability when applied apically either at the same time as the cytokines or after inflammation had been induced. The benefits of MGLL inhibition on TNBS colitis involved both CB1 and CB2, as inhibition of either abrogated the effects of JZL184. However the CB2 antagonist used, AM630, has recently been shown to have off-target effects, and so it would be useful to have data using alternative CB2 antagonists to confirm the role of this receptor in mediating the effects seen. It is also worth noting that the dose of JZL184 used here is high [32 mg/kg daily in divided doses] and is within the dose to desensitise CB1, although it is not clear whether this happens within the 3-day time frame used in this model.

6.3.2. FAAH
Although only one in vivo study has been performed using MGLL inhibition, several studies have investigated FAAH inhibition. First, FAAH−/− mice exhibit less severe chemical colitis. Use of pharmacological FAAH inhibitors corroborates the findings from the genetic study with amelioration of disease in a CB1- and CB2-dependent manner. A combined FAAH/COX inhibitor, ARN2508, was effective in a CB1- and PPARα-dependent manner. Inhibition using FAAH-II not only improved colitis and reduced pro-inflammatory cytokine production, but also impaired infiltration by immune cells and affected expression of micro-RNAs in mesenteric lymph nodes and Peyers patches of colitic mice as a potential mechanism of action. One study has suggested that therapeutic FAAH inhibition may have additional benefits over prophylactic administration, although this remains to be replicated. Despite several studies demonstrating a benefit of FAAH inhibition, findings are not unanimous. One study demonstrated that PF3845 is effective at ameliorating colitis in the TNBS model but not the DSS model. The beneficial effects on TNBS were not replicated by a second group.

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6.4. Other ECS targets
It is not well understood to what extent the non-CB1, non-CB2 cannabinoid receptors have roles in colitis. The atypical cannabinoid O-1602 is a derivative of CBD and is known to bind to GPR55. Although it exerts anti-inflammatory effects on both DSS and TNBS colitis, this is independent of GPR55 as genetic knockout of this receptor, and indeed of both CB1 and CB2, does not alter the effect of O-1602. It is not known therefore by which pathways this compound acts on in colitis. The GPR55 antagonist CID16020046 and GPR555 mice exhibit less severe colitis in response to DSS or TNBS, contrary to genetic knockout or pharmacological inhibition of CB1/2. Therefore, whereas CB1/2 are likely to have anti-inflammatory effects, GPR55 triggers a pro-inflammatory cascade.

Inhibition of AEA reuptake using the endocannabinoid membrane transport inhibitor VDM11 has also been shown to improve experimental colitis.

6.5. Exocannabinoids
Further to the studies modulating endocannabinoid levels, administration of exocannabinoids in experimental colitis has largely demonstrated similar benefit. B-caryophyllene is available orally and acts via CB2 and PPARγ to limit colitis. Similarly, α6-amyrisol ameliorates both TNBS and DSS colitis and acts, at least in part, through cannabinoid receptors. The phytocannabinoid CBD has been most extensively studied. It reduces intestinal inflammation induced by LPS as measured by TNFα. A reduction in inducible nitric oxide synthase [iNOS] expression has been demonstrated by CBD which also reduces IL-1β and increases IL-10 levels. The effect of CBD is maintained when administered intraperitoneally or rectally but not orally. It has also been shown to potentiate the anti-inflammatory effects of THC in chemical colitis. Synthetic derivatives such as abnormal CBD ameliorate colitis independently of CB1/2 and possibly through GPR18, whereas the highly concentrated CBD, known as CBD botanical drug substance [a major ingredient of nabiximols], was effective orally and intraperitoneally unlike pure CBD. Reference has been made to benefit of CBD in the IL10−/− mouse in a related publication, but this study has not.
been published independently. Despite these promising findings, CBD has been shown to worsen LPS-induced pulmonary inflammation in mice\textsuperscript{115} and so some caution should be maintained.

Other exocannabinoids which have been beneficial at ameliorating colitis include MPF [an extract of medicinal cannabis],\textsuperscript{112} cannabigerol,\textsuperscript{113} and cannabichromene.\textsuperscript{114}

Of all experiments within the published literature assessing modulation of ECS components on experimental ileitis/colitis, few have used non-chemical models. These include IL\textsuperscript{10} colitis [one published study,\textsuperscript{19} and one referenced within the text of another\textsuperscript{106}] and TNF\textsuperscript{100} ileitis.\textsuperscript{16} Several studies have used oil of mustard, croton oil, and LPS to induce colitis [detailed within a recent systematic review\textsuperscript{117}] but these models are not classically felt to be experimental models of human IBD, although they may provide insight into intestinal inflammation in general.

Better understanding of the molecular wiring of the endocannabinoid system in the mammalian intestine may enable development of more specific models capable of interrogating the potential protective effects of this pathway via genetic ablation approaches. Further investigation of ECS modulation in other models of IBD and intestinal inflammation, such as Citrobacter rodentium and Helicobacter hepaticus, would be informative. The development of a first-in-class MGLL inhibitor for human clinical trials, ABX-1431 [Abide Therapeutics\textsuperscript{1}], provides opportunity to investigate this in human IBD if preclinical study was supportive.

7. Clinical studies and trials in human IBD

Several questionnaire-based studies have confirmed current use of cannabis in 6.8-15.9% of adult patients with IBD, with lifetime use in 48.1–67.3% of patients.\textsuperscript{116-120} A Canadian study found that 17.6% of IBD patients were current or previous users of cannabis specifically for IBD.\textsuperscript{10} Among an adolescent cohort, 32% of patients with IBD had ever used marijuana, 57% for medicinal purposes.\textsuperscript{121} The most common reasons given for cannabis use were to alleviate abdominal pain, diarrhoea, or anorexia\textsuperscript{10} and use is higher in patients with previous surgery or chronic analgesic requirements.\textsuperscript{117,118,121} Improvements in quality of life have also been demonstrated\textsuperscript{118} along with a reduction in Harvey-Bradshaw Index.\textsuperscript{122}

A large population-based survey confirmed a younger age of onset of cannabis use in patients with IBD and overall heavier consumption.\textsuperscript{123} Another group has identified an association between prolonged cannabis use [>6 months] in CD and a higher incidence of previous surgery (odds ratio 5.030 [95% confidence interval 1.449–17.459]).\textsuperscript{123} The studies are summarised in Figure 2.

An open-label, single-arm study of inhaled THC used ‘as required’ for pain in IBD patients [n = 13] demonstrated effects on analgesia, improved quality of life, and increased body mass index.\textsuperscript{124} A reduction in Harvey-Bradshaw Index from 11.36 to 5.72 was seen in patients with CD [11/13] and a slight decrease in partial Mayo score for patients with UC [2/13]. Subsequently a prospective, placebo-controlled trial of inhaled THC [23% THC, <0.5% CBD] in CD failed to meet its primary endpoint of increased clinical remission.\textsuperscript{124} However, effects were seen on clinical response as assessed by the CD Activity Index [CDAI]—a scoring system based on largely clinical parameters with no objective assessment of inflammation.\textsuperscript{125} Patients reported improved quality of life and reduced pain, which likely accounts for the reduction in CDAI without there necessarily being an effect on inflammation. Alternatively, the choice of patients with medically refractory disease in this trial may mask subtle benefits.

The same group performed a double-blind randomised controlled trial [RCT] of oral CBD in CD.\textsuperscript{126} There were no safety issues identified, but they failed to meet the primary endpoint of a reduction in CDAI after 8 weeks. Whereas this may represent a failure of CBD to exert anti-inflammatory effects in human IBD, the randomisation procedure resulted in 6/10 [60%] of those treated with CBD versus 0/9 [0%] of placebo being current smokers. Smoking is well known to be associated with a more difficult-to-treat disease, and this may mask an effect of CBD.

Inhaled Cannabis sativa containing THC has been trialled in patients with extensive or left-sided UC refractory to medications, including thiopurines and biological agents, and published in abstract form.\textsuperscript{127} The placebo arm was inhaled Cannabis sativa from which THC had been extracted. This demonstrated no effect of THC on C-reactive protein [CRP] or calprotectin levels compared with placebo. A modest effect was seen on disease activity index and Mayo endoscopic subscore [reduction from 2 to 1 in the intervention group, p <0.01]. Improvements were seen in terms of abdominal pain, appetite, and general satisfaction, with no clear safety signals.

A double-blind, placebo-controlled, RCT of GW42003 [approximately 96% CBD, 4% THC] in active UC has recently been published.\textsuperscript{128} The trial recruited 60 patients with mild-moderate UC, excluding isolated proctitis, and remains the largest clinical trial of cannabinoids in human IBD to date. Participants were required to be on either no or stable dose of 5-aminosalicylic acid [5ASA] before entry. Importantly, this trial incorporated endoscopic evaluation and measurement of CRP and fecal calprotectin as objective measures of inflammation, alongside clinical scoring. The trial failed to meet the primary endpoint of clinical remission, but a reduction in Mayo score and improvement in quality of life were favoured by GW42003. However, only 59% of those treated complied with protocol, due to adverse effects likely due to the THC component. There is a need for cannabinoids which do not have neuropsychiatric side effects—THCA, discussed earlier, may be beneficial in this regard.

It is interesting to hypothesise why the experimental data are not yet translating into meaningful improvements for patients. This may simply represent immunological differences between species; or that chemical experimental colitis models are insufficient to accurately model human IBD; or that the route of drug administration is wrong; or that the inclusion criteria for patients in some of these trials generally selected for patients with more advanced, and therefore inherently more difficult to treat, disease. Replication of experimental findings in alternative animal models such as T cell transfer or IL10 \textsuperscript{56} Helicobacter hepaticus should be encouraged. Interestingly, a recent study has shown that the Jurkat cell line [T cell line] is more resistant to the effects of THC than CBD;\textsuperscript{129} and so some caution should be maintained.

The same group performed a double-blind randomised controlled trial [RCT] of oral CBD in CD.\textsuperscript{126} There were no safety issues identified, but they failed to meet the primary endpoint of a reduction in CDAI after 8 weeks. Whereas this may represent a failure of CBD to exert anti-inflammatory effects in human IBD, the randomisation procedure resulted in 6/10 [60%] of those treated with CBD versus 0/9 [0%] of placebo being current smokers. Smoking is well known to be associated with a more difficult-to-treat disease, and this may mask an effect of CBD.

Inhaled Cannabis sativa containing THC has been trialled in patients with extensive or left-sided UC refractory to medications, including thiopurines and biological agents, and published in abstract form.\textsuperscript{127} The placebo arm was inhaled Cannabis sativa from which THC had been extracted. This demonstrated no effect of THC on C-reactive protein [CRP] or calprotectin levels compared with placebo. A modest effect was seen on disease activity index and Mayo endoscopic subscore [reduction from 2 to 1 in the intervention group, p <0.01]. Improvements were seen in terms of abdominal pain, appetite, and general satisfaction, with no clear safety signals.

A double-blind, placebo-controlled, RCT of GW42003 [approximately 96% CBD, 4% THC] in active UC has recently been published.\textsuperscript{128} The trial recruited 60 patients with mild-moderate UC, excluding isolated proctitis, and remains the largest clinical trial of cannabinoids in human IBD to date. Participants were required to be on either no or stable dose of 5-aminosalicylic acid [5ASA] before entry. Importantly, this trial incorporated endoscopic evaluation and measurement of CRP and fecal calprotectin as objective measures of inflammation, alongside clinical scoring. The trial failed to meet the primary endpoint of clinical remission, but a reduction in Mayo score and improvement in quality of life were favoured by GW42003. However, only 59% of those treated complied with protocol, due to adverse effects likely due to the THC component. There is a need for cannabinoids which do not have neuropsychiatric side effects—THCA, discussed earlier, may be beneficial in this regard.

It is interesting to hypothesise why the experimental data are not yet translating into meaningful improvements for patients. This may simply represent immunological differences between species; or that chemical experimental colitis models are insufficient to accurately model human IBD; or that the route of drug administration is wrong; or that the inclusion criteria for patients in some of these trials generally selected for patients with more advanced, and therefore inherently more difficult to treat, disease. Replication of experimental findings in alternative animal models such as T cell transfer or IL10 \textsuperscript{56} Helicobacter hepaticus should be encouraged. Interestingly, a recent study has shown that the Jurkat cell line [T cell line] is more resistant to the effects of THC than CBD;\textsuperscript{129} and so some caution should be maintained.

8. Conclusion

Research into the distribution and function of the endocannabinoid system in IBD and models of intestinal inflammation is increasing. There is accumulating evidence that enhancing signalling through cannabinoid receptors 1 and 2 has anti-inflammatory potential in the intestine in vivo. This was the subject of a recent systematic review and
Clinical studies of cannabis use in IBD

| Country | Source of data | Number of IBD patients | Key findings | Source of data | Number of IBD patients |
|---------|----------------|------------------------|--------------|----------------|------------------------|
| Spain   | IBD referral centre | 214 patients | 10% cannabis but only 1/3 inform their physician | Canada | IBD referral centre |
| Canada  | IBD referral centre | 292 patients (191 CD, 101 UC) | Current users: 15.9% CD, 31.6% UC | USA | IBD referral centre |
| USA     | Population-based case-control | 319 patients (211 CD, 108 UC) | Current or past users: 17.6% (75% of these had CD) | USA | Survey of self-identified IBD patients |
| USA     | Retrospective observational | 1666 patients | Life times: 6.7% vs 60% | USA | 99 patients (62 CD, 27 UC, 10 IBD-U) |

Clinical trials of cannabinoids in IBD

| Country | Type of trial | Patients | Intervention | Primary outcome (intervention vs placebo) | Other outcomes |
|---------|---------------|----------|--------------|------------------------------------------|---------------|
| Israel  | Retrospective observational | 30 patients (all CD) | Cannabis sativa for pain, lack of response to treatment or recreational | Clinical remission (CDAI ≤ 150): 5/11 vs 17/20 (p<0.05) | Reduction in HBI 1.6% at 6 months (before cannabis vs after) |
| Israel  | Prospective, open label, single-arm | 13 patients (CD, 2 UC) | Inhaled cannabis sativa PRN for pain for 3 months | Clinical response (reduction in CDAI of >30%): 74.3% vs 31.5% (p<0.05) | Improved pain, QoL, and BMI (11.36 ± 3.17 to 7.32 ± 2.68, increased BMI) |
| Israel  | Prospective, double-blind, placebo-controlled RCT | 21 patients (all CD) | Inhaled cannabis (23% THC, 0.5% CBD) | Improved QoL and no side effects | Clinical response (reduction in CDAI of >100): 10/31 vs 4/10 (p<0.05) |
| Israel  | Prospective, double-blind, placebo-controlled RCT | 19 patients (all CD) | Oral CBD | Clinical remission (total Mayo score ≤ 2): 28% vs 26% (p=0.02) | Only 59% protocol compliance, PP analysis of Mayo score and QoL in favour of CBD BDS |
| UK      | Prospective, double-blind, placebo-controlled RCT | 60 patients (all UC) | Oral CBD BDS (96% CBD, 4% THC) | Good tolerability and safety | Medical (e.g. pain, nausea, appetite, weight) 57% Smoking or oral ingestion were the commonest route |

Figure 2. A summary of clinical studies and trials of cannabis and cannabinoids in human IBD. Studies consistently demonstrate use of cannabis in patients with IBD, frequently for symptom relief. As yet, no clinical trials of cannabinoids in IBD have met their primary endpoints but demonstrate improvements in symptoms, quality of life, and clinical severity scores. Data extracted from references 60, 116–124, 126, 128. IBD, inflammatory bowel disease; CD, Crohn’s disease; UC, ulcerative colitis; OR, odds ratio; HBI, Harvey-Bradshaw Index; CDAI, Crohn’s Disease Activity Index; QoL, quality of life; CBD, cannabidiol; CBD BDS, cannabinoids botanical drug substance; THC, tetrahydrocannabinol; PP, per protocol; RCT, randomised-controlled trial; ns, not significant.

meta-analysis, although this paper did not include studies of cannabinoid receptor antagonists and, as mentioned above, did include studies of LPS, oil of mustard, and croton oil-induced colitis. Critically though, this article confirms the bias towards chemical models of colitis. Although cannabis use is fairly common in patients with IBD, particularly to relieve symptoms, the limited number of trials of exocannabinoids in IBD have not met their primary endpoints [Figure 2].

Before novel therapies targeting endocannabinoids, rather than exocannabinoids, can be translated into the clinical setting for IBD, it is essential that sufficient preclinical work is completed. There is an urgent need for better reagents to interrogate the system in vitro and in vivo. Antibodies are often non-specific, and small molecules do not necessarily target the receptor appropriately, potentially resulting in misleading results. Many ECS enzymes, including MGLL, FAAH, and DAGL, are serine hydrolases. Activity-based protein profiling [ABPP] can be used not only to profile activity of these enzymes in cells and tissues, but also identify off-target effects of inhibitors on other enzymes within this family, but has not yet been employed in human IBD.

The development of single-cell techniques opens up the possibility of better understanding ECS tone in individual cells. It is entirely plausible that the ECS functions in the gut in a similar way to the central nervous system where signals are sent between cells to modify neurotransmission. Improved understanding of the effect of inflammation on the ECS in different cell types might lead to a better understanding of how to translate this into meaningful therapies. At present though, it is difficult to make firm recommendations on the benefit or risk of cannabinoids in the management of the inflammation associated with IBD.

It should not be overlooked, however, that a beneficial effect of cannabinoids on symptom control in patients with IBD is possible. There are well-documented effects of ECS modulation on gastrointestinal motility. Polymorphisms in FAAH and CB1 have been associated with subtypes of irritable bowel syndrome in humans, and in vivo administration of CB1 antagonists reverses the inhibition of gastrointestinal motility seen with cannabinoid agonists. Dronabinol, a non-selective cannabinoid agonist, reduces gastric emptying, with a gender bias towards females. In addition to roles in gastric emptying, cannabinoids [including dronabinol, nabnilone, and nabiximols] exert an anti-emetic effect likely mediated through central effects on CB1 and possibly CB2. The beneficial effects on nausea and vomiting may be lost, however, when cannabis is used chronically—resulting in the cannabis hyperemesis syndrome. This has many features similar to cyclic vomiting syndrome, a condition which has associations with CB1 polymorphisms.

Although the exact mechanisms are poorly understood, it is reproducibly observed that symptoms may be relieved by hot bathing. The ECS, cannabinoids, and modulation of pain, including in visceral hypersensitivity associated with chronic stress, are inextricably
linked and have been the subject of many reviews [including and]. Indeed, nabiloximol [a combination of THC and CBD] is licensed for the treatment of spasticity and spams in multiple sclerosis, with some effects on pain in this condition. To this end and with relevance for IBD, a phase 2a clinical trial of orlinabin [APD371], a full CB2 agonist, for visceral pain in Crohn’s disease is under way but has not yet reported [ClinicalTrials.gov Identifier: NCT03155945]. Any benefit of cannabis, cannabinoids, and ECS modulation in IBD has to be carefully balanced against the potential myriad negative, including neuropsychiatric, side effects.

Relevant to the rise in addiction to prescribed and illicit opiates, and the associated adverse health outcomes, there are valuable preclinical data suggesting overlap between the endocannabinoid and opioid systems. The MGLL inhibitor MJN110 and morphine act synergistically via μ-opioid and cannabinoid receptors to relieve neuropathic pain, but without the unwanted side effects of reduced gastrointestinal motility and cannabimimetic side effects. MGLL mice are hypersensitive to the μ-opioid agonist, loperamide and CB2 agonists have been shown to induce μ-opioid receptor transcription in Jurkat cells. Modulation of the ECS may increase sensitivity to opioids and therefore may be a strategy to reduce opioid requirements if the evidence translates to human disease.

As calls for medicinal cannabis for treatment of epilepsy and other conditions intensify, it is all the more pressing that we better understand the effects of cannabinoids on human diseases—not just to identify novel applications, whether for symptomatic relief or as anti-inflammatory agents, but also to reduce the risk of exposing our patients to harm.

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**Conflict of Interest**

TA and AS have received research funding from Abide Therapeutics. Abide Therapeutics have not contributed to any aspect of this manuscript.

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**Author Contributions**

TA: concept, writing, approval of final manuscript. AS: concept, revised manuscript for intellectual content, approval of final manuscript.

**References**

1. Friedman D, Sirven JI. Historical perspective on the medical use of cannabis for epilepsy: Ancient times to the 1980s. *Epilepsy Behav* 2017;70:298–301.
2. O’Shaughnessy WB. Extract from a memoir on the preparations of the indian hemp, or ganjha, [cannabis indica] their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases. *J Asiat Soc Bengal* 1839;93:732–45.
3. O’Shaughnessy WB. On the preparations of the indian hemp, or ganjha - cannabis indica, their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases. *Proc Med J Retrop Med Sci* 1843;5:363–9.
4. Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of a active constituent of hashish. *J Am Chem Soc* 1964;86:1646.
5. Mechoulam R, Hanouï LO, Pertwee R, Howlett AC. Early phytochan
binnoid chemistry to endocannabinoids and beyond. *Nat Rev Neurosci* 2014;15:757–64.
6. Herkenham M, Lynn AB, Little MD, et al. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A* 1990;87:1932–6.
7. Bouaboula M, Rinaldi M, Carayon P, et al. Cannabinoid-receptor expres
sion in human leukocytes. *Eur J Biochem* 1995;214:173–80.
8. Galègue S, Mary S, Marchand J, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* 1995;232:54–61.
9. Daaka Y, Friedman H, Klein TW. Cannabinoid receptor proteins are increased in Jurkat, human T-cell line after nitrogen activation. *J Pharmacol Exp Ther* 1996;276:776–83.
10. Sugamura K, Sugiyama S, Nozaki T, et al. Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation* 2009;119:28–36.
11. Krishnan G, Chatterjee N. Endocannabinoids alleviate proinflammatory conditions by modulating innate immune response in muller glia during inflammation. *Glia* 2012;60:1629–45.
12. de Campos-Carli SM, Araújo MS, de Oliveira Silveira AC, et al. Cannabinoid receptors on peripheral leukocytes from patients with schizophrenia: Evidence for defective immunomodulatory mechanisms. *J Psychiatr Res* 2017;87:44–52.
13. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993;365:61–5.
14. Graham ES, Angel CE, Schwarz LE, Dunbar PR, Glass M. Detailed characterisation of CB2 receptor protein expression in peripheral blood immune cells from healthy human volunteers using flow cytometry. *Int J Immunopharmacol Pharmacol* 2010;23:25–34.
15. Console-Beam L, Brailiou E, Braiouou GC, Sharit H, Aboud ME. Activation of GPR18 by cannabinoid compounds: a tale of biased agonism. *Br J Pharmacol* 2014;171:3908–17.
16. Ryberg E, Larsson N, Siigren S, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152:1092–101.
17. Hansen KB, Rosenkilde MM, Knop FK, et al. 2-Oleoyl glycerol is a GPR119 agonist and signals GLP-1 release in humans. *J Clin Endocrinol Metab* 2011;96:E1409–17.
18. De Petrocellis L, Ligresti A, Moriello AS, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRPV channels and endocannabinoid metabolic enzymes. *Br J Pharmacol* 2011;163:1479–94.
19. Pertwee RG, Howlett AC, Aboud ME, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacol Res* 2010;62:588–631.
20. Rodríguez de Fonseca F, del Arco I, Bermudez-Silva FJ, et al. The endocannabinoid system: physiology and pharmacology. *Alcohol Alcohol* 2005;40:2–14.
21. Lambert DM, Di Marzo V. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? *Curr Med Chem* 1999;6:757–73.
22. Ben-Shabat S, Frude E, Sheskin T, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol* 1998;353:23–31.
23. Murataeva N, Dhopeshwarkar A, Yin D, et al. Where's my entourage? The curious case of 2-arachidonoylglycerol, 2-linolenoylglycerol, and 2-palmitoyleglycerol. *Pharmacol Res* 2016;110:173–80.
24. Cohen LJ, Esterhazy D, Kim SH, et al. Commensal bacteria make GPCR ligands that mimic human signalling molecules. *Nature* 2017;549:48–53.
25. Rouzer CA, Marnett LJ. Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chem Rev* 2011;111:5899–921.
26. Zelanko S, Arnold WR, Das A. Endocannabinoid metabolism by cytochrome P450 monoxygenases. *Prostaglandins Other Lipid Mediat* 2015;116:117:112–23.
27. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol* 2007;14:1347–56.

28. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NR. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 1996;384:83–7.

29. Nomura DK, Morrison BE, Blankman JL, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science* 2011;334:809–13.

30. Savinaizen JR, Kansanen E, Pantzar T, et al. Robust hydrolysis of prostaglandin glycerol esters by human monoglyceride lipase [MAGL]. *Mol Pharmacol* 2014;86:522–35.

31. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012;142:46–54.e2; quiz e30.

32. Jess T, Rius L, Vind I, et al. Changes in clinical characteristics, course, and prognosis of inflammatory bowel disease during the last 5 decades: a population-based study from Copenhagen, Denmark. *Inflamm Bowel Dis* 2007;13:481–9.

33. Lofts CG, Lofts EV Jr, Harmsen WS, et al. Update on the incidence and prevalence of Crohn’s disease and ulcerative colitis in Olmsted County, Minnesota, 1940-2000. *Inflamm Bowel Dis* 2007;13:234–61.

34. Ahuja V, Tandon RK. Inflammatory bowel disease in the Asia-Pacific area: a comparison with developed countries and regional differences. *J Dig Dis* 2010;11:134–47.

35. Ananthakrishnan AN. Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol* 2015;12:205–17.

36. Jostins L, Ripke S, Weersma RK, et al.; International IBDS Genetics Consortium [IIBDGC]. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;491:119–24.

37. Liu JZ, van Someren S, Huang H, et al.; International Multiple Sclerosis Genetics Consortium; International BD Genetics Consortium. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet* 2015;47:979–86.

38. Huang H, Fang M, Jostins L, et al.; International Inflammatory Bowel Disease Genetics Consortium. Fine-mapping inflammatory bowel disease loci to single-variant resolution. *Nature* 2017;547:173–8.

39. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature* 2011;474:307–17.

40. Van Limbergen J, Radford-Smith G, Satsangi J. Advances in IBD genetics. *Nat Rev Gastroenterol Hepatol* 2014;11:372–85.

41. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009;361:2066–78.

42. de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol* 2016;13:13–27.

43. Lee JC, Bianci D, Roberts R, et al.; UKIBD Genetics Consortium. Genomewide association study identifies distinct genetic contributions to prognosis and susceptibility in Crohn’s disease. *Nat Genet* 2017;49:262–8.

44. Spie JC, Arbour N, Gerber A, Beutler E. Reduced endocannabinoid immune modulation by a common cannabinoid 2 [CB2] receptor gene polymorphism: possible risk for autoimmune disorders. *J Leukoc Biol* 2005;78:231–8.

45. Strisciuglio C, Bellini G, Miele E, et al. Cannabinoid receptor 2 functional variant contributes to the risk for pediatric inflammatory bowel disease. *J Clin Gastroenterol* 2018;52:e37–43.

46. Yonal O, Eren F, Yilmaz A, Atuğ Ö, Över HH. No association between the functional cannabinoid receptor type 2 Q63R variants and inflammatory bowel disease in Turkish subjects. *Turk J Gastroenterol* 2014;25:63–49.

47. Storr M, Emmerding D, Diegelmann J, et al. The cannabinoid 1 receptor [CNBR] 1359 G/A polymorphism modulates susceptibility to ulcerative colitis and the phenotype in Crohn’s disease. *PLoS One* 2010;5:e9453.

48. Storr M, Emmerding D, Diegelmann J, et al. The role of fatty acid hydrolyse gene variants in inflammatory bowel disease. *Aliment Pharmacol Ther* 2009;29:542–51.

49. Storr MA, Keenan CM, Emmerding D, et al. Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. *J Mol Med [Berl]* 2008;86:925–36.

50. Camilleri M, Carlson P, McKinzie S, et al. Genetic variation in endocannabinoid metabolism, gastrointestinal motility, and sensation. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G13–9.

51. Marquez L, Suárez J, Igeosia M, Bermedo-Silva FJ, Rodríguez de Fonseca F, Andreu M. Ulcerative colitis induces changes on the expression of the endocannabinoid system in the human colonic tissue. *PLoS One* 2009;4:e6893.

52. Wright K, Rooney N, Feeney M, et al. Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology* 2005;129:437–53.

53. Harvey BS, Nicotra LL, Vu M, Smid SD. Cannabinoid CB2 receptor activation attenuates cytokine-evoked mucosal damage in a human colonic explant model without changing epithelial permeability. *Cytokine* 2013;63:209–17.

54. Di Sabatino A, Battista N, Biancheri P, et al. The endogenous cannabinoid system in the gut of patients with inflammatory bowel disease. *Mucosal Immunol* 2011;4:574–83.

55. Sintzing S, Wissniowski TT, Lohwasser C, Alinger B, Neureiter D, Ocker M. Role of cannabinoid receptors and RAGE in inflammatory bowel disease. *Histol Histopathol* 2011;26:735–45.

56. Leinwand KL, Jones AA, Huang RH, et al. Cannabinoid receptor-2 ameliorates inflammation in murine model of Crohn’s disease. *J Crohns Colitis* 2017;11:1369–80.

57. D’Argenio G, Valenti M, Scaglione G, Cosenza V, Sorentini I, Di Marzo V. Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. *FASEB J* 2006;20:568–70.

58. Darmann NA, Izzo AA, Degenhardt B, et al. Involvement of the cannabinomimetic compound, N-palmitoyl-ethanolamine, in inflammatory and neuropathic conditions: review of the available pre-clinical data, and first human studies. *Neuropsychopharmacology* 2005;48:1154–63.

59. Nicotra LL, Vu M, Harvey BS, Smid SD. Prostaglandin ethanolamides attenuate damage in a human explant colitis model. *Prostaglandins Other Lipid Mediat* 2013;100:101:22–9.

60. Storr M, Devlin S, Kaplan GG, Panaccione R, Andrews CN. Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease progression in patients with Crohn’s disease. *Inflamm Bowel Dis* 2014;20:472–80.

61. Duncan M, Thomas AD, Cluny NL, et al. Distribution and function of monoacylglycerol lipase in the gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol* 2008;295:G1253–65.

62. Hanlon EC, Tassali E, Leproult R, et al. Circadian rhythm of circulating levels of the endocannabinoid 2-arachidonoylglycerol. *J Clin Endocrinol Metab* 2015;100:220–6.

63. Kantae V, Nahon KJ, Straat ME, et al. Endocannabinoid tone is higher in healthy lean South Asian than white Caucasian men. *Sci Rep* 2017;7:7558.

64. Storr MA, Yuce B, Andrews CN, Sharkey KA. The role of the endocannabinoid system in the pathophysiology and treatment of irritable bowel syndrome. *Neurogastroenterol Motil* 2008;20:857–68.

65. De Filippis D, Esposito G, Cirillo C, et al. Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. *PLoS One* 2011;6:e28159.

66. Nallathambi R, Mazuz M, Ion A, et al. Anti-inflammatory activity in colon cancer cells. *Circadian rhythm of circulating levels of the endocannabinoid 2-arachidonoylglycerol. J Clin Endocrinol Metab* 2015;100:220–6.

67. Camilleri M, Carlson P, McKinzie S, et al. Genetic variation in endocannabinoid metabolism, gastrointestinal motility, and sensation. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G13–9.

68. Engel MA, Kellermann CA, Burnat G, Hahn EG, Rau T, Konturek PC. Mice lacking cannabinoid CB1, CB2-receptors or both receptors show increased susceptibility to trinitrobenzene sulfonic acid [TNBS]-induced colitis. *J Physiol Pharmacol* 2010;63:89–97.

69. Ke P, Shao BZ, Xu ZQ, et al. Activation of cannabinoid receptor 2 ameliorates DSS-induced colitis through inhibiting NLRP3 inflammasome in macrophages. *PLoS One* 2016;11:e0155076.

70. Kimball ES, Schneider CR, Wallace NH, Hornby PJ. Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of...
mustard and by dextran sulfate sodium. *Am J Physiol Gastrointest Liver Physiol* 2006;291:G64–71.

71. El Rakkad J, Muccioli GG, Body-Malapel M, et al. Conformational restriction leading to a selective CB2 cannabinoid receptor agonist orally active against colitis. *ACS Med Chem Lett* 2015;6:198–203.

72. Storr MA, Keenan CM, Zhang H, Patel KD, Makriyannis A, Sharkey KA. Activation of the cannabinoid 2 receptor [CB2] protects against experimental colitis. *Inflamm Bowel Dis* 2009;15:1678–85.

73. Singh UP, Singh NP, Singh B, Price RL, Nagarkatti M, Nagarkatti PS. Cannabinoid receptor-2 [CB2] agonist ameliorates colitis in IL-10(-/-) mice by attenuating the activation of T cells and promoting their apoptosis. *Toxicol Appl Pharmacol* 2012;258:256–67.

74. Tourteau A, Andrezejak V, Body-Malapel M, et al. 3-Cardboxamido-5-aryl-isoxazoles as new CB2 agonists for the treatment of colitis. *Bioorg Med Chem* 2013;21:338–94.

75. Soetthoudt M, Gerther U, Fingerle J, et al. Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity. *Nat Commun* 2017;8:13958.

76. Cluny NL, Keenan CM, Duncan M, Fox A, Lutz B, Sharkey KA. Naphthalen-1-yl-[4-pentyloxynaphthalen-1-yl]methanone [SAB378], a peripherally restricted cannabinoid CB1/CB2 receptor agonist, inhibits gastrointestinal motility but has no effect on experimental colitis in mice. *J Pharmacol Exp Ther* 2010;334:973–80.

77. Li YY, Yuece B, Cao HM, et al. Inhibition of p38/MK2 signaling pathway improves the anti-inflammatory effect of WIN55 on mouse experimental colitis. *Lab Invest* 2013;93:322–33.

78. Feng YJ, Li YY, Lin XH, Li K, Cao MH. Anti-inflammatory effect of cannabinoid agonist WIN55,212 on mouse experimental colitis is related to inhibition of p38/MAPK. *World J Gastroenterol* 2016;22:9515–24.

79. Fichna J, Bawa M, Thakur GA, et al. Cannabidiol alleviates experimental induced intestinal inflammation by acting at central and peripheral receptors. *PLoS One* 2014;9:e109115.

80. Lin S, Li Y, Shen L, et al. The anti-inflammatory effect and intestinal barrier protection of HU210 differentially depend on TLR4 signaling in dextran sulfate sodium-induced murine colitis. *Dig Dis Sci* 2017;62:372–86.

81. Dzidudlewicz EK, Bevan SJ, Brain CT, et al. Naphthalen-1-yl-[4-pentyloxynaphthalen-1-yl]methane: a potent, orally bioavailable human CB1/CB2 dual agonist with antihyperalgesic properties and restricted central nervous system penetration. *J Med Chem* 2007;50:3851–6.

82. Hassanzadeh P, Arabi E, Aryabi F, Dinarvand R. Application of carbon nanotubes as the carriers of the cannabinoid, 2-arachidonoylglycerol: Towards a novel treatment strategy in colitis. *Life Sci* 2017;179:66–72.

83. Engel MA, Kellermann CA, Rau T, Burnat G, Hahn EG, Konturek PC. Ulcerative colitis in AKR mice is attenuated by intraperitoneally administered anandamide. *J Physiol Pharmacol* 2008;59:673–89.

84. Sarnelli G, D’Alessandro A, Iuvone T, et al. Palmitoylethanolamide modulates inflammation-associated vascular endothelial growth factor [VEGF] signaling via the Akt/mTOR pathway in a selective peroxisome proliferator-activated receptor-alpha [PPAR-α] dependent manner. *PLoS One* 2016;11:e0156198.

85. Alhamoruni A, Lee AC, Wright KL, Larvin M, O’Sullivan SE. Pharmacological effects of cannabinoids on the Caco-2 cell culture model of intestinal permeability. *J Pharmacol Exp Ther* 2010;335:92–102.

86. Karwad MA, Couch DG, Theophilidou E, et al. The role of CB1 in intestinal permeability and inflammation. *FASEB J* 2017;31:3267–77.

87. Alhamoruni A, Wright KL, Larvin M, O’Sullivan SE. Cannabinoids mediate opposing effects on inflammation-induced intestinal permeability. *Br J Pharmacol* 2012;165:2598–610.

88. Kinsey SG, Wise LE, Ramesh D, et al. Repeated low-dose administration of the monooacylglycerol lipase inhibitor JZL184 retains cannabinoid receptor type 1-mediated antinoceptive and gastroprotective effects. *J Pharmacol Exp Ther* 2013;345:492–501.

89. Andrezejak V, Muccioli GG, Body-Malapel M, et al. New FAAH inhibitor based on 3-carboxamido-5-aryl-isoxazole scaffold that protect against experimental colitis. *Bioorg Med Chem* 2011;19:3777–86.

90. Tourteau A, Leleu-Chavain N, Body-Malapel M, et al. Switching cannabinoid response from CB2[α] agonists to FAAH inhibitors. *Bioorg Med Chem Lett* 2014;24:1322–6.
Cannabinoids in IBD

110. Mallat AM, Gallily R, Sumariwalla PF, et al. The nonpsychoactive canna-
binoid constituent cannabidiol is an oral anti-arthritis therapeutic in murine
colagen-induced arthritis. Proc Natl Acad Sci U S A 2000;97:561–6.

111. Karmaus PW, Wagner JG, Hartkema JR, Kaminski NE, Kaplan BL. Can-
nabidiol (CBD) enhances lipopolysaccharide (LPS)-induced pulmo-
nary inflammation in C57BL/6 mice. J Immunotoxicol 2013;10:321–8.

112. Wallace JL, Flannigan KL, Mc Knight W, Wang L, Ferraz JG, Tuitt D. Pro-
resolution, protective and anti-nociceptive effects of a cannabis extract in
the rat gastrointestinal tract. J Physiol Pharmacol 2013;64:167–75.

113. Borrelli F, Fassolino I, Romano B, et al. Beneficial effect of the non-py-
chotropic plant cannabinoid cannabigerol on experimental inflammatory
bowel disease. Biochem Pharmacol 2013;85:1306–16.

114. Romano B, Borrelli F, Fassolino I, et al. The cannabinoid TRPA1 agonist
cannabichromene inhibits nitric oxide production in macrophages and
ameliorates murine colitis. Br J Pharmacol 2013;169:213–29.

115. Couch DG, Maudslay H, Doleman B, Lund JN, O’Sullivan SE. The use of
cannabinoids in colitis: a systematic review and meta-analysis. Inflamm
Bowel Dis 2018;24:680–97.

116. García-Planella E, Martín L, Doménech E, et al. Use of complementary
and alternative medicine and drug abuse in patients with inflammatory
bowel disease. Med Clin [Barc] 2007;128:45–8.

117. Lal S, Prasad N, Ryan M, et al. Cannabis use amongst patients with inflam-
matorv bowel disease. Eur J Gastroenterol Hepatol 2011;23:891–6.

118. Ravikoff Allegretti J, Courtwright A, Lucci M, Korzenik JR, Levine J.
Treatment of Crohn’s disease with cannabis: an observational study.
Pediatr Gastroenterol Nutr 1999;19:105–8.

119. Hoffman GJ, McWilliams SK, Mikulich-Gilbertson SK, et al. Marijuana
use patterns among patients with inflammatory bowel dis-
 ease. Inflamm Bowel Dis 2013;19:2809–14.

120. Weiss A, Friedenberg E. Patterns of cannabis use in patients with
Inflammatory Bowel Disease: a population based analysis. Drug Alcohol
 Depend 2015;156:94–9.

121. Kerlin AM, Long M, Kappelman M, Martin C, Sandler RS. Profiles of
patients who use marijuana for inflammatory bowel disease. Dig Dis Sci
2018;63:1600–4.

122. Hoffenberg EJ, McWilliams SK, Mikulich-Gilbertson SK, et al. Marijuana
use by adolescents and young adults with inflammatory bowel disease. J
Pediatr 2018;199:99–105.

123. Naftali T, Lev LB, Yablecovitch D, Yablekovitz D, Half E, Konikoff FM.
Treatment of Crohn’s disease with cannabis: an observational study. Isr
Med Assoc J 2011;13:455–8.

124. Lahat A, Lang A, Ben-Horin S. Impact of cannabis treatment on the qual-
ity of life, weight and clinical disease activity in inflammatory bowel dis-
ease patients: a pilot prospective study. Digestion 2012;85:1–8.

125. Naftali T, Bar-Lev Schleider L, Dotan I, Lansky EP, Sklerovsky
Benjaminov F, Konikoff FM. Cannabis induces a clinical response in
patients with Crohn’s disease: a prospective placebo-controlled study.
Clin Gastroenterol Hepatol 2013;11:1276–80.e1.

126. Walsh AJ, Bryant RV, Travis SP. Current best practice for disease activity
assessment in IBD. Nat Rev Gastroenterol Hepatol 2016;13:567–79.

127. Naftali T, Mechoulam R, Mari A, et al. Low-dose cannabis is safe but
not effective in the treatment for Crohn’s disease, a randomized con-
trolled trial. Dig Dis Sci 2017;62:1615–20.

128. Naftali T, Bar-Lev Schleider L, Sklerovsky Benjaminov F, et al. P398 can-
nabis induces clinical and endoscopic improvement in moderately active
ulcerative colitis (UC). J Crohn’s Colitis 2018;12[Suppl 1]:S306.

129. Kalenderoglu N, Macpherson T, Wright KL. Cannabidiol reduces leuko-
emic cell size but is it important? Front Pharmacol 2017;8:144.

130. Colgan SP, Taylor CT. Hypoxia: an alarm signal during intestinal inflam-
mation. Nat Rev Gastroenterol Hepatol 2010;7:281–7.

131. Grimsey NL, Goodfellow CE, Scotter EL, Dowie MJ, Glass M, Smith SE.
Specific detection of CB1 receptors; cannabinoid CB1 receptor antibodies
are not all created equal! J Neurosci Methods 2008;171:78–86.

132. Marchalant Y, Brownjohn PW, Bonnet A, Kleffmann T, Ashton JC.
Validating the cannabinoids CB2 receptor: antibody sensi-
tivity is not evidence of antibody specificity. J Histochim Cytoch-
em 2016;62:395–404.

133. Bachovchin DA, Cravatt BF. The pharmacological landscape and
therapeutic potential of serine hydrolases. Nat Rev Drug Discov
2012;11:52–68.

134. Di Marzo V, Stella N, Zimmer A. Endocannabinoid signalling and the
deteriorating brain. Nat Rev Neurosci 2015;16:30–42.

135. Camilleri M, Kolar GJ, Vazquez-Roque MI, Carlson P, Burton DD,
Zinsmeister AR. Cannabinoid receptor 1 gene and irritable bowel syn-
drome: phenotype and quantitative traits. Am J Physiol Gastrointest
Liver Physiol 2013;304:G553–60.

136. Pinto I, Izzo AA, Cascio MG, et al. Endocannabinoids as physi-
ological regulators of colonic propulsion in mice. Gastroenterology 2002;
123:227–34.

137. Esfandyari T, Camilleri M, Ferber I, Burton D, Baxter K, Zinsmeister
AR. Effect of a cannabinoid agonist on gastrointestinal transit and post-
prandial satiation in healthy human subjects: a randomized, placebo-
controlled study. Neurogastroenterol Motil 2006;18:831–8.

138. Sharkey KA, Darmani NA, Parker LA. Regulation of nausea and vomit-
ing by cannabinoids and the endocannabinoid system. Eur J Pharmacol
2014;722:134–46.

139. Wieslewska A, Lewandowska U, Mosinska P, et al. Cannabinoid recep-
type 1 and mu-opioid receptor polymorphisms are associated with cys-
ic vomiting syndrome. Am J Gastroenterol 2017;112:933–9.

140. Sorensen CJ, DeSanto K, Borgelt L, Phillips KT, Monte AA. Cannabinoid
hyperemesis syndrome: diagnosis, pathophysiology, and treatment a sys-
tematic review. J Med Toxicol 2017;13:71–87.

141. Sharkey KA, Wiley JW. The role of the endocannabinoid system in the
brain-gut axis. Gastroenterology 2016;151:252–66.

142. Izoo AA, Sharkey KA. Cannabinoids and the gut: new developments and
emerging concepts. Pharmacol Ther 2010;126:21–38.

143. Rice J, Cameron M. Cannabinoids for treatment of MS symptoms: state
emerging concepts. Neurology 2009;72:4419–29.

144. Zinsmeister AR. Cannabinoid receptor 1 gene and irritable bowel syn-
drome: phenotype and quantitative traits. Am J Physiol Gastrointest
Liver Physiol 2013;304:G553–60.

145. Taschler U, Eichmann TO, Radner FP, et al. Monoglyceride lipase defi-
cy clinical relevance is not evidence of antibody specificity. J Neurosci Methods 2016;257:145–56.

146. Borner C, Holll V, Kraus J. Cannabinoid receptor type 2 agonists induce
transcription of the mu-opioid receptor gene in Jurkat T cells. Mol
Pharmacol 2006;69:1486–91.