CANNABINOID-BASED DRUGS AS ANTI-INFLAMMATORY THERAPEUTICS

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Abstract | In the nineteenth century, marijuana was prescribed by physicians for maladies ranging from eating disorders to rabies. However, as newer, more effective drugs were discovered and as the potential for abuse of marijuana was recognized, its use as a therapeutic became restricted, and only recently has its therapeutic potential been re-evaluated. Recent studies in animal models and in humans have produced promising results for the treatment of various disorders — such as obesity, cancer, and spasticity and tremor due to neuropathology — with drugs based on marijuana-derived cannabinoids. Moreover, as I discuss here, a wealth of information also indicates that these drugs have immunosuppressive and anti-inflammatory properties; therefore, on the basis of this mode of action, the therapeutic usefulness of these drugs in chronic inflammatory diseases is now being reassessed.

CANNABINOID RECEPTORS
G-protein-coupled receptors for Δ^9-tetrahydrocannabinol, its synthetic analogues and endocannabinoids. They have been identified in most vertebrate phyla. Two subtypes are known: cannabinoid receptor 1 (CB_1) and CB_2.

ENDOCANNABINOIDS
Endogenous agonists for cannabinoid receptors that are present in animals. They are metabolites of eicosanoid fatty acids.

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Cannabis sativa — also known as marijuana — is the most frequently used illicit drug in the United States, particularly among young people, with up to 46.1% of those aged 17–18 being users. Although marijuana-usage rates are lower than those for legal drugs, such as cigarettes and alcohol, they are significantly higher than those for other illicit drugs, such as cocaine and ecstasy. As a therapeutic, marijuana has been recognized for centuries, and in the nineteenth century, it was recommended as an analgesic, muscle relaxant, appetite stimulant and anticonvulsant[1]. Its therapeutic applications continued to grow such that, by the early twentieth century, therapy with marijuana was used to ease the symptoms of a broad spectrum of diseases, ranging from rheumatism and epilepsy to tetanus and gonorrhoea. However, the popularity of marijuana as a therapeutic declined as newer, more effective drugs were discovered for these ailments and as the potential for abuse of marijuana was recognized, so by the early 1940s, it was removed from use as a pharmaceutical in the United States. From that time until the 1980s, marijuana and its derivatives, such as Δ^9-tetrahydrocannabinol (THC), have been viewed as both scientific curiosities and illegal drugs with a high potential for abuse; however, this now seems to be changing. In the past few years, numerous publications have reported the potential use of marijuana-based medicines for the treatment of diseases ranging from cancer to glaucoma[1–8], and one compound, SR141716A (also known as rimonabant or Acomplia; Sanofi-Synthélabo)[9], has been widely publicized as the next wonder drug for promoting weight loss and smoking cessation. Here, I review the effects of marijuana and related compounds on the adaptive and innate immune systems and provide some perspective concerning the therapeutic potential of marijuana-derived cannabinoids and related compounds for the treatment of chronic inflammatory diseases.

Cannabinoid-based drugs and receptors

The main psychoactive component in marijuana is the 'classical' cannabinoid THC[10] (FIG. 1a). Similar to its synthetic analogues (FIG. 1b,c), it functions by activating specific cell-surface CANNABINOID RECEPTORS, which are normally engaged by a family of endogenous ligands — the ENDOCANNABINOIDS (FIG. 1g,h). Compounds that bind these receptors induce CANNABIMIMETIC responses

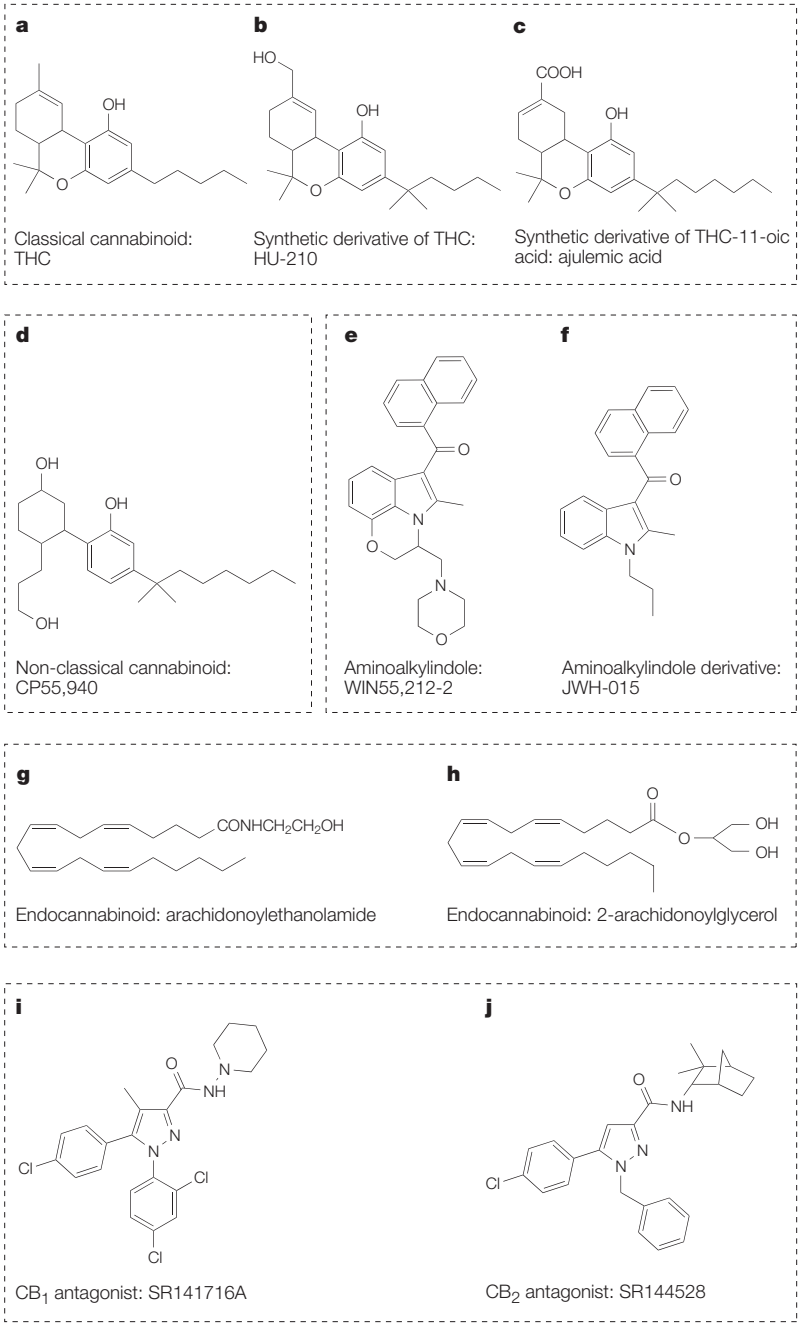

Figure 1 | **Drugs based on marijuana-derived cannabinoids are divided into various groups.** There are four groups of cannabinoids or cannabimimetic drugs that have cannabinoid-receptor-binding activity. The first group contains the 'classical' cannabinoids, which are tricyclic dibenzopyran derivatives. They occur naturally in the marijuana plant — for example, Δ^9-tetrahydrocannabinol (THC) (**a**). Also, there are synthetic analogues of these natural compounds — for example, HU-210 (**b**). These cannabinoids bind relatively non-selectively to both cannabinoid receptor 1 (CB_1) and CB_2, with HU-210 having a higher affinity than THC. Ajulemic acid (**c**) is another analogue; it is a classical cannabinoid derived from THC-11-oic acid. Ajulemic acid binds both cannabinoid receptors but has a lower affinity for CB_2. The second group contains the non-classical cannabinoids, which are synthesized analogues that lack the dihydropyran ring of THC. This group contains compounds such as CP55,940 (**d**). The third group contains the aminoalkylindoles, such as WIN55,212-2 (**e**). Derivatives of WIN55,212-2, such as JWH-015 (**f**), have been shown to selectively bind CB_2. The fourth group contains the endocannabinoids, which are eicosanoid compounds rather than cannabinoid compounds. The most studied members of this group are arachidonoylethanolamide (**g**) and 2-arachidonoylglycerol (**h**). Finally, several cannabinoid-receptor antagonists have been synthesized, and the most widely studied are SR141716A (**i**), which selectively binds CB_1, and SR144528 (**j**), which selectively binds CB_2.

in vivo and *in vitro*[11]. Research on the structure–activity relationships of THC led to the synthesis of 'non-classical' cannabinoid analogues. The most widely studied of this group is CP55,940 (FIG. 1d), a high-affinity ligand with potent biological effects that are mediated through binding both cannabinoid receptor 1 (CB_1) and CB_2. Another class of cannabimimetic agents is the aminoalkylindoles, such as WIN55,212-2 (FIG. 1e), which also has high affinity for both receptors and has potent activity. By contrast, some derivatives of this class, such as JWH-015 (FIG. 1f), have been shown to selectively bind CB_2. Such derivatives might be of therapeutic value, because psychoactive effects are mediated through binding CB_1 and not through binding CB_2. Other plant-derived cannabinoids (such as cannabidiol) and synthetic derivatives (such as HU-211; also known as dexanabinol; Pharmos Corporation) bind CB_1 and CB_2 with very low affinity, so they have low cannabimimetic activity and might function by binding different receptors, such as the NMDA (*N*-METHYL-D-ASPARTATE) RECEPTOR[5] or other unknown receptors. Furthermore, a derivative of THC-11-oic acid, ajulemic acid (also known as CT-3) (FIG. 1c), has low affinity for CB_2 (REF. 12) but has anti-inflammatory activity[13], which might be mediated through disruption of the arachidonic-acid cascade[14] or through activation of peroxisome-proliferative-activated receptor-γ (PPAR-γ)[15]. Finally, receptor antagonists have been synthesized, such as SR141716A and SR144528 (REF. 11) (FIG. 1i,j), which inhibit or reverse the biological effects of CB_1 and CB_2 agonists. These antagonists have been used experimentally to determine the receptor-binding activities of various putative agonists and are now also being used clinically to inhibit CB_1 activity. For example, SR141716A is given to suppress appetite during the treatment of obesity[9].

The naturally occurring endocannabinoids are produced by the cleavage of membrane fatty acids[16,17], in particular arachidonic acid, and have varying specificities for the two cannabinoid receptors. Arachidonoylethanolamide (AEA; previously known as anandamide) is an endogenous fatty-acid amide and was the first endocannabinoid to be discovered[18] (FIG. 1g). It has less intrinsic activity when bound to CB_2 than to CB_1, and it has also been shown to bind VANILLOID RECEPTORS, which are ligand-gated cation channels that are sensitive to capsaicin and related analogues[19]. Several other endocannabinoids have also been described, including 2-arachidonoylglycerol (2-AG)[20] (FIG. 1h) and 2-arachidonylglyceryl ether (also known as noladin ether)[21], with the former being a full agonist of CB_2 and the latter showing a higher affinity for CB_1. The endocannabinoids are produced by various cells, including cells of the immune system and the brain (discussed later).

In addition to the two cannabinoid receptors that have been characterized[22,23], there might be others, particularly receptors that interact with the endocannabinoids, which would be consistent with the observation that AEA also binds vanilloid receptors[19]. CB_1 and CB_2 are seven-transmembrane, G-protein-coupled receptors that are coupled to G_i or G_o heterotrimeric proteins and adenylyl cyclases, but other second messengers and

CANNABIMIMETIC
Δ⁹-Tetrahydrocannabinol (THC)-like in pharmacological terms. A compound is usually accepted to be cannabimimetic if it produces four characteristic effects of THC in an *in vivo* assay known as the 'mouse tetrad model'. These effects are hypomotility, hypothermia, analgesia and a sustained immobility of posture (catalepsy).

signalling components are also involved in their activity[24,25]. The tissue distribution of CB_1 and CB_2 accounts for the well-known psychotropic and peripheral effects of cannabinoids (reviewed in REFS 11,25–28). CB_1 is abundant in the central nervous system (CNS), in particular in the basal ganglia, cerebellum, hippocampus and cortex, where the receptors and endocannabinoids are implicated in retrograde neurotransmitter regulation of synaptic transmission[28,29]. CB_1 is also expressed in the periphery, as is CB_2, where they are mainly restricted to immune cells and tissues (discussed later).

Endocannabinoids in innate immunity

Production of endocannabinoids. Microbial pathogens that invade the tissues are recognized by host cells and host factors that trigger the activation of both innate and adaptive immune responses[30]. Activation of the inflammatory response to infection largely depends on the release of pro-inflammatory cytokines and chemokines. However, in addition to cytokines and other proteins, various metabolic products of immune cells — including membrane fatty acids, such as arachidonic acid[31] — have also been implicated in the inflammatory response to infection. It is therefore not surprising that chemically similar metabolites — such as the endocannabinoid AEA — are produced and released by activated immune cells[32] (FIG. 2). *In vitro* studies have shown that stimulation with bacterial lipopolysaccharide (LPS) increases the production of AEA and 2-AG by immune cells (FIG. 2), including macrophages[33], peripheral-blood mononuclear cells (PBMCs)[34] and dendritic cells[35]. In addition, LPS-activated PBMCs show reduced expression of the AEA-degrading enzyme fatty-acid amide hydrolase (FAAH)[36]. Given that inflammatory responses must be tightly controlled to avoid extensive tissue damage[37] and to allow a return to homeostatic conditions, FAAH-mediated degradation is one of the mechanisms for the inactivation of endocannabinoids. In addition, as-yet-uncharacterized endocannabinoid membrane transporters seem to facilitate both the release and the subsequent uptake of endocannabinoids by neurons and glial cells, thereby contributing to the regulation of endocannabinoids. So, immune cells from humans and animals increase the production of endocannabinoids in response to LPS and in response to activation by other stimuli. Additional studies are needed to determine the range of immune and microbial stimuli that induce endocannabinoid production and to further define the mechanisms that regulate this effect.

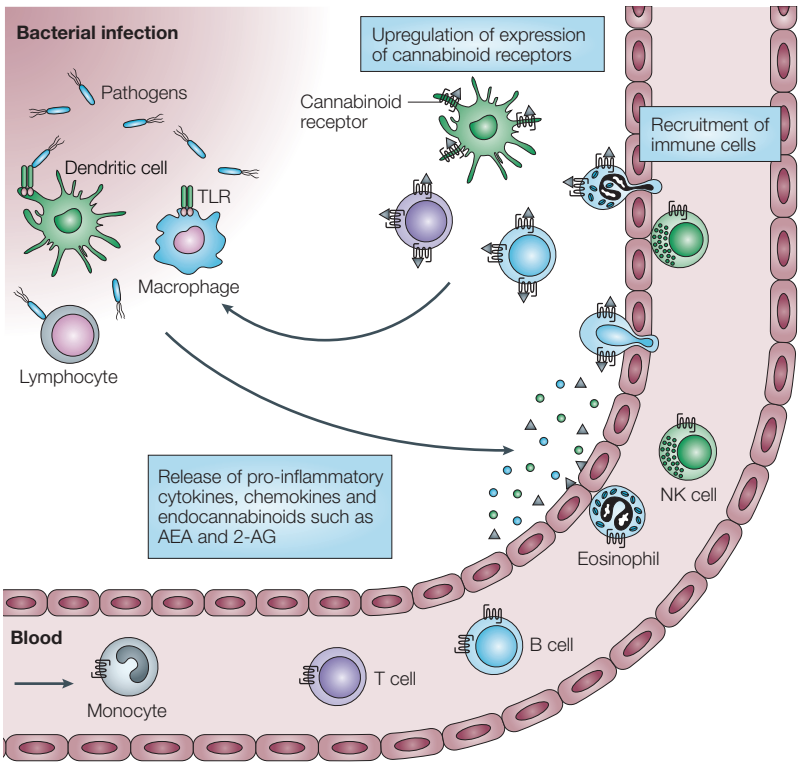

Figure 2 | **The endocannabinoid system and innate immunity.** Bacteria stimulate lymphocytes, dendritic cells (DCs) and macrophages — through pattern-recognition receptors, such as Toll-like receptors (TLRs) — to release cytokines and chemokines, which attract leukocytes to the site of infection. It is now known that stimulation of these cells also induces the release of endocannabinoids — such as arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) — that are also chemotactic for leukocytes. Leukocytes — including T cells, B cells, eosinophils, natural killer (NK) cells, DCs and macrophages — invade the tissues, promoting the elimination of the microorganisms and the development of an adaptive immune response. These activated cells seem to upregulate the expression of both types of cannabinoid receptor — cannabinoid receptor 1 (CB_1) and CB_2 — and these are then available for participation in immune regulation.

Endocannabinoids as chemotactic agents. After recognition of an invading pathogen, the release of cytokines and chemokines by cells involved in the innate immune response triggers an influx of lymphoid and myeloid cells from the blood to the site of infection[38]. Recent evidence indicates that the endocannabinoid 2-AG also induces the migration of various cell types (FIG. 2). For example, in transwell cultures, 2-AG attracted human eosinophils[39], as well as Raji B cells[40] and mouse bone-marrow-derived dendritic cells[41]. Furthermore, CB_2 seems to be overexpressed in several myeloid leukaemias, and these leukaemic cells are induced to migrate after stimulation with 2-AG[42]. It therefore seems that endocannabinoids might be involved in cellular migration by functioning as chemotactic agents, together with cytokines and proteins that have classically been defined as chemokines. It is interesting to note that a similar function has been reported for opioids, such as morphine, which have neuroimmune functions in common with cannabinoids[43].

Expression of cannabinoid receptors. In addition to the induction of endocannabinoid expression and the subsequent chemotaxis of immune cells, there is evidence that activation of immune cells by LPS or other stimuli also modulates the expression of the cannabinoid receptors CB_1 and CB_2 by these cells (FIG. 2). For example, the initial report on the cloning of CB_2 also showed that stimulation of the human pro-myelocytic leukaemia cell line HL-60 with phorbol 12-myristate 13-acetate (PMA) caused an increase in the level of mRNA encoding CB_2 (REF. 23); similar results were also obtained in mouse splenocyte cultures stimulated with CD40-specific antibody[44]. In addition to CB_2, the levels of mRNA encoding CB_1 were also increased in

both Jurkat T cells[45] and mouse splenocytes[46] after stimulation. However, other studies have shown that stimulation decreased the expression of these receptors[46,47], which most probably reflects differences in the stimulatory substance used or the cell type studied. LPS has also been shown to modulate cannabinoid-receptor expression, although findings from separate studies are inconsistent, with some reports showing upregulation of receptor expression[48] and others showing downregulation[35,44,49].

Consistent with a possible role for endocannabinoids and CB_2 in chemotaxis and other immune-activation events, a recent study of mouse brain microglial cells showed that, under chemotactic conditions, the expression of CB_2 was specifically localized to the leading edge of the cell[50], indicating that there is receptor modulation similar to that observed for immunoreceptors during chemotaxis or during the formation of the immunological synapse[51]. Taken together, these studies indicate that microbial antigens or other stimuli that induce immune activation influence cannabinoid-receptor expression by immune cells; however, the factors that are involved in increased or decreased expression are far from understood, and little is known about the molecular regulation of the genes encoding these receptors in immune cells. Further research is needed to take full advantage of manipulating this potentially important immunomodulating system for therapeutic purposes (discussed later).

Cannabinoids in inflammation

Cannabinoids modulate cytokine production. In the mid-1980s, it was shown that mouse cells treated with the drug THC produced decreased levels of type I interferons (IFN-α and IFN-β) after stimulation with LPS or polyinosinic–polycytidylic acid (polyI:C)[52,53], providing the first evidence that cannabinoids might modulate cytokine production. Many subsequent studies have shown that cannabinoids, for the most part, suppress the production of cytokines in innate and adaptive immune responses, both in animal models and in human cell cultures[26,27,54]. Their suppression of pro-inflammatory cytokine and chemokine production indicates that these drugs might have anti-inflammatory effects and could therefore be used for the treatment of chronic inflammatory diseases. Consistent with this, serum levels of tumour-necrosis factor (TNF) and interleukin-12 (IL-12) were shown to be decreased in mice that were primed by infection with *Propionibacterium acnes* (*Corynebacterium parvum*) and stimulated with an injection of LPS (conditions that promote optimum cytokine upregulation) then treated with the synthetic THC derivative HU-210 or the aminoalkylindole WIN55,212-2 (REF. 55). The cannabinoids also protected these mice from the lethal effects of LPS in this model, and this protection might have resulted from, at least in part, a concomitant drug-induced increase in the levels of the regulatory cytokine IL-10 (TABLE 1). TNF production was also suppressed in the brain of rats subjected to closed head injury, after treatment with the non-psychoactive cannabinoid HU-211, and this treatment was also neuroprotective and resulted in a better clinical outcome[56]. Although HU-211 does not bind cannabinoid receptors and is therefore not cannabimimetic, it seems to function as an NMDA-receptor antagonist, thereby preventing excitotoxicity and neuronal death[57]. In another mechanistically complex mouse model, treatment with WIN55,212-2 decreased tissue damage after myocardial ischaemia–reperfusion injury[58]. Treatment of animals before ischaemia and reperfusion considerably reduced the size of the infarct, and this was paralleled by lower levels of production of IL-1β and CXC-chemokine ligand 8 (CXCL8) in the injured tissue. In human studies, lung alveolar macrophages removed from marijuana smokers were compromised in their ability to produce TNF, granulocyte/macrophage colony-stimulating factor and IL-6 in response to LPS stimulation[59] (TABLE 1). It therefore seems that cannabinoids can inhibit the production of TNF and other cytokines in several different models and by several different mechanisms (TABLE 1), not all of which depend on interaction with cannabinoid receptors.

As well as suppressing the production of cytokines, cannabinoids have been shown to increase the production of cytokines (including TNF, IL-1, IL-6 and IL-10) when they are administered together with bacteria or other antigens[60–62], or in some cases, when cannabinoids are administered alone[63,64]. So, *in vivo*, cannabinoids might either suppress or enhance the production of these pro-inflammatory agents, depending on either the nature of the pro-inflammatory stimulus or the type of cannabinoid used (TABLE 1).

Cannabinoids modulate inflammatory-cell migration. The effect of cannabinoids has also been analysed in the experimental allergic encephalomyelitis (EAE) model of multiple sclerosis (a neuroinflammatory disease), and they have been shown to suppress disease progression[65,66] and inflammatory reactions[66]. In one study, mice were induced to develop EAE and given WIN55,212-2 alone or WIN55,212-2 and then either a CB_1 or CB_2 antagonist every 4 days. After several weeks, disease progression was assessed, together with leukocyte–endothelial-cell interactions, using intravital microscopy. Treatment with WIN55,212-2 suppressed the rolling and adhesion of venous leukocytes and improved neurological function in mice with EAE, compared with control animals; furthermore, the suppressive effect was attenuated by co-treatment with a CB_2 antagonist but not a CB_1 antagonist, indicating that CB_2 is involved in these processes[66]. The reduction in DIAPEDESIS presents a paradox, because cannabinoids have been shown to enhance chemotaxis and chemokine production (as discussed earlier). Although the levels of cytokines and adhesion molecules were not measured in this study, it is possible that cannabinoid-mediated suppression of leukocyte adhesion results from an inhibition of T helper 1 (T_H1)-cell cytokine production: for example, of IFN-γ, which facilitates transendothelial cell trafficking[67,68] (discussed later).

NMDA RECEPTOR
(*N*-methyl-D-aspartate receptor). NMDA is a synthetic amino acid with affinity for NMDA receptors, which mediate excitatory effects in the brain when they are stimulated by endogenous ligands such as glutamic acid. Overstimulation can lead to neuronal excitotoxicity.

VANILLOID RECEPTORS
Cation channels that are expressed by nerve sensory fibres and are involved in the perception of pain. These receptors are ligand-, proton- and heat-activated and are targets for capsaicin — the hot component of chillies.

DIAPEDESIS
The last step in the leukocyte–endothelial-cell adhesion cascade. This cascade includes tethering, triggering, tight adhesion and transmigration. Diapedesis is the migration of leukocytes across the endothelium, which occurs by squeezing through the junctions between adjacent endothelial cells.

Table 1 | **Cannabinoid effects on pro-inflammatory cytokines**

Cannabinoid	Receptor	Cell or tissue type	Cytokine stimulant or inflammation model	Effect	Reference
Mice					
WIN55,212-2 or HU-210	ND	Spleen	LPS and *Propionibacterium acnes**	Decreases TNF and IL-12 and increases IL-10	55
THC	ND	Macrophage cell line (RAW264.7)	LPS	Decreases TNF	122
HU-211	NMDA receptor	Brain	Closed head injury	Decreases TNF	56
WIN55,212-2	CB_2 dependent	Heart	Ischaemia–reperfusion	Decreases IL-1β and CXCL8	58
THC, AEA or 2-AG	ND	Macrophage cell line (J774)	LPS	Decreases IL-6	123
THC	ND	Spleen	*Legionella pneumophila*	Increases TNF and IL-6	60
THC	ND	Peritoneal macrophages	LPS	Increases IL-1α and IL-1β	61
Humans					
Marijuana smoking	ND	Lung alveolar macrophages	LPS	Decreases TNF, GM-CSF and IL-6	59
Ajulemic acid	ND	Peripheral-blood and synovial monocytes	LPS	Decreases IL-1β	100
2-AG	CB_2 dependent	Pro-myelocytic leukaemia cell line (HL-60)	2-AG	Increases CXCL8 and CCL2	64
CP55,940	CB_2 dependent	Pro-myelocytic leukaemia cell line (HL-60) (CB_2 transfected)	CP55,940	Increases TNF, CXCL8, CCL2 and CCL4	63
Rats					
AEA, 2-AG, WIN55,212-2 or HU-210	CB_1 and CB_2 independent	Microglial cells	LPS	Decreases TNF	124

**Propionibacterium acnes* was previously known as *Corynebacterium parvum*. AEA, arachidonoylethanolamide; 2-AG, 2-arachidonoylglycerol; CB, cannabinoid receptor; CCL, CC-chemokine ligand; CXCL8, CXC-chemokine ligand 8; GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; LPS, lipopolysaccharide; ND, not determined; NMDA, *N*-methyl-D-aspartate; THC, Δ^9-tetrahydrocannabinol; TNF, tumour-necrosis factor.

An increasing number of studies indicate that cannabinoids and related compounds modulate pro-inflammatory cytokines in various systems, ranging from infection to tissue injury. So far, the mechanisms of this modulation are unclear, but mechanisms that involve cannabinoid receptors, as well as other mechanisms, seem to be involved. For therapeutic applications, compounds that function either by binding CB_2 or by by-passing cannabinoid receptors altogether would be of benefit, because CB_1-mediated psychoactive side-effects would be diminished.

Cannabinoids modulate T helper cells

Cannabinoid treatment has been shown to suppress both innate immunity and adaptive immunity, and the effects of cannabinoids on humoral and cellular immunity have been extensively reviewed[26,27,69]. So, here I focus on the more recent studies that indicate that cannabinoids have a T_H-cell biasing effect, in which T_H1-cell activity is suppressed and T_H2-cell activity is increased (TABLE 2). The first indications of this effect can be traced to the observation that treatment with THC induces suppression of both T_H1-cell activity and cell-mediated immunity in mice infected with *Legionella pneumophila*[70]. Additional studies showed that treatment of mice with THC not only decreased the production of IFN-γ and the levels of IL-12 and the IL-12 receptor (IL-12R) but also increased the production of IL-4 (REF. 71) (TABLE 2), showing that the immune response to the drug was biased towards the production of T_H2 cells. Cannabinoid receptors were shown to be involved in the effects of THC, and it is possible that signalling through these G-protein-coupled receptors suppresses the expression of IL-12, as has been shown in other systems[72]. In addition to this infection model, the T_H-cell-biasing effect was also shown in a mouse model of lung cancer, in which IFN-γ production was decreased but IL-10 and transforming growth factor-β (TGF-β) production were increased[73]. The presence of higher levels of these cytokines indicates that THC either biases the immune response towards T_H2 cells or activates regulatory T cells, either T regulatory 1 (T_R1) cells or T_H3 cells[74,75]. These regulatory T-cell populations have been shown to produce IL-10 and TGF-β, respectively, so they could be the source of these cytokines following treatment with THC. THC was also shown to bias T_H-cell differentiation towards T_H2 cells during an allogeneic response to human peripheral-blood T cells[76]. Furthermore, PBMCs isolated from marijuana smokers proliferated less and produced less IL-2 in response to mitogens, but they produced more IL-10 and TGF-β than PBMCs isolated from non-marijuana smokers, indicating a T_H-cell bias towards T_H2 cells[77] (TABLE 2). Animal models of multiple sclerosis have also shown that treatment with cannabinoids attenuates cell-mediated immunity and T_H-cell activity, concomitant with reducing disease symptoms. For example, in a model in which demyelinating disease is induced by Theiler's murine

encephalomyelitis virus, cannabinoid-receptor ligands — such as WIN55,212-2 and JWH-015 — were shown to ameliorate disease progression by reducing the delayed-type hypersensitivity response, by decreasing IFN-γ production[78] and microglial-cell activation, and by suppressing the number of $CD4^+$ T cells infiltrating the brain[79].

From these studies, it seems that cannabinoids bias the immune response away from T_H1-cell immunity and that cannabinoid receptors are involved in this process. It is possible that signalling through cannabinoid receptors expressed by T and B cells, as well as by antigen-presenting cells, suppresses the expression of T_H1-cell-promoting cytokines and increases the expression of T_H2-cell-promoting cytokines. Indeed, treatment with cannabinoids has been shown to alter the expression of specific transcription factors: the expression of the T_H2-cell-promoting transcription factor GATA-binding protein 3 (GATA3) has been found to be increased by treatment with THC[80,81], whereas the production of IL-2-expression-promoting transcription factors is suppressed by treatment with cannabinoids[82]. The selective suppression of T_H1-cell immunity by these drugs supports their potential use in the treatment of chronic inflammatory diseases.

Therapeutics for inflammatory diseases

Recent work has led researchers to consider that cannabinoid-based drugs have therapeutic potential for the treatment of a variety of disorders. For example, the CB_1 antagonist SR141716A has been shown to effectively promote weight loss and smoking cessation in preclinical and clinical trials[9,83,84]. Furthermore, cannabinoids seem to be neuroprotective in models of inflammatory neurodegenerative disease, possibly because some derivatives function as inhibitors of NMDA receptors and as antioxidants[5,7] and because they function to control spasticity and tremor that result from neurodegeneration[85]. These drugs have also been proposed for the treatment of tumours, owing to their antiproliferative and pro-apoptotic effects[2,86–88]. However, as cannabinoids can also modulate innate and adaptive immune responses, their therapeutic potential is now being evaluated on the basis of their immunomodulatory and anti-inflammatory actions.

Nervous-tissue inflammation. Injection of THC was initially shown to suppress the neurological signs and symptoms of EAE in rat and guinea-pig models[89] (TABLE 3). A marked reduction in inflammation in the CNS of cannabinoid-treated animals was observed, and this was later suggested to result from a THC-induced increase in the serum levels of the steroid corticosterone, which is known to be anti-inflammatory and immunosuppressive[90]. However, this explanation might be an oversimplification, because, as described earlier, cannabinoids attenuate demyelinating disease in these models by suppressing the differentiation of T_H cells into T_H1 cells[78,79], and this suppression can be uncoupled from THC-induced steroid mobilization in treated animals[91]. Another consequence of neurodegeneration is neuropathic or central pain, which results from sclerotic plaques that affect pain pathways in the CNS[92]. These lesions and central pain are observed in various chronic conditions, including trauma, type 2 diabetes and multiple sclerosis. Although cannabinoids have a well-recognized analgesic effect that is mediated by signalling through CB_1 (REF. 93), compounds that selectively bind CB_2 and act in the periphery have recently been shown to suppress experimentally induced central pain[94]. The aminoalkylindole derivative AM1241 was shown to reverse tactile and thermal hypersensitivity produced in rats by spinal nerve ligation, and this effect was blocked by a CB_2 antagonist, but not a CB_1 antagonist, and occurred in CB_1-deficient mice. This almost completely CB_2-dependent effect has been proposed to occur in the periphery because of the paucity of these receptors in the CNS; the mechanism of action has been suggested to be indirect, with the drug acting on local mast cells and immune cells to suppress the release of mediators (such as histamines and TNF) that can sensitize primary afferent neurons, thereby rendering them more sensitive to pain[94]. This anti-inflammatory mechanism has yet to be established; however, it is

Table 2 | **Cannabinoid effects on adaptive immunity and T helper cells**

Cannabinoid	Receptor	Cell or tissue type	Cytokine stimulant or inflammation model	Effect	Reference
Mice					
THC	ND	Spleen	*Legionella pneumophila*	Decreases IFN-γ and IgG2a	70
THC	CB_1 and CB_2 dependent	Spleen	*Legionella pneumophila*	Decreases IL-12 and IL-12R and increases IL-4	71
THC	CB_2 dependent	Spleen	Tumour model	Decreases IFN-γ and increases IL-10 and TGF-β	73
WIN55,212-2	ND	Spleen	Theiler's murine encephalomyelitis virus	Decreases IFN-γ	78
Humans					
THC	CB_2 dependent	Peripheral-blood T cells	Allogeneic dendritic cells	Decreases IFN-γ	76
Marijuana smoking	ND	Peripheral-blood mononuclear cells	Phytohaemagglutinin and concanavalin A	Decreases IL-2 and increases IL-10 and TGF-β	77

CB, cannabinoid receptor; IFN-γ, interferon-γ; IL, interleukin; IL-12R, IL-12 receptor; ND, not determined; TGF-β, transforming growth factor-β; THC, Δ^9-tetrahydrocannabinol.

interesting that, in a recent small clinical trial, the THC-based drug dronabinol (Marinol; Unimed Pharmaceuticals, Inc.) was shown to have a small but clinically relevant analgesic effect on central pain in patients with multiple sclerosis; this is possibly associated with, in part, suppression of release of these sensitizing mediators through a CB_2-dependent mechanism[95]. From these findings and other data, it seems that cannabinoids have several mechanisms of action for the attenuation of symptoms and disease progression of multiple sclerosis and other neurodegenerative diseases. These mechanisms might include the inhibition of T_H1-cell responses in the CNS, owing to increased steroid production and other mechanisms, and the suppression of neuroimmune mediators of neuropathic pain that are locally released (FIG. 3). In addition to these mechanisms, the action of cannabinoids in reducing spasticity, which does not seem to be mediated by inflammatory

Table 3 | **Preclinical and clinical studies examining the anti-inflammatory effects of cannabinoids**

Preclinical model	Drugs	Outcome or mechanism	Clinical trials	Drug	Outcome	Refs
Nervous-tissue inflammation						
EAE in rats and guinea pigs	THC	Decreased CNS inflammation	–	–	–	89
EAE in rats	THC	Reduced disease progression and increased corticosterone production	–	–	–	90
Theiler's murine encephalomyelitis-virus-induced EAE in mice	WIN55,212-2	Decreased DTH responses and IFN-γ production	–	–	–	78
Theiler's murine-encephalomyelitis-virus-induced EAE in mice	WIN55,212-2, ACEA or JWH-015	Decreased $CD4^+$ T-cell influx to CNS	–	–	–	79
Neuropathic pain in rats	AM1241	Decreased nerve hypersensitivity (CB_2 involved) and pro-inflammatory-mediator production	Central pain in patients with multiple sclerosis	Dronabinol	Analgesia	94,95
Closed head injury in mice	2-AG	Decreased brain oedema and improved clinical outcome (CB_1 involved)	Cerebral pressure and clinical outcome in patients with head injury;	HU-211	Favourable	100,101
			Phase III trial for patients with traumatic brain injury	HU-211	No efficacy	102
Closed head injury in rats	HU-211	Decreased TNF production and neuropathology	–	–	–	56
Inflammatory bowel disease						
LPS-induced gastrointestinal transit in rats	ACEA or JWH-133	Decreased gastrointestinal transit (CB_2 involved) and pro-inflammatory-mediator production	–	–	–	107
Chemically induced colitis in mice	HU-210	Decreased colonic inflammation (CB_1 involved) and inflammatory-cell influx	–	–	–	108
Arthritis						
Leukocyte influx in mice and adjuvant-induced arthritis in rats	Ajulemic acid	Decreased granulocyte influx, joint inflammation and prostaglandin production	–	–	–	13
PBMCs and synovial-fluid monocytes	Ajulemic acid	Decreased IL-1β production	–	–	–	110
Collagen-induced arthritis in mice	Cannabidiol	Decreased arthritis, cell-mediated immunity, and IFN-γ and TNF production	–	–	–	112
Collagen-induced arthritis in mice	HU-320	Decreased arthritis, cell-mediated immunity and TNF production	–	–	–	114
Vascular inflammation						
LPS-induced hypotension in rats	SR141716A	Decreased hypotension (2-AG and CB_1 mediate hypotension)	–	–	–	115
Myocardial ischaemia–reperfusion injury in mice	WIN55,212-2	Decreased tissue injury and cytokine production (CB_2 involved)	–	–	–	58
Septic shock in mice	HU-211	Decreased lethality and TNF production	–	–	–	117

ACEA, arachidonoyl-2′-chloroethylamide; 2-AG, 2-arachidonoylglycerol; CB, cannabinoid receptor; CNS, central nervous system; DTH, delayed-type hypersensitivity; EAE, experimental allergic encephalomyelitis; IFN-γ, interferon-γ; IL-1β, interleukin-1β; LPS, lipopolysaccharide; PBMC, peripheral-blood mononuclear cell; THC, Δ^9-tetrahydrocannabinol; TNF, tumour-necrosis factor.

changes[96], has been analysed in clinical trials and has been shown to have efficacy in reducing spasms and stiffness[97–99].

Several studies have shown that endocannabinoids (such as AEA and 2-AG), as well as synthetic cannabinoids that bind cannabinoid receptors with very low affinity (such as HU-211), are neuroprotective after traumatic brain injury. The mechanisms that mediate the protective effects involve cannabinoid-receptor function, as well as NMDA receptors, inhibition of GLUTAMATERGIC SYNAPTIC TRANSMISSION, and reduction of TNF production and oxidative stress[5]. For example, levels of endogenous 2-AG were found to be markedly increased in mice with closed head injury, and administration of 2-AG following injury reduced brain oedema (a consequence of inflammation) and led to a better clinical outcome[100] (TABLE 3). The effect of 2-AG was attenuated by pretreatment with a CB_1 antagonist, indicating the involvement of cannabinoid receptors. However, receptor-independent mechanisms also seem to be involved. For example, administration of HU-211 attenuated neuropathology in rats with closed head injury and decreased TNF production in the brain, indicating that the suppression of cytokine production by HU-211 was at least partly responsible for the neuroprotection[56]. Similarly, administration of HU-211 to patients with severe closed head injury was initially shown to result in a better clinical outcome[101] but was recently reported not to have any clinical efficacy[102]. As well as TNF, other pro-inflammatory mediators, such as the IL-1R antagonist, might also be involved in the neuroprotective effects of cannabinoids[103]. So, it seems that cannabinoids and endocannabinoids regulate some of the inflammatory aspects of brain injury and that this occurs by both cannabinoid-receptor-mediated and non-cannabinoid-receptor-mediated mechanisms. It is possible that these drugs reduce brain oedema and other aspects of neuroinflammation by inhibiting NMDA receptors, by functioning as antioxidants and by reducing the levels of pro-inflammatory cytokines in the brain (FIG. 3).

Inflammatory bowel disease. Inflammatory bowel disease, which includes ulcerative colitis and Crohn's disease, affects millions of individuals. Better therapies are needed to control the chronic inflammation that is associated with this disease, which leads to increased intestinal motility and faecal transit, resulting in pain, diarrhoea and poor ability to digest food[3]. The endocannabinoid system seems to be involved in the inhibition of intestinal motility, because functional CB_1 is expressed in the human ileum and colon[104], and its expression is increased during inflammation[105]. Furthermore, AEA and 2-AG can be present in high levels in the gut[105] and can inhibit colonic propulsion in mice[106]. Also, activation of CB_2 was recently shown to attenuate LPS-induced increases in gastrointestinal transit[107] (TABLE 3), and CB_1 agonists were shown to be suppressive — but only for basal, and not for LPS-induced, gastrointestinal transit. Interestingly, CB_2-agonist-mediated inhibition of LPS-induced gastrointestinal transit was associated with the attenuation of inflammatory changes, such as suppressing the levels of cyclooxygenases and inducible nitric-oxide synthase, indicating that prostaglandins and/or nitric oxide mediate the effects of CB_2 agonists on gastrointestinal transit[107]. Anti-inflammatory effects of cannabinoids were also observed in a mouse model of chemically induced colitis[108]. Both colonic-tissue ulceration and the inflammatory response were attenuated by cannabinoid treatment, as well as in FAAH-deficient mice, as assessed histologically and by measuring tissue myeloperoxidase activity (which correlates with neutrophil influx). By contrast, ulceration and inflammation were increased in mice deficient in CB_1 and in mice treated with a CB_1 antagonist. From these studies, it seems that cannabinoids regulate the tissue response to inflammation in the colon. It is possible that this regulation occurs on two levels: the first, involving the smooth-muscle response to pro-inflammatory mediators that affect gastrointestinal transit time; and the second, involving the direct suppression of pro-inflammatory-mediator production (FIG. 3). Further studies will be needed to determine the relative contribution of each of these possibilities.

Arthritis. Several cannabinoids have been shown to be anti-inflammatory in animal models of arthritis. The first of these is the dimethylheptyl homologue of the natural *C. sativa* plant product THC-11-oic acid[13]. This derivative, known as ajulemic acid, binds cannabinoid receptors with very low affinity. It was initially tested for its effects on the AIR-POUCH INFLAMMATORY RESPONSE in mice and on adjuvant-induced arthritis in rats[13] (TABLE 3). Daily feeding of ajulemic acid suppressed leukocyte accumulation after injection of IL-1β and TNF into the pouch. In the adjuvant-induced arthritis model, chronic administration (every third day) of ajulemic acid attenuated inflammation in the joints, compared with animals that did not receive ajulemic acid. In terms of the mechanism of action, ajulemic acid was shown in *in vitro* studies to suppress prostaglandin production to a greater extent than the anti-inflammatory drug INDOMETHACIN[13]. In other studies, ajulemic acid was shown to be more potent than common non-steroidal anti-inflammatory drugs in suppressing adjuvant-induced arthritis, with less gastrointestinal ulceration occurring[109], and it was shown to decrease the LPS-induced production of IL-1β in cultures of human PBMCs or synovial-fluid monocytes[110]. So, ajulemic acid might have therapeutic potential for patients with arthritis or other chronic inflammatory diseases, and it could have fewer side-effects than conventional therapies; however, there is some controversy concerning its psychoactive potential[111].

The second non-psychoactive component of *C. sativa* that has anti-inflammatory potential is cannabidiol[112,113]. This drug was tested in a mouse model of collagen-induced arthritis, and when administered either intraperitoneally or orally (every day), beginning at the first signs of arthritis, it effectively blocked progression of the disease. It seemed to function as an

GLUTAMATERGIC SYNAPTIC TRANSMISSION
Glutamic acid is the main excitatory transmitter in the central nervous system, where it mediates fast synaptic transmission. It is released from the terminal of a glutamatergic nerve, crosses the synaptic cleft and acts on postsynaptic receptors.

AIR-POUCH INFLAMMATORY RESPONSE
An experimental model of acute inflammation. Skin pouches are established on the backs of mice, by subcutaneous injection of air on several consecutive days. Subsequently, inflammation is induced by injection of interleukin-1β and tumour-necrosis factor into the pouch cavity.

INDOMETHACIN
A cyclooxygenase inhibitor and thereby a non-steroidal anti-inflammatory drug.

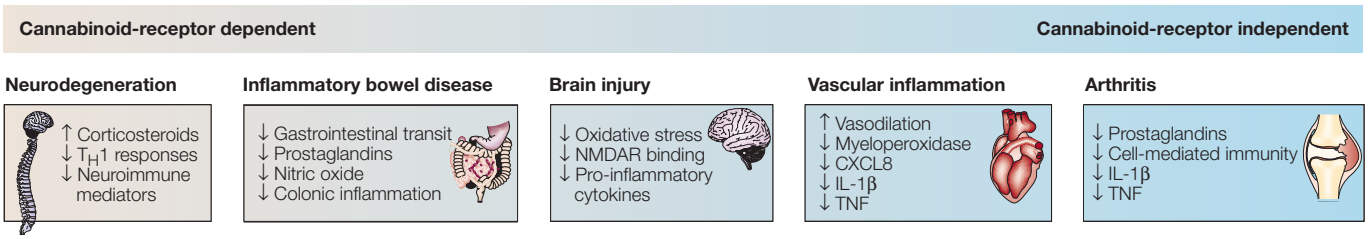

Figure 3 | **Anti-inflammatory effects of cannabinoid-based drugs.** Symptoms of neurodegeneration are attenuated by treatment with cannabinoid-based drugs. This process is mediated, at least in part, through binding of cannabinoid receptors. It involves an increase in corticosteroid release and a decrease in both T helper 1 (T_H1)-cell responses in the central nervous system and in neuroimmune-mediator production, including histamine and tumour-necrosis factor (TNF). Similarly, brain oedema that occurs after injury is suppressed by treatment with cannabinoid-based drugs. This process is also mediated, at least in part, through binding of cannabinoid receptors. It involves a decrease in oxidative stress, NMDA (*N*-methyl-D-aspartate) receptor (NMDAR) binding and cytokine production, including TNF. The pathophysiology of inflammatory bowel disease is also suppressed by cannabinoids. This involves binding to cannabinoid receptors, and there is a concomitant decrease in gastrointestinal-tract faecal transit, prostaglandin and nitric-oxide production, and colonic-tissue inflammation. The inflammation that is associated with arthritis is suppressed by cannabinoids. This occurs through cannabinoid-receptor-independent mechanisms, which involve a decrease in prostaglandin production and cell-mediated immunity, together with a decrease in cytokine production, including interleukin-1β (IL-1β) and TNF. Finally, vascular inflammatory disease might be partly regulated through the binding of cannabinoids to cannabinoid receptors and through vasodilation effects. This occurs together with a decrease in local pro-inflammatory-mediator production, including myeloperoxidase, CXC-chemokine ligand 8 (CXCL8), IL-1β and TNF, through cannabinoid-receptor-dependent or -independent mechanisms.

immunosuppressant, because cells from the draining lymph node of treated mice showed reduced cell-mediated immune functions and IFN-γ production in response to stimulation with antigen. Furthermore, treatment with cannabidiol blocked the LPS-induced increase in serum TNF and other immune responses. In a subsequent study, similar results were obtained at lower doses using a more potent dimethylheptyl derivative of cannabidiol[114]. These data indicate that plant-derived cannabinoids and their synthetic derivatives are anti-inflammatory and immunosuppressive; their mechanisms of action are independent of cannabinoid receptors and are mediated, in part, by suppression of pro-inflammatory-cytokine production by lymphocytes and macrophages (FIG. 3).

Vascular inflammation. Many of the features of cardiovascular disease and atherosclerosis include the elements and mechanisms of the inflammatory cascade, and this contributes to thrombosis and tissue destruction. This is also true of the pathophysiology of septic shock, in which pro-inflammatory mediators are released into the blood in excess, leading to thrombosis, vasodilation, capillary leakage and organ-system failure. Recent evidence indicates that cannabinoids might influence vascular inflammatory disease in several ways. One way is as a mediator of vasodilation, particularly LPS-induced hypotension[4,115]. Levels of the endocannabinoids AEA and 2-AG were found to increase in the sera of patients with endotoxic shock[116], and CB_1 antagonists were shown to prevent LPS-induced hypotension in animals, indicating that endocannabinoids and CB_1 might function as an endogenous system that promotes LPS-induced hypotension[115] (TABLE 3). In addition to this effect of CB_1, there is also evidence of a second way in which vascular inflammatory disease might be influenced, because CB_2 is involved in vascular changes during inflammation. Myocardial ischaemia–reperfusion injury in mice, as measured by infarct size, was considerably reduced by pretreatment with WIN55,212-2, and the protective effect was attenuated by a CB_2 antagonist but not a CB_1 antagonist[58]. Treatment with WIN55,212-2 also reduced the levels of myeloperoxidase, IL-1β and CXCL8 in injured tissue, indicating that it suppresses the mobilization of pro-inflammatory mediators. The authors of this study speculated that WIN55,212-2 might have exerted its effect by binding CB_2 at the cell surface of macrophages in the injured tissue[58]. There is also a third mechanism in that cannabinoid effects that are independent of both CB_1 and CB_2 have been shown to attenuate septic shock[117]. The non-psychoactive cannabinoid HU-211, when given as single pretreatment dose, reduced mouse and rat lethality in response to a challenge with LPS. In addition, similar to effects in models of closed head injury, HU-211 markedly suppressed TNF production, which might explain the attenuation of septic shock. So, it seems that cannabinoids have both a hypotensive effect that is CB_1 mediated and an anti-inflammatory effect that is either CB_2 mediated or independent of cannabinoid receptors (FIG. 3). This complexity provides both challenges and opportunities in the management of cardiovascular diseases.

Conclusions

Several general principles emerge from the studies that are discussed in this Review. The first is that endocannabinoids are expressed by immune cells and that cannabinoid receptors at the surface of immune cells are activated after infection or immune stimulation. The consequences of this for the immune response are not fully understood, but regulation of cellular chemotaxis seems to be involved. Furthermore, because cannabinoid receptors are G-protein-coupled receptors (similar to receptors for chemokines and lipid mediators), their ligation during immune stimulation probably leads to regulation of gene products that are

required for immune-cell function. The second general principle is that the main immune targets of cannabinoid-based drugs involve the suppression of cytokines and cell-mediated immunity, through cannabinoid-receptor-dependent and -independent mechanisms. The cannabinoid-receptor-dependent mechanisms most probably involve G-protein signalling and the regulation of numerous cytokine genes. Regarding cannabinoid-receptor-independent mechanisms, in the case of endocannabinoids, their effects could be mediated through vanilloid receptors, which are known to bind these compounds[118], or they might result from the binding of as-yet-uncharacterized receptors[119]. Cannabinoid-receptor-independent mechanisms might also involve effects on lipid-raft structure and function[120], which are known to be important for immune-cell function[121]. The third general principle involves the suppression, by marijuana-based drugs, of the chronic inflammatory response and the subsequent attenuation of disease processes and symptoms. These anti-inflammatory effects are undoubtedly, in part, associated with the ability of these drugs to suppress the expression of cytokines, as well as other endogenous pro-inflammatory mediators. In addition, these drugs might also function by increasing the production of anti-inflammatory mediators. Further elucidation of the effect of marijuana-based drugs on pro-inflammatory and anti-inflammatory mechanisms will provide the basis for the formulation of more effective drugs for the management of chronic inflammatory diseases.

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Competing interests statement
The author declares no competing financial interests.

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