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# Cannabinoid receptors and immunity

**Thomas W. Klein, Cathy Newton and Herman Friedman**

*Marijuana cannabinoids are both psychoactive and immunoactive. Here, we will review evidence that cannabinoids modulate immunity and that cannabinoid receptors and endogenous ligands are expressed in immune tissues. Clues will also be presented concerning the role of the cannabinoid system in immune regulation and the possible molecular mechanisms involved.*

Cannabis smoke contains many different chemicals; however, the main psychoactive component, $\Delta^9$-tetrahydrocannabinol (THC), is in the group called cannabinoids (reviewed in Ref. 1). It is known that THC binds receptors in the brain and that these receptors appear also to be present in tissues outside the central nervous system (CNS), including those of the immune system. It is therefore likely that THC modulates the function of various peripheral tissues as well as CNS function. In addition to cannabinoids, an endogenous compound with affinity for cannabinoid receptors (CBRs) has been described. This substance, anandamide, is believed to be an endogenous ligand for CBRs; therefore, humans and other animals possess a cannabinoid system comprising receptors and ligands that possibly regulate brain and other organ system homeostasis. In this article, we will review the major aspects of the cannabinoid system, including the evidence that cannabinoids modulate the immune response, and present evidence that the immune modulation is related in part to CBR activity (the physiology of CBRs is reviewed in Refs 2, 3).

## Cannabinoid ligands and receptors

### *Ligands*

Over 60 cannabinoids, each possessing a multi-ring structure, have been identified in extracts of the cannabis plant[1]. The major psychoactive cannabinoid is THC **(Fig. 1)**, which was first purified and structurally described in 1964 (Ref. 4) and allowed the subsequent chemical synthesis of structural analogues for use in structure–activity studies[1]. These studies provided evidence for specific binding sites linked to G proteins, especially $G_i$ (Ref. 5). The synthesis of analogues also led to the introduction of

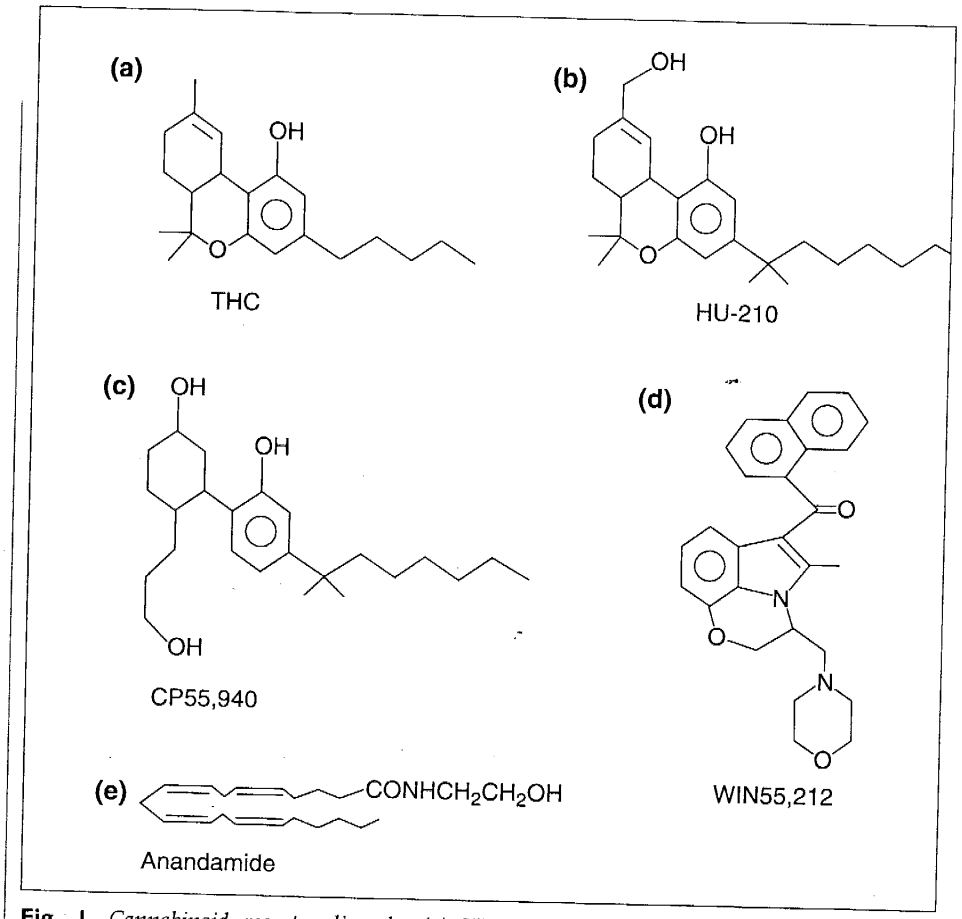


**Fig. 1.** *Cannabinoid receptor ligands. (a) THC, (−)-Δ⁹-6a,10a-*trans*-tetrahydrocannabinol. (b) HU-210, (−)-11-hydroxy-Δ⁸-tetrahydrocannabinol-dimethylheptyl. (c) CP55,940, (−)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-[3-hydroxypropyl]cyclohexan-1-ol. (d) WIN55,212, (+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate. (e) Anandamide,* cis*-5,8,11,14-eicosatetraenoylethanolamide.*

newer, more-potent cannabimimetic agents such as the bicyclic derivative CP55,940 (Ref. 5) and the dimethylheptyl derivative HU-210 (Ref. 6) (Fig. 1). In addition, aminoalkylindole compounds such as WIN55,212 were described and reported to have potent cannabimimetic activity[7]. All of these compounds mimic the action of THC in human and animal studies, and are believed to function through ligation of CBRs.

**Table 1. Cannabinoid effects on immunity in humans**

| Studies in | Cell type | Function | Effect | Refs |
|---|---|---|---|---|
| Human subjects | T cells | Proliferation | Decrease | 19, 20 |
| | | | No effect | 21, 22 |
| | | Rosette formation | Decrease | 20, 23 |
| | | CD4:CD8 ratio | Increase | 24 |
| | B cells | IgE | Increase | 32 |
| | | IgG | Decrease | 31 |
| | Macrophages | Phagocytosis | No effect | 40, 41 |
| | NK cells | Cytolysis | No effect | 49 |
| Human cell culture | T cells | Proliferation | Decrease | 25 |
| | B cells | Proliferation | Increase | 39 |
| | Macrophages | NO release | Increase | 48 |
| | | TNF-α | Decrease | 60 |
| | NK cells | Cytolysis | Decrease | 50 |

Abbreviations: NK, natural killer; NO, nitric oxide; TNF-α, tumor necrosis factor α.

## Receptors

The first CBR, CB1, was cloned in 1990 from a rat brain cDNA library[8]. The isolated cDNA coded for a 473 amino acid protein with the features of a G-protein-coupled receptor (GPCR) (Fig. 2). The protein sequence of CB1 suggested it differed from other neurotransmitter receptors and the correct ligand was shown to be THC (Ref. 8). CB1 was also shown to be negatively coupled to adenylate cyclase and to be expressed primarily in brain[8]. Human CB1 was cloned in 1991, and encoded a protein with 472 amino acids. Interestingly, in addition to brain, the human protein was reported to be expressed in testis[9]. The mouse CB1 sequence has also been cloned[10], and showed 99% and 97% identity to rat and human CB1, respectively, at the amino acid level. CB1 genes have been demonstrated in other species including the tetraodontoid fish, *Fugu rubripes*[11], suggesting the conservation and importance of the CBR system in evolution.

The second CBR, CB2, was cloned by the polymerase chain reaction (PCR) from a human promyelocytic cell line (HL60) cDNA library, and showed 44% amino acid identity to the rat CB1 protein[12]. Interestingly, the cDNA encoded a protein of only 360 amino acids (substantially shorter than CB1) although this did include the typical seven-transmembrane structure of a GPCR (Fig. 2). Another interesting aspect of CB2 was its predominant expression in the periphery, rather than in the brain, and particularly in cells of the immune system (Ref. 12 and see below). Receptor subtypes in addition to CB1 and CB2 have been sought; however, with the exception of a CB1 isoform called CB1A (Ref. 13), no additional types have been found, leading to recent speculation that there are only two CBRs (Ref. 14). The reasons for the asymmetrical distribution of these two receptors between brain and periphery is unknown at this time.

## Endogenous ligands

The demonstration of CBRs in humans and animals predicted that an endogenous

ligand for the receptors would be found. Indeed, the isolation from porcine brain of an active substance was reported in 1992 (Ref. 15). The structure of the substance was determined to be arachidonylethanolamide (Fig. 1), and it was named anandamide. Since then, several other related compounds have been reported and anandamide has been shown to have many of the biological effects associated with cannabinoids[16]. At this time, the cannabinoid system is known to contain two receptors and several endogenous ligands widely distributed throughout the body. Yet despite these structural details, the physiological role of this system remains unclear. In this regard, it has been recently speculated to be involved in the coordination of movement, short-term memory, and titration of mood and emotions[17]. It is tempting to speculate that it may also be involved in immune homeostasis and control.

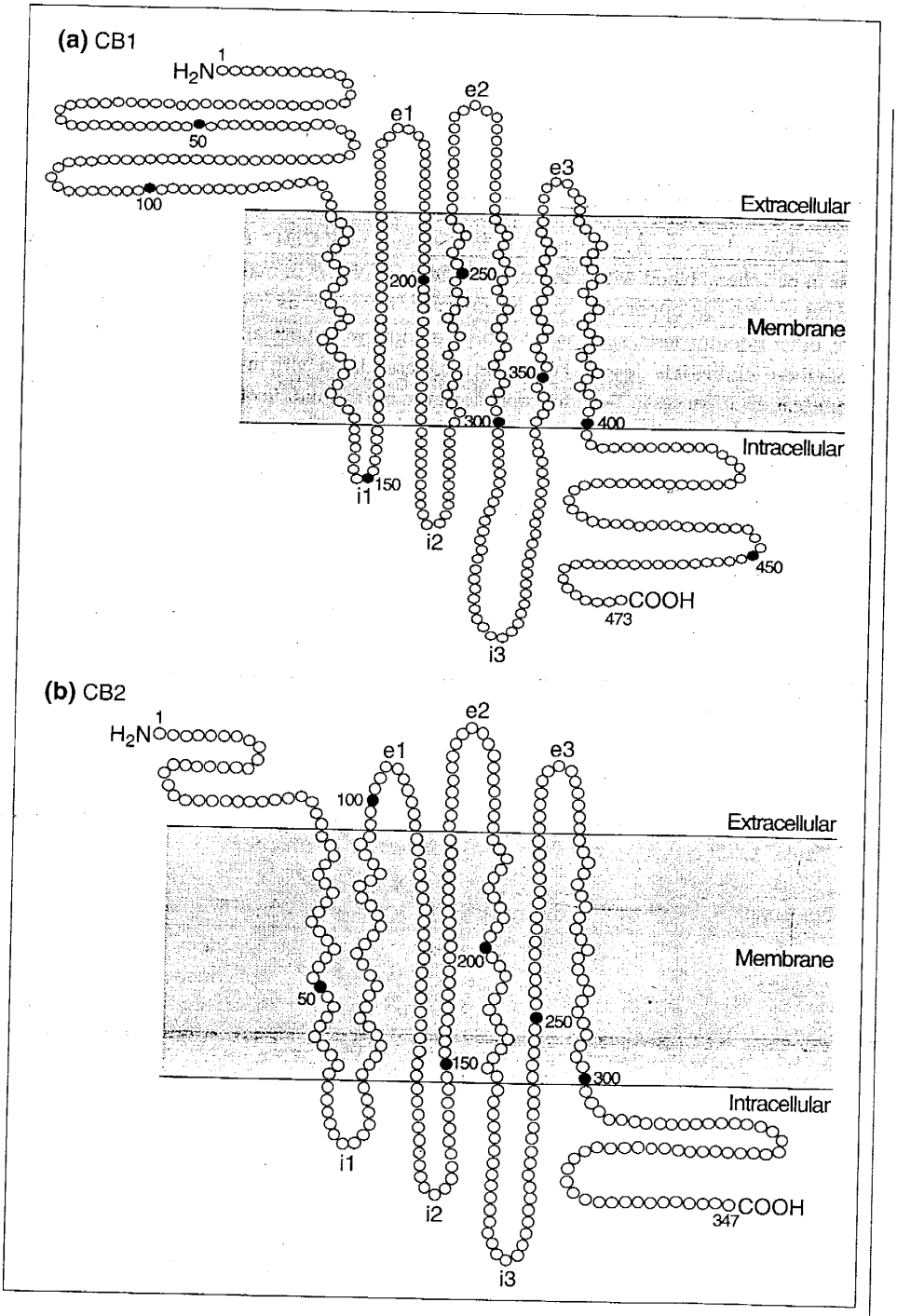


**Fig. 2.** *Mouse cannabinoid receptor CB1 (a) and CB2 (b) proteins based on GenBank sequences U22948 and X86405, respectively. Both receptors are single polypeptides with seven transmembrane α-helices, and have an extracellular, glycosylated N-terminus and an intracellular C-terminus. CB1 has longer extra- and intracellular tails than CB2. CB1 is 66% similar to CB2 at the amino acid level overall; however, in the transmembrane regions the two chains are 78% similar. Agonists may bind to the e2 domain in CB1 and CB2 (Ref. 73) as well as the third transmembrane domain in CB1 (Ref. 74). e1–e3 are extracellular loops 1–3; while i1-i3 are the respective intracellular loops.*

## Cannabinoid effects on immune cells

The main body of literature involving marijuana and immune modulation dates back to the 1970s. At that time, a few reports suggested that cannabis use was associated with an increased incidence of viral infections as well as allergic symptoms (reviewed in Ref. 18). Subsequent animal studies with herpes simplex virus, Friend leukemia virus, *Listeria monocytogenes*, *Staphylococcus albus*, *Treponema pallidum* and *Legionella pneumophila* have shown that cannabinoids suppress host resistance to infection[18]. Because of the diversity of immune cells and mechanisms associated with these infections, it was hypothesized that cannabinoids might either directly or indirectly affect the function of various immune cell subpopulations. Studies were designed to test drug effects on various types of human and animal immune cells.

### *T cells*

T cells are important regulators and effectors of immune responses to viruses and other microbes. Several groups studied the proliferation response of peripheral blood T cells obtained from marijuana smokers (Table 1). The results of these studies were varied, with some showing that marijuana use correlated with decreased proliferation[19,20] while others showed no effect[21,22]. These studies also had varied parameters including population size and the amount and type of drug exposure. In addition to T-cell proliferation, cannabis use was studied in relation to T-cell rosette formation with sheep red blood cells (SRBCs), and T-cell subsets. Impaired rosette

formation was observed in peripheral blood cells taken from chronic marijuana users[20,23] but it should be noted that the subjects were mostly healthy and that other immune tests such as proliferation[20] and skin testing[23] were within normal limits. Cannabis use was also associated with an increase in the percentage of $CD4^+$ T cells in peripheral blood with a mean CD4:CD8 ratio of 1.95 in marijuana smokers as opposed to 1.27 in controls[24]. However, as before, other immune tests, such as T-cell proliferation, were normal. From these studies it is clear that cannabis use is associated with intermittent disturbances in T-cell function; however, the magnitude of the change is often small and not obviously sufficient to suppress resistance to infection.

The effect of THC on human T cells in culture was also reported (Table 1). Proliferation in response to phytohemagglutinin and concanavalin A was suppressed by various cannabinoids, both psychoactive and nonpsychoactive, and at drug concentrations in the μM range[25]. *In vitro* studies using mouse cell cultures (Table 2) showed that mouse splenocyte proliferation to T-cell mitogens, as well as the B-cell mitogen lipopolysaccharide (LPS), were suppressed by THC concentrations in the 10 μM range and that B cells appeared to be more sensitive than T cells[26]. These studies were confirmed and extended to T-cell stimulation with anti-CD3 antibody[27]. Interestingly, the THC effect was biphasic, with lower drug doses increasing proliferation and higher doses suppressing the response. This biphasic effect has been seen in other studies[26,28] and its meaning is still unclear. In addition to THC and other cannabinoids, anandamides and other endogenous CBR ligands have been shown to modulate T-cell proliferation responses to T- and B-cell mitogens[29]. Cytotoxic T-lymphocyte (CTL) activity in mice was also shown to be suppressed in animals injected with THC followed by sensitization to alloantigens, as well as in splenocyte cultures stimulated with alloantigens and then drug treated[30]. THC treatment did not inhibit binding of the CTLs to the target cell but decreased the cytolytic activity subsequent to binding. These *in vitro* studies with human and mouse cells demonstrate that CBR ligands can suppress T- and B-cell proliferation and CTL activity. It is tempting to extrapolate these *in vitro* observations to whole-animal infection paradigms; however, the effective drug concentrations observed *in vitro* are in the μM range and therefore at least tenfold higher than the blood concentration observed in marijuana smokers.

**Table 2. Cannabinoid effects on immunity in animals**

| Studies in | Cell type | Function | Effect | Refs |
|---|---|---|---|---|
| Animal subjects | T cells | Cytolysis | Decrease | 30 |
| | | Th1-cell activity | Decrease | 64 |
| | B cells | Antibody formation | Decrease | 33, 34, 38 |
| | Macrophages | Protein production | Decrease | 43 |
| | | IL-1, IL-6, TNF-α | Increase | 59 |
| | | Cytolysis | Decrease | 44 |
| | NK cells | Cytolysis | Decrease | 51, 52 |
| Animal cell culture | T cells | Proliferation | Decrease | 26, 27 |
| | | Cytolysis | Decrease | 30 |
| | | Th1-cell activity | Decrease | 64 |
| | | Th2-cell activity | Increase | 64 |
| | | IL-2 | Varies | 56 |
| | B cells | Antibody formation | Decrease | 35–38 |
| | | Proliferation | Decrease | 26, 29 |
| | Macrophages | Phagocytosis | Decrease | 42 |
| | | Antigen presentation | Decrease | 45 |
| | | Arachidonic acid and anandamide release | Increase | 46, 72 |
| | | IL-1 | Increase | 58 |
| | | TNF-α | Decrease | 60, 61 |
| | | NO release | Decrease | 47 |
| | NK cells | LAK-cell activity | Decrease | 53 |
| | | IL-2 receptor | Varies | 57 |
| | | Cytolysis | Decrease | 51, 52 |

Abbreviations: IL-1, interleukin 1; LAK, lymphokine-activated killer; NK, natural killer; NO, nitric oxide; Th, T helper; TNF-α, tumor necrosis factor α.

### B cells

Many reports show that cannabis and CBR ligands suppress serum immunoglobulin (Ig) levels and antibody formation (Table 1). For example, cannabis use was associated with a decline in serum IgG, although IgD and IgE were increased[31,32]. Studies in mice (Table 2) showed that CBR ligands, either injected into mice or added to splenocyte cultures, suppressed the development of plasma cells in response to SRBCs (Refs 33–37). However, although extensive, these studies failed to show which cell was the target of the drug effect. This question was addressed in a study using T-cell-dependent and T-cell-independent antigens[38] wherein THC given either *in vivo* or *in vitro* suppressed the antibody response to SRBCs (T-cell-dependent) but not DNP-Ficoll (T-cell-independent). This observation coupled with the finding that THC suppressed the proliferation of T cells suggested that the drug was targeting an accessory cell such as the T cell rather than directly affecting B cells[38]. However, other studies have shown that cannabinoids can suppress the proliferation response to the B-cell mitogen LPS (Refs 26, 29), and more recently, using B cells purified from human tonsillar tissue, it was shown that CBR ligands increased rather than decreased cell proliferation[39] at drug concentrations in the nM range. These studies suggest that cannabinoids alter antibody formation by affecting either B cells or

accessory cells, and more studies are needed to define the drug effects as well as the relative CBR expression on these immune subpopulations.

### *Macrophages*

Macrophages play major roles in both innate and acquired immunity to infections. For innate immunity, macrophages produce acute-phase cytokines (see below), phagocytose and kill microbes, and release inflammatory mediators such as nitric oxide and arachidonic acid metabolites; for acquired immunity, macrophages present antigen and release cytokines. Cannabis use and effects on innate macrophage functions were initially studied in pulmonary models (Tables 1 and 2). Studies of lung alveolar macrophages from humans and rats showed that both tobacco and marijuana smoking had little effect on phagocytic capacity but did cause some metabolic and morphological changes in the cells[40,41]: the significance of these changes was not clear. Other studies, on mouse peritoneal macrophages and cell lines in culture (Table 2), showed that various CBR ligands in the μM concentration range consistently suppressed various functions including cell spreading and phagocytosis[42], protein expression[43], cytolysis[44] and antigen presentation[45]. More recently, molecular changes in macrophages have been reported following CBR ligand treatment (Table 2). For example, arachidonate release from mouse peritoneal macrophage cultures was reported following treatment with THC by mechanisms involving several phospholipases and CBRs (Ref. 46), and nitric oxide release was suppressed by THC and other agonists by mechanisms involving CBRs (Ref. 47). However, others have reported that CBR agonists increase nitric oxide in human monocyte cultures[48]. This effect was inhibited by the CB1 antagonist SR141716A, suggesting that CBRs were involved. Although these studies show that a variety of macrophage functions important in host immunity are modulated *in vitro* by cannabinoids, the effective drug concentrations are high relative to *in vivo* values and therefore, as with T-cell effects, extrapolation to results obtained in humans and animals is difficult.

### *Natural killer cells*

Natural killer (NK) cells help to control infections by killing infected target cells and as a source of cytokines for upregulating immune function. Only a few reports have examined NK-cell activity in marijuana users (Table 1). For example, in one study, subjects were given THC for several weeks and hormone levels and immune tests were performed including peripheral blood NK-cell lytic activity against K562 targets[49]. Although no significant changes were reported in any of the test results, it should be noted that the THC was given orally and in a low dose and that NK-cell activity did vary with time from controls but not with statistical significance. In other human studies, THC was effective in suppressing the lytic activity of cultured NK cells in both a time- and dose-dependent manner and at concentrations of 10 μM (Ref. 50). Suppressive lytic effects were also seen in studies with rats[51] and mice[52] (Table 2). Injection of THC suppressed subsequent splenocyte NK-cell activity, and drug addition to cultured splenocytes was also suppressive. In addition, it was determined that THC treatment did not interfere with NK-cell binding to targets but prevented killing mechanisms post-binding[52]. Lymphokine-activated killer (LAK) cell activity was also shown to be suppressed by cannabinoids[53], demonstrating that these drugs can alter the immunomodulating effects of cytokines (see below).

## Cannabinoid effects on cytokines

Cytokines play a major role in mediating the antimicrobial effects of immune cells. For example, interferons (IFNs) are powerful antiviral agents as well as immunomodulators, and interleukin 2 (IL-2) is a major growth factor for the development of immunity. If cannabinoids are immunomodulatory, they would be expected to exert some of that effect through modulation of cytokine production and function. Mouse splenocyte cultures treated with THC (10 μM range) produced less IFN, while chronic injection of mice with THC (40 mg $kg^{-1}$) caused reduced IFN production in splenocytes tested *ex vivo* (Ref. 54). The type of IFN and its cellular source were not reported in this study. Suppression of IFN-α/β production was observed in an infection model consisting of mice injected with THC (15–100 mg $kg^{-1}$) and subsequently infected with herpes simplex virus[55]. Serum IFN levels were significantly reduced following drug treatment; however, the concomitant effect of the drug on virus susceptibility was not reported. Production and function of IL-2 has also been shown to be affected by cannabinoids. LAK-cell activity was suppressed by THC treatment[53] as was IL-2 production[56]. Recent evidence suggests that the molecular effect of the drug on the IL-2 system involves a modulation of the expression of IL-2 receptor proteins, resulting in a downregulation of high-affinity IL-2 receptors[57].

The modulation of production of acute-phase cytokines such as tumor necrosis factor (TNF), IL-1 and IL-6 might also be responsible for cannabinoid effects on antimicrobial immunity (Table 2). When added to macrophage cultures, THC (10–30 μM) increased supernatant IL-1 activity by a mechanism related to processing and release of the IL-1 proteins[58]. An increase in IL-1 mobilization was also observed following THC injection into mice in conjunction with an *L. pneumophila* infection[59]. The mobilization of other acute-phase cytokines such as TNF-α and IL-6 was also observed in this study, leading to a drug-induced enhanced mortality of the animals following infection. Microbes such as *L. pneumophila* can induce the mobilization of cytokines in the host and it was concluded that THC augmented this normal host response and, in some way, increased the cytokine response to a toxic level[59]. Acute-phase cytokine production has also been observed to be decreased by cannabinoids (Table 2). Mouse and human macrophage cultures treated with THC produced less TNF-α in response to LPS and IFN-γ (Ref. 60), and in the macrophage cell line, RAW264.7, supernatant TNF-α levels were decreased by THC treatment due to an inhibition of conversion of the pro-mature form of TNF to the secreted 17 kDa form[61]. The molecular mechanisms of these effects are not yet clear. It is possible that drug-induced changes in arachidonic acid metabolites or cyclic AMP (cAMP) levels are involved and linked

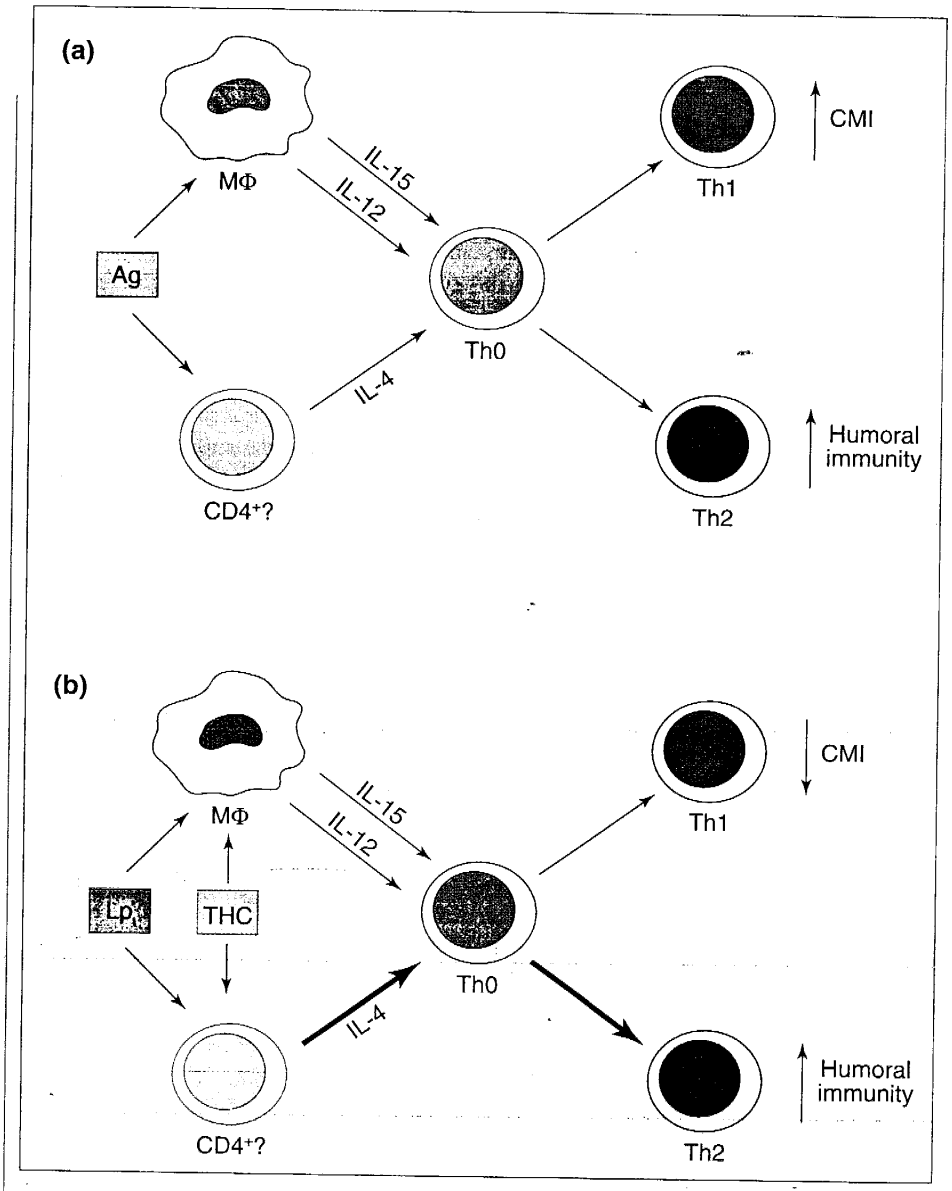


**Fig. 3.** *THC ($\Delta^9$-tetrahydrocannabinol) treatment disrupts the balance between T helper 1 (Th1)- and Th2-cell activity, suppressing the development of cell-mediated immunity (CMI). (a) Under normal conditions of antigen stimulation, various cell types contribute to the development of CMI and humoral immunity. For example, macrophages (MΦs) become stimulated by antigen (Ag) and produce cytokines such as interleukin 15 (IL-15) and IL-12 supportive of Th1 cells and CMI. Stimulated CD4+ T cells and other cells produce IL-4 supportive of Th2 cells and humoral immunity. Certain infections manipulate this balance and cause the preferential development of one type of immunity over the other*[75]*. (b) THC treatment suppresses the Th1 arm of immunity while increasing the Th2 arm. This comes from data showing that THC decreases CMI to* Legionella pneumophila *(Lp) infection as well as Th1-type cytokines such as interferon γ (IFN-γ), IL-12 and IL-15, while increasing the level of serum anti-*L. pneumophila *IgG1 and Th2-type cytokines such as IL-4 and IL-10 (Ref. 64; T. Klein* et al., *unpublished).*

somehow to CBR ligation; however, these mechanisms have yet to be established.

### *T helper 1 (Th1)- and Th2-type cytokines*

Regulation of host resistance depends on a number of factors including a major role played by CD4+, Th1 and Th2 cells[62]. Several years ago, it was reported that mice failed to develop immunity to infection with *L. pneumophila* when exposed to THC (Ref. 63). Because immunity to *L. pneumophila*, an intracellular parasite, depends heavily on Th1 cells, an effect of THC on Th1-cell development was examined[64]. The results showed that a single injection of THC (4 mg $kg^{-1}$) into mice 24 h before a sublethal infection with *L. pneumophila* suppressed the normal development of Th1-cell activity as measured by the *ex vivo* production of IFN-γ. Furthermore, THC suppressed the expression of IgG2a anti-*L. pneumophila* antibodies but increased the expression of the IgG1 subclass in the serum of infected mice. Additional results obtained in this model as well as an *in vitro* model of drug treatment showed that, besides suppressing the Th1 cytokine IFN-γ, THC treatment suppressed the production of IL-12 and IL-15. Furthermore, in addition to increasing the Th2-associated, IgG1 antibody response, THC treatment increased the production of IL-4 and IL-10 (Ref. 64; T. Klein *et al.*, unpublished).

From these results, it is speculated that cannabinoid treatment directs the cytokine network away from cell-mediated immunity by somehow suppressing Th1-cell activity, and towards humoral immunity through the overproduction of Th2-type cytokines such as IL-4 (Fig. 3). This shift in the Th1/Th2 balance might then cause a deficiency in host resistance to certain pathogens, especially those controlled by Th1 responses such as viruses, intracellular bacteria and parasites. Interestingly, such an imbalance has been proposed to occur in the development of AIDS (Ref. 65).

## Cannabinoid receptors and immunity

### *Receptor expression and immune cells*

Despite the many early studies showing that THC modulates immune function, evidence that CBRs were involved was lacking and the drug effects were believed to be mainly nonspecific interactions with lipids in cell membranes affecting the function of integral membrane proteins[2]. However, in 1992, the first report appeared showing that splenocytes expressed CB1 mRNA as measured by reverse transcriptase (RT)–PCR (Ref. 37). A modest structure–activity relationship was shown in this study using three different cannabinoid agonists tested in an *in vitro* antibody-forming system with mouse splenocytes. The active analogues THC,

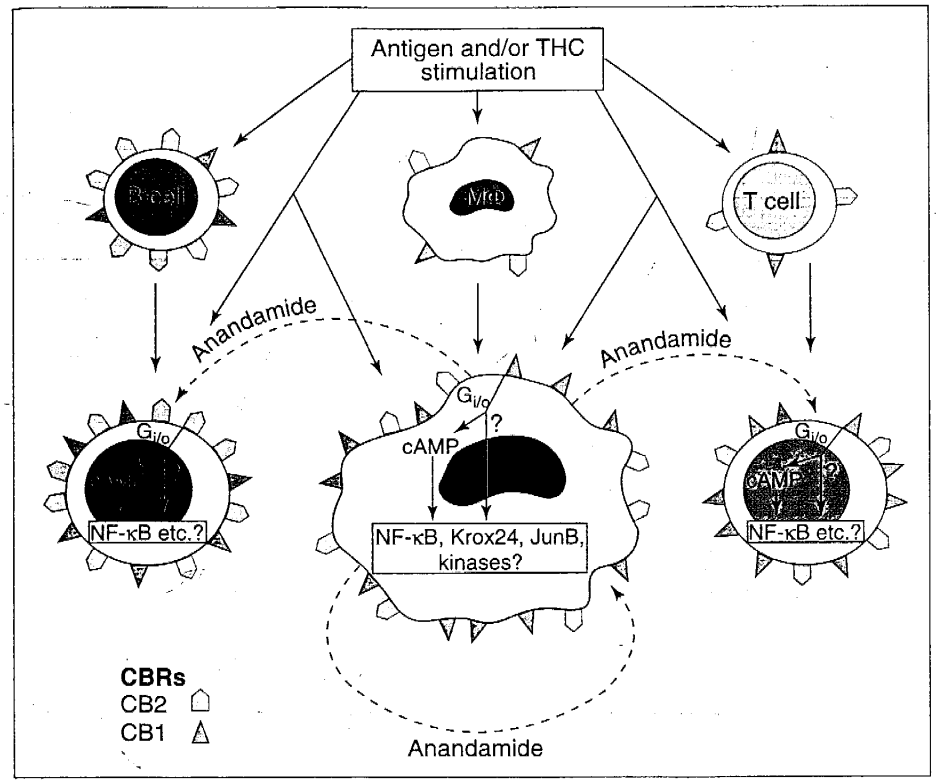


**Fig. 4.** *Possible mechanism for the regulation of immune cells by cannabinoid receptors (CBRs) and anandamide. Resting T cells and macrophages (MΦs) express less CB1 and CB2 mRNA than B cells, and CB2 expression predominates. However, stimulating the cells causes an increase in CBR mRNA expression and possibly protein, as well as the generation of the endogenous CBR ligand anandamide. The cannabinoid system is now positioned to modulate immunity resulting from incoming signals from antigen and other immune factors, as well as THC. Modulation of immunity through CBR ligation involves $G_i/G_o$ mechanisms and cyclic AMP (cAMP) (Ref. 2). However, cAMP-independent mechanisms might also be involved[2]. CBR ligation also leads to activation of transcription factors (e.g. NF-κB, Krox24, JunB) and kinases, resulting in changes in gene expression in the cells[3].*

CP55,940 and HU-210 were more potent in suppressing antibody formation than the inactive ones. Specific equilibrium binding of [$^3$H]CP55,940 in splenocyte preparations was shown with a $K_d$ of 910 pM, and CB1 mRNA was demonstrated in splenocyte RNA extracts. This important report suggested that immune cells transcribed one of the CBR genes and expressed high-affinity cannabinoid-binding sites on the cell surface. The mRNA findings were extended to human immune tissues, leukocyte subpopulations, and leukocyte cell lines[66]. Although present in lower abundance than in brain, CB1 transcripts were demonstrated in spleen, tonsils, peripheral blood mononuclear cells and polymorphonuclear leukocytes (PMNs). In addition, quantitative PCR revealed that CB1 mRNA was present in different amounts in different leukocyte subpopulations with the levels in B cells>NK cells>PMNs >CD8$^+$ cells> monocytes>CD4$^+$ cells. Purified mouse splenic B cells were also reported to have higher levels of CB1 mRNA than either T cells or macrophages[67], and the level of message was higher in spleen than in thymus[68]. This same rank order (with minor variation) was observed for CB2 in human blood cells and, in addition, the level of CB2 mRNA was found to be higher than that of CB1 in immune cells from humans[69] and mouse[68]. These findings coupled with the recent report that human tonsillar B-cell proliferation was shown to be enhanced by cannabinoids in a CB1-independent way[39] suggest that CB1 and CB2 are differentially expressed and functional on cells of the immune system, with CB2 being the prominent receptor subtype, especially on B cells (Fig. 4).

### *Receptor modulation*

The above finding concerning the rank order of CBR mRNA expression in different immune subsets suggested that receptor expression was related to the stage of cellular differentiation and therefore possibly related to cell function. If this is true, activating the cells to drive differentiation might lead to a change in the level of receptor message. This hypothesis was tested in T-cell[70] and macrophage[67] cell lines, and CB1 message was increased substantially within hours after stimulation suggesting the gene product was activated as part of the immune cell activation program[71]. In addition, at least in the macrophage cell line, the message level increased to a maximum at 6 h after stimulation and then declined by 24 h, suggesting that the gene was turned on and off during leukocyte activation[67]. Besides increasing mRNA, T-cell stimulation also increased the number of cannabinoid ligand-binding sites and the amount of membrane immunoreactive protein[70]; from these data, it is speculated that the level of cell-surface CBR is increased following cell activation (Fig. 4) and therefore the receptors play a role in the activation process. Other evidence supports this concept. Immune cells, especially macrophages, are known to release arachidonic acid metabolites upon stimulation, and THC treatment of these cells leads to release through CBR mechanisms[46]. Interestingly, one of the metabolites released by macrophages is anandamide[72], suggesting that activated cells respond by releasing the endogenous cannabinoid ligand, thus positioning it for interaction with CBRs on immune cells and consequent immunomodulation (Fig. 4). Although more experiments are needed to establish these mechanisms, they depict a possible immunoregulatory role for the cannabinoid system, which has been suggested by others[17,72].

## Concluding remarks

In summary, the evidence to date suggests that a CBR–ligand system exists in the immune system and has some role in immune homeostasis. However, the function and distribution of receptor subtypes in the various immune compartments is far from clear at

this time. CB2 appears to be readily expressed but this subtype, as well as CB1, is probably upregulated in various immune cells depending upon the level of cell differentiation and/or activation. The immune system, because of its capacity to generate arachidonic acid, probably also produces endogenous CBR ligands. In addition, although CBRs are linked to $G_i/G_o$ proteins and cAMP, they might also be linked to other signaling cascades. Clearly, many basic issues need clarification. For example, the CBR phenotype of immune cell subsets needs to be determined. Furthermore, what are the conditions leading to CBR expression as well as expression of the cellular genes activated by CBR ligation? Finally, the role of the cannabinoid system in immune regulation, health and disease needs to be clarified not only in individuals who smoke marijuana and ingest THC but in nonusers as well.

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