The cannabinoid system and immune modulation

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Abstract: Studies on the effects of marijuana smoking have evolved into the discovery and description of the endocannabinoid system. To date, this system is composed of two receptors, CB1 and CB2, and endogenous ligands including anandamide, 2-arachidonoyl glycerol, and others. CB1 receptors and ligands are found in the brain as well as immune and other peripheral tissues. Conversely, CB2 receptors and ligands are found primarily in the periphery, especially in immune cells. Cannabinoid receptors are G protein-coupled receptors, and they have been linked to signaling pathways and gene activities in common with this receptor family. In addition, cannabinoids have been shown to modulate a variety of immune cell functions in humans and animals and more recently, have been shown to modulate T helper cell development, chemotaxis, and tumor development. Many of these drug effects occur through cannabinoid receptor signaling mechanisms and the modulation of cytokines and other gene products. It appears the immunocannabinoid system is involved in regulating the brain-immune axis and might be exploited in future therapies for chronic diseases and immune deficiency. *J. Leukoc. Biol.* 74: 486–496; 2003.

Key Words: *marijuana · Th1 cells · chemotaxis · tumor · G protein · CB1*

INTRODUCTION

The normal immune system is modulated and regulated by foreign antigens that induce the production and secretion of a plethora of proteins from immune and nonimmune cells. These proteins include cytokines such as interleukin (IL)-2, chemokines such as monocyte chemotactic protein-1 [1], and surface receptors and other membrane proteins. In addition to these primary immune modulators, other humoral factors and metabolic products also modulate immunity. For example, prostaglandins [2], steroids [3], and histamine [4] are known to exert powerful effects on the quantitative and qualitative aspects of immunity. In addition, mediators of the brain-immune axis such as catecholamines [5], endogenous opioids [6], and cannabinoids [7, 8] are also capable of modulating immune function. It is reasonable to speculate that these latter factors serve as secondary modulators, which when mobilized coincident with or shortly after first-line immune modulators such as lymphokines, increase or decrease immune activity. These secondary mediators are relatively simple in structure and therefore have been synthesized. This, along with the fact that the pharmacology of these agents in many cases has been thoroughly studied, makes these compounds good candidates for use in immunotherapy.

The medicinal uses of marijuana were described centuries ago for diseases such as asthma, migraine, pain, convulsions, and anxiety (reviewed in ref. [9]). More recently, emphasis has been placed on marijuana's putative, beneficial effects on appetite, glaucoma, spasticity in multiple sclerosis, pain, and inflammation [10]. Recent experimental evidence supports marijuana's therapeutic potential in some of these maladies [11]. The active plant ingredients in marijuana belong to the C21-cannabinoid compounds including the primary psychoactive compound, Δ^9-tetrahydrocannabinol (THC). This cannabinoid along with others such as Δ^8-THC, cannabidiol, and cannabinol, as well as chemical analogs, have been extensively studied over the years for their biological and therapeutic properties [8]. Some of the properties of these agents have included effects on immunity ranging from suppression of resistance to infection to enhancement of IL-1 production by macrophages. These early studies about the immunomodulating effects of these drugs have been the subject of previous overviews [7, 12–18] and will not be reviewed here. Instead, we will briefly summarize the general features of the cannabinoid system and review recent findings on the structure and function of the cannabinoid system components in the immune system. For convenience, we will refer to this as the "immunocannabinoid" system.

CANNABINOID SYSTEM

Marijuana cannabinoids, analogs, and endocannabinoids

The flowering tops and leaves of the marijuana plant contain many chemicals including the C21, tricyclic, cannabinoids, the most widely studied of these is THC, which is also the most potent in terms of psychoactivity (for review of structures, see ref. [8]). Cannabinoids are lipophilic and therefore difficult to work with; however, several were isolated in the 1940s, and by 1964, THC was isolated and synthesized [19]. Elucidating the

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structure allowed for the subsequent synthesis and testing of active analogs with varying cannabimimetic potencies leading to the pharmacological demonstration of cannabinoid receptors [9, 20]. Of the many analogs synthesized, several have been used extensively over the years. One widely studied analog is the dimethylheptyl derivative of 11-hydroxy-Δ^8-THC (HU210), which has a much higher affinity for cannabinoid receptors than THC (ref. [21]; **Table 1**). In addition to the tricyclic cannabinoids, bicyclic analogs, such as CP55,940 [29], and aminoalkylindoles, such as *R*-(+)-WIN55,212 [30], have also been synthesized with cannabimimetic activity and with affinities higher than THC (Table 1). All of these agents have been widely used in examining the biology of the cannabinoid system and as can be seen from Table 1, these agents are relatively nonselective for cannabinoid receptors in that their K_is are similar for both. More recently, CB2-selective agonists have been synthesized, such as JWH-015, which displays a higher affinity for CB2 than for CB1 (ref. [31]; Table 1).

Besides the classical agonists, such as THC, and nonclassical agonists, such as HU210, other ligand groups have been described. One of these, the so-called endocannabinoid group, is chemically eicosanoid in structure, and its members are derived from free arachidonic acid (ref. [32]; **Fig. 1**). The first of this group was isolated from pig brain and was determined to be an arachidonyl ethanolamide. This compound was named anandamide [33] and was shown in some systems to be relatively nonselective for either of the cannabinoid receptors (Table 1). The existence of this endogenous ligand had been predicted by the discovery of cannabinoid receptors, and its demonstration established the existence of the endocannabinoid system of receptors and ligands. More recently, several other endocannabinoids have been isolated from tissues, such as 2-AG [26] and 2-AGE [34], the latter of which appears to be relatively selective for CB1. Anandamide and 2-AG are produced in brain as well as spleen and other peripheral tissues [35, 36] and catabolized by the membrane-bound serine hydrolase, fatty acid amide hydrolase [37], and monoacylglycerol lipase [36], respectively. In addition to binding to cannabinoid receptors, anandamide also binds to vanilloid receptors, which

TABLE 1. Binding Affinities of Cannabinoid Receptor Ligands

Ligand	K_i (nM) CB1	CB2	Cell type[a]	Reference
Agonists				
Δ^9THC	53.3	75.3	Transfected	[22]
HU-210	0.06	0.5	Transfected	[22]
CP-55940	1.3	1.3	Both	[23]
R-(+)-WIN55212	9.9	16.2	Normal	[23]
JWH-015	383	13.8	Transfected	[24]
ACEA	1.4	>2000	Normal	[25]
Anadamide	252	581	Transfected	[26]
Atagonists				
SR 141716A	11.8	13,200	Both	[27]
SR 144528	437	0.6	Both	[28]

[a] Studies were done with receptor-expressing membranes from cells transfected with cloned receptors, normal cells, or both types of samples. K_i, Mean inhibition constant; CB1/2, cannabinoid receptor 1/2; ACEA, arachidonoyl-2-chloroethylamide.

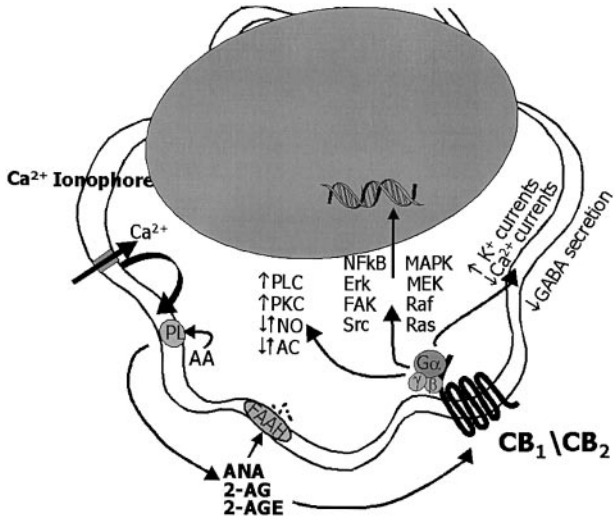

Fig. 1. The endocannabinoid system and cell function. An influx of Ca^{2+} activates phospholipases (PL), which drive the conversion of membrane arachidonic acid (AA) to endocannabinoids such as anandamide (ANA), 2-arachidonoylglycerol (2-AG), and 2-arachidonylglyceryl ether (2-AGE). The endocannabinoids can be taken up and metabolized by fatty acid amide hydrolase (FAAH) or can bind to CB_1 or CB_2. These are G protein-coupled receptors (GPCR) capable of signaling through G protein subunits, Gα, -β, and -γ, leading to a modulation of nitric oxide (NO) and adenylyl cyclase (AC). In neurons, this signaling can lead to a change in K^+ and Ca^{2+} currents and secretion of transmitters such as γ-aminobutyric acid (GABA). In addition, CB1 and CB2 signaling can lead to the activation of many other factors, including PLC, protein kinase C (PKC), nuclear factor-κB (NF-κB), extracellular signal-regulated protein kinase (ERK), focal adhesion kinase (FAK), steroid receptor coactivator (Src), mitogen-activated protein kinase (MAPK), and MAPK kinase (MEK).

are ligand-gated cation channels sensitive to capsaicin and related analogs [38].

Another group of cannabinoid receptor ligands is the diarylpyrazole group, which has antagonist/inverse agonist activity when tested in a variety of tissues. The first of these described is the CB1-selective compound, SR141716A [23], which has potent antagonist activity for the CB1 receptor. The second described is the CB2-selective compound, SR144528, which has potent CB2 antagonism (ref. [28]; Table 1). Since the description of these two, other antagonists have been reported, and these have recently been reviewed [8].

Cannabinoid receptors

Pharmacological evidence initially supported the existence of cannabinoid receptors of high affinity in brain tissue and with specificity sufficient to distinguish different enantiomers of cannabinoid analogs [20, 39]. Subsequent to this, the first receptor, CB1, was cloned from a rat brain cDNA library using a probe derived from the sequence of bovine substance-K receptor [40]. The translated sequence of the gene yielded a protein of 473 amino acids and the structure of a 7-transmembrane, GPCR. Since then, homologues of CB1 have been cloned from humans [41] and mice [42], displaying 97–99% amino acid sequence identity from one species to the other. CB1 mRNAs are expressed in brain, especially in the basal ganglia, as well as several tissues in the periphery including immune tissue (see below). Polymorphisms in the human gene

have been reported, including a G-to-A silent mutation at position 1359 in the coding exon and an $(AAT)_n$ repeat polymorphism outside of the exon [43]. The significance of these changes in terms of gene function and disease is not clear at this time. The CB1 coding sequence is contained in a single exon, but other cDNA segments have been reported, such as a 5′-untranslated first-exon and a large 3′-untranslated sequence. The second receptor, CB2, was cloned from a cDNA library from the promyelocytic, human cell line, HL60 [44]. The protein encoded by this gene was reported to contain only 360 amino acids and showed only 44% identity with the human CB1 receptor. The mouse [45] and rat [46] CB2 genes also have been cloned and encode proteins of 347 and 410 amino acids, respectively. The variation in length among these proteins occurs in the carboxy end with the rat protein containing an extended carboxy terminus [46]. Amino acid identity of CB2 from species to species is lower than for CB1, and the mRNAs for CB2 are found in peripheral tissues, such as spleen, but not in brain and tissue, such as heart.

Function of cannabinoid system

Cannabinoid receptors are GPCR, and very early on, it was recognized that they were linked to inhibition of adenylyl cyclase (ref. [20]; Fig. 1). In addition, GPCR are known to be linked to a variety of other second messengers and signaling components [47–49], and ligation of CB1 and CB2 has been shown to not only suppress adenylyl cyclase but also to activate it [50], suggesting these receptors activate factors other than $G\alpha_i$ [51]. In addition to adenylyl cyclase, cannabinoid receptor ligation has been shown to activate PLC [52], which in turn, might activate diacylglycerol and inositol triphosphatase, increasing intracellular calcium and PKC. Ligation also has been shown to inhibit Ca^{2+} currents and activate K currents [53, 54] and to increase NO [55] synthesis (Fig. 1). Cannabinoid receptor ligation also has been shown to lead to the activation of transcription factors such as members of the MAPK [56] and NF-κB [57, 58] families. From this and other evidence, it is clear that signaling through cannabinoid receptors and other GPCR involves numerous second messengers and signaling factors and that a variety of cellular and genetic activities are regulated by these kinds of receptors, including neuronal excitability [59] and IL-12 gene expression [49].

Cannabinoid receptors and ligands are found in the brain and peripheral tissues, and our current understanding of its function, at least in the brain, is beginning to take shape. The role of endocannabinoids and cannabinoid receptors in modulation of neurotransmitter release recently has been reviewed [59–61]. Several reports in 2001 showed that CB1 is located on presynaptic nerve terminals, that endocannabinoids are released from depolarized postsynaptic neurons, and that CB1 agonists and antagonists can modulate presynaptic neurotransmitter release [62–64]. Stimulation of the CB1 receptors was shown to suppress the release of neurotransmitters, such as GABA, thus allowing the cannabinoid system to turn down the activity of the GABA terminal in a retrograde manner back across the synapse. In the case of GABA release in hippocampal cells, the cannabinoid system appears to mediate depolarization-induced suppression of inhibition and may account for the inhibitory effects of cannabinoid ligands on memory and movement [60].

IMMUNE MODULATION

Cellular effects

Prior to the discovery of the cannabinoid receptors and the endocannabinoids, numerous studies examined the effects of marijuana smoking or THC on immune cell function. These studies have been extensively reviewed over the years [7, 12–18] and support several conclusions. The first of these is that moderate marijuana smoking has little in the way of acute effects on systemic immunity but may have significant suppressive effects on the function of immune cells directly exposed to the smoke. For example, marijuana smoking was associated with suppressing the antimicrobial activity, cytokine production, and cytokine responsiveness of pulmonary alveolar macrophages [65, 66]. However, regarding heavy or chronic use, sufficient studies have not been done to conclude that intense drug exposure is not without consequence on immune cell function. In fact, several studies suggest that long-term use in humans may be associated with increased incidence of infection [67, 68], squamous cell carcinoma of the head and neck [69, 70], and modulation of cannabinoid receptors in immune cells [71]; in nonhuman primates, heavy use may be associated with suppression of lymphocyte [72] and alveolar macrophage [73] function. Another conclusion from early studies is that treatment of mice and rats with THC or treatment of immune cell cultures from humans and rodents has, for the most part, a suppressive effect on T and B cells, natural killer (NK) cells, and macrophages. Various functions from cytotoxic T cell killing to antibody production by B cells to phagocytosis and killing by macrophages have been studied. Most functions were suppressed by drug treatment, and the effective drug concentrations in vitro were in the μM range. As this concentration range was higher than the reported receptor affinities, it was concluded by many that nonspecific effects of these lipophilic drugs on cell membranes primarily mediated the effects of cannabinoids on immune cell function. However, since the cloning of the receptors, it is now recognized that immune cells do express cannabinoid receptors and therefore, the modulating effects of cannabinoids are being reexamined in the context of cannabinoid receptor function. In addition, questions are being asked concerning the possible role of the immunocannabinoid system of receptors and ligands in the normal regulation of immunity.

Cannabinoid receptor expression in immune cells

By Northern blotting, the initial report on the cloning of CB1 failed to detect the expression of CB1 mRNA in tissues outside of the brain [40]. However, the cloning of the human gene demonstrated the message in testis tissue, but again, other tissues, including immune system tissues, were negative [41]. In 1992, Kaminski et al. [74] reported the demonstration of CB1 mRNA in mouse splenocytes using the more sensitive method of reverse transcriptase-polymerase chain reaction (RT-PCR). The expression of receptors by these cells was also

supported by pharmacological studies using cannabinoid agonist effects on in vitro antibody production by the splenocytes. Several agonists, such as THC, CP55,940, and HU210, suppressed antibody production in a dose-dependent manner when added to sheep red blood cell-stimulated splenocyte cultures. However, the affinity and efficacy of these agonists were lower with spleen cells than reported for behavioral studies and studies involving neural tissue, suggesting a lower density of surface receptors and variation from neural tissue in receptor coupling and affinity in immune cells. Also, as immune cells express CB2 receptors in addition to CB1 (see below), it is not clear whether CB1 or CB2 was being stimulated in these studies. Subsequent studies showed that CB1 mRNA levels varied among cell subpopulations. In human peripheral blood cells, message was reported to be most abundant in B cells, and the rank order was B cells > NK cells > polymorphonuclear neutrophils (PMNs) > CD8 cells > monocytes > CD4 cells [75]. The level of message also varied among different leukocyte cell lines; Jurkat cells were negative, and THP1 cells were highly positive [75]. CB1 message levels also varied in mouse immune subpopulations, and consistent with human cells, splenic B cells expressed more message than macrophages or T cells [76–78]. In addition to mouse splenocytes, CB1 message has been detected in rat brain microglia cells [79] and in human peripheral blood cell-derived dendritic cells (DC) [80]. We also have observed that CB1 and CB2 mRNAs are expressed in bone marrow-derived DC from mice (unpublished results).

Antibodies to CB1 have been produced and used to examine the expression of CB1 protein on immune cells. Rabbit polyclonal antiserum to a CB1–glutathione-*S*-transferase (GST) fusion protein demonstrated several immunoreactive proteins in membrane preparations from Jurkat and a CB1-positive neuroblastoma cell line. Proteins of 45, 60, 70, and 87 kDa were consistent with CB1 expression [81]. Similarly, a 58-kDa protein was demonstrated in membranes from rat microglia cells using an antiserum to CB1–GST fusion proteins [79], and proteins of 64 and 83 kDa were demonstrated in human DC [80]. We have prepared polyclonal rabbit antiserum to the 14 terminal amino acids of CB1 conjugated to keyhole limpet hemocyanin (KLH) and used this to analyze cell membrane proteins from mouse splenocyte immune cell subpopulations. **Figure 2** shows the antiserum detected a major band at 80 kDa in splenocyte subpopulations with B cells and macrophages showing more protein than T/NK cells. In addition, the neuroblastoma cell line, N18TG2, showed several bands, and the predominant one was at 67 kDa, suggesting a variation in immunoreactive protein size between brain cells and immune cells. We also tested several commercial polyclonal antisera prepared to CB1 peptides. **Figure 3, A** and **B**, shows these antibodies demonstrate major bands at 65 kDa (Company A) and 75 kDa (Company B) and that these bands are also the major ones demonstrated in the neuroblastoma cell line. Curiously, all three antisera (Fig. 3, A–C) demonstrate analogous protein bands in spleen cell preparations from CB1 homozygous knockout mice provided by Dr. Andreas Zimmer and co-workers [82]. This could result from several factors. The first is that other proteins expressed in mouse immune and neural tissue share epitopes with the CB1 protein. A search of the mouse genome using the website <http://www.ensembl.org/> and the 14 amino acids of the amino terminal end of the mouse CB1 protein shows scores of identical matches of five amino acids or less in the translated portion of the genome, suggesting that these CB1 epitopes are shared by many proteins of the mouse. A second possibility stems from the fact that in the knockout mice, the CB1 gene is disrupted with the *neo*^{r} cassette in the middle of the single-coding exon, leaving short 5′ and 3′ portions of this exon intact [82]. In fact, mRNA containing the 5′ end of the coding exon up to position 93 (accession #U22948) can be detected by RT-PCR in splenocytes from these mice at a higher level than in wild-type mice (unpublished results). It is possible, therefore, that fusion proteins containing amino and carboxy fragments of CB1 plus the *neo*^{r} insert are expressed in these mice and are being detected by the antisera. We are currently testing antiserum to epitopes coded by the deleted portion of the CB1 gene to see whether it can detect proteins of comparable size in immune tissue from knockout and wild-type mice and N18 cells. It is possible that this antiserum will detect comparable bands in only wild-type and N18 cells, suggesting the possibility of expression of CB1 fusion proteins in knockout mice. At this time, the specificity of available anti-CB1 antibodies remains in question.

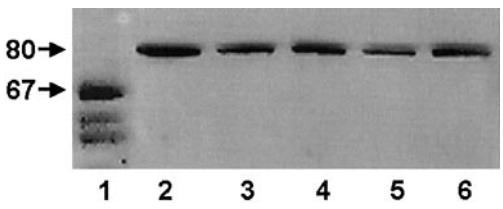

Fig. 2. Western blotting of membrane proteins from splenocytes using anti-CB1 antibody. Affinity-purified, polyclonal antiserum to CB1 peptide (MKSILDGLADTTFR-C) was used (160 ng/ml) to probe membrane proteins from splenocyte subpopulations and N18TG2 neuroblastoma cells. Lane 1, N18 cells; lane 2, whole splenocytes; lane 3, nonadherent splenocytes; lane 4, adherent splenocytes; lane 5, T cells; lane 6, B cells.

Unlike CB1, CB2 was cloned from a leukocyte cell line rather than brain tissue [44]. CB2 transcripts were detected in HL60 cells and rat spleen but not in other tissues such as thymus and brain. mRNA for the receptor was also detected by RT-PCR in macrophages from rat spleen but not in T cells [44]. CB2 mRNA expression was extended to human tissues, wherein by Northern blotting, it was demonstrated in only spleen and peripheral blood mononuclear cells (PBMC) and not in other tissues including brain; however, by RT-PCR, it was demonstrated in immune tissues including PBMC, thymus, spleen, and tonsils [83]. When examining PBMC subpopulations, CB2 message was in the rank order of B cells > NK cells > monocytes > PMNs > T cells. These and other studies suggested that CB2 mRNA was expressed primarily in immune cells but not in brain and that the message was most abundant in B cells [46, 77, 83, 84]. Rodent peritoneal macrophages, cell lines, and microglia cells were also shown to be positive for CB2 mRNA when examined by mutational RT-PCR [85]. As in other studies, the CB2 message was more abundant than CB1 and was readily expressed in cortical primary microglia.

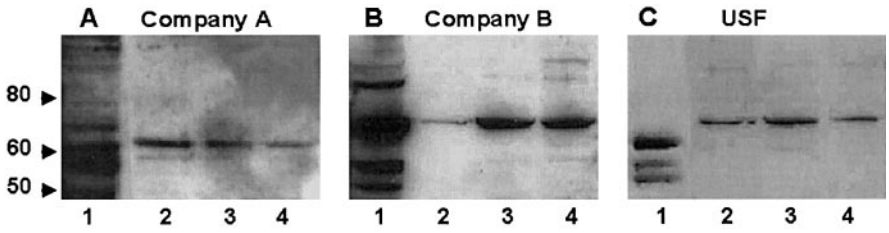

Fig. 3. Analysis of CB1 by Western blotting of splenocyte membrane proteins from wild-type and knockout mice. (A) Membrane proteins probed with anti-CB1 antibody from Company A: lane 1, N18TG2 neuroblastoma cells; lane 2, wild-type splenocytes; lane 3, wild-type splenocytes; lane 4, homozygous knockout splenocytes. (B) Membrane proteins probed with anti-CB1 antibody from Company B: lane 1, N18 cells; lane 2, wild-type splenocytes; lane 3, wild-type splenocytes; lane 4, homozygous knockout splenocytes. (C) Membrane proteins probed with antibody prepared as in Figure 2: lane 1, N18 cells; lane 2, homozygous knockout splenocytes; lane 3, heterozygous knockout splenocytes; and lane 4, wild-type splenocytes. USF, University of South Florida.

As with CB1, antibodies also have been used to examine the expression of CB2 protein on immune cells. Polyclonal antiserum to a C-terminal peptide of CB2 was used for immunofluorescence studies in human tonsillar tissue [83]. Immunoreactive tissue was highest in germinal centers, suggesting B cells or antigen-presenting cells (APCs) were expressing the receptor. Polyclonal antiserum produced to amino acids 320–336 of the mouse protein conjugated to KLH was used to examine CB2 protein expression in homogenates of cultured rat peritoneal macrophages and brain microglia [85]. The main immunoreactive protein in peritoneal macrophages was ~40 kDa, and in microglia preparations, the protein was 27 kDa. The reason for the relatively small size of the microglia protein was not clear but was suggested to be a result of a degradation product of the predicted 40-kDa protein or a variant form unique to microglia [85]. In addition to these studies, commercial antibodies were used to analyze CB2 protein in rat spleen and human blood-derived DC [80]. In these samples, immunoreactive bands of 39, 47, and 59 kDa were observed, although the reason for the multiple bands was not clear. As with anti-CB1 antibodies, the specificity and reliability still remain in question.

Modulation of cannabinoid receptor expression by cell activation

Immune cells frequently express new gene products when stimulated with antigens and other bioactive substances. This also appears to be the case with cannabinoid receptors. The initial suggestion of this came with the cloning of CB2, wherein it was shown that CB2 mRNA was increased in HL60 cells by treatment with agents such as phorbol myristate acetate (PMA) [44]. At basal levels, HL60 cells were negative for CB2 mRNA by Northern blotting but became positive by 24 h following 12-*O*-tetradecanoylphorbol 13-acetate treatment. Since this report, stimuli, ranging from lipopolysaccharide (LPS) to anti-CD40 antibodies (**Fig. 4**), have been shown to increase CB2 expression. For example, the level of CB2 mRNA and protein in human tonsillar B cells [86] and mRNA in mouse splenocytes [84] was increased following activation by anti-CD40 antibody, and CB2 expression was increased by IFN-γ in mouse macrophages and microglia [85]. In addition, we recently showed that chronic marijuana smoking increased the CB2 mRNA in PBMC [71], suggesting that drug exposure may cause a change in receptor expression in humans as has been reported with CB1 in animal models [87]. Stimulation of immune cells also leads to a suppression of CB2 expression (Fig. 4). For example, the differentiation of B cells in tonsils was accompanied by decreased expression of CB2 mRNA and protein in centroblasts as opposed to virgin B cells [86], and the stimulation by LPS of mouse splenocytes, macrophages, rat microglia, and human DC resulted in decreased expression of CB2 message [80, 84, 85]. In addition, TGF-β treatment of cultured human peripheral blood lymphocytes led to a decrease in CB2 protein with increasing concentration of the cytokine [88].

CB1 expression is also modulated in immune cells by various stimuli (Fig. 4). Initially, it was reported that PHA treatment in Jurkat cells [81] and LPS treatment of the mouse macrophage cell line, RAW 264.7 [89], induced an increase in CB1 message within several hours following treatment. However, studies with other cell types such as rat microglia [85]

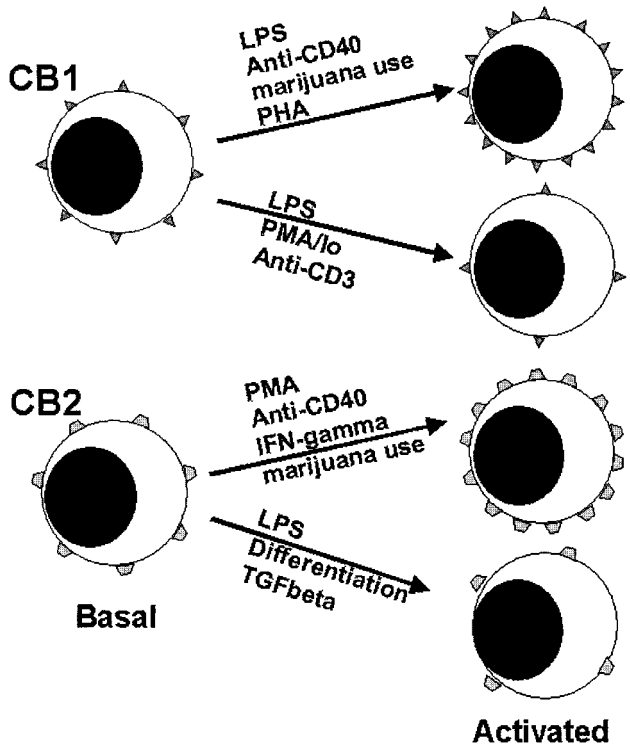

Fig. 4. CB1 and CB2 expression is increased or decreased following cell activation. Stimuli reported to increase CB1 are LPS, anti-CD40 antibody, PHA, and marijuana use, and agents suppressing CB1 are LPS, PMA/ionomycin (Io), and anti-CD3 antibody. Stimuli reported to increase CB2 are PMA, anti-CD40 antibody, interferon-γ (IFN-γ), and marijuana use, and agents suppressing CB2 are LPS, differentiation, and transforming growth factor-β (TGF-β). PHA, Phytohemagglutinin.

and human DC [80] showed that LPS treatment decreased rather than increased mRNA. The results with mouse splenocytes were even more complex with PMA/ionomycin or anti-CD3 antibody treatment, causing a decrease in CB1 message, and anti-CD40 treatment caused an increase [78]. It also has been reported that chronic marijuana smoking led to increased CB1 mRNA in PBMC, suggesting that drug use not only increased CB2 (see above) but also CB1 receptor expression [71]. From these studies, it is likely that the level of CB1 and CB2 receptors is modulated like many other immune gene products to varying degrees in various immune cell subpopulations. Although a few general themes are emerging in this regard, additional studies are needed using purified cells and detection reagents to define receptor expression in the immunocannabinoid system.

Cannabinoid receptor signaling in immune cells

A number of studies have defined various signaling events associated with CB1 and CB2 (see Function of cannabinoid system section). Many of these results were obtained in receptor-transfected cell systems or primary cells other than immune cells. However, a few reports have examined signaling events in immune cells in response to cannabinoid treatment. For example, intracellular calcium mobilization was studied in concanavalin A (Con A)-treated mouse thymocytes using fluorescent indicators and drug treatment with THC [90]. Cytosolic-free calcium was increased by Con A treatment, and this effect was suppressed by treatment with THC. The suppression of cytosolic calcium mobilization was a result of a drug-induced inhibition of extracellular influx as well as inhibition of calcium release from intracellular stores [90]. THC was also shown to suppress forskolin-stimulated adenylyl cyclase activity in mouse spleen cells [91] and purified splenic T cells [77]. The suppression of adenylyl cyclase by THC and CP55,940 was shown to be attenuated by treatment with pertussis toxin, suggesting that the cannabinoids were acting through G_i proteins [92]. Members of the NF-κB family of proteins also have been shown to be modulated in immune cells. The NK-like cell line, NKB61A2 [93], when treated with THC, was shown to increase the transcription of the IL-2Rα gene and increase the nuclear level of NF-κB protein [58]. However, in the macrophage cell line, RAW 264.7, THC was shown to inhibit NF-κB activity in response to treatment with LPS [57], suggesting that the type of cannabinoid effect on this family of proteins may be cell type- and stimulus-dependent. Several other immune cell lines have been tested for signaling modulation following cannabinoid treatment. The HL60 cell line was treated with CP55,940 and was shown to express higher levels of MAPK and Krox-24 gene activation [56], suggesting these signaling properties were increased by the drugs. In addition, EL4.IL2 mouse lymphoma cells treated with cannabinol or THC showed a decrease in PKA activity, activated protein-1, and NF-activated T cells binding [94, 95]; furthermore, treatment of splenocytes with cannabinol decreased ERK–MAPK activity [96]. From these results, it appears that cannabinoid treatment of immune cells leads to modulation of various cannabinoid receptor-signaling pathways. This fact, coupled with immune modulation effects of these agents, suggests that cannabinoids may modulate immune cell function through CB1 and CB2 signaling mechanisms.

Modulation of T helper (Th) cells

Th cells are powerful regulators of cell-mediated (Th1) and humoral (Th2) adaptive immunity. Regulation of the development of these cells and the link between innate and adaptive immunity are areas of intense investigation [97] to better understand how infectious agents and other environmental factors affect the processes fundamental to activating host immunity. It is now clear that in addition to microbial products, drugs and endogenous factors regulate the development of Th1 and Th2 cells (**Fig. 5**). For example, prostaglandins increase Th2 activity and decrease Th1 activity [2]. In addition, glucocorticoids [98], morphine [99], and certain chemokines [49] are reported to suppress Th1 immunity, and adrenergic agents such as norepinephrine increase Th1 immunity [100].

Cannabinoids were observed to suppress cell-mediated immunity to *Legionella pneumophila* infection when injected into mice shortly before infection. In addition, immunoglobulin G_1 antibodies to *Legionella* were also increased in drug-treated mice, suggesting that THC caused, besides a decrease in cell-mediated immunity, an increase in Th2 activity and therefore a change in the balance of Th1 and Th2 normally observed in this infection model [101]. Additional studies using this model showed that THC injection suppressed Th1 immunity by inhibiting the production of IFN-γ and IL-12 as well as the expression of IL-12 receptors, and in addition, drug treatment increased the expression of the Th2-promoting cytokine, IL-4 [102]. It was also demonstrated, using CB1 and CB2 antagonists, that both receptors were involved in this THC-induced Th cell biasing. Other laboratories have reported Th biasing

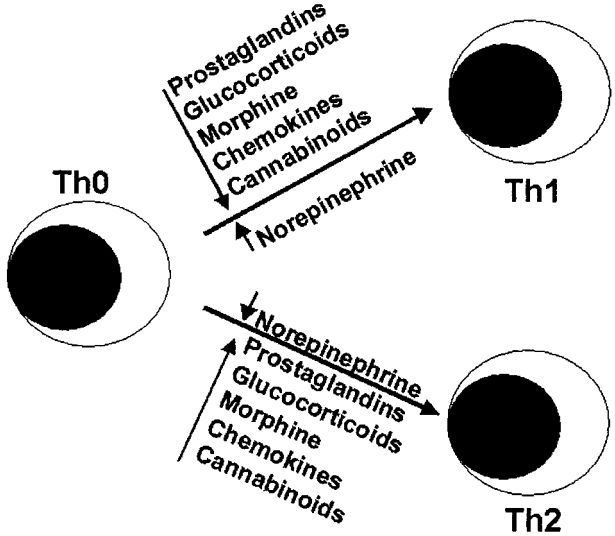

Fig. 5. Drugs and endogenous factors regulate the development of Th cells. Naïve CD4 Th (Th0) receive immune signals and in addition, receive signals from drugs and endogenous factors in the selective differentiation to Th1 or Th2 cells. Th1 development is decreased (↓) by prostaglandins, glucocorticoids, morphine, chemokines, and cannabinoids and is increased (↑) by norepinephrine. In contrast, Th2 development is decreased (↓) by norepinephrine and increased (↑) by prostaglandins, glucocorticoids, morphine, chemokines, and cannabinoids.

effects of cannabinoids (Fig. 5). Splenocytes taken from THC-injected mice and stimulated in vitro with Con A were shown to produce reduced amounts of IL-2 and IFN-γ [103] and *Propionibacterium acnes*-primed mice injected with LPS showed a decreased production of IL-12 and increase of IL-10 when pretreated with HU210 and other cannabinoids [104]. Biasing toward Th2 immunity was also reported in a murine tumor model [105]. Mice injected with tumor cells and THC showed enhanced tumor growth, depressed cell-mediated immunity, and increased Th2 activity such as production of IL-10 and TGF-β, and these effects were attenuated by treatment with the CB2 antagonist, SR144528. A similar shift to Th2 cytokines was demonstrated in response to THC in activated peripheral blood T cell cultures [106]. In drug-treated cultures, proliferation was inhibited along with Th1 cytokines; Th2 cytokines were increased, and the CB2 antagonist inhibited the effects [106]. There is now ample evidence that cannabinoids and other GPCR agonists can modulate the development of Th (Fig. 5). In the case of cannabinoids, it is possible that CB1 and CB2 may be differentially expressed on different subpopulations of APCs and Th cells. This selective expression could lead to an increase in Th2 development and a decrease in the development of Th1 cells, resulting in decreased cell-mediated immunity and increased antibody immunity. Continued research on the distribution and function of cannabinoid receptors as well as the production of endocannabinoids by different immune cells will provide greater insight into these mechanisms.

Cannabinoids and chemotaxis

Chemokine receptors, as noted above, are GPCR. It appears that other receptors of this group such as those for opioids and cannabinoids also are linked to cell-migration gene programs. For example, opioids are chemotactic when added to cultures of human blood monocytes and neutrophils, and these drugs can cross-desensitize cells to chemotaxis in combination with chemokines such as CCL5 [107]. These and other results provided evidence that the process of heterologous desensitization was occurring among chemotactic receptors that have varying affinities for different ligands such as chemokines and opioids. Cannabinoids also appear to have chemotactic activity for various cell types. HL60 cells expressing CB2 receptors were shown to migrate in response to CP55,940, and the migration could be demonstrated in experiments measuring chemotaxis and chemokinesis [108]. Drug treatment was also shown to increase the production of various cytokines and chemokines. Other cell types of immune lineage have been shown to migrate in response to cannabinoids. Myeloid leukemia cells expressing CB2 receptors displayed chemotaxis and chemokinesis in response to 2-AG but not in response to a variety of other cannabimimetic agents, and the migration was enhanced in the presence of IL-3 or granulocyte macrophage-colony stimulating factor [109]. 2-AG was also chemotactic for mouse spleen cells, and it was concluded that a major function for the CB2 receptor on these cells was the regulation of migration [109]. Mouse microglia cells also were shown to migrate in response to 2-AG through a CB2-mediated mechanism, and this effect was antagonized by the nonpsychotropic cannabinoids, cannabinol and cannabidiol [110]. Finally, in contrast to these immune cell studies, nonimmune cell migration is suppressed in response to cannabinoids. Human umbilical vein endothelial cells, when precultured with cannabinoids for 18 h, showed reduced chemotaxis in response to lysophosphatidic acid [111]. The CB2 antagonist inhibited this effect, and cannabinoid treatment was shown to suppress other functions involved in angiogenesis. From these studies, it appears that cannabinoids are similar to opioids in modulating cell migration and suggest that at least one function of the immunocannabinoid system is to regulate migration of immune cells.

Cannabinoids and tumor suppression

Marijuana smoking has been related to an increased incidence of head and neck cancer (see Cellular effects section); however, paradoxically, cannabinoids have been shown to suppress tumor development. Anandamide and other cannabinoids induce apoptosis in human PBMC, and this was suggested to account for the drug-induced suppression of lymphocyte proliferation [112]. These drugs were also shown to induce apoptosis and decrease Bcl-2 in macrophages and splenocytes, and this was suggested to contribute to increased processing and release of IL-1 [113]. Anti-tumor effects also have been linked to cannabinoid induction of apoptosis. Established gliomas in rats regressed in response to intra-tumor injection of THC and WIN55,212 [114]. Treatment of C6 glioma cells in culture with cannabinoids induced apoptosis by a pathway involving cannabinoid receptors and discrete signaling pathways involving ceramide and ERK activation. Similar experiments were performed using the CB2-selective agonist, JWH-133 [115]. Again, regression of established C6 tumors was observed following intra-tumor injection. In addition, a CB2 antagonist inhibited the effects, and cultures of C6 cells were induced to apoptosis in response to treatment with cannabinoids [115]. In other studies, proliferation of C6 glioma cells was suppressed by cannabinoids, an effect mediated by cannabinoid and vanilloid receptors [116]; furthermore, a variety of human and mouse malignant immune cell lines were induced to apoptosis by cannabinoid treatment, were suppressed in vivo by cannabinoids, and were inhibited through CB2-mediated mechanisms [117]. From these studies, it appears that cannabinoids can induce apoptosis and the inhibition of tumors through CB1 and CB2 mechanisms, suggesting their potential as anti-cancer drugs.

In addition to effects mediated by apoptosis, cannabinoids appear to suppress tumor cell growth by several other mechanisms. One of these involves the suppression of prolactin receptors. Endocannabinoids and synthetic cannabinoids were observed to inhibit the proliferation of human breast cancer cells [118]. The effect was CB1-dependent and resulted from the down-regulation of the long form of the prolactin receptor. Subsequent studies showed that endocannabinoids such as anandamide suppress the receptor by inhibiting adenylyl cyclase and MAPK and also can inhibit nerve growth factor receptor by CB1- but not CB2-mediated mechanisms [119, 120]. From this, it appears that in some tumor suppression systems, both cannabinoid receptors are not involved, as in the case of apoptosis, suggesting cannabinoids affect tumor growth by mechanisms other than programmed cell death. Finally,

cannabinoids have been reported to suppress tumor growth in several other ways, such as inducing the accumulation of lipid droplets in the tumor cells [121] and the suppression of angiogenesis [111], again suggesting that these drugs have the potential to affect tumor growth in a variety of ways. In contrast, the CB2 receptor has been reported to have oncogenic activity by inhibiting neutrophil differentiation through MEK/ERK and phosphatidylinositol-3 kinase signaling pathways [109]. It appears that the effect of marijuana and cannabinoids on tumor growth and development is exceedingly complex, showing enhancing and suppressive effects. This would suggest that the application of these agents to tumor therapy should proceed slowly and with consideration of all possible outcomes.

SUMMARY

There is increasing effort to define and understand the mechanisms of the brain-immune connection. Many immune cytokines and brain neurochemicals engage in cross-talk between the immune and brain systems, orchestrating the regulation of immunity and chronic inflammatory diseases of the brain. The immunocannabinoid system of receptors and endogenous ligands appears to be a component of the brain-immune connection, and the details of its structure and function are now being elucidated. Immune cells express CB2 receptors and most probably, CB1 receptors, but considerable work is still needed to understand the distribution of these receptors among the various immune subpopulations, and in addition, a greater understanding is needed to define the factors that regulate receptor expression. It also is apparent that cannabinoid receptor signaling regulates the expression of various genes in immune cells; however, the scope and mechanisms of these gene activation programs await further definition. Cannabinoid receptors are GPCR, and the importance of this family of receptors in immune cell function is increasingly recognized. Regulatory functions are emerging for these receptors involving the mechanisms of Th cell biasing as well as chemotaxis of leukocytes and the regulation of apoptosis and tumor suppression. These developments involving the cannabinoid system and immune regulation suggest the possibility that the components of this system will be exploited in the future for therapy of chronic diseases and immune deficiencies. Furthermore, continued work in this area will lead to a better understanding of immune regulation in general and the brain-immune connection in particular.

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