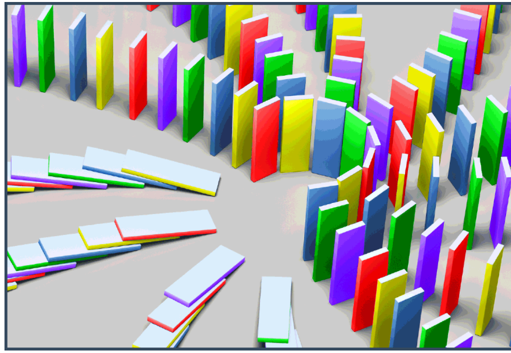

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Δ^9-Tetrahydrocannabinol Treatment Suppresses Immunity and Early IFN-γ, IL-12, and IL-12 Receptor β2 Responses to *Legionella pneumophila* Infection[1]

Thomas W. Klein,[2] Catherine A. Newton, Noryia Nakachi, and Herman Friedman

The marijuana cannabinoid, Δ^9-tetrahydrocannabinol (THC), suppresses immunity to *Legionella pneumophila* and development of Th1 activity and cell-mediated immunity. In the current study, THC effects on cytokines regulating the development of Th1 cells were examined. BALB/c mice showed significant increases in serum IL-12 and IFN-γ within hours of infection; however, the levels of these Th1-promoting cytokines as well as resistance to a challenge infection were suppressed by THC (8 mg/kg) injected 18 h before priming. The Th2-promoting cytokine, IL-4, was increased within hours of a *Legionella* infection and was further increased by THC treatment. These results suggested that THC injection suppressed the cytokine environment promoting Th1 immunity. In additional experiments, THC pretreatment and infection of IL-4 knockout mice showed that serum IL-12 and IFN-γ were suppressed equally in both knockout and normal mice. This suggested that the drug-induced increase in IL-4 was not responsible for the decreases in serum IL-12 and IFN-γ. However, THC treatment was shown to suppress the expression of IL-12 receptor β2 mRNA, indicating that, in addition to suppression of IL-12, THC injection suppressed the expression of IL-12 receptors. Finally, the role of cannabinoid receptors in Th1-promoting cytokine suppression was examined, and results with receptor antagonists showed that both cannabinoid receptors 1 and 2 were involved. It is suggested that suppression of Th1 immunity to *Legionella* is not due to an increase in IL-4 production but to a decrease in IFN-γ and IL-12. Furthermore, both types of cannabinoid receptors are involved. *The Journal of Immunology,* **2000, 164: 6461–6466.**

The psychoactive effect of marijuana smoke is caused primarily by the cannabinoid, Δ^9-tetrahydrocannabinol (THC)[3] (1, 2). This drug and various analogues produce effects by binding to cannabinoid receptors in the brain and periphery, and recent evidence suggests that cannabinoid receptor 1 (CB1) is expressed in the former while cannabinoid receptor 2 (CB2) is expressed in the latter (3). Endogenous ligands for cannabinoid receptors derived from arachidonic acid have also been described, thus supporting the existence of a complete endogenous cannabinoid system of receptors and ligands (4). The purpose and function of this system is currently under investigation.

Besides psychoactivity, THC and other cannabimimetic agents have been shown to modulate immune function in animals and humans (5). However, the impact of these effects on human health and disease is unclear, and the involvement of cannabinoid receptors is also unclear. The possible adverse effects of marijuana and cannabinoids on immunity to infection is of particular concern, and we have begun testing the possibility that THC might compromise host resistance to infection with opportunistic bacterial agents such as *Legionella pneumophila*. We have developed a mouse model of *Legionella* infection that displays many of the cellular and cytokine features involved in immunity to many other intracellular bacteria including the activation of Th1 cells and cell-mediated immunity (CMI) (6–8). For example, splenocytes from sublethally infected mice become sensitized and proliferate to a greater extent and produce more IFN-γ when stimulated with *Legionella* Ags. Furthermore, reinfection of the mice induces an increase in these responses and an increase in splenic and PBL T cells (6). Th1 activity is also increased following *Legionella* infection (8). Within 4–5 days, IgG2a Ab and splenocyte IFN-γ production are on the rise, and these responses precede the development of immune memory to *Legionella* infection. Previously, we reported that the injection of THC in this model suppressed the memory response and the development of Th1 activity and increased the Th2-associated response, IgG1 Abs to *Legionella* (8). Recent evidence has now established that the relative differentiation of Th1 and Th2 cells depends in part on the action of either IL-12 or IL-4 (9), with IL-12 causing the generation of Th1 cells while IL-4 inducing Th2 cells. In the current report, we show that suppression by THC of Th1-promoting cytokines is not due to an increase in IL-4 but more likely due to suppression in the production of IFN-γ, IL-12, and IL-12 receptor β2 (IL-12Rβ2). In addition, we show that CB1 and CB2 antagonists attenuate the drug effects on cytokine production, suggesting that both receptor subtypes are involved in the modulation of immunity.

Materials and Methods

Bacteria and drugs

Serogroup 1, *L. pneumophila*, isolated from a case of Legionnaires' disease at Tampa General Hospital was grown on buffered charcoal yeast extract plates (Difco, Detroit, MI) for 48 h from a passage no. 3 stock maintained at −80°C. This strain is highly virulent for guinea pigs and readily grows

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[3] Abbreviations used in this paper: THC, Δ^9-tetrahydrocannabinol; CMI, cell-mediated immunity; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; IL-12Rβ2; IL-12 receptor β2.

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in macrophage cultures (10). SR141716A, a CB1 antagonist, was a generous gift from M. Rinaldi-Carmona (Sanofi Recherche, Montpellier, France), and SR144528, a CB2 antagonist, as well as THC were obtained from the Research Technology Branch of the National Institute on Drug Abuse. The antagonists and THC were initially diluted in DMSO to either 50 mg/ml (THC) or 25 mg/ml (antagonist). They were then diluted 1:25 in normal mouse serum to either 2 mg/ml (THC) or 1 mg/ml (antagonist). These were then injected i.v. into mice to yield doses of either 8 or 4 mg/kg. For vehicle control injections, mice received i.v. 0.1 ml of DMSO diluted 1:25 in normal mouse serum.

Animal injections and tissue sampling

Female BALB/cByJ mice and BALB/c-IL-4$^{-tm2Nnt}$ knockout mice (11) (The Jackson Laboratory, Bar Harbor, ME), 7–8 wk of age, were housed and cared for in the University of South Florida animal facility, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care. Normal mice were primed via the tail vein with $\sim 7 \times 10^6$ *Legionella* suspended in saline, while knockout mice, which were more susceptible to the priming infection with *Legionella,* were infected with fewer bacteria (3×10^6). For challenge infection, mice were injected with 5×10^7 *Legionella*. For drug treatment, THC (8 mg/kg) or the equivalent amount of drug vehicle (DMSO) diluted in serum was injected i.v. 18 h before infection, and the antagonists (4 mg/kg) diluted in serum, when used, were injected i.v. 30 min before THC. For mortality tests, the mice were observed continuously following *Legionella* infection, and those that became moribund were euthanized by CO_2 asphyxiation and added to the mortality total. Blood, collected by cardiac puncture, and spleens were obtained at the times indicated after infection and processed for cytokine analysis. Single-cell suspensions of splenocytes were cultured in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated FCS (HyClone, Logan, UT), 2-ME (5 μM), and antibiotics for 3 h for the IL-4 determinations.

ELISA

IL-4, IL-12, and IFN-γ assays were determined using sandwich ELISAs with Ab pairs from PharMingen (San Diego, CA). Medium-bind, 96-well enzyme immunoassay plates (Costar, Cambridge, MA) were coated with 50 μl of capture Ab in PBS (anti-IFN-γ, 4 μg/ml; anti-IL-4, 2 μg/ml) or in 0.1 M $NaHCO_3$, pH 8.2 (anti-IL-12 p40/p70, 10 μg/ml), overnight at 4°C. The plates were blocked for 30 min with 150 μl of 0.5% BSA/0.05% Tween 20/PBS (IFN-γ, IL-4) or for 2 h with 3% BSA in PBS (IL-12). The culture supernatants, sera, or serial dilutions of cytokine standards were added for 1–2 h. Biotinylated detection Abs (IFN-γ and IL-12, 2 μg/ml; IL-4, 1 μg/ml) were added in 50-μl volumes for 1 h, followed by streptavidin-HRP (1:1000 in 50 μl) for 30 min. After the substrates were added, plates were developed for 5–45 min. The HRP reaction was stopped with 1 N sulfuric acid. The reactions were read at 450 nm (HRP) on an Emax microplate reader (Molecular Devices, Menlo Park, CA). Sample cytokine concentrations were generated from standard curves.

RT-PCR

Total RNA was extracted from mouse spleens by standard techniques using TriReagent (Sigma; 1 ml per 2×10^7 cells) and quantitated using RiboGreen RNA Quantitation Kit (Molecular Probes, Eugene, OR). The cDNAs were synthesized at 42°C for 45 min from 1 μg of total RNA by priming with 0.5 μg oligo$(dT)_{15}$ primer, 20 nmol each deoxynucleoside triphosphate, 0.5 U RNase inhibitor, and 15 U avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) in a final volume of 25 μl. The reverse transcriptase product, equivalent to 0.1 μg starting RNA, was used for PCR with mouse primers for IL-12Rβ2 (12) (5′-GAGTACAT AGTGGAATGGAGAG-3′ and 5′-TCACAGCTGTCATCCATAGGAC-3′) and β-actin (13) (5′-ATGGATGACGATATCGCT-3′ and 5′-ATGAG GTAGTCTGTCAGGT-3′). The PCR was performed in a Minicycler (MJ Research, Watertown, MS) for either 27 cycles and 63°C annealing (IL-12R) or 23 cycles and 63°C annealing (β-actin). PCR products were analyzed on ethidium bromide-stained, 2% agarose gels and quantitated and normalized to β-actin using densitometry readings (Bio-Rad, Hercules, CA). The data are presented as the ratio of IL-12R to β-actin densities for pools of mice.

Results

THC suppresses immunity to Legionella

BALB/c mice are relatively resistant to a challenge infection with *Legionella*. For example, they will survive bacterial doses of up to 10^7 bacteria administered i.v. However, they will succumb to higher doses unless they have been primed or immunized by a prior Ag exposure (8). In the current studies, individual mice were either nonprimed or primed with an injection of 7×10^6 viable *Legionella* following DMSO treatment. The mice were rested for 3 wk and challenged with a lethal dose of 5×10^7 bacteria. Fig. 1 shows that this dose of bacteria was uniformly lethal in the nonprimed group, with 50 of 50 mice dying. However, mice primed with a *Legionella* infection were totally resistant to the challenge infection, with 0 deaths occurring among 35 animals. To test the effect of THC on this immunization, animals were injected i.v. with THC (8 mg/kg) 18 h before the immunizing infection. As seen in Fig. 1, these animals were very susceptible to the challenge infection with approximately two-thirds (23 of 35 mice) succumbing to the infection. Because CMI must develop in the mice between the priming and challenge infections for the animals to survive (7, 8), these results suggested that THC treatment suppressed this response.

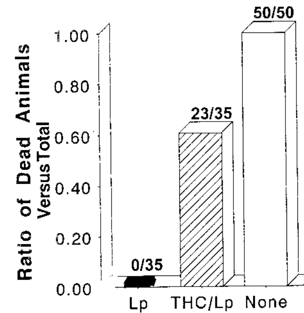

FIGURE 1. THC increases mortality to *Legionella* infection. Mice were either nonprimed (None), injected with DMSO and primed with *Legionella* (7×10^6; Lp group), or injected with THC followed 18 h later by *Legionella* (THC/Lp group). All groups were challenged 3 wk later with *Legionella* (5×10^7) and observed for mortality. The data are expressed as the ratio of the number of dead mice to the total tested. The totals in each of the groups were 50 for None, 35 for Lp, and 35 for THC/Lp.

THC injection increases Th2- and decreases Th1-promoting cytokines

Immunity to *Legionella* infection, as with many other facultative intracellular microbes, depends on the preferential development or polarization of Th1 cells over Th2 cells (14, 15). Mice infected with *Legionella* have been shown to preferentially mobilize Th1 cytokines in their immune tissues (8), and humans recovering from *Legionella* pneumonia show a predominance in serum of Th1-promoting cytokines such as IL-12 and IFN-γ over Th2-promoting cytokines such as IL-4 and IL-10 (16). We wanted to see if THC injection altered the cytokine environment promoting Th1 development. Mice were injected with either THC or DMSO, infected with *Legionella* as in Fig. 1 above, and bled at various times after infection. Serum was analyzed for IL-12 and IFN-γ by ELISA, and it was observed that in control mice both cytokines peaked in serum between 3 and 24 h; furthermore, it was observed that drug treatment decreased this cytokine mobilization (Fig. 2, *A* and *B*). IL-4 was not detected in serum at this time but could be demonstrated in splenocytes removed from the mice and cultured in vitro. Interestingly, instead of THC treatment decreasing IL-4 production, it increased the production (Fig. 2*C*). These results suggested that THC treatment caused an immune deviation from the expected Th1-promoting environment.

THC decreases IL-12 and IFN-γ in IL-4 knockout mice

The decrease observed in IL-12 and IFN-γ production could stem from a variety of causes including an increase in IL-4 production

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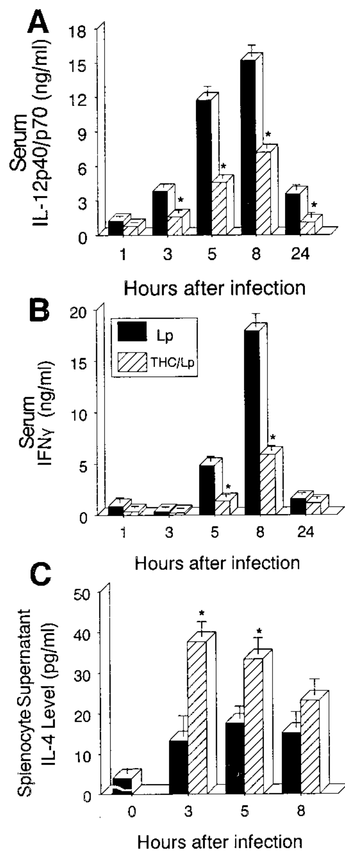

FIGURE 2. THC decreases IFN-γ and IL-12 and increases IL-4. Mice were injected with DMSO or THC (8 mg/kg) followed 18 h later by a *Legionella* infection. Within hours after infection, blood and spleen were removed and the cytokines measured by ELISA in serum (*A* and *B*) and in splenocyte supernatants (*C*). Splenocytes were incubated for 3 h, and the supernatants were removed for ELISA. Data are presented as the mean ± SEM, n = 8–20 mice per group.

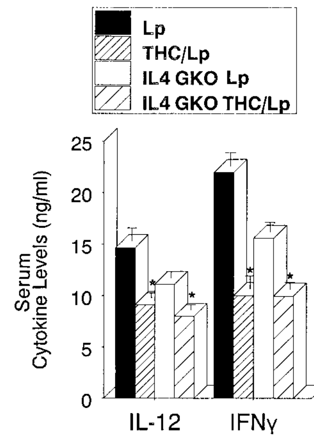

FIGURE 3. THC decreases IFN-γ and IL-12 in IL-4 knockout mice. Normal and IL-4 knockout BALB/c mice were injected with DMSO or THC followed 18 h later by a *Legionella* infection (7×10^6 normal and 3×10^6 knockout). Eight hours following infection, the mice were bled and the levels of IL-12 and IFN-γ in sera were measured by ELISA. The data are presented as mean ± SEM, n = 5 mice per group.

that has been shown to suppress the production of IFN-γ (17, 18). Indeed, THC-treated animals display heightened IL-4 activity in the spleen (Fig. 2*C*). Disruption of the IL-4 gene has been used to study the role of IL-4 in the differentiation of Th cells (19), and we employed this model to study the role of IL-4 in the THC-induced decrease in IFN-γ and IL-12 production. In our hands, the knockout mice differed from wild-type animals only in their sensitivity to the priming infection with *Legionella*. This appears to be due to the inability to regulate acute-phase cytokine mobilization (C. Newton, manuscript in preparation). Otherwise, the knockouts developed Th1 immunity and presented the same disease symptoms when challenged with *Legionella* or treated with THC. IL-4 knockout mice were infected with *Legionella* and treated with THC as above. At 5 and 8 h following infection, bloods were removed and IFN-γ and IL-12 were measured in the sera by ELISA. Fig. 3 shows that both cytokines were suppressed by THC treatment at 8 h after infection in both knockout mice as well as in mice containing the intact IL-4 gene. Similar results were obtained at 5 h (data not shown). Because the knockout mice produced no IL-4 (data not shown), it was concluded that the suppression of Th1-promoting cytokines was not due to heightened production of IL-4.

THC decreases IL-12Rβ1 mRNA

The development of Th1 cells is dependent on the expression and function of the IL-12 system including both the cytokine produced by macrophages and NK cells (20) and IL-12 receptors composed of two subunits, β1 and β2 (21). Both of these subunits appear to be important in the functioning of the IL-12 receptor (21, 22), and therefore we tested to see if THC altered the production of IL-12Rβ2. Groups of mice were either noninjected or injected with either DMSO or THC followed 18 h later by *Legionella* infection. At various times after infection, individual spleens were removed and processed for RT-PCR to measure mRNA for IL-12Rβ2 and the control gene, β-actin. Fig. 4 shows pooled data from 81 individual mice treated in the ways indicated, and data are presented as the means ± SEM of the densitometry ratios for each group of animals. These normalized results showed that *Legionella* infection rapidly increased the expression of IL-12Rβ2 message in the spleen up through 5 h after infection. However, prior THC treatment significantly suppressed the message expression at all time points following infection. These results suggested that THC treatment suppressed the expression of the IL-12Rβ2 subunit gene and therefore possibly suppressed the receptor expression and function in the spleen of infected mice.

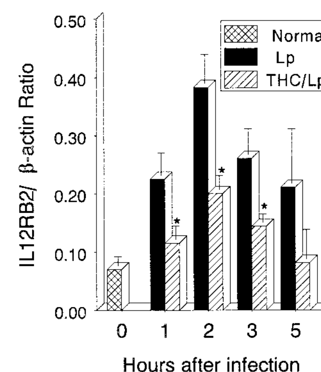

FIGURE 4. THC decreases the level of IL-12Rβ2 mRNA. Mice were either not injected (Normal group) or injected with either *Legionella* (Lp) or *Legionella* plus THC (THC/Lp). The spleens were then removed at various times and processed for RT-PCR. The figure shows results from six different experiments involving 81 individual mice treated in the ways indicated. The data are presented as the mean ± SEM of the densitometry ratios (IL-12R:β-actin) for each group of animals (average of nine mice per group).

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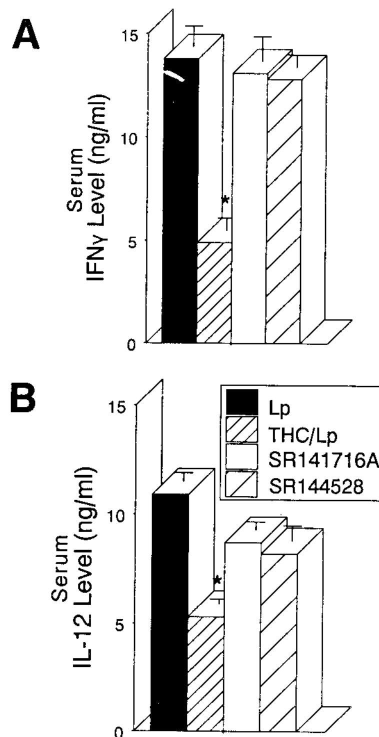

FIGURE 5. Cannabinoid receptor antagonists attenuate the THC effect on Th1-promoting cytokines. Groups of mice were injected with saline and *Legionella* (Lp group), saline, THC, and *Legionella* (THC/Lp group), or SR141716A or SR144528 followed by THC and *Legionella* (SR groups). Eight hours following *Legionella* infection, mice were bled for serum cytokine analysis by ELISA. The data are presented as the mean ± SEM, n = 7–13 mice per group.

Suppression of Th1-promoting cytokines is cannabinoid receptor mediated

Controversy exists concerning the role of cannabinoid receptors in THC-induced immunomodulation (5). Therefore, it was important to examine the involvement of CB1 and CB2 in the observed drug-induced suppression. Specific antagonists have been described that block the activity of cannabinoid receptors. For example, SR141716A inhibits CB1 (23) and SR144528 inhibits CB2 (24). Therefore, we injected mice with either antagonist compound (4 mg/kg) 30 min before THC treatment. As expected, the CB1 antagonist suppressed the catalepsy response (25) of the mice, while the CB2 antagonist had no effect in this regard (data not shown). Fig. 5, *A* and *B* show that both antagonists reversed at 8 h the suppressive effect of THC on the serum levels of IFN-γ and IL-12, respectively, suggesting that both receptors were involved. Similar results were observed at 5 h (data not shown).

Discussion

A number of studies dating back several decades have suggested that marijuana components such as THC modulate the resistance of animals to various infectious agents (26). However, only recently has it been reported that THC suppresses CMI to facultative, intracellular bacteria (8). Because of intense interest in determining possible harmful effects of marijuana (27) we have begun a reexamination of the effects and mechanisms of THC in an animal model of CMI to *L. pneumophila*. The immune system of mice can be sensitized to bacterial Ags by infection with a sublethal dose of viable bacteria. For example, infection of mice with *Legionella* results in the sensitization of splenocytes to *Legionella* Ags and mobilizes various immune cells during exposure to these Ags (6). If the immune response is sensitized sufficiently, the animals will become immune or resistant to subsequent challenge infection as shown in Fig. 1. Mice that were sublethally infected were shown to be completely resistant to a lethal challenge infection 3 wk later (Fig. 1). Using this animal model, we asked the question what effect THC administration would have on CMI development if given around the time of immune priming and activation. Our results definitively show that THC injection into mice shortly before the priming infection inhibits the development of protective immunity, thus leaving the animal susceptible to a subsequent challenge infection with a lethal dose of bacteria (Fig. 1). These results support our previous findings (8) and firmly establish that a relatively low dose of THC (8 mg/kg) can suppress the development of a protective immune response.

Immunity to *Legionella* infection, as with many other facultative intracellular bacteria, depends on the preferential development or polarization of Th1 cells over Th2 cells (14, 15). Our previous work (8) suggested that THC treatment suppressed Th1 activity, and therefore we wanted to examine this model further to determine whether the drug treatment suppressed the formation of cytokines that promote Th1 activity. In vivo cytokine levels can be analyzed in several ways in animals and humans undergoing immune stimulation. For example, tissues such as spleen and liver (28) can be removed and tested in culture; alternatively, serum can be removed and tested by ELISA (16, 29). Fig. 2 shows that mice infected with *Legionella* displayed readily detectable levels of IL-12 and IFN-γ in serum, reaching a peak between 8 and 24 h after infection. The cellular sources of these Th1-biasing cytokines were probably macrophages and NK cells as shown in other infection models (15, 20); in addition, T cells might also contribute as a source of these cytokines (our unpublished observation). Our IFN-γ results differ from a previous report that showed serum IFN-γ reached a peak at 24 h after *Legionella* infection (29). However, this difference is probably related to either variations in mouse strain or route of infection. We used the less susceptible strain, BALB/c, and our infections were done i.v., while the previous study used the more susceptible A/J strain of mice and inoculated the mice intratracheally (29). We also tested for the Th2-biasing cytokine, IL-4, but this was not detectable in the serum samples; however, it was detected in supernatants of cultured splenocytes removed within hours following infection (Fig. 2*C*) and was observed to increase through 5 h. This early increase in IL-4 has been observed by others (30), and although IL-4 generally biases toward the development of Th2 cells it can have a positive effect on Th1 development under the right conditions (31, 32). Regarding the effect of THC injection, it was observed to significantly decrease the serum level of IL-12 and IFN-γ but increase the supernatant level of IL-4 (Fig. 2). These results coupled with our previous findings (6, 8) show that treatment suppresses the development of Th1 immunity leading to increased susceptibility to *Legionella* infection.

Th1 cells produce cytokines such as IFN-γ that promote the resistance to and elimination of intracellular bacteria such *Legionella* (15). The development of Th1 cells from Ag-activated precursor cells requires the presence of IL-12 and IFN-γ as well as the presence of specialized dendritic cells (33). However, the presence of IL-4 can have an inhibitory effect on Th1 cell development (17, 18, 34), and because drug treatment increased the splenocyte production of IL-4 (see Fig. 2) we wanted to determine whether THC suppression of IL-12 and IFN-γ occurred in IL-4 knockout mice. The results showed (Fig. 3) that knockout mice were similar to

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intact mice in that the drug treatment reduced Th1-biasing cytokines, suggesting that THC was suppressing immunity by a mechanism other than increasing IL-4 production.

Th1 cell development is also attenuated by a down-regulation of IL-12R subunits (21, 22), and so we tested if THC treatment was in some way inhibiting the expression of these receptors. We found that splenocytes removed from mice after drug treatment and infection showed less IL-12Rβ2 mRNA by RT-PCR than did nontreated animals (Fig. 4), suggesting that the expression of this receptor subunit is inhibited in some way by the drug. Although, in this study, we did not define the cell type or types with depressed IL-12R mRNA, it is possible that both T cells and splenic adherent cells are affected because in preliminary purification studies (not shown) both cell types were observed to express IL-12R mRNA within hours of infection. The IL-12R complex is composed of two subunits, β1 and β2, belonging to the β-type cytokine receptor group (35). Both receptor proteins must be expressed for high-affinity binding of IL-12, and both are of importance in cell signaling following binding of IL-12. A decrease in expression of the β2 subunit mRNA as observed in our study has been correlated with a decrease in responsiveness to IL-12 and Th1 activation (22), and therefore it is possible that this effect of the drug is at least partially responsible for the decrease in CMI. However, demonstration of a decrease in subunit protein on splenocyte subsets is needed and is currently under investigation. As to how the drug is suppressing the expression of β2 mRNA, the induction of inhibitory cytokines such as IL-4, TGF-β, and IL-10 (22, 35) are a possibility, and we have observed that THC increases IL-4 (Fig. 2) as well as TGF-β and IL-10 in mice (our unpublished observations).

THC can exert its effects by both cannabinoid receptor- and nonreceptor-mediated mechanisms (36). The nonreceptor mechanism might involve insertion of the THC into the cell membrane lipid bilayer altering the function of membrane proteins (37). Receptor mechanisms would be mediated through the action of either CB1 or CB2 expressed in both the brain and peripheral tissues including the immune system (3). Elucidating the role of receptor involvement has been facilitated by the discovery of CB1 and CB2 antagonists (23, 24), and we employed these drugs to determine whether either receptor was involved in the THC effect. The results showed that both antagonists attenuated the drug effects on IL-12 and IFN-γ, supporting the conclusion that both receptors are involved (Fig. 5). Because there is evidence that CB1 and CB2 are expressed on cells of the immune system (5), it is possible that THC is acting directly on these cells (see below). However, CB1 receptors are also expressed in various regions of the brain such as the hippocampus and amygdala (38). These regions can regulate the hypothalamus (39) and thus regulate the hypothalamo-pituitary-adrenal axis, a known modulator of immune function (40). Therefore, it is possible that THC-induced increases in pituitary and adrenal hormones are modulating in our system. Indeed, injection of THC into rats causes a rapid increase in serum adrenocorticotropic hormone and corticosterone as well as a depletion of hypothalamic corticotropin-releasing factor, demonstrating this hypothalamo-pituitary-adrenal effect (41), and this steroid mobilization was implicated in immune modulation (42). Corticosteroids have also been reported to have effects on Th cells, favoring the development of Th2 responses over Th1 (43–45), which is consistent with our findings. Also consistent is a recent report (46) showing that dexamethasone treatment of immune cells suppressed the expression of IL-12R subunits similar to what we have observed. We are currently testing for an increase in corticosterone in our animals and will also look for an inhibitory function of RU486 in attenuating the THC effect on cytokine production.

THC injection might also be affecting immune cells directly through interaction with CB1 and CB2. These receptors are G protein-coupled receptors that are negatively coupled to adenylyl cyclase (47), and this coupling occurs in cells of the immune system (48). However, more recently, receptor ligation of CB1 in the presence of the G_i inhibitor, pertussis toxin, uncovered a stimulatory effect on adenylyl cyclase and an increase in cellular cAMP (49, 50). Therefore, it is possible that stimulation of immune cell CB1 or CB2 might either increase or decrease cAMP and various associated functions. For example, others have shown that increasing cAMP decreases IL-12 and IL12R proteins (46, 51, 52) but increases IL-10 (53). This is similar to what we have observed. Increasing cAMP can also interrupt the JAK-STAT signaling pathway important in cytokine cell signaling (54). In contrast, the production of IL-4 by lymphocytes can be suppressed by cAMP (55), which is the reverse of what we observed following THC injection.

Finally, another mechanism that might account for the effects observed in our studies involves the mobilization of arachidonic acid and PGE following THC injection. This effect was noted a number of years ago (56) and has more recently been shown to occur by cannabinoid receptor-mediated mechanisms (57) in cells of the immune system (58). PGE has been shown to either increase or decrease Th cytokines depending upon the protocol employed (46, 55, 59), and therefore mobilization by THC injection might account for some of the effects we have observed. We are currently examining the effect of indomethacin injection on the THC-induced modulation of Th cytokine.

In summary, the effect of THC on cytokines promoting the development of Th1 cells involves cannabinoid receptors and probably a variety of other physiologic and molecular mechanisms. This complexity of mechanisms for THC is significantly compounded when trying to understand the effects and mechanisms of action of marijuana smoke in various immune systems. Clearly, more studies are needed to understand these drug effects as well as understand the role of cannabinoid receptors in host defenses.

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