Psychoactive Cannabinoids Increase Mortality and Alter Acute Phase Cytokine Responses in Mice Sublethally Infected with *Legionella pneumophila*

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Abstract. **Marijuana contains both psychoactive and nonpsychoactive cannabinoids which have varying effects on the immune response system. Previous studies with δ-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, showed that this substance augmented the susceptibility of mice to infection with the opportunistic pathogen *Legionella pneumophila*. The present study compared the enhancement of Legionella-induced mortality in mice due to two other major of marijuana components, cannabinol and cannabidiol, as well as the synthetic psychoactive cannabinoid CP 55,940. Inbred BALB/c mice, relatively resistant to infection with Legionella, were given the marijuana component 1 day before and 1 day after a sublethal intravenous infection with Legionella. Unlike the effect of THC, an 8 mg/kg dose of either cannabinol or cannabidiol did not affect mortality of the mice sublethally infected with Legionella. Mice given a 16 mg/kg dose of these components of marijuana, however, showed a slight to moderately increased mortality following the sublethal infection with Legionella. In contrast, a dose of 6 mg/kg of the synthetic psychoactive cannabinoid CP 55,940 given 1 day before and 1 day after infection with Legionella resulted in about 50% of the animals dying, the same level of augmentation of lethality induced by THC. Liver, spleen, and lung tissues were removed from the surviving mice 24 hr after the second THC injection and tested for the presence of viable Legionella using a standard CFU assay. The mice injected with THC before and after infection showed significantly higher levels of bacteria in their lung than mice that were not given any cannabinoid but were infected sublethally with the Legionella. Mice injected with the other cannabinoids, including the synthetic cannabinoid, showed a much smaller increase in the number of Legionella in their lung when infected with Legionella and treated with the drug. The number of bacteria recovered from the kidney and liver of the mice regardless of treatment with a cannabinoid, including with THC, did not show significant changes. RNA isolated from the spleen of the THC- and Legionella-treated animals was examined to determine at the molecular level whether acute phase pro-inflammatory cytokines (i.e., IL-1, IL-6 and TNF-α) were altered following drug treatment and infection, since previous studies had shown there were increased serum levels of these cytokines in the mice. It was found that the mRNA levels for IL-1 remained generally constant regardless of wheth-**

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er the infected animals were treated with a cannabinoid. However, the mRNA level for IL-6 was markedly increased following treatment of the infected animals with THC, suggesting the possible involvement of this pro-inflammatory cytokine in increased mortality. The mRNA level for TNF-α was generally low and not significantly altered in the drug treated animals. Mice given other cannabinoids, including cannabinol and cannabidiol, as well as the synthetic CP 55,940, showed no significant change in the level of mRNA for any of the cytokines tested. [P.S.E.B.M. 1997, Vol 214]

Marijuana is a widely used psychoactive recreational drug. A number of laboratories, including ours, have shown that this illicit drug, as well as its components, are markedly immunosuppressive. For example, δ-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, has been shown in numerous studies to have a variety of immunosuppressive properties. This marijuana component can alter functional activity of immune cells, including suppression of the function of T cells, B cells, macrophages, and natural killer cells (1–5). It is known that over 40 different cannabinoids are present in marijuana, and THC is only one of these. However, THC and its homologs are the most psychoactive of the natural cannabinoids. Several other major components in cannabinis are moderately psychoactive. These are cannabinol (CBN) and cannabidiol (CBD). Previous studies have shown that systemic availability of CBD and CBN is approximately one-third greater than that of THC and both have comparable half-lives. Thus, in marijuana smokers immune cells are probably exposed to CBD or CBN longer than to THC (6). The cannabimimetic CP 55,940 compound is a synthetic cannabinoid known to be a potent psychoactive agent (7). The order of cannabinoid binding activity to binding sites on tissue cells is as follows: CP 55,940, THC, CBN, and CBD (8, 9).

Previous studies in this laboratory showed that THC increases the mortality of BALB/c mice to infection with a sublethal dose of *Legionella pneumophila,* an ubiquitous gram-negative opportunistic intracellular pathogen (10). It is widely known that gram-negative bacterial infections can induce an acute-phase reaction. For example, pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor–α (TNF-α) are produced in large amounts in infected individuals and may enhance the immune responses to the bacteria. Some of the systemic effects of IL-1 include hepatic acute-phase protein synthesis, fever, increased arachidonic acid metabolites, etc. Administration of exogenous IL-6 or TNF-α induces many of the same effects *in vivo* (11). We previously reported that infection of mice with a sublethal concentration of Legionella and injection with THC increases serum levels of IL-6 and TNF-α, and this is related to toxic shock–like death in the animals, suggesting that induction of these cytokines was related to increased mortality (10). Furthermore, treatment of the mice with antibody to IL-6, TNF-α, or even IL-1 abrogated this enhanced mortality. The present study investigated the likelihood that altered proliferation of the Legionella in cannabinoid-treated mice contributes to their increased mortality. We also examined whether development of acute-phase cytokine message in the spleen of treated animals correlated with the increased levels of pro-inflammatory cytokine in the serum of the animals and the increased mortality of the mice following exposure to various marijuana components.

Methods and Materials

Bacteria. A virulent strain of *L. pneumophila,* serogroup 1, was used for this study. The organism was isolated from a fatal case of legionellosis at Tampa General Hospital and cultured as previously described (12). Forty-eight–hour bacterial cultures were diluted in pyrogen-free saline and suspended to a concentration of 8×10^7 CFU/ml as determined by standard spectrophotometry.

Animals. All experiments were performed using female BALB/c mice 8–9 weeks of age. The animals were housed in groups of six to eight in plastic mouse cages at ambient temperature and given food and water *ad libitum.*

Cannabinoids. δ-9-THC in alcohol and CBN and CBD in powder form were provided by the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD). The alcohol was evaporated from the THC residue with nitrogen gas, and the residue dissolved in dimethylsulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO). A powder of the synthetic analog CP 55,940 was generously provided by Dr. Lawrence Melvin (Pfizer Central Research, Groton, CT). The powdered cannabinoids were suspended in DMSO for use. The cannabinoids were further diluted in sterile, heat-inactivated normal mouse serum (Harlan Biproducts for Science, Inc., Indianapolis, IN) for use *in vivo.*

Cytokine Assays. TNF was measured with a ^{51}Cr release assay. WEHI 164 cells were labeled with sodium ^{51}Cr (100 μCi/10^6 cells) for 1 hr. Serum samples and TNF standard (Genzyme, San Francisco, CA), serially diluted in 96-well plates (Costar, Cambridge, MA), were incubated with the labeled WEHI cells (5×10^3/well) for 18 hr at 37°C. Supernatants were collected and counted in a Packard Cobra gamma counter (Sterling, VA). The number of units in the serum samples was compared to a recombinant TNF standard curve. Interleukin-6 activity in the serum was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) technique (10). Medium binding 96-well EIA plates (Costar) were coated with 50 μl of 6 μg/ml

anti-murine IL-6 for 2 hr at 37°C. The wells were blocked for 30 min with 0.15 ml of phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin and 0.05% Tween 20. Serial dilutions of serum samples or murine IL-6 standard (2–200 μg/ml; Pharmingen, San Diego, CA) were added and the plates incubated for 1 hr. All plates were rinsed and biotinylated anti-murine IL-6 added (0.05 ml of 2 μg/ml) for 1 hr, followed by streptavidin-alkaline phosphate (1:1000 triethyanolamine, 0.05 ml) for 30 min. After substrate 1 mg/ml of nitrophenyl phosphate and buffer (pH 9.8) was added, the plates were allowed to develop for 30–45 min. Units of IL-6 were calculated from IL-6 standard curves from standards in each plate. Interleukin-1 was determined by a standard ELISA method. Microtiter plates (Costar) were coated with 50 μl of hamster anti–IL-1 antibody (10 μg/ml) in saline for 2 hr at 70°C. The plates were then washed with saline and blocked with saline plus 0.05% Tween 20 containing 0.5% bovine serum albumin for 30 min at 37°C. The samples (50 μl) and serial diluted anti–IL-aα were incubated for 2 hr at 37°C. Rabbit anti–IL-1α (50 μl) antibody was added and incubated for 1 hr at 37°C. Then 50 μl of goat anti-rabbit immunoglobulin antibody conjugated to alkaline phosphatase was added for 30 min at 37°C. Each of these steps was followed with three washes with distilled water. Finally the plates were developed with 1 mg/ml of *p*-nitrophenol phosphate and read in a microtiter reader.

Drug Administration and Bacterial Infection. Groups of mice were injected intravenously (iv) with various doses of the different cannabinoids 24 hr prior to and 24 hr after sublethal infection with Legionella, as previously described (10). The mice were given 4 to 16 mg/kg of the cannabinoid in mouse serum in 0.1 ml volume. The mice were infected with 8×10^6 CFU of Legionella iv in a volume of 0.1 ml. Mortality was recorded over the next 2 days, but death of the cannabinoid-treated mice always occurred between 30 min and 20 hr.

CFU Analysis. The mice were sacrificed by CO_2 asphyxiation. Peripheral blood was obtained by cardiac puncture with heparinized syringes (0.1 ml, 1000 U/ml sodium heparin sulfate, Sigma). The spleen, liver, and lung were obtained from the mice and placed into Stomacher bags containing 2 ml of Hanks' balanced salt solution (HBSS, Sigma). The Stomacher bags were weighed before and after the tissues were placed inside to determine tissue weight. The tissue was then disrupted in a Stomacher 80 Lab blender (Tekmar Co., Cincinnati, OH). The supernatants from the disrupted tissue were diluted appropriately in HBSS and plated onto buffered charcoal yeast extract agar (BCYE; Difco, Detroit, MI) to determine CFUs (13). Plates were incubated for a minimum of 4 days before being counted electronically with an Autocount plate reader (Artek Systems, Corp., Dynatech Labs Inc., Chantilly, VA).

RNA Isolation. Total RNA was isolated from splenocytes disrupted in the Stomacher blender (14). The supernatant was removed, washed once with HBSS and centrifuged at 200 *g*. The pelleted cells were resuspended in 3 ml of ACK lysing buffer (0.1 *M* NH_4Cl, 1.0 *M* Na_2 and 0.01 *M* Na_2 EDTA) for 5 min to remove contaminating erythrocytes. Then, 27 ml of HBSS were added to the suspension followed by centrifugation. The cells were washed again with HBSS and all remaining supernatant removed from the pellet. Tri-reagent (Molecular Research Center, Cincinnati, OH) was used to isolate the RNA, which was then dissolved in 30 μl of RNAase-free water (Sigma) and the concentration quantitated spectrophotometrically (14). Intact 18S and 28S RNA bands were visualized by agarose electrophoresis with ethidium bromide.

Reverse transcriptase–Polymerase Chain Reaction. Reverse transcriptase (RT) of total RNA (1 mg of RNA) was performed using avian myeloblastosis virus reverse transcriptase in RT buffer with recombinant RNAase inhibitor in Mg Cl_2 deoxynucleoside triphosphate (dNTP) mixture and oligo $(dT)_{15}$ primer (Reverse Transcription System; Promega, Madison, WI) in a final volume of 10 μl. The cDNA was then subjected to polymerase chain reaction (PCR) using primers for β_2-microglobulin (BMG). IL-1β, IL-6, and TNF-α PCR was performed with two primer sets with BMG as the endogenous standard and cytokine primers in the same tube as previously described (14). The primers were synthesized on a DNA synthesizer (PS-250; Cruachem, Dulles, VA) using published sequences or those used by Stratagene (Ehlers, 1992; Stratagene, La Jolla, CA). Four microliters of RT product were amplified using Taq DNA polymerase, primer sets, and dNTPs. The final volume of 50 μl was overlaid with mineral oil and the PCR performed using a minicyler (MJ Research, Watertown, MA) for 30 cycles. The annealing temperatures varied from 49°–60°C, depending upon the primers used. The first cycle was a 5-min denaturation at 94°C and a 5-min annealing, followed by 30 cycles of 1.5 min each at 72°C, 45 sec at 94°C, and 45 sec at the annealing temperature. The final extension was 10 min at 72°C.

HPLC Analysis. The PCR products were analyzed and quantitated as previously described (14). Then 10 μl of the PCR products were diluted in 60 μl of starting buffer (30% Solution A: 1.0 *M* NaCl, 25 m*M* Trizma Base, pH 9.0; and 70% Solution B: 25 m*M* Trizma Base, pH 9.0 (Sigma), and applied to a high-performance liquid chromatograph (Series 410BIO; Perkin Elmer, Norwalk, CT) with a precolumn filter and 0.5-μm scinter, a TSK DEAE-NPR guard column (5 × 4.6 mm), and a 35 × 4.6 mm column (Toso Haas, Montgomeryville, PA). The gradient program utilized was as follows: 30%–40% Solution A for 0.1 min, 40%–52% Solution A for 2.9 min, 52%–60% Solution A for 7 min, 60%–100% Solution A for 0.5 min, 100% Solution A for 1 min, and 100%–30% Solution A for 0.2 min, then 10 min with the initial mobile phase composition for the next injection at the flow rate of 1.0 ml/min. Chromatographic measurements were performed with a diode array detection system (LC-235; Perkin Elmer) operated at 260 nm. The absorbance data were analyzed by computer using PE Nel-

son software (Perkin-Elmer Nelson Systems, Cupertino, CA).

Statistical Analysis. The computer software SigmaStat (Jandel Scientific, San Rafael, CA) was used to compute Student's *t* tests and analysis of variance.

Results

Administration of the natural psychoactive cannabinoid THC and the synthetic psychoactive CP 55,940 1 day before and 1 day after the sublethal infection of the mice with Legionella resulted in about 50% mortality (Table I). This occurred when the mice were given the 6 mg/kg dose, which was nonlethal for control mice not infected with Legionella. None of the control noninfected animals given the same doses of any of the cannabinoids tested, including THC, died in a 2-week period. Moreover, mice given 4 mg/kg doses at the same time before and after infection with the same low dose of Legionella did not die. Furthermore, none of the Legionella-infected mice given cannabinol at the 8 mg/kg dose died, but approximately a third of the animals given twice as much (i.e., 16 mg cannabinol/kg body wt) died (Table I). It was noteworthy that mice given the 16 mg/kg dose of cannabidiol 1 day before and 1 day after infection with Legionella evinced only about 10% mortality (Table I).

Mice treated with the various cannabinoids 1 day before and 1 day after infection with Legionella were assayed for bacterial load in their tissue. There was no evidence of bacterermia in the mice infected with Legionella only or those given any one of the cannabinoids tested only. Furthermore, mice treated with the different cannabinoids did not show statistically higher levels of bacteria in their liver or spleen compared with control mice infected with Legionella only (Table II). Thus, there did not appear to be any enhanced sequestration of the bacteria in these tissues of the cannabinoid-treated mice. However, when mice were injected with the psychoactive cannabinoid THC, there was a greater number of Legionella in the lung of surviving mice compared with nontreated mice (Table II). For example, the mean Legionella CFU per mg lung tissue in mice treated with THC was approximately 50, while mice infected with bacteria only and not treated with the cannabinoid had a CFU level averaging less than 5 bacteria per mg lung. However, it is notworthy that this was only observed 24 hr after infection, not at 48 or 72 hr. CFU could only be determined in the animals that survived infection and treatment with THC. Obviously, animals that died less than 1 day after the second injection of THC could not be tested for bacteria in their lung or any other tissues. Nevertheless, the results showed there was an increased number of bacteria in the lung of mice given THC 1 day before and 1 day after infection with Legionella. Mice given other cannabinoids, including CP 55,940 as well as the cannabinol or cannabidiol, had bacterial levels in the lung somewhat less than those given THC but more than in control animals infected with Legionella only.

Table I. Toxic Shock Lethality by Cannabinoids in Mice Infected with *Legionella*

Cannabinoid[a]	Dose (mg/kg)	Survivors/ infected[b]	Percentage deaths (%)
None (control)	—	0/24	0
THC	6	14/27	52
CP 55,940	6	12/24	50
Cannabinol	8	0/16	0
	16	3/8	37.5
Cannabidiol	16	2/16	12.5

[a] Indicated cannabinoid given to mice by iv injection 1 day before and 1 day after sublethal infection with *L. pneumophila* (5×10^6 bacteria).
[b] Indicated number of animals surviving versus number infected after sublethal injection of *Legionella* and cannabinoid treatment; deaths occurred within 0.5–24 hr after infection.

Table II. *L. pneumophila* Growth in Various Organs in Mice Given Cannabinoids

Cannabinoid[a]	CFU/mg tissue[b]		
	Spleen	Lung	Liver
None	473 ± 65	4 ± 1	443 ± 42
THC	458 ± 52	48 ± 12[c]	494 ± 72
CP 55,940	391 ± 36	38 ± 7[c]	348 ± 68
Cannabinol	407 ± 59	24 ± 6[c]	295 ± 48
Cannabidiol	416 ± 32	15 ± 4	312 ± 52

[a] Indicated cannabinoid given mice iv 1 day before and 1 day after sublethal infection with Legionella. THC and CP 55,940 given at a 6 mg/kg dose and cannabinol and cannabidiol given at a 16 mg/kg dose.
[b] Legionella CFU per mg mean ± SEM of liver, lung or spleen in surviving cannabinoid treated mice 24 hr following second cannabinoid injection; data is mean for four experiments with at least five mice per group for each drug ($n = 20$ mice).
[c] $P < 0.01$.

As shown in Table III, the serum levels of IL-1, IL-6, and TNF-α activity increased in the Legionella-infected animals treated with THC 1 day before and 1 day after infection. For example, within 10 min after the second injection of THC the serum level of IL-6 nearly doubled, and an hour later the level of this cytokine activity increased almost 3-fold. There was also an increase in the level of serum TNF-α. The level of serum IL-1 also increased in the THC-treated mice infected with Legionella. The increase was essentially similar to that previously reported following THC treatment of spleen cells or macrophages *in vitro*, with or without Legionella (15). Treatment of the Legionella-infected mice with the psychoactive synthetic cannabinoid CP 55,940 also increased the levels of these cytokines, but to a lesser extent. There was an even smaller increase in the serum level of the cytokines in the mice given cannabinol or cannabidiol and Legionella infection. For example, the levels of IL-1, IL-6, and TNF-α in the CP 55,940 treated mice injected with Legionella increased from an initial level of approximately 10 ng/ml of IL-1, 500 pg/ml of IL-6, and 100 units/ml of TNF-α to levels of only approximately 15 ng/ml of IL-1, 600 pg/ml of IL-6, and 150 units of TNF-α/ml,

Table III. Serum Levels of Acute-Phase Cytokines after Sublethal Infection of Mice with *L. pneumophila* and Treatment with THC

Time[a] (min)	Cytokine level[b] (mean ± SEM)		
	IL-1 (ng/ml)	IL-6 (pg/ml)	TNF-α (units/ml)
0	14.5 ± 2	495 ± 24	142 ± 37
10	22.6 ± 5	782 ± 148[c]	274 ± 258[c]
60	28.5 ± 5[c]	1490 ± 325[c]	582 ± 119[c]

[a] Mice infected with *L. pneumophila* and given THC (6 mg/kg) 1 day before and 1 day afterward and tested for serum cytokine levels 0, 10, and 60 min after second injection of THC.
[b] Cytokine level as average mean/ml ± SEM in serum assayed by standard methods (IL-1 or IL-6 protein determined by ELISA and TNF-α activity determined by WEHI-164 ^{51}Cr release assay). Mice infected with Legionella only or given DMSO vehicle, but no THC, had low levels of serum cytokines (less than 50 units TNF-α/ml, less than 400 pg IL-6/ml and less than 10 ng IL-1/ml. Data show mean average for 16–30 mice in three to five experiments ($n = 65$). Control normal mice not infected with *L. pneumophila* given one of the cannabinoids tested, including THC, had less than 10 units of TNF-α/ml, 20 pg of IL-6/ml, or 2 ng of IL-1/ml in their serum.
[c] $P < 0.01$.

respectively, within 40–60 min after the second injection of the synthetic cannabinoid. In addition, mice injected with cannabinol or cannabidiol before and after Legionella infection had only a maximum of 12–15 ng/ml of IL-1, 600 pg/ml of IL-6, and 150 units/ml of TNF-α in their serum within 60–100 min after injection of these cannabinoids. None of these changes seemed significant in comparison to the changes of acute-phase cytokine levels in Legionella-infected mice injected with THC. Mice injected with these cannabinoids alone, but not infected with Legionella, did not show any significant level of IL-1, IL-6, or TNF-α cytokine activity in their serum.

Since the pro-inflammatory cytokines tested were found to increase in the serum of mice given Legionella plus THC, compared with control animals infected with Legionella only, it was of interest to assess the production of these cytokines at the molecular level. For this purpose cytokine mRNA was measured using the RT-PCR method and quantified using HPLC (14). The message for IL-1 production was constitutively produced in normal mice not infected with Legionella or treated with a cannabinoid (15). For example, normal mice showed a ratio of approximately 5–6 for IL-1 mRNA–to–BMG mRNA expression. The level of mRNA for TNF-α was relatively low in all treatment groups and was not significantly altered by sublethal infection with Legionella and treatment with THC (Table IV). The mRNA level for IL-6 production was not increased by treatment with THC alone (ratio less than 2), but increased about 3-fold by 60 min after infection with Legionella and THC treatment only (Table IV). For example, mice infected with Legionella and given THC before and after such infection had a marked increase in IL-6 mRNA production. Animals infected with Legionella only and given one of the other cannabinoids, including the synthetic cannabinoid, showed a lower increase in the mRNA for the cytokines tested. For example, unlike the situation with the THC-treated mice, where the mRNA reached a level of about 8 units, the level of the mRNA remained lower (i.e., a ratio of about 2–3) when mice were treated with the CP 59,940 compound, cannabinol, or cannabidiol. It was also found noteworthy that the ratio for IL-6 mRNA did not increase more than 2-fold in 60–120 min in spleen cells of mice given any of the three cannabinoids but not infected with Legionella.

Table IV. Effect of THC on Splenic Acute-Phase Cytokine mRNA Levels

Cytokine[a]	Time after THC treatment[b] (min)			
	0	10	30	60
IL-1	6.9	6.7	6.3	5.8
IL-6	2.9	4.6[c]	7.8[c]	8.2[c]
TNF-α	2.8	2.7	3.1	4.8

[a] Cytokine mRNA level for IL-1, IL-6, or TNF-α in spleen cells of mice sublethally infected with Legionella (5×10^6 bacteria iv) and given THC (6 mg/kg iv) at indicated time in minutes prior to assay.
[b] Mice injected iv with THC 24 hr prior and 24 hr after infection with Legionella and then tested to determine mRNA expression for the indicated cytokine; data presented as average ratio of cytokine to BMG mRNA expression for spleen cells from five mice per experiment (three experiments) at indicated time in minutes after second injection of THC ($n = 15$). SEM < ±10%.
[c] $P = 0.01$.

There was no significant change in the level of mRNA for the other cytokines tested (i.e., TNF and IL-1) in the spleen cells of the mice treated with the synthetic cannabinoid, cannabinol, or cannabidiol when given 1 day before and 1 day after infection with Legionella. Although there was a higher endogenous level of IL-1 mRNA in mice infected with Legionella and given a drug 24 hr before and afterwards, there was no consistent change in the level of the mRNA to these cytokines.

Discussion

Previous studies had shown that mice sublethally infected with *L. pneumophila* developed a toxic shock–like death within 0.5–20 hr after treatment with the marijuana component THC 1 day before and 1 day after infection (16). It was noteworthy that mice infected with the same sublethal dose of Legionella but treated with THC only once, either before or even after infection, did not evince this enhanced mortality. Furthermore, when THC was given at even a higher dose either several times before or after infection, none of the mice displayed this enhanced mortality to the Legionella (16). For example, we reported that mice infected with Legionella and given THC either at the same or higher doses two to four times before or after infection did not show evidence of the rapid toxic shock–like death. The animals given THC at a dose and time associated with the rapid toxic shock–like death (i.e., treated with THC 1 day before and 1 day after infection) showed a simultaneous

increase in serum levels of the pro-inflammatory cytokines TNF-α and IL-6, suggesting that these cytokines, known to be involved in toxic shock–like deaths in a variety of situations, might be involved in this augmented death (16). Those previous studies showed an increase in the systemic level of IL-6 and TNF-α in the serum of the mice within 30–60 min after the second injection of THC (10). Furthermore, administration of antibodies to IL-1, IL-6, and TNF-α prevented death. However, additional experiments should be performed to evaluate the dose-response relationship between cannabinoid injection and sublethal infection with Legionella. Such experiments are presently under way.

The production of these cytokines systemically was examined at the message level in the present study and found to be only moderately affected. This is not surprising, since much of the production of pro-inflammatory cytokines, including IL-6, takes place in cells not directly related to immune functions such as fibroblasts, astrocytes, keratocytes, and endothelial cells. Although cells of the immune system such as monocytes and T cells also produce IL-6, such production does not necessarily occur in the spleen. Although TNF-α can be produced by cells of the immune system such as macrophages, T cells, and NK cells (17), much of this production is localized and likely occurs at the site of immunological insult, not necessarily in the spleen. Furthermore, IL-1, another important pro-inflammatory cytokine, is produced by virtually the same cells as IL-6 and therefore should not be expected to increase significantly. IL-1 is known to be produced in the spleen at constitutively high levels, unlike the other acute-phase cytokines examined.

It seems important to note that the serum levels of the pro-inflammatory cytokine IL-6 appeared to increase 3-fold within 1 hour after treatment of Legionella-infected mice with the second injection of THC. TNF-α serum levels also increased approximately 3-fold, while IL-1 level increased about 2-fold. On the other hand, the level of mRNA for IL-1 and TNF-α in the spleen did not significantly change. Furthermore, when the less active cannabinol or cannabidiol was used, there was no significant change in the serum levels of the splenic mRNA activity for these pro-inflammatory cytokines. The synthetic cannabinoid also did not significantly increase the serum or the messenger RNA levels for those pro-inflammatory cytokines. However, it is apparent from the data obtained that the CP 55,940, given at the same dose as THC, resulted in a similar toxic shock–like death activity (i.e., 50% of the mice died). On the other hand, cannabinol or cannabidiol only caused mortality at a much higher dose (i.e., 16 mg/kg). Therefore, it is likely that the lesser ability of these cannabinoids to induce the pro-inflammatory cytokines as evidenced by a much smaller effect on mRNA levels to these cytokines could be related to their lower biologic activity in terms of mortality. However, this probably was not the case with CP 55,940, since this synthetic cannabinoid also induced low levels of pro-inflammatory cytokines in the serum, as well as low levels of mRNA for these cytokines. It is possible that the acute death induced by this synthetic cannabinoid might be due to other factors, including induction of other cytokines. Additional studies are necessary to show whether the enhancement of mortality induced by this synthetic cannabinoid or even other cannabinoids are due to similar or different mechanisms.

The cytokines IL-1 and IL-6 affect not only B- and T-cell responses but also hepatic acute-phase protein synthesis. These cytokines are involved in increased levels of arachidonic acid metabolites such as prostaglandins (18). Prostaglandin production can profoundly affect the immune response, and it is possible that prostaglandins play a role in the increased mortality of the Legionella-infected animals given the psychoactive cannabinoids. It is of interest that THC not only had the greatest effect in inducing augmented mortality, compared with the less psychoactive cannabinol or cannabidiol, but also had a greater effect in inducing increased levels of the acute-phase cytokines than did the less psychoactive cannabinoids. The natural ligand for THC, as well as presumably for the other cannabinoids and CP 55,940, is anandamide, an arachidonic acid–like compound. Thus, it appears plausible that the immunomodulatory activities of cannabinoids like THC, as well as other components of marijuana, may be associated with the cannabinoid receptor present on immune cells and that the interaction of cannabinoids with this receptor may be related to the effects noted.

It is important to note that the psychoactive cannabinoid examined in this study (i.e., THC) was more active in augmenting mortality in the sublethal Legionella-infected mouse model than were cannabidiol and cannabinol. The numbers of Legionella found in the tissue of treated animals were lower after treatment of the mice with the less psychoactive marijuana components compared with those in mice infected with Legionella and given THC. Mice treated with THC showing the augmented mortality to Legionella had a higher level of bacteria in their lung compared with the mice sublethally infected with Legionella only. From previous studies and the results of the present infectivity studies, it was apparent that these mice would have died upon administration of a second dose of THC and that this might be not only related to increased cytokine levels induced by the rapidly replicating Legionella but also due to increased bacterial burden in the lung of these animals.

The precise cause of increased death in the THC-treated mice still must be elucidated. Nevertheless, the increased acute-phase cytokine levels found in the circulation of these mice, including the increased message for the production of the acute phase cytokine IL-6, coupled with the increased number of bacteria in the lung of these animals, may all have contributed to the toxic shock–like death. Synergy of the acute-phase cytokine reaction and the septic state of these mice could be related to the increased mortality of the animals given the sublethal infection and the psychoactive cannabinoid.

It is also possible that increased mortality associated with greater psychoactivity of the marijuana component may be indirectly due to effects on the central nervous system rather than the periphery, since the less psychoactive cannabinoids studied (i.e., cannabinol and cannabidiol) had less effect not only on mortality but also in altering cytokine levels and message for the cytokines. Further studies to determine the mechanisms of this increased mortality seem important, especially since otherwise sublethal infections in drug abusers may be related to increased morbidity and mortality in individuals who would normally be expected to resist infection.

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