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Secondary Immunity to *Legionella pneumophila* and Th1 Activity Are Suppressed by Delta-9-Tetrahydrocannabinol Injection

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Resistance to infection with *Legionella pneumophila* is primarily dependent upon cell-mediated immunity rather than humoral immunity. Recent evidence suggests that activation of cell-mediated immunity depends on Th1 cells and activation of humoral immunity depends on Th2 cells. In this report, Δ^9-tetrahydrocannabinol (THC), the major psychoactive cannabinoid of marijuana and an immunomodulator, suppressed development of secondary immunity to *L. pneumophila*, which correlated with a reduction in Th1 activity. BALB/c mice, infected with a primary sublethal dose of *L. pneumophila*, developed resistance to a larger challenge infection 3 to 4 weeks later. However, intravenous injection of THC (4 mg/kg of body weight) 1 day prior to primary infection resulted in increased mortality after the challenge infection. The level of anti-*L. pneumophila* antibodies in serum increased in both THC-treated and control mice; however, in the THC group IgG1 antibodies which are stimulated by Th2 cells were elevated while Th1-regulated, IgG2a antibodies were depressed. Furthermore, cultured splenocytes from THC-treated mice had less *L. pneumophila*-specific lymphoproliferation, indicating a deficiency in cell-mediated immunity. Normal mouse splenocytes treated in vitro with THC and pokeweed mitogen showed suppressed production of gamma interferon, a cytokine associated with Th1 cells, but increased production of interleukin 4, a cytokine produced by Th2 cells. Splenocytes from THC-treated mice, stimulated in vitro with either pokeweed mitogen or anti-CD3 antibodies, also produced less gamma interferon, indicating less Th1 activity in these mice. These results suggest that THC decreases the development of anti-*L. pneumophila* immunity by causing a change in the balance of Th1 and Th2 activities.

The major psychoactive component of marijuana, Δ-9-tetrahydrocannabinol (THC), has been shown to have marked effects on in vitro and in vivo immune mechanisms, including antibody formation, cell-mediated immunity, and cytokine production (11, 19, 24). This immunomodulation is due to various effects of THC on T cells, B cells, NK cells, and macrophages. In previous studies, we showed that a single injection of THC prior to a primary sublethal infection with *Legionella pneumophila* did not reduce resistance to the primary infection (21) but did reduce resistance to a secondary infection (22). This increased mortality appeared to involve THC suppression of cell-mediated immunity rather than humoral immunity.

Protective immunity to *L. pneumophila*, a facultative, intracellular bacterial pathogen (16), has been demonstrated to involve cell-mediated immunity (4, 13, 15, 40) and probably $CD4^+$ T cells (18). Recently, two subsets of these cells, Th1 and Th2, have been defined on the basis of the cytokine profiles of $CD4^+$ clones (28). These subsets have contrasting roles in the immune response (5, 28). Th1 cells produce interleukin 2 (IL-2) and gamma interferon (IFN-γ) and are involved in cell-mediated immunity, while Th2 cells produce IL-4, IL-5, IL-6, and IL-10 and are associated with humoral immunity. Additionally, these two T-cell subsets appear to regulate the production of different immunoglobulin G (IgG) isotypes by B cells (9, 37). Th1 cells regulate IgG2a antibody production, while Th2 cells regulate IgG1 antibodies. Because of the importance of Th1 cells in cell-mediated immune resistance to bacteria (25, 33, 41) and the finding that THC injection suppresses IFN levels (3) and immunity to *Legionella* infection (22), we hypothesized THC injection might influence the relative activities of Th1 and Th2 cells. Our results suggest this does indeed occur and may account for the inability of the THC-treated animals to develop protective immunity and survive a second challenge infection with *L. pneumophila*.

MATERIALS AND METHODS

Animals. Female BALB/c mice (National Cancer Institute-Harlan, Frederick, Md.) 8 to 9 weeks of age were used for these experiments. They were housed and cared for in our animal facility, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

THC. Δ-9-THC was provided as a 98.6% pure tar by the Research Technology Branch of the National Institute on Drug Abuse (Rockville, Md.). The drug was initially dissolved in dimethyl sulfoxide (DMSO [Sigma Chemical Co., St. Louis, Mo.]) to a concentration of 150 mg/ml and stored under nitrogen gas at −20°C. We periodically analyzed the drug by using reverse-phase high-performance liquid chromatography to verify that it contained only one peak with a retention time consistent with a THC standard (Sigma). At the time of experiments, the drug was diluted (50 mg/ml) in DMSO. For injections, THC (1 mg/ml) and DMSO (20 μl/ml) as a control were diluted in heat-inactivated normal mouse serum. The endotoxin contamination of the THC was assayed by the *Limulus* amebocyte lysate test (Biowhittaker, Walkerville, Md.) and found to be <0.1 pg per injection. For in vitro

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studies, the THC-DMSO was suspended in warm RPMI medium (200 μg/ml).

Bacteria. The *L. pneumophila* strain used for these studies was a virulent serogroup 1 isolate obtained from a case of legionellosis and maintained at −70°C. Prior to use, *L. pneumophila* was cultured on buffered yeast extract agar for 48 h, as described previously (2). Bacteria were suspended in pyrogen-free saline, and the concentration was adjusted by spectrophotometry.

Infection. For primary infection, groups of BALB/c mice were injected intravenously with either THC (100 μg per mouse) or DMSO (2 μl per mouse) 24 h prior to an intravenous infection with a sublethal dose (7×10^6) of bacteria. For secondary or challenge infection, the animals were infected intraperitoneally with 5×10^7 *L. pneumophila* organisms 3 weeks after the primary infection. This concentration was greater than a 90% lethal dose for normal, nonimmunized BALB/c mice (90% lethal dose, 3×10^7). Similar results with challenge infection were obtained with either intravenous or intraperitoneal injections (data not shown); therefore, intraperitoneal injection was selected for these studies since it appears to be less stressful to the mice. Animals were monitored for morbidity and mortality for 7 days after challenge.

Anti-*L. pneumophila* antibody isotypes. On days 2, 4, 8, and 11 after infection, mice were sacrificed, blood was collected by cardiac puncture, and the serum was processed from the blood samples. Enzyme immunoassay plates were coated overnight at 4°C with formalin-killed *L. pneumophila* (10^6 bacteria per well) in phosphate-buffered saline (PBS). The plates were blocked with PBS containing 0.5% bovine serum albumin and 0.05% Tween 20 for 30 min at 37°C. Serial dilutions of mouse sera were incubated for 60 min, washed, and then treated for 60 min with alkaline phosphatase-labelled rabbit antibodies against murine Ig, IgG1, or IgG2a antibodies (1:100; Southern Biotechnology). The substrate (1 mg of *p*-nitrophenyl phosphate per ml in diethanolamine buffer [49 mg of $MgCl_2$ per liter–96 ml of diethanolamine per liter] [pH 9.8]) was added and the plates were developed for 20 min prior to being read at 405 nm. Titers were determined from the linear portion of the serum serial dilution curve and were the reciprocal of the serum dilution that gave an optical density (A_{405}) reading of 0.100.

Lymphoproliferation assay. Spleens were collected from mice at day 0, 8, or 21 postinfection, single-cell suspensions were prepared, and cells were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum (HyClone, Logan, Utah) plus penicillin-streptomycin (10% fetal calf serum–RPMI medium). The cells (10^6 cells per ml) were cultured in 96-well plates (Costar, Cambridge, Mass.) with formalin-killed *L. pneumophila* as antigen (10^8 bacteria per ml) for 116 h, pulsed with [^{3}H]thymidine (1 μCi per well) for 4 h and harvested on glass-fiber filters, and the counts per minute were determined by standard scintillation counting.

IFN-γ and IL-4 production by splenocytes. Single-cell suspensions of splenocytes from normal mice were prepared, washed, and resuspended in 10% fetal calf serum–RPMI medium. Cells (3×10^6/ml) were cultured in 24-well Costar plates with pokeweed mitogen (PWM [10 μg/ml]) and/or THC (3 to 7 μg/ml) for 24, 48, or 72 h. Supernatants were collected and assayed by enzyme-linked immunosorbent assay (ELISA). In other studies, the splenocytes from THC-treated and infected mice were collected on days 0, 1, 4, 6, 11, and 13 and cultured in 24-well plates with either PWM (10 μg/ml) or anti-CD3 antibody (5 μg/ml) for 24 or 72 h, and supernatants were collected and analyzed.

IFN-γ and IL-4 ELISA. Medium-bind, 96-well Costar enzyme immunoassay (EIA) plates were coated with anti-murine IFN-γ (200 ng per well; clone R4-6A2; Pharmingen, San Diego, Calif.) in PBS. After 2 h at 37°C, the plates were blocked for 30 min at 37°C. Culture supernatants or serial dilutions of murine IFN-γ standard (Pharmingen) were added for 1 h, followed by biotinylated anti-murine IFN-γ (100 ng per well; clone XMG1.2; Pharmingen) for 1 h, and then streptavidin-alkaline phosphatase (1:1,000; Southern Biotechnology, Birmingham, Ala.) for 30 min. After the substrate was added, plates were allowed to develop for 15 to 45 min. The plates were washed between additions with three to five changes of nanopure water. Units were calculated from the IFN-γ standard curve, which was performed for each plate. IL-4 ELISA was performed by the same protocol with anti-IL-4 antibody (100 ng per well; clone BVD4-1D11), biotinylated anti-IL-4 antibody (50 ng/ml; clone BVD6-24G2), and recombinant IL-4 (all from Pharmingen).

Statistical analysis. Data were analyzed by the Student *t* test for comparing two groups, one-way analysis of variance with Dunnett's test for comparing individuals, and SigmaStat (Jandel Scientific, San Rafael, Calif.) for comparing multiple groups.

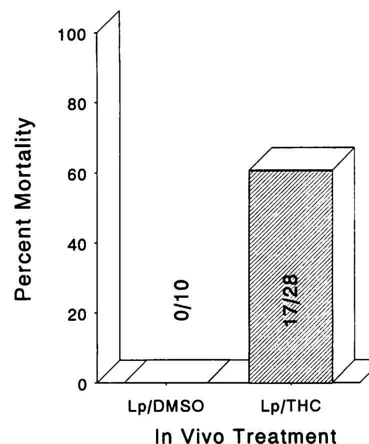

FIG. 1. THC injection 1 day prior to an immunizing infection with *L. pneumophila* (7×10^6 bacteria per animal) increases mortality in response to a second infection (5×10^7 bacteria per mouse) 3 weeks later. Each bar is the percentage of mortality, and the ratios are the number of deaths versus the total number challenged. Lp/DMSO, pretreatment with DMSO and infection with *L. pneumophila*; Lp/THC, pretreatment with THC (4 mg/kg of body weight) and infection with *L. pneumophila*.

RESULTS

In BALB/c mice, a primary, sublethal infection (7×10^6 to 8×10^6) with *L. pneumophila* results in the development of immunity against a secondary, lethal challenge of bacteria (4×10^7 to 5×10^7). This immunity is accompanied by the development of a strong antigen-specific lymphoproliferation response (32). With this paradigm, the effect of THC on protective immunity was examined.

Mortality. Previous studies (21, 22) suggested THC injection into mice suppressed both primary and secondary immunity to infection with *L. pneumophila*; however, secondary immunity was suppressed by a lower drug dose. Figure 1 shows that mice given only a single injection of THC (4 mg/kg of body weight) 24 h prior to primary infection (7×10^6 bacteria) displayed increased mortality (i.e., 0% versus 60%) when challenged 3

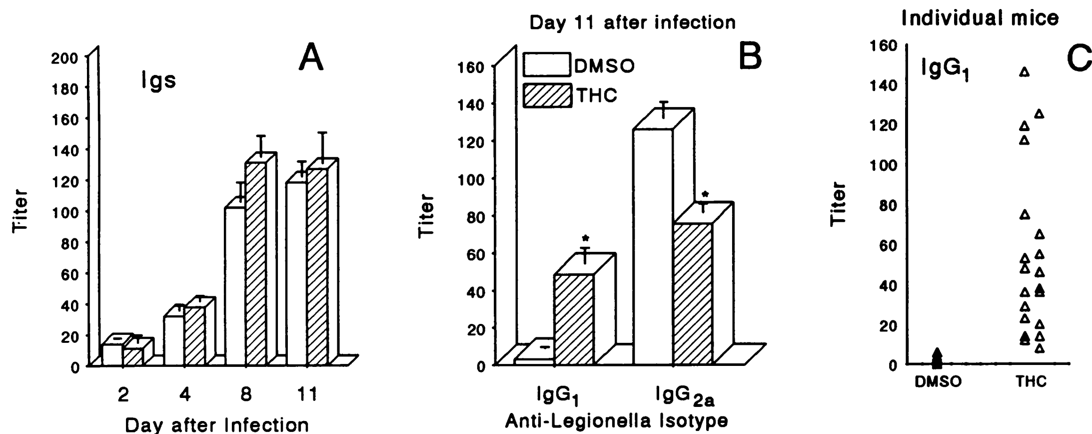

FIG. 2. THC injection prior to *L. pneumophila* infection increases the concentration of anti-*L. pneumophila* antibodies of the IgG1 isotype and decreases the IgG2a isotype in serum. Mice were bled at various days after *Legionella* infection and injection with either DMSO or THC and the serum was processed for ELISA measurement. Total antibodies to *L. pneumophila* (A) and antibodies of IgG2a and IgG1 isotypes (B) were measured. Data are mean titers ± standard errors for four to six experiments with a total of 8 to 30 animals. Panel C displays the IgG1 titers of individual mice from representative groups at day 11. *, $P \leq 0.05$ versus DMSO control.

weeks later with 5×10^7 bacteria. The mice showed no mortality during the primary infection unless the drug dose was increased (21).

Anti-*L. pneumophila* antibodies in serum. Primary infection of mice with *L. pneumophila* causes an increase in specific serum antibodies (22), although the role of these antibodies in host immunity is unclear (15, 16). To study THC effects on antibody isotype production, serum was obtained from individual animals in both DMSO-treated infected mice (Lp/DMSO) and THC-treated infected mice (Lp/THC) on days 2, 4, 8, and 11 after primary infection. The total antibody content of anti-*L. pneumophila* antibodies was analyzed by ELISA as well as the content of IgG1 and IgG2a isotypes. As seen in Fig. 2A, the level of specific anti-*L. pneumophila* antibodies in serum increased within several days after primary infection and continued to rise through day 11. THC treatment had little effect on the total Ig response. However, the drug did affect the relative concentration of IgG1 versus IgG2a in the serum. The Lp/THC animals had significantly elevated levels of IgG1 antibodies and reduced levels of IgG2a (Fig. 2B), although the IgG1 levels varied from one animal to another (Fig. 2C). Interestingly, in one study, mice challenged at day 21 survived the infection if their day 11 IgG1 titer was less than 50, while animals with titers greater than 100 died (data not shown). Although preliminary, this observation supports a negative correlation between the level of IgG1 antibody in serum and host resistance to infection.

Anti-*L. pneumophila* lymphoproliferation. Infection of mice with *L. pneumophila* results in increased splenocyte proliferation after exposure to *L. pneumophila* antigens (32). To study THC effects on the development of cell-mediated immunity, the splenocyte proliferation response to killed *L. pneumophila* was examined. Cultures were pulsed on day 5 rather than day 2 to separate the mitogenic response from the antigen-specific response (12). As expected, cultured splenocytes from infected mice (Lp/DMSO group) showed an elevated proliferation response to killed bacteria compared with splenocytes from normal, uninfected mice (Fig. 3). The response was higher at 1 week than at 3 weeks after infection (Fig. 3). The Lp/THC group, however, showed a significantly reduced antigen-specific proliferation both at day 8 and at day 21 compared with the Lp/DMSO control group (Fig. 3). Thus, THC given just prior to a primary infection reduced the cell-mediated response, as indicated by the lower level of antigen-specific lymphoproliferation.

THC effects on normal splenocytes. To determine if THC could directly influence the Th1 and Th2 activities of cultured cells, splenocytes from normal mice were cultured and treated with PWM, a T-cell mitogen, and various concentrations of THC. Supernatants from 24, 48, and 72 h in culture were collected and assayed by ELISA for IFN-γ and IL-4. Under these conditions, the cytokine levels in 24- and 48-h supernatants were very low, especially for IL-4. However, as shown in Fig. 4, by 72 h PWM stimulation increased the cytokine levels

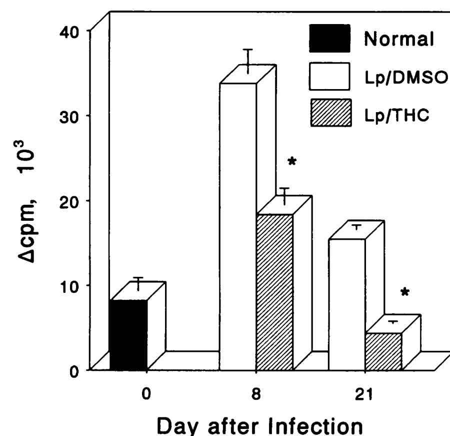

FIG. 3. THC injection decreases anti-*L. pneumophila*-specific proliferation of splenocytes. Mice were injected with THC (4 mg/kg of body weight), followed by *L. pneumophila* infection. Splenocytes were removed and cultured on day 0, 8, or 21 after infection. The splenocyte cultures were stimulated with killed *L. pneumophila* cells, and [^{3}H]thymidine incorporation was determined. Day 0 data represent those from normal, uninfected mice. Each bar represents the net incorporation of [^{3}H]thymidine for the various groups expressed as mean counts per minute ± standard error for three experiments, with six to eight animals per experiment. *, $P \leq 0.05$ versus *L. pneumophila* control.

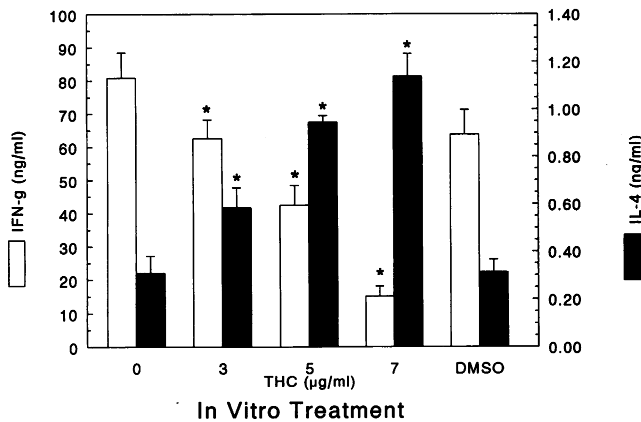

FIG. 4. THC treatment of normal, cultured splenocytes decreases the production of IFN-γ but increases the production of IL-4. Splenocytes were cultured for 72 h with PWM and either medium, THC, or DMSO. Supernatants were harvested, and the levels of IFN-γ and IL-4 were determined by ELISA. Data are presented as the mean concentration ± standard error for four experiments. *, $P \leq 0.05$ versus the 0 THC group.

in the cultures and THC suppressed this IFN-γ production. In contrast, THC treatment increased IL-4 levels in these supernatants. These results suggested that THC could cause in cultured cells a change in the relative activities of Th1 and Th2 cells.

THC suppresses IFN-γ during infection. To examine if THC injection could suppress cytokine production during infection, groups of mice were treated with THC (100 μg per mouse) or DMSO (2 μl per mouse) 24 h prior to an immunizing infection with *L. pneumophila* (7×10^6 organisms). The mice were sacrificed at various times after treatment, and spleens were removed for further study. Cultured splenocytes were stimulated with anti-CD3 antibody or PWM for 24 h, and the supernatants were collected and assayed for IFN-γ and IL-4. As shown in Fig. 5A and B, splenocytes from *L. pneumophila*-primed mice had elevated IFN-γ levels in response to both anti-CD3 antibody and PWM compared with normal, uninfected mice. This increase was greatest at day 6 and waned thereafter. In splenocyte cultures from Lp/THC mice, the IFN-γ levels, while slightly elevated from normal levels, were significantly suppressed compared with those of the Lp/DMSO group (Fig. 5), suggesting the THC treatment caused a decrease in the splenocyte Th1-type activity. IL-4 was not consistently detected in supernatants by ELISA during the course of infection even after 72 h of mitogen stimulation. This low level of IL-4 was also observed in normal splenocyte cultures (described above) and was not increased by drug injection in infected mice (data not shown).

DISCUSSION

A variety of studies have shown THC is an immunomodulator (11, 19, 24); however, only a few studies have shown modulation of bacterial infection (6, 26). We have initiated THC and infection studies with a model employing mice and the bacterial pathogen *L. pneumophila* (21, 22). As seen in the present study, a primary infection with a sublethal dose of *L. pneumophila* leads to the development of protective immunity to reinfection (Fig. 1) which is accompanied by increased antigen-specific splenocyte proliferation (Fig. 3), IFN-γ production by splenocytes (Fig. 5), and levels of anti-*L. pneumophila* IgG2a antibodies in serum (Fig. 2). The sublethal, primary infection appears, therefore, to induce an increase in cellular activity associated with Th1 cells and activation of cell-mediated immunity (18, 28, 39).

A single injection of THC (4 mg/kg) 24 h prior to the primary infection disrupted the development of this normal Th1-type response. This was evidenced by increased mortality to the challenge infection (Fig. 1) and the accompanying relative decreases in antigen-specific splenocyte proliferation (Fig. 3), serum IgG2a levels (Fig. 2), and splenocyte IFN-γ production (Fig. 5) and the increase in serum IgG1 levels (Fig. 2). Therefore, it appears the THC injection affected the developing Th1 activities, which contribute to protective immunity, and possibly affected Th2 activity.

To determine if THC could directly cause a Th1/Th2 imbalance in splenocytes and to further explore the drug's effects on subset activity, normal splenocytes were cotreated with THC and mitogen, followed by analysis of supernatants for the Th1-associated cytokine IFN-γ and the Th2 cytokine IL-4. These results showed that THC directly suppressed

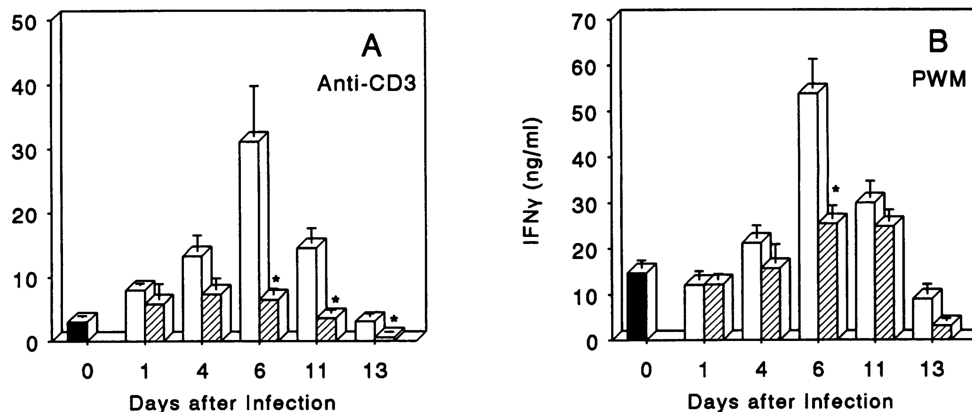

FIG. 5. THC injection prior to *Legionella* infection reduces the capacity of splenocytes to produce IFN-γ. Splenocytes were removed and prepared for culture from normal mice (solid bars) or on various days after mice were injected with DMSO and infected (Lp/DMSO [open bars]) or injected with THC and infected (Lp/THC [hatched bars]). Cultures were stimulated for 24 h with either anti-CD3 antibody (A) or PWM (B), and the supernatants were tested by ELISA for IFN-γ. Data are presented as the mean concentration ± standard error for three to four experiments with two to six mice per experiment. *, $P \leq 0.05$ versus the Lp/DMSO control.

mitogen-induced Th1 activity (i.e., IFN production), supporting the in vivo findings presented above, and increased Th2 activity (i.e., IL-4 production), consistent with the drug-induced increase in serum IgG1 levels (Fig. 2). However, increased IL-4 production was not observed in splenocytes from THC-treated and infected mice (Fig. 5). This discrepancy is partly due to the low levels of IL-4 detected in our system, and we are currently examining the production of other Th2 cytokines (e.g., IL-6 and IL-10) to see whether their levels are increased after drug treatment and infection. It is possible, however, that THC primarily decreases Th1 activity and that this effect accounts for the increase in serum anti-*L. pneumophila* IgG1 antibodies, since IFN-γ is inhibitory for IgG1 (37). Whatever the exact mechanism, our results support a THC-induced decrease in activities of Th1 cells, with a possible increase of Th2 activities, being associated with reduced resistance to *L. pneumophila* infection. This association between the decrease in Th1 levels and the increase in Th2 levels has been noted in several other infection models, including AIDS (7, 8, 27, 41).

Several possible mechanisms might account for the THC-induced Th1/Th2 imbalance. These involve the effects of THC on the cytokine environment controlling the maturation of Th0 cells to Th1 and Th2 (10). Strong evidence implicates IL-4 as a primary inducer of Th2 cells (10, 36, 38), while IL-12 in conjunction with IFN-γ stimulates Th1 cells (17, 35). NK cells when stimulated by IL-12 have been shown to be the source of IFN-γ for Th1 development in *Leishmania major* infections (34). We have previously shown that NK cells are a source of IFN-γ in *L. pneumophila* infection (2), and THC injection decreases IFN production (3) and NK activity (20, 31). It is possible, therefore, that THC suppresses the maturation of Th0 to Th1 cells by interfering with NK cells and IFN-γ production. On the other hand, THC effects on IL-1 production might also be involved. We have demonstrated that THC treatment of LPS-stimulated macrophage cultures increases the release of IL-1 (19, 42) and that THC injection increases the level of IL-1 in serum in infected mice (21). Because IL-1 is costimulatory for Th2 cells and not Th1 cells (14, 23), the THC treatment could augment Th2 cell activation and proliferation through increased IL-1 levels.

A final point concerns the relationship between the THC dose used in our study and the doses associated with human use. Nahas et al. reported several years ago that human THC consumption could approach 2 to 8 mg/kg of body weight per day if the cannabis used was 10 to 40% THC (29). Recently, similar doses, averaging 3.5 mg/kg/day, were reported in volunteers allowed to control their daily cigarette usage (30). In another study, mice were exposed to either 40 or 80 cigarette puffs per day, giving estimated THC doses of 3.2 to 6.4 mg/kg/day (1). These reports suggest the dose of 4 mg/kg/day used by us is not outside the scope of human experience. Finally, our dose is much lower than that used in other infection studies (6, 26), and our THC-treated mice show only a transient catalepsy of less than 2 h and excrete cannabinoid metabolites in urine at concentrations similar to those of humans using THC.

In summary, our results suggest that THC treatment of mice causes a decrease in Th1 activity, leading to a relative imbalance in Th1 and Th2 activities, decreased cell-mediated immunity, and enhanced susceptibility to infection with *L. pneumophila*. Future studies will be aimed at defining the drug's effect on Th2 activity and establishing the mechanism of the drug-induced imbalance.

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REFERENCES

1. **Ashfaq, M. K., E. S. Watson, and H. N. ElSohly.** 1987. The effect of subacute marijuana smoke inhalation on experimentally induced dermonecrosis by *S. aureus* infection. Immunopharmacol. Immunotoxicol. **9:**319–331.
2. **Blanchard, D. K., H. Friedman, W. E. Stewart II, T. W. Klein, and J. Y. Djeu.** 1988. Role of gamma interferon in induction of natural killer activity by *Legionella pneumophila* in vitro and in an experimental murine infection model. Infect. Immun. **56:**1187–1193.
3. **Blanchard, D. K., T. W. Klein, C. Newton, W. E. Stewart II, and H. Friedman.** 1986. In vitro and in vivo suppressive effect of delta-9-tetrahydrocannabinol on interferon production by murine spleen cells. Int. J. Immunopharmacol. **8:**819–824.
4. **Blander, S. J., and M. A. Horwitz.** 1991. Vaccination against *Legionella pneumophila* membranes induces cell-mediated and protective immunity in a guinea pig model of Legionnaires' disease. J. Clin. Invest. **87:**1054–1059.
5. **Bottomly, K.** 1988. A functional dichotomy in $CD4^+$ T lymphocytes. Immunol. Today **9:**268–274.
6. **Bradley, S. G., A. E. Munson, W. L. Dewey, and L. S. Harris.** 1977. Enhanced susceptibility of mice to combinations of Δ^9-tetrahydrocannabinol and live or killed gram-negative bacteria. Infect. Immun. **17:**325–329.
7. **Clerici, M., and G. M. Shearer.** 1993. A $T_H1 \rightarrow T_H2$ switch is a critical step in the etiology of HIV infection. Immunol. Today **14:**107–111.
8. **Clerici, M., T. A. Wynn, J. A. Berzofsky, S. P. Blatt, C. W. Hendrix, A. Sher, R. L. Coffman, and G. M. Shearer.** 1994. Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus. J. Clin. Invest. **93:**768–775.
9. **Coffman, R. L., B. W. P. Seymour, D. A. Lebman, D. D. Hiraki, J. A. Christiansen, B. Shrader, H. M. Cherwinski, H. F. J. Savelkoul, F. D. Finkelman, M. W. Bond, and T. R. Mosmann.** 1988. The role of helper T cell products in mouse B cell differentiation and isotype regulation. Immunol. Rev. **102:**5–28.
10. **Coffman, R. L., K. Varkila, P. Scott, and R. Chatelain.** 1991. Role of cytokines in the differentiation of $CD4^+$ T-cell subsets in vivo. Immunol. Rev. **123:**189–207.
11. **Friedman, H., T. Klein, and S. Specter.** 1991. Immunosuppression by marijuana and components, p. 931–953. *In* R. Alder, D. L. Felton, and N. Cohen (ed.), Psychoneuroimmunology. Academic Press, Inc., San Diego, Calif.
12. **Friedman, H., R. Widen, T. W. Klein, L. Searls, and K. Cabrian.** 1984. *Legionella pneumophila*-induced blastogenesis of murine lymphoid cells in vitro. Infect. Immun. **43:**314–319.
13. **Friedman, H., R. Widen, I. Lee, and T. Klein.** Cellular immunity to *L. pneumophila* in guinea pigs assayed by direct and indirect migration inhibition reactions in vitro. Infect. Immun. **41:**1132–1137.
14. **Greenbaum, L. A., J. B. Horowitz, A. Woods, T. Pasqualini, E.-P. Reich, and K. Bottomly.** 1988. Autocrine growth of $CD4^+$ T cells—differential effects of IL-1 on helper and inflammatory T cells. J. Immunol. **140:**1555-1560.
15. **Horwitz, M. A.** 1983. Cell-mediated immunity of Legionnaires disease. J. Clin. Invest. **71:**1686–1697.
16. **Horwitz, M. A., and S. G. Silverstein.** 1981. Interaction of the Legionnaires' disease bacterium (*Legionella pneumophila*) with human phagocytes. I. *L. pneumophila* resists killing by polymorphonuclear leukocytes, antibody, and complement. J. Exp. Med. **153:**386–397.
17. **Hsieh, C.-S., S. E. Macatonia, C. S. Trip, S. F. Wolf, A. O'Garra, and K. M. Murphy.** 1993. Development of T_H1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. Science **260:**547–549.
18. **Kaufmann, S. H. E.** 1993. Immunity to intracellular bacteria. Annu. Rev. Immunol. **11:**29–63.
19. **Klein, T. W., and H. Friedman.** 1990. Modulation of murine

immune cell function by marijuana components, p. 87–111. *In* R. R. Watson (ed.), Drugs of abuse and immune function. CRC Press, Boca Raton, Fla.

20. **Klein, T. W., C. Newton, and H. Friedman.** 1987. Inhibition of natural killer cell function by marijuana components. J. Toxicol. Environ. Health **20:**321–332.
21. **Klein, T. W., C. Newton, and H. Friedman.** 1993. Δ9-Tetrahydrocannabinol injection induces cytokine-mediated mortality of mice infected with *Legionella pneumophila*. J. Pharmacol. Exp. Ther. **267:**635–640.
22. **Klein, T. W., C. Newton, and H. Friedman.** 1994. Resistance to *Legionella pneumophila* suppressed by the marijuana component, tetrahydrocannabinol. J. Infect. Dis. **169:**1177–1179.
23. **Lichtman, A. H., J. Chin, J. A. Schmidt, and A. K. Abbas.** 1988. Role of interleukin 1 in the activation of T lymphocytes. Proc. Natl. Acad. Sci. USA **85:**9699–9703.
24. **Martin, B. R.** 1986. Cellular effects of cannabinoids. Pharmacol. Rev. **38:**45–74.
25. **Mills, K. H. G., A. Barnard, J. Watkins, and K. Redhead.** 1993. Cell-mediated immunity to *Bordetella pertussis*: role of Th1 cells in bacterial clearance in a murine respiratory infection model. Infect. Immun. **61:**399–410.
26. **Morahan, P. S., P. C. Klykken, S. H. Smith, L. S. Harris, and A. E. Munson.** 1979. Effects of cannabinoids on host resistance to *Listeria monocytogenes* and herpes simplex virus. Infect. Immun. **23:**670–674.
27. **Morris, L., A. B. Troutt, K. S. McLeod, A. Kelso, E. Handman, and T. Aebischer.** 1993. Interleukin-4 but not gamma interferon production correlates with the severity of murine cutaneous leishmaniasis. Infect. Immun. **61:**3459–3465.
28. **Mosmann, T. R., and R. L. Coffman.** 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. **7:**145–173.
29. **Nahas, G. G., A. Morishima, and B. Desoize.** 1977. Effects of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes. Fed. Proc. **36:**1748–1752.
30. **Nahas, G. G., and E. F. Ossweman.** 1991. Altered serum immunoglobulin concentration in chronic marijuana smokers, p. 25–35. *In* H. Friedman, S. Specter, and T. W. Klein (ed.), Drugs of abuse, immunity, and immunodeficiency. Plenum Press, New York.
31. **Newton, C., H. Friedman, and T. W. Klein.** Unpublished data.
32. **Newton, C., R. Widen, H. Friedman, and T. W. Klein.** Submitted for publication.
33. **Orme, I. M., A. D. Roberts, J. P. Griffin, and J. S. Abrams.** 1993. Cytokine secretion by CD4 T lymphocytes acquired in response to *Mycobacterium tuberculosis* infection. J. Immunol. **151:**518–525.
34. **Scharton, T. M., and P. Scott.** 1993. Natural killer cells are a source of interferon γ that drives differentiation of $CD4^+$ T cell subsets and induces early resistance to *Leishmania major* in mice. J. Exp. Med. **178:**567–577.
35. **Scott, P.** 1991. IFNγ modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. J. Immunol. **147:**3149–3155.
36. **Seder, R. A., W. E. Paul, M. M. Davis, and B. Fazekas de St. Groth.** 1992. The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of $CD4^+$ T cells from T cell receptor transgenic mice. J. Exp. Med. **176:**1091–1098.
37. **Snapper, C. L., and W. E. Paul.** 1987. Interferon-γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science **236:**944–947.
38. **Swain, S. L., A. D. Weinburg, M. English, and G. Huston.** 1990. IL-4 directs the development of Th2-like effectors. J. Immunol. **145:**3796–3806.
39. **Winn, W. C., Jr.** 1988. Legionnaires disease: historical perspective. Clin. Microbiol. Rev. **1:**60–81.
40. **Yamamoto, Y., T. W. Klein, C. A. Newton, and H. Friedman.** 1992. Differing macrophage and lymphocyte roles in resistance to *L. pneumophila* infection. J. Immunol. **148:**584–589.
41. **Yamamura, M., K. Uyemura, R. J. Deans, K. Weinberg, T. H. Rea, B. R. Bloom, and R. L. Modlin.** 1991. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science **254:**277–279.
42. **Zhu, W., C. Newton, Y. Daaka, H. Friedman, and T. K. Klein.** Δ^9-Tetrahydrocannabinol (THC) enhances the secretion of interleukin 1 (IL1) from endotoxin stimulated macrophages. J. Pharmacol. Exp. Ther., in press.