Enhanced Growth of *Legionella pneumophila* in Tetrahydrocannabinol-Treated Macrophages (43330)

SATORU ARATA, CATHERINE NEWTON, THOMAS KLEIN, AND HERMAN FRIEDMAN[1]
Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, Florida 33612

Legionella pneumophila is an important intracellular pathogen that causes pulmonary, as well as systemic, infections in susceptible individuals (1). A defect in the immune response system is considered an important contributor to heightened susceptibility in this organism. For example, previous studies in this laboratory have shown that suppression of immune capability in normally resistant mice using immunosuppressive drugs such as cyclophosphamide makes the animals more susceptible to infection with this bacterium. These bacteria grow readily in macrophages not only from humans, but also from susceptible guinea pigs, as well as from a susceptible mouse strain (2–4). Macrophages from most mouse strains are highly resistant to growth of these bacteria, as are the mice themselves in terms of susceptibility to infection by Legionella (5). Nevertheless, Legionella replicate readily in macrophages from susceptible A/J mice and can infect this mouse strain (4). When these mice are treated with immunostimulators like bacterial endotoxin, as well as products of the immune response, such as cytokines, resistance is enhanced and macrophages from the animals do not readily replicate Legionella (unpublished observation). In the present study, we demonstrate that Δ^9-tetrahydrocannabinol (THC) treatment of macrophage cultures that are normally permissive for Legionella growth appears to make them even more permissive. Furthermore, this THC effect of promoting intracellular growth occurs in macrophage cultures made less permissive by pretreatment with bacterial lipopolysaccharide as an activating agent.

Methods and Materials

Bacteria. A virulent strain of *L. pneumophila* was obtained from a case of fatal legionellosis at Tampa General Hospital, Tampa, FL, and cultured on buffered charcoal yeast agar exactly as described previously (4). The organisms obtained from 48-hr cultures were suspended in pyrogen-free saline and diluted to 2×10^7 colony-forming units per milliliter with RPMI 1640 medium supplemented with 10% fetal calf serum.

Animals. A/J mice were used for these studies. They were obtained from Jackson Laboratories, Bar Harbor, ME at 6–8 weeks of age, kept in groups of 6–8 in plastic mouse cages with wire mesh lids, and fed Purina mouse chow and water *ad libitum*.

Macrophages. Peritoneal exudates were obtained after injection of mice 4 days earlier with 3 ml of thioglycollate medium, as described previously (4). The harvested peritoneal cells were adhered to 96-well plates (Costar, Cambridge, MA) for 2 hr and the resulting macrophage monolayers (10^5/well) were washed with the Hanks' balanced salt solution. Then, the monolayers were precultured with RPMI-fetal calf serum for 24 hr. After preculture, the monolayers were infected with 2×10^6 Legionella and incubated for 1 to 3 days at 37°C. The number of viable Legionella within the cells (colony-forming units per well) was determined in lysates prepared by treatment with 0.1% Saponin using standard plate assays with buffered charcoal yeast agar medium (4). Each culture was performed in triplicate with cells from two or more animals and each experiment was repeated at least three times.

Tetrahydrocannabinol. Δ^9-THC was obtained from the National Institute on Drug Abuse, Rockville, MD. The stock THC was dissolved in alcohol for use and was placed in dimethylsulfoxide to prepare solutions, as described previously (6). For each experiment, THC was freshly prepared at the appropriate concentration.

Lipopolysaccharide. Lipopolysaccharide (LPS) from *Salmonella minnesota* R595 was prepared as described previously (7). Stock solutions were prepared by dissolving 1 mg in 5 ml of pyrogen-free distilled water containing 0.05% triethylamine and diluted with RPMI-fetal calf serum.

[1] To whom requests for reprints should be addressed at Department of Medical Microbiology and Immunology, University of South Florida College of Medicine, Tampa, FL 33612.

Received February 1, 1991. [P.S.E.B.M. 1992, Vol 199]
Accepted July 17, 1991.

0037-9727/92/1991-0065$3.00/0

Experiment Results

As is apparent in Table I, *L. pneumophila* replicated vigorously by 48 hr after infection of peritoneal exudate macrophages from normal A/J mice. There was a peak of over 1 million bacteria per culture. This was about 1000-fold more Legionella in the cultures of these cells at this time as compared with the number at Time 0, and this number of bacteria decreased about two thirds 1 day later. Treatment of the cell cultures with THC for 1 or 2 days or throughout the entire time period of culture had little effect on cell viability or total number of macrophages over a 3-day period using concentrations less than 10 μg/ml (>90% viability). Higher concentrations, such as 20 μg, reduced the number of cells and their viability (24% for Day 2 viability). Therefore, 10-μg/ml doses or less were used in the experiments reported here because there was little effect on cell number or viability.

When the peritoneal cell cultures were infected with Legionella and treated simultaneously with THC, there was no significant alteration of growth of bacteria in the first 48 hr after infection. However, unlike growth in the nontreated controls, the number of bacteria in the THC-treated cultures stayed constant or increased at 72 hr (Table II). Similar results were observed with less permissive macrophages from C3H/HeJ and BDF_1 mice (data not shown). The increase in bacterial number varied from 2- to 4-fold.

Bacterial LPS is a known activator of macrophages and increases the ability of A/J peritoneal cells to kill Legionella *in vitro*. As is apparent in Table III, the addition of THC to LPS-activated macrophages infected with Legionella altered the ability of the cells to inhibit Legionella growth. In control cultures without THC, activated with LPS alone, there was much less growth of the Legionella in the A/J macrophages. For example, macrophage cultures treated with 10 ng of LPS became markedly nonpermissive for growth of Legionella, as compared with untreated cultures. THC, at a dose of either 2.5 or 5.0 μg, abrogated much of the effect of the LPS in converting the permissive A/J mouse macrophages to a state of nonpermissiveness.

Table I. Growth of Legionella in A/J Mouse Macrophages

Time[a] (hr)	Infected cultures: No. of bacteria[b] (CFU × 10^3 ± SE)
0	1.6 ± 0.4
7	3.2 ± 0.7
24	120 ± 21
48	1400 ± 510
72	410 ± 220

[a] Cultures of thioglycollate-induced peritoneal macrophages from A/J mice infected with *L. pneumophila*.
[b] Growth of Legionella ± SE determined for three experiments, with triplicate cultures at indicated times, CFU, colony-forming units.

Table II. Effect of THC on Legionella Growth in Peritoneal Macrophages after 3 Days of Culture

THC (μg/ml)[a]	Infected cultures: Noninfected cultures (% viable cells)[b]	Infected cultures: No. of bacteria (CFU × 10^3 ± SE)[c]
None (control)	95.6	410 ± 220
2.5	94.2	1100 ± 892[d]
5.0	94.3	2000 ± 850[d]
10.0	91.8	1100 ± 350[d]

[a] Indicated concentration of Δ^9-THC added to thioglycollate-induced peritoneal macrophages from A/J mice infected with Legionella; control cultures treated with dimethylsulfoxide.
[b] Viability determined at 3 days of culture by trypan blue dye exclusion assay.
[c] Average number of Legionella determined for three or more experiments from indicated group at 3 days of culture.
[d] $P \leq 0.01$.

Table III. THC Effects on LPS-Induced Nonpermissiveness of A/J Mouse Macrophages Infected with *L. pneumophila*

LPS[a] (ng/ml)	THC[b] (μg/ml)	*L. pneumophila* growth[c] (CFU × 10^3 ± SE): Day 2	Day 3
0	0	1400 ± 510	410 ± 220
1.0	0	120 ± 22	230 ± 34
1.0	2.5	350 ± 68[d]	870 ± 140[d]
1.0	5.0	200 ± 51	820 ± 95[d]
10.0	0	19 ± 9	57 ± 12
10.0	2.5	47 ± 15	730 ± 60[d]
10.0	5.0	31 ± 10	550 ± 95[d]

[a] Cultures treated with indicated concentrations of LPS for 24 hr prior to infection.
[b] Indicated concentration of Δ^9-THC added to triplicate macrophage cultures infected with Legionella.
[c] Growth of Legionella ± SE in triplicate cultures 2 or 3 days after infection; average of three experiments.
[d] $P \leq 0.01$.

Discussion and Conclusions

L. pneumophila is a well-known intracellular opportunistic pathogen that can grow in macrophages from humans, guinea pigs, and a susceptible mouse strain. The results of this study indicate that nontoxic doses of THC enhance the growth of Legionella in macrophages from A/J mice when added to the cultures subsequent to infection. Extensive growth of Legionella is toxic for the macrophage cultures and it could be argued that THC interferes with growth early during infection, thereby resulting in a greater number of healthy and competent macrophages late in the culture period. However, this does not appear to be the case, because the extent of Legionella growth is parallel in the treated and untreated groups during the first 48 hr of culture and a difference between the two groups was

noted even in less permissive macrophage cultures from C3H/HeJ and BDF_1 mice, in which Legionella growth is so low that toxic changes in macrophage cultures are not observed. Furthermore, the experiments with LPS-activated macrophages showed that the macrophage cultures appear very healthy, but, as can be seen from Table III, anti-Legionella activity induced by LPS was inhibited by the drug treatment. For these reasons, it seems likely that the augmentation of Legionella growth was caused by decreased resistance of macrophages against these bacteria induced by THC treatment. This view seems plausible, since previous studies in this and other laboratories have shown that THC depresses not only immune responses mediated by B and T cells, but also affects some activities of macrophages. For example, cell spreading and phagocytosis of peritoneal macrophages are markedly inhibited in mice treated with THC *in vivo*, and this drug of abuse also affects these functional activities of macrophages when added to cultures *in vitro* (8, 9). Thus, the increase in the number of Legionella in culture at 72 hr of growth was not related to macrophage number or viability.

Previous studies by others have shown that THC can depress resistance to bacteria or virus infections in whole animals, and this may be due to effects on factors, such as interferons, that are involved in antimicrobial resistance (10–12). There have been no reports to date on cells exposed to THC and infected with a microorganism similar to this study. Studies are in progress to determine mechanisms involved concerning differences in the growth of Legionella in THC-treated macrophage cultures versus untreated normal cultures. It is important to note, however, that THC has no detectable effect on the viability of bacteria, including Legionella. A possible direct effect on the ability of macrophages to be infected by Legionella could be important in defining the host/parasite relationship to this and other opportunistic intracellular bacterial infections. Nevertheless, it is noteworthy that there is little indication that marijuana use increases susceptibility to opportunistic bacterial infections, such as that caused by Legionella. However, it is possible that marijuana use or use of other drugs of abuse may alter susceptibility to infectious agents, especially those that cause chronic infections, in the presence of other immunosuppressive modalities, including those associated with immunosuppressive viruses. Studies to examine this possibility are in progress.

Summary

Legionella pneumophila is an opportunistic intracellular pathogen that infects macrophages, both *in vivo* and *in vitro*. Tetrahydrocannabinol is a major psychoactive component of marijuana and can affect the functional activity of macrophages. In the present study, it was found that the treatment of macrophage cultures from permissive A/J mice with THC enhanced the growth of Legionella in these cells. Legionella grew much better in macrophages treated with low doses of THC, which caused no alteration in the number or viability of macrophages, as compared with growth in untreated cells. Furthermore, lipopolysaccharide-treated A/J mouse macrophages restricted the growth of Legionella, but this growth restriction was overcome by the addition of THC to LPS-treated macrophage cultures after infection. Thus, it is apparent that THC has the ability to enhance the growth of the intracellular opportunistic pathogen Legionella that grows in A/J mouse macrophages.

1. Eisenstein TK, Friedman H. Immunity to legionella. In: Katz SM, Ed. Legionellosis, Vol. II. Boca Raton, FL: CRC Press, pp159–169, 1985.
2. Horowitz MA, Silvorstein SC. Legionnaires' disease bacterium (*Legionella pneumophila*) multiplies intracellularly in human monocytes. J Clin Invest **66**:441–450, 1980.
3. Kishimoto RA, White JD, Shirey FG, McGann VG, Berendt RH, Larson EW, Hedlund KW. *In vitro* response of guinea pig peritoneal macrophages to *Legionella pneumophila*. Infect Immun **31**:1209–1213, 1981.
4. Yamamoto Y, Klein TW, Newton CA, Widen R, Friedman R. Growth of *Legionella pneumophila* in thioglycollate-elicited peritoneal macrophages from A/J mice. Infect Immun **56**:370–375, 1988.
5. Yoshida S, Mizuguchi Y. Multiplication of *Legionella pneumophila* Philadelphia-1 in cultured peritoneal macrophages and its correlation to susceptibility of animals. Can J Microbiol **32**:438–442, 1986.
6. Klein TW, Newton CH, Widen R, Friedman H. The effect of delta-9-tetrahydrocannabinol and 11-hydroxy-delta-9-tetrahydrocannabinol on T-lymphocyte and B-lymphocyte mitogen responses. J Immunopharmacol **7**:451–466, 1988.
7. Kasai N, Arata S, Mashimo J, Okuda K, Aihara Y, Kotani S, Takada L, Shiba T, Kusumoto S. *In vitro* antigenic reactivity of synthetic lipid A analogues as determined by monoclonal and conventional antibodies. Biochem Biophys Res Commun **128**:607–612, 1985.
8. Lopez-Cepero M, Friedman M, Klein TW, Friedman H. Tetrahydrocannabinol-induced suppression of macrophage spreading and phagocytic activity *in vitro*. J Leukocyte Biol **39**:679–686, 1986.
9. Klein TW, Friedman H. Modulation of murine immune cell function by marijuana components. In: Watson RR, Ed. Drug of Abuse and Immune Function. Boca Raton, FL: CRC Press, pp87–111.
10. Morahan PS, Klykken PC, Smith SH, Harris LS, Munson AE. Effects of cannabinoids or host resistance to *Listeria monocytogenes* and herpes simplex virus. Infect Immun **3**:670–674, 1979.
11. Munson AE, Sanders VM, Bradley SJ, Loveless SE, Harris LS. Lethal interactions of bacterial lipopolysaccharide and naturally occurring cannabinoids. J RES **24**:642–655, 1978.
12. Cabral GA, Lockmuller JC, Mishkin EM. Delta-9-tetrahydrocannabinol decreases alpha/beta interferons response to herpes simplex virus type 2 in the B6C3F1 mouse. Proc Soc Exp Biol Med **181**:305–311, 1986.