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Effects of Cannabinoids on Host Resistance to *Listeria monocytogenes* and Herpes Simplex Virus

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Previous investigations from our laboratories have demonstrated that cannabinoids possess immunosuppressive properties. The present studies were designed to determine whether these agents decrease host resistance to infections with *Listeria monocytogenes* and herpes simplex virus type 2. Host resistance was measured by changes in the 50% lethal dose of the pathogen in cannabinoid-treated and control mice. The effect of cannabinoids on resistance to *L. monocytogens* was dose dependent. Delta-9-tetrahydrocannabinol at doses of 38, 75, and 150 mg/kg suppressed resistance to infection by 10-, 17-, and 657-fold, respectively. Marijuana extract was less active but significantly reduced resistance to *L. moncytogenes* at all tested doses. Resistance to systemic herpes simplex virus type 2 infection was decreased 96-fold by delta-9-tetrahydrocannabinol, although marijuana extract was inactive. The doses and regimen of treatment with cannabinoids that produced significant decreases in host resistance were similar to those which caused suppression of delayed-type hypersensitivity to sheep erythrocytes. The possible mechanisms and public health aspects of the decreased host resistance produced by marijuana extract and its cannabinoids are discussed.

Considerable evidence has emerged which implicates marijuana and its major psychoactive constituents as immunosuppressive agents. Clinical investigations have revealed that marijuana impairs lymphocyte responsiveness to mitogens and particulate antigens (17), decreases T-cell rosette formation (6, 8), suppresses leukocyte migration (20), and alters alveolar macrophage morphology, function, and mobility (14). We and others have previously documented immunosuppressant activities of Δ^9-tetrahydrocannabinol (THC) and selected congeners in animal models, as evidenced by prolongation of skin allograft survival (16), suppression of lymphocyte reactivity to T- and B-cell mitogens (16), reduction of antibody responsiveness to T-dependent antigens (7, 10, 11), and depletion of splenic and peripheral nucleated cells (10, 16). Our available evidence suggests that possibly all of the immunosuppressant activities of the cannabinoids reside in their action on T lymphocytes (10, 21).

The present studies were undertaken to determine whether several naturally occurring and synthetic cannabinoid compounds changed host resistance to infections. As an index of T-cell function the delayed-type hypersensitivity (DTH) response to sheep erythrocytes (SRBC) was employed. Assessment of host resistance to *Listeria monocytogenes* and herpes simplex virus type 2 (HSV-2), pathogens known to be infectious in both mouse and man, was determined by measuring changes in the 50% lethal dose (LD_{50}) of the pathogen in cannabinoid-treated and control mice.

MATERIALS AND METHODS

Mice. BALB/c mice, 6 to 8 weeks old, were used for all experiments. Animals were acclimated for 1 week after arrival before use in experiments.

Drugs. Delta-9-THC, delta-8-THC, cannabidiol, and marijuana extract were supplied by the National Institute of Drug Abuse, Bethesda, Md. Twenty percent of the marijuana extract was delta-9-THC, but drug doses indicated refer to the total extract. 1-Methyl-delta-8-THC was synthesized by I. M. Uwaydah of the Medical College of Virginia by the method of Wildes et al. (22). The cannabinoids were dissolved in a mixture of Emulphor (EL-360; GAF Corp., New York, N.Y.) and ethanol (1:1, wt/vol) at a concentration of 100 mg/ml. The cannabinoid solutions were diluted with 0.15 M sodium chloride, all doses were adjusted to equivalent concentrations of vehicle, and the drugs were administered intraperitoneally (i.p.) or subcutaneously. Flumethazone pivalate (Locorten) was kindly supplied by Charles A. Brownley, CIBA, and dissolved in Emulphor-ethanol vehicle. Cyclophosphamide (NSC 26261) was supplied by the Na-

tional Cancer Institute, Bethesda, Md. Sodium pentobarbital (Diabutal) was obtained from Diamond Laboratories, Des Moines, Iowa. All drugs were administered in a volume of 0.01 ml/g of body weight. Flumethazone was administered i.p. or subcutaneously, and cyclophosphamide was administered i.p.

Measurement of DTH. The DTH response was quantitated by a modification of a radioisotope footpad assay orginally developed by Parajpe and Boone (19). Mice were sensitized to SRBC by injection of 10^7 cells into the rear left footpad. Unsensitized controls received physiological saline. On days 1 through 3 after sensitization the animals were treated subcutaneously with cannabinoids or the potent immunosuppressive steriod flumethazone. A challenge dose of 10^8 SRBC was administered into the same footpad on day 4 or 5. At 22 h after challenge the mice were injected intravenously (i.v.) with 1 μCi of ^{125}I-labeled human serum albumin, and 2 h thereafter the test foot and contralateral foot were excised and placed in a gamma counter. A positive footpad reaction was accompanied by an extravasation of the radiolabeled serum albumin from the blood stream into the intercellular space of the footpad. The DTH reactivity was calculated as a stimulation index, which is equal to the difference in the ratios of the radioactivity in the test foot divided by the radioactivity in the contralateral foot between sensitized and unsensitized animals. The stimulation indexes ranged from 2.0 to 4.0. The data were analyzed by analysis of variance with the Dunnett *t* test. We have determined that the footpad swelling response is a DTH reaction by the classic kinetics (21) and the mononuclear infiltration observed during histopathological examination (unpublished data).

Host resistance studies. The pool of HSV-2 was prepared as previously described (15). For experiments, the viral stock was diluted in diluent containing Hanks balanced salt solution, 0.5% gelatin, 0.25% lactalbumin hydrolysate, and 50 μg of gentamicin (Schering Laboratories, Kenilworth, N.J.) per ml.

L. monocytogenes strain 19303, obtained from H. Welshimer, Medical College of Virginia, was grown in brain heart infusion broth at 36°C overnight; the bacteria were centrifuged and suspended in 0.5% peptone broth, and the suspension was lyophilized. For experiments, the lyophilized bacteria were suspended in distilled water and diluted in distilled water to provide the appropriate dilutions. The bacteria were titrated for colony-forming units (CFU) on tryptose blood agar with 1% glucose. Mice were inoculated i.v. with 0.2 ml of appropriate dilutions of the organisms in the lateral tail vein. Each group consisted of 8 to 10 mice. Mortality was recorded daily for 14 days for *L. monocytogenes* and for 21 days for HSV-2 infection. The LD_{50} and 95% confidence limits were determined by the method of Litchfield and Wilcoxan (12), modified for computer calculation, and the data were analyzed statistically by analysis of variance. The LD_{50} for HSV-2 infection was expressed as the $\log_{10}$ of the dilution that resulted in 50% lethality. The LD_{50} for *L. monocytogenes* was expressed as the $\log_{10}$ CFU in the dilution that resulted in 50% mortality.

RESULTS

Effects of cannabinoids on resistance to *L. monocytogenes*.

Mice were infected i.v. with dilutions of bacteria and treated i.p. on days 1 and 2 after infection with various cannabinoids or with the known immunosuppressive steroid flumethazone (Table 1). The LD_{50} for untreated control mice (5.2 $\log_{10}$ CFU) was similar to that of mice treated with Emulphor–saline (5.4 $\log_{10}$ CFU), the vehicle in which the cannabinoids were suspended. However, treatment of mice with marijuana extract and delta-9-THC markedly suppressed resistance to the bacterial infection by 88- and 365-fold, respectively. The decreased resistance produced by delta-9-THC was similar to the 385-fold decrease produced by flumethazone.

The effects on host resistance were dose dependent (Fig. 1). Doses as low as 38 mg of delta-9-THC per kg significantly reduced resistance of mice to *Listeria*, although doses of 150 to 200 mg/kg were required for maximal suppression. Marijuana extract was less active, but also significantly reduced resistance to the bacterial infection.

The decreased host resistance appeared to be due to the immunosuppresssive properties of the drugs rather than the central nervous system (CNS) effects. Mice treated with sodium pentobarbital, which causes CNS depression and anesthesia, for 10 days during *Listeria* infection exhibited only a slightly decreased LD_{50} compared with that for control mice (Table 2). In contrast, treatment of mice with 1-methyl-delta-8-THC or with cannabidiol, which exhibit immunosuppressive effects without CNS activity (9), markedly decreased host resistance, 1-methyl-delta-8-THC by 34-fold and cannabidiol by 13-fold. Delta-9-THC was the most active, suppressing resistance by 107-fold.

TABLE 1. *Effect of cannabinoids on resistance to L. monocytogenes infection*[a]

Treatment		$\log_{10}$ CFU/ LD_{50}	95% confidence limits	Resistance decrease[b]
Drug	Dose (mg/kg)			
None		5.2	4.8–5.5	
Vehicle		5.4	4.8–5.9	2
Delta-9-THC	200	2.6	2.1–3.1	365[c]
Marijuana extract	200	3.2	2.8–3.6	88[c]
Flumethazone	5	2.6	2.2–2.9	385[c]

[a] Mice were inoculated i.v. with dilutions of bacteria and treated i.p. on days 1 and 2 of infection with the indicated doses of drugs; mortality was recorded, and the LD_{50} was calculated.

[b] Resistance decrease equals the arithmetic difference between the LD_{50} values of the control and treated groups.

[c] $P < 0.05$.

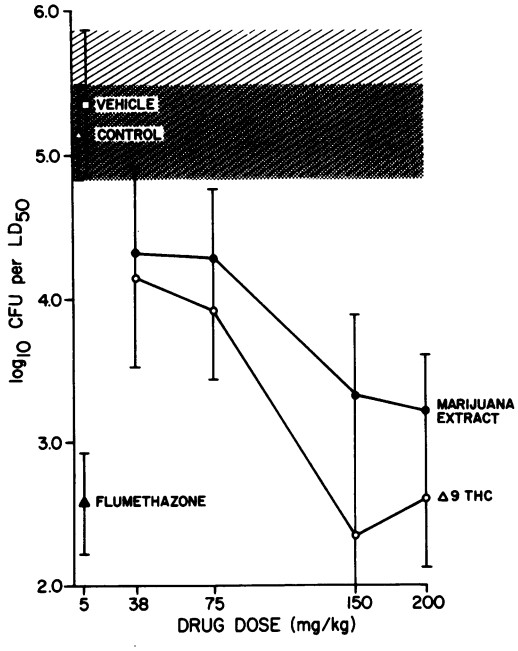

FIG. 1. *Dose response effects of cannabinoids on host resistance to L. monocytogenes. Mice were infected with dilutions of bacteria and treated i.p. with various doses of drugs on days 1 and 2 of infection; mortality was recorded, and the LD_{50} was calculated. The bars indicate the 95% confidence limits. All treated groups, except vehicle alone, were significantly different from the untreated control group.*

Effects of cannabinoids on resistance to HSV-2 infection. Delta-9-THC also decreased host resistance to systemic HSV-2 infection by 96-fold, although the marijuana extract was not active (Table 3). The inactivity of the marijuana extract may be related to insufficient active drug, because only 20% of the extract was delta-9-THC. The decreased host resistance produced by the cannabinoid was between that produced by flumethazone (10-fold) and that produced by cylophosphamide (260-fold).

Effects of cannabinoids on the DTH response. To confirm that cell-mediated immune responses were suppressed by cannabinoid treatment, the DTH response to SRBC was measured in treated and control animals. Treatment of mice with flumethazone at concentrations of 1 to 5 mg/kg produced a marked dose-dependent suppression of the DTH response (Table 4). The 50% effective dose was 0.5 mg/kg. Treatment of mice with marijuana extract produced significant immunosuppression at day 4 which waned somewhat by day 5 after antigen administration. Delta-9-THC was also effective, as were the two cannabinoids that do not have CNS activity. The 50% effective doses of the cannabinoids were all approximately 100 mg/kg. Other experiments have shown that the inhibition was not the result of inhibition of mobilization of an inflammatory response, but an effect of T lymphocytes (21).

DISCUSSION

The present studies clearly demonstrate that cannabinoids have the capacity to suppress host resistance to *L. monocytogenes* and herpes sim-

TABLE 2. *Relationship between immunosuppression and CNS activity on resistance to L. monocytogenes*[a]

Treatment		$\log_{10}$ CFU/LD_{50}	95% confidence limits	Resistance decrease[b]
Drug	Dose (mg/kg)			
None		6.2	5.8–6.5	
Vehicle		5.7	5.3–6.2	3
Delta-9-THC	150	4.2	3.8–4.5	107[c]
Sodium pentobarbital	60	5.5	5.1–6.0	4[c]
1-Methyl-delta-8-THC	200	4.6	3.6–5.7	34[c]
Cannabidiol	200	5.0	4.6–5.5	13[c]

[a] Mice were inoculated i.v. with dilutions of bacteria. Animals were treated i.p. with the indicated doses of cannabinoids on days 1 and 2 after infection and on days 1 to 10 with sodium pentobarbital. Mortality was recorded, and LD_{50} was calculated.

[b] Resistance decrease equals the arithmetic difference between the LD_{50} values of the untreated and treated groups.

[c] $P < 0.05$.

TABLE 3. *Effects of cannabinoids on resistance to HSV-2 infection*[a]

Treatment		$\log_{10}$ LD_{50}	95% confidence limits	Resistance decrease[b]
Drug	Dose (mg/kg)			
None		1.9	1.4–2.4	
Vehicle		2.6	2.2–3.0	6
Delta-9-THC	200	3.9	3.1–4.7	96[c]
Marijuana extract	200	2.3	1.8–2.9	3
Flumethazone	5	2.9	2.5–3.3	10[c]
Cyclophosphamide	200	4.3	3.0–5.6	260[c]

[a] Mice were inoculated i.v. with dilutions of HSV-2 and treated i.p. on days 1 and 2 with the indicated doses of drugs; mortality was recorded, and LD_{50} values were calculated.

[b] Resistance decrease equals the arithmetic difference between the LD_{50} values of the untreated and treated groups.

[c] $P < 0.05$.

TABLE 4. *Suppression of DTH response to SRBC by cannabinoids*[a]

Treatment		No. of mice per group	% Inhibition of footpad swelling on:	
Drug	Dose (mg/kg)		Day 4	Day 5
Delta-9-THC	1	32	28 ± 9[b]	13 ± 9
	10	52	38 ± 16	33 ± 8[b]
	100	32	53 ± 9[b]	50 ± 9[b]
1-methyl-delta-8-THC	1	32	27 ± 7[b]	9 ± 6
	10	32	30 ± 9[b]	26 ± 16
	100	32	53 ± 7[b]	37 ± 4
	200	32	88 ± 14[b]	59 ± 7[b]
Cannabidiol	1	16	31 ± 21	26 ± 10
	10	16	52 ± 13[b]	20 ± 10
	100	16	44 ± 10[b]	52 ± 11[b]
Marijuana extract	1	16	0 ± 9	0 ± 10
	10	16	0 ± 8	0 ± 12
	100	16	43 ± 9[b]	25 ± 9
	200	16	70 ± 9[b]	48 ± 13[b]
Flumethazone	0.1	32	ND[c]	0 ± 18
	1	32	73 ± 8[b]	75 ± 6[b]
	2	32	93 ± 3[b]	ND
	5	32	ND	100 ± 3[b]

[a] Mice were immunized in the footpad with 10^7 SRBC, treated subcutaneously with the indicated doses of drugs on days 1 to 3, and challenged on day 4 or 5 with SRBC in the footpad. The values are reported as mean ± standard error for the number indicated.

[b] $P < 0.05$.

[c] ND, Not determined.

plex virus, infections in which macrophages and immune T cells are involved in elimination of the microorganisms (1, 2, 13, 18). The degree of suppression produced by delta-9-THC, a major psychoactive component of marijuana, was similar to that of flumethazone and cyclophosphamide, potent immunosuppressants known to decrease host resistance to infection (3).

These data also indicate that a correlation may exist between the ability of cannabinoids to depress cell-mediated immunity and increase susceptibility to these infectious agents. The doses and regimen of treatment with cannabinoids that produced significant decreases in host resistance were similar to those which caused suppression of DTH, treatment with 100 mg/kg for 2 to 3 days after antigen stimulation or microbial infection. Moreover, Munson et al. (16) have demonstrated that single or multiple doses of delta-9-THC do not alter the phagocytic activity of the fixed macrophage system.

The mechanism whereby the cannabinoids suppress lymphocyte responsiveness and host resistance remains to be established. We have previously reported that delta-9-THC does not produce humoral immunosuppression by release of adrenal steroids because delta-9-THC maintained its immunosuppressant activity in adrenalectomized mice (S. H. Smith, J. A. Levy, and A. E. Munson, Abstr. Virg. J. Sci. 27:87, 1976). The present studies indicate that the immunosuppression is not related to marijuana-like CNS activity; the nonpsychoactive 1-methyl-delta-8-THC and cannabidiol displayed comparable immunosuppressant activity to delta-9-THC and delta-8-THC. Furthermore, treatment for 10 days with sodium pentobarbital, which causes CNS and pulmonary suppression, was only slightly active in decreasing host resistance to *L. monocytogenes*. Although the possibility exists that the cannabinoids increase susceptibility to infection by their action on T lymphocytes (21), the interaction of the cannabinoids with other biological components cannot be excluded. Recent studies from our laboratories demonstrate that a lethal synergism exists between naturally occurring cannabinoids and bacterial lipopolysaccharides (4, 16a) and that the lethality resulting from this interaction is a function of cannabinoid activity and not lipopolysaccharide activity. Therefore, the possibility exists that infections may increase the toxicities manifested by cannabinoids.

On the basis of the data presented herein we do not propose at the present time that individuals exposed to marijuana are more susceptible to infectious agents. The route of administration and dosages of cannabinoids employed in these investigations are not representative of actual human exposure. Thus, the potential risk of increased infections by human marijuana users cannot be reliably predicted by the present data. Definitive answers to this question will only come from well-structured prospective investigations, such as the one now being carried out in Costa Rica on the susceptibility of human marijuana users to various infectious diseases (5).

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