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Suppressive Effect of Δ^9-Tetrahydrocannabinol *in Vitro* on Phagocytosis by Murine Macrophages (42332)

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Abstract. Incubation of normal mouse peritoneal cells consisting of over 90% phagocytizing macrophages with Δ^9-tetrahydrocannabinol (THC) resulted in a inhibition of phagocytic function. The THC in a dose-related manner suppressed the percentage of macrophages per culture which ingested yeast and the average number of yeast particles ingested by the phagocytizing macrophages. The vehicle used to suspend the THC *in vitro,* i.e., DMSO, had no detectable effect on macrophage function. Suppression of phagocytosis with no effects on viability or cell number occurred with doses of 10 μg or less THC per milliliter culture medium. Measurable suppression also occurred after 24- to 48-hr treatment of the macrophages with the THC. This compound had little if any detectable effect on phagocytosis when added directly to the cultures shortly before testing for phagocytosis. Further studies concerning the effects of THC on macrophage function appear warranted.

Mononuclear phagocytes serve an important role in nonspecific defenses against infectious agents such as bacteria, viruses, and fungi (1, 2). Previous studies have shown that some activities of these cells may be influenced by Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychogenic constituent of marijuana. For example, it has been observed that alveolar macrophages from rats exposed to aerosols of THC for varying lengths of time are depressed in their ability to kill *Staphylococcus aureus in vitro* and also show marked alterations in the activity of enzymes known to be important in phagocytic function (3, 4). However, there have been no studies reported concerning the direct effects of THC on macrophages in culture. In the present study, graded concentrations of THC were added to cultures of resident murine peritoneal macrophages which were then examined for their ability to phagocytize yeast particles *in vitro.* Whereas dimethyl sulfoxide (DMSO), the vehicle used to suspend THC, had no detectable effect, the cannabinol suppressed phagocytosis in a dose-related manner.

Methods and Materials. *Tetrahydrocannabinol.* Δ^9-THC was obtained from the National Institutes on Drug Abuse, Rockville, Maryland. Stock solutions of graded concentrations were prepared by dissolving 1 mg in 1% DMSO at room temperature and then by preparing graded serial dilutions in saline.

Peritoneal macrophages. Normal BALB/c mice, 6–8 weeks of age, were obtained from Jackson Laboratories, Bar Harbor, Maine. They were sacrificed by cervical dislocation; the peritoneal membrane was exposed and an intraperitoneal injection of 5 ml sterile medium 199 (DMEM) was administered. The peritoneum was gently massaged and cells were obtained by needle and syringe. The cells were sedimented by gentle centrifugation at 1000*g* at 4°C and washed several times by sequential centrifugation with DMEM at 4°C fortified with 10% FCS supplemented with antibiotics and suspended to a concentration of 2.5×10^6 viable macrophages per milliliter as determined by staining with trypan blue and microscopic examination (4). Over 90% of the resulting cells were capable of ingesting heat killed yeast particles within 15–30 min by incubation at a ratio of 10 killed yeast per viable peritoneal cells at 37°C in medium containing 10% sterile rabbit serum as the opsonin.

Phagocytic assay. Suspensions of 2.5×10^6 peritoneal macrophages in 0.1 ml of medium were placed on coverslips in microwells of 24-well Linbro plates. The cells were allowed to adhere for 2 hr at 37°C and the nonadherent cells decanted. Washed suspensions of 10^6 heat

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killed *Saccharomyces cerevisiae* (18 hr washed overnight growth in broth heated for 30 min at 80°C) were added to each culture. The cultures were incubated at 37°C in an atmosphere of 95% air and 5% CO_2. At the time of assay (15 min), the coverslip was removed from each well, stained with Wright's stain, and the number of cells with two yeast particles per cell and the average number of yeast per positive cell were determined by microscopic examination of at least 300 cells per coverslip. In order to assure that the yeast were phagocytized, viable yeast (nonheat killed) were used in similar experiments. The number of viable yeast per culture, both in distilled water lysates of the 10^6 macrophages and the cell-free supernatant medium, was determined by direct plating technique on Sabouraud's agar. Results obtained were essentially similar to those obtained by direct microscopic examination. For experimental cultures, graded amounts of THC in 0.1 ml medium were added at the time of culture initiation and addition of yeast, either killed or live, or, in some experiments, at various times thereafter. Controls concentrated of similar cultures were incubated in medium alone or medium with 0.1% DMSO, the final concentration for the 10-μg dose of THC. In all cases each experiment was repeated at least three times.

Experimental Results. Treatment of normal mouse peritoneal cells with graded doses of THC resulted in a dose-dependent suppression of the ability of the macrophages to ingest yeast particles (Table I). Within 24–48 hr after incubation of the macrophage cultures with 1–20 μg THC there was an alteration of the percentage of macrophages which had ingested two or more yeast particles each in many of the cultures. Control cultures incubated with the DMSO vehicle alone or medium alone readily ingested the yeast. About 50% of the peritoneal cells phagocytized two or more yeast particles at 48 hr of incubation. About 25% of the cells in the control cultures were phagocytic at 24 hr. In contrast, only about 10% of the cells pretreated with 10 μg DMSO ingested more than two yeast particles each at 24 hr. About 20% were positive at 48 hr.

Twenty micrograms THC resulted in an even greater inhibition, but with this dose the cells in culture, after 24 hr incubation, lost about 40–50% of their viability as compared to those in control cultures (Table I). The trypan blue stain, however, showed only a moderate increase in blue positive cells (apparently dead cells) at 24 hr as compared to untreated cell cultures. By 48 and 96 hr even larger number cells treated with 20 μg THC were nonviable as compared to control cultures. There were marked cytologic changes evident also.

Lower doses of THC had a moderately less effect on phagocytic activity of the peritoneal cells. Addition of 7.5 μg THC to 10^6 cells resulted in moderate inhibition of phagocytosis

TABLE I. EFFECT OF THC ON PHAGOCYTOSIS OF YEAST PARTICLES BY NORMAL MOUSE PERITONEAL CELLS

THC Dose (μg/culture)[a]	Percentage viable cells per culture[d]	Phagocytosis on day[b]		
		+1	+2	+4
Medium (control)	86.3 ± 5.8	25 ± 6	49 ± 9	79 ± 6
20.0	47.8 ± 4.9	14 ± 1	18 ± 2[c]	24 ± 3[c]
10.0	82.1 ± 3.6	11 ± 2[c]	28 ± 5[c]	45 ± 4[c]
7.5	84.3 ± 4.8	15 ± 3[c]	30 ± 5[c]	55 ± 5[c]
5.0	83.5 ± 5.3	19 ± 5[c]	39 ± 5[c]	58 ± 5[c]
2.5	89.2 ± 4.1	22 ± 3	42 ± 3	66 ± 6
1.0	86.3 ± 5.1	29 ± 3	48 ± 8	72 ± 4
DMSO (control)	82.4 ± 3.2	26 ± 5	51 ± 7	87 ± 5

[a] Indicated concentration of THC in DMSO added to suspensions of 10^6 mouse peritoneal cells on day of culture initiation.

[b] Average percentage of macrophages ± standard deviation at indicated time of culture which phagocytized two or more killed yeast cells after 15 min incubation.

[c] Statistically significant ($P = 0.02$ by student's t test vs medium control).

[d] Average percentage trypan blue negative cells after 24 hr incubation with indicated dose of THC for 1 day.

at 24 to 72 hr, as compared to untreated control cultures or cultures treated with DMSO alone (Table I). Addition of 5 μg THC per culture resulted in a much less effect and 2.5 μg and lower doses of THC had only a slightly detectable effect (Table I).

There was a marked difference in the total number of yeast particles phagocytized by cells in culture treated with different doses of THC. As is evident in Table II, the control cultures which were untreated or treated with DMSO only showed that about 12% had eight or more yeast particles per cell and about 25% showed only one yeast per cell. A smaller percentage of cultures treated with 10 or 5 μg THC had eight or more yeast per cell and more cells had none or only one yeast per cell. A majority of the cells had two to four yeast particles each. One microgram THC had no effect.

A greater suppression occurred when THC was added at the same time of culture initiation as compared to 24 or 48 hr later (Table III). Addition of the THC immediately before assay (i.e., 15 min before testing) did not result in suppression of the number of yeast per macrophage (data not shown). Similar results were observed in experiments where viable yeast was used and phagocytosis was determined by plating distilled water lysates of washed macrophage cultures in Sabouraud's medium 24 or 48 hr after incubation with viable yeast and graded doses of THC (data not presented).

TABLE III. SUPPRESSIVE EFFECT OF THC ON UPTAKE OF YEAST PARTICLES ADDED TO MOUSE PERITONEAL CELLS AT VARIOUS TIMES

	Phagocytosis[b]	
Time of addition of THC[a]	Percentage macrophages with yeast	Percentage of control
None (control)	76.5 ± 13.3	—
0 time	25.3 ± 4.8	33
+1 day	49.3 ± 6.2	64
+2 day	70.3 ± 11.3	92

[a] Addition of 10.0 μg THC to cultures of 10^6 normal mouse peritoneal cells at indicated time after culture initiation.

[b] Average number of macrophages, ±SE, with two or more yeast particles 3 days after culture initiation.

Discussion and Conclusions. Macrophages are considered a class of reticuloendothelial cells which are involved in nonspecific host resistance to a wide variety of substances, including microorganisms such as bacteria, fungi, and viruses, as well as to tumor cells (1, 2). These cells may be readily activated by soluble mediators elaborated by other lymphoid cells, such as sensitized T cells interacting with antigen or mitogen, as well as with antibody itself. A variety of substances, both specific and nonspecific, may activate macrophages to evince activities such as phagocytosis. Various studies in recent years have also been concerned with inhibitory effects of different substances on macrophage functions.

In the present study it was observed that Δ^9-THC, the active psychogenic moiety present

TABLE II. EFFECT OF VARYING CONCENTRATIONS OF THC ON UPTAKE OF YEAST PARTICLES BY MACROPHAGES FROM NORMAL MICE

		Percent of macrophages ingesting indicated number of yeast[c]			
THC dose[a] (μg/culture)	Percentage viable cells/cultures[b]	0–1	2–4	5–7	8 or more
None (control)	66.3 ± 4.6	25.5	23.4	38.6	12.5
10.0	63.2 ± 3.3	35.4[d]	34.2[d]	25.3[d]	5.1[d]
5.0	61.5 ± 6.5	28.8	32.6[d]	29.8[d]	8.8[d]
1.0	62.5 ± 4.8	29.5	30.9[d]	30.3[d]	15.3
DMSO (control)	70.9 ± 6.3	26.3	22.7	40.5	10.5

[a] Indicated concentration of THC or DMSO added to cultures of 10^6 peritoneal cells from normal mice.

[b] Viability determined by trypan blue dye exclusion test after 3 days culture.

[c] Average percentage ± standard macrophages ingesting indicated number of yeast particles in three to four cultures 3 days after culture initiation.

[d] Statistically significant ($P = 0.02$ by student's t test of THC-treated cultures vs medium control).

in marijuana, inhibits in a dose-related manner the phagocytic activity of macrophages challenged *in vitro* with killed yeast particles. A dose of THC achievable *in vivo* (i.e., approximately 5 μg/ml) suppressed the percentage of macrophages capable of ingesting yeast particles as well as affected the total number of yeast cells ingested by individual macrophages.

Previous studies have suggested that macrophages may be influenced by THC or at least by marijuana smoke. Exposure of rats to marijuana smoke for as little as 15 min resulted in marked histological alteration of alveolar macrophages as determined by microscopic examination (6, 7). Biochemical alterations have also been observed in alveolar macrophages from rats which have been exposed to marijuana smoke (6, 7). There have been no reported studies on the effects of THC itself on macrophage function *in vitro.* It is widely recognized that many factors may influence the apparent phagocytic functions of macrophages *in vivo,* including cell migration, as well as other lymphoid cells such as T lymphocytes, soluble mediators released by such lymphocytes, antibody itself, and antibody–antigen complexes, as well as complement and other factors in serum. Thus analysis of effect of a substance such as THC on the function of macrophages *in vitro,* i.e., yeast phagocytosis, may be of value in attempts to assess the direct effects of the substance on an important cellular component of the defense system. The results of the present studies provide evidence that THC, even at doses which are not considered toxic and which do not affect viability or total number of macrophages *in vitro,* affects the ability of these cells to ingest yeast particles. Other studies in this laboratory have shown that the spreading ability of macrophages on glass surfaces, considered an important indicator of the biologic activity of "healthy" macrophages, is also inhibited by THC treatment *in vitro* (in preparation).

It is of interest to note that although DMSO was used as a vehicle for suspending THC, this substance by itself had no effect on macrophage phagocytosis. Thus it appears likely that THC in some way influences the macrophages so they either ingest fewer yeast particles per cell or the percentage of cells which can ingest the yeast is markedly diminished. This may reflect an altered capability of these important cells to function in this phagocytic capability to opportunistic pathogens.

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