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Enhanced Suppression of Blastogenic Responses by Weanling Mouse Lymphoid Cells Treated with Tetrahydrocannabinol *in Vitro* (42577)

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Abstract. The suppressive effects of Δ^9-tetrahydrocannabinol (THC) on the proliferation of lymphocytes from the spleen, lymph node, and thymus of weanling animals vs adult animals to the T-cell mitogen PHA were examined. THC had a suppressive effect on thymus cells from animals of both younger and older mice. THC suppressed spleen and lymph node cells responses to phytohemagglutinin (PHA) more readily when the cells were obtained from young mice rather than older animals. Suppression by THC in the adult mice was greater in an organ containing fewer mature T lymphocytes such as the thymus in comparison to lymphocytes in secondary organs such as the spleen and lymph nodes which contain more mature lymphocytes. © 1987 Society for Experimental Biology and Medicine.

Δ^9-Tetrahydrocannabinol (THC) is considered the major psychoactive substance present in marijuana (1). Many reports, including those from this laboratory, indicate that THC, as well as other psychoactive components of marijuana, influence the function of lymphocytes and macrophages *in vitro* and *in vivo* without causing any detectable toxicity (2–8). Only a few reports have failed to substantiate the observation that marijuana smoking is immunosuppressive (9–10). Many investigators have reported suppressed immunologic parameters in marijuana smokers including fewer rosette-forming cells (11–12), a diminished delayed type hypersensitivity reaction (13), and a reduced response to mitogens (2, 14, 15). Discrepancies in the literature concerning the effects of THC on lymphocyte blastogenesis have been related to methodological variations of culture conditions (including protein concentration, cannabinoid concentration and form, and species differences) (14, 16).

Previous studies in this laboratory have shown that murine spleen cells treated with THC or its metabolic product 11-hydroxy THC suppressed proliferative responses when stimulated with T-cell mitogens such as phytohemagglutinin (PHA) or concanavalin A (Con A) (16, 17). Other recent studies in this laboratory have shown a lesser effect of THC and 11-OH THC on lymph node cells. In the present study, a differential effect was noted for lymphoid cells from different organs. Thymus cells were more readily suppressed than cells from either the spleen or lymph nodes in adult mice. This appeared to be due to the maturity of T lymphocytes in the different organs. If this view is correct, then it is possible that there may be a differential effect of THC on lymphoid cells from younger individuals as compared to those from more mature ones. In order to test this possibility, the effects of THC on the responsiveness of lymphoid cells from younger animals to PHA were studied. For this purpose the effect of THC on blastogenic responsiveness of spleen, lymph node, and thymus cells from weanling mice 3–4 weeks of age was compared to the effect on cells from adult animals 10–12 weeks of age.

Methods and Materials. *Animals.* Weanling Balb/c mice, 3–4 weeks of age, were obtained from Jackson Laboratories, Bar Harbor, Maine. They were kept in groups of six to eight in pathogen-free conditions in plastic mouse cages. For comparative purposes, adult Balb/c mice 10–12 weeks of age were similarly obtained and studied. All the animals were fed commercial mouse pellets and water *ad libitum.*

THC. This marijuana component was obtained from the National Institute on Drug Abuse, Rockville, Maryland, in ethanol. For use, the THC preparation was reconstituted in dimethyl sulfoxide (DMSO), which was also used as a control.

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Blastogenic response. The spleen, mesenteric lymph nodes, and thymus were obtained from weanling and adult mice after cervical dislocation and single cell suspensions were prepared with a Stomacher 80 lab blender (Tekmar Companies, Cincinnati, OH). The cells were washed several times by centrifugation with Hanks' balanced salt solution and resuspended to a concentration of 4×10^6 nucleated cells/ml in RPMI 1640 medium containing 10% fetal calf serum, antibiotics and 2-mercaptoethanol (5×10^{-5} *M*). For assay 0.1 ml of a cell suspension was suspended in individual wells of 96-well flat bottom plates (Costar). PHA (Burroughs-Wellcome Co., Greensville, NC) was prepared in medium at a final concentration of 5 μg/ml using a 0.05-ml volume of medium. Graded doses of THC were added in 0.05-ml volumes to each well. The cultures were incubated at 37°C for 48 hr in an atmosphere of 5% CO_2 and air. The plates were then pulsed with 0.5 μCi [^{3}H]thymidine for 18 hr. The cells were harvested on glass fiber filters and radioactivity in the cell preparation was determined by standard liquid scintillation counting. Counts per minute ± standard deviation were calculated for triplicate cultures and in all cases each experiment was performed at least three times. The groups were averaged and data are expressed as counts per minute ± standard error of the mean (cpm ± SEM). Data were calculated using Student's *t* test.

Experimental Results. As noted previously, there was a dose-related effect of THC on the proliferative response of lymphoid cells from mice incubated *in vitro* with this marijuana component and stimulated with a constant amount of PHA (Table I). DMSO, at the concentration used for the 10 micrograms per milliliter THC dose, had no effect on the blastogenic response of lymphoid cells from either young or adult mice as compared to cultures in DMSO-free medium only. It is evident that cultures of thymocytes from both age groups showed the most marked depression of PHA-induced blastogenesis following treatment *in vitro* with 10 μg THC. However, there was somewhat greater suppression of thymus cell blastogenesis from younger animals with lower doses of THC as compared to the responses of thymus cells from adult animals. For example, the 7-μg dose of THC resulted in a 50%

TABLE I. THC-INDUCED EFFECT ON PROLIFERATION OF SPLEEN, LYMPH NODE, AND THYMUS CELLS FROM WEANLING VS ADULT MICE STIMULATED *IN VITRO* WITH PHYTOHEMAGGLUTININ

THC concentration (μg/ml)[a]	Weanling mouse						Adult mouse					
	Spleen cpm[b]	% of control	Lymph node cpm	% of control	Thymus cpm	% of control	Spleen cpm	% of control	Lymph node cpm	% of control	Thymus cpm	% of control
None (medium control)	92.6 ± 15	—	95.2 ± 2	—	39.2 ± 5	—	101.3 ± 4	—	75.9 ± 14	—	21.7 ± 3	—
DMSO in medium	87.1 ± 3	—	75.8 ± 19	—	32.5 ± 4	—	83.5 ± 5	—	78.2 ± 14	—	21.5 ± 2	—
1.0	77.5 ± 5	83	81.4 ± 3	85	34.1 ± 3	87	94.7 ± 8	93	76.9 ± 17	101	27.1 ± 3	125
5.0	81.4 ± 3	88	84.8 ± 4	89	36.7 ± 3	94	97.0 ± 8	96	83.7 ± 9	110	23.8 ± 4	110
7.0	53.3 ± 8	58[c]	55.5 ± 4	39[c]	19.7 ± 4	50[c]	93.0 ± 4	92	73.2 ± 4	96	17.7 ± 3	82
10.0	6.1 ± 2	6[c]	8.4 ± 3	9[c]	3.8 ± 1	10[c]	37.8 ± 2	37[c]	59.3 ± 3	78[c]	3.8 ± 2	18[c]

[a] Indicated dose of THC added to cultures of 4×10^6 lymphoid cells/ml from spleen, lymph node, or thymus from 3- to 4-week-old weanling or 10- to 12-week-old adult mice.
[b] Average blastogenic response as Δ CPM ± SEM ($\times 10^{-3}$) for three to four cultures per group after stimulation with 5.0 μg PHA.
[c] Statistically significant as compared to DMSO control responses at $P \leq 0.05$.

suppression of thymus cells from weanling mice vs approximately a 20% suppression of the response of thymus cells from adult mice.

Susceptibility of the lymphoid cells from the spleen and lymph nodes to suppression by THC was most evident when the cells were derived from the young mice as compared to those from adult animals (Table I). The 7-μg dose resulted in a 40–60% suppression of the response of these cells from younger mice but had no effect on cells from older mice. Even the 10-μg dose of THC had only a slight to moderate effect on the response of lymph node cells from adult mice but resulted in about a 90% suppression of the response of the same cell populations from weanling mice. The 10-μg dose of THC resulted in a 63% suppression of adult spleen cells and a 94% suppression of spleen cells from young animals.

Discussion and Conclusions. It is widely acknowledged that proliferation of lymphoid cells is considered a fundamental and necessary event in activation of the immune response mechanism. Previous studies in this and other laboratories have shown that THC, the major psychoactive component of marijuana, has suppressive effects on the proliferation of both human and animal lymphoid cells to a T-cell mitogen such as PHA as well as to Con A or to a B-cell mitogen such as *Escherichia coli* LPS (3, 16). In the present study THC was found to have a greater ability to suppress the blastogenic response of lymph node and spleen cells from weanling mice as compared to the response of similar lymphoid cells from older animals.

It is noteworthy that thymus cells were readily suppressed when derived from either younger or adult animals. These results are in contrast to those obtained from lymph node cells. This supports the view that THC has a differential effect on lymphoid cells from different organs, possibly because of the differential content of mature T lymphocytes. It is widely assumed that thymus cells represent a more immature cell population as compared to spleen or lymph node cell populations. Thus the results of these experiments indicate that THC and possibly other psychoactive agents may have a greater activity at the level of developing lymphocytes such as immature thymocytes as compared to more mature lymphocytes. If in fact THC does have a greater effect on immature T cells, the age of an individual exposed to marijuana and its components may prove critical. The susceptibility of lymphoid cells from young vs adult animals in regard to susceptibility to suppression by THC thus appears due to qualitative as well as possibly quantitative variations in cellular composition of these organs. In this regard, it has been reported that younger mice are very susceptible to THC following *in vivo* administration (17). These studies, as in our own, used mice 3–4 weeks old, a time before sexual maturity.

It seems likely from the results of these experiments that younger animals may have more THC-susceptible lymphoid cells in the secondary lymphoid organs (spleen and lymph node) than do the adults. The concept that the cannabinoids may preferentially affect certain T-cell subsets was recently mentiond (18), but not directly investigated. Thus it appears important to compare lymphoid cell populations in secondary organs with those in primary lymphoid organs, as well as to compare lymphoid organs from young vs adult animals.

Similar studies in progress are being pursued with other purified components of marijuana as well as other psychoactive drugs. Studies concerning the possible differential effect of THC and other psychoactive drugs on immune responses *in vivo* are also under way with varying doses of the drugs administered by various routes. If these drugs indeed have a greater suppressive effect on more immature cells present in younger individuals, this may have implications concerning the effects of psychoactive agents such as marijuana in younger individuals.

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