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Influence of Maturity on Immunosuppression by Δ^9-Tetrahydrocannabinol (40202)

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Evaluation of marihuana's potential hazards has indicated possible interference with immunological defense mechanisms. Peripheral blood lymphocytes from regular users of marihuana demonstrated an impaired capacity to proliferate in the presence of phytohemagglutinin (PHA) (1). However, other reports failed to substantiate an association between chronic marihuana smoking and a depressed cell-mediated immunity (2–4). No impairment was seen in marihuana smokers subjected to a skin test measuring delayed-type hypersensitivity (5). Further assessment of T-cell function revealed a decrease in the formation of rosettes with sheep erythrocytes (SRBC) (6–8). Splenic lymphocytes obtained from mice treated with Δ^9-Tetrahydrocannabinol (THC) have been shown to be impaired in their blastogenic response to PHA and *E. coli* lipopolysaccharide (LPS) (9). Treatment of mice with THC has also been responsible for a reduction in the production of circulating antibody to SRBC (9, 11) and in the number of IgM plaque-forming cells (PFC) following SRBC challenge (10, 12).

Since chronic marihuana use has extended to children, teenagers and young adults, immunological studies in animals should include drug regimens in young, immature animals. It was therefore of interest to determine if younger mice might be more sensitive to the effects of THC.

Materials and methods. THC, provided by the National Institute on Drug Abuse, was prepared and administered in a vehicle of 5% Tween 80 in phosphate buffered saline (PBS). Male BDF_1 hybrid mice, 4–14 weeks of age were injected intraperitoneally (ip) with 10–40 mg/kg of THC. Age-matched controls received vehicle only. The total number of daily injections varied depending on the parameter measured in order to accommodate immunization schedules and peak responses.

Lymphocyte reactivity to PHA was measured by the method described by Adler *et al.* (13). For all assays, single cell suspensions were prepared by gentle teasing of the spleen. Cells were washed and counted on a Coulter Counter, Model ZBI. Hemagglutination titers to SRBC were performed using a "micro" system. Mice were injected for 4 days with THC. On the first day of injection, in addition to drug or vehicle, mice received 0.2 ml of a 50% suspension of SRBC. Following the procedure of Zimmerman *et al.* (12), blood was collected by cardiac puncture 4 days after antigen injection, allowed to clot, and the sera removed. To avoid error seen with doubling dilutions, previously inactivated sera were initially diluted 1:2, 1:3, 1:5 and 1:15. Each initial dilution was then serially diluted in microtiter plates and incubated for 1 hour at 37° with SRBC. Antibody titers were read as the highest serum dilution demonstrating definite agglutination. The IgG plaque PFC response to SRBC was determined by the localized hemolysis in gel method described by Dresser and Wortis (14). Mice were injected ip with 4×10^8 SRBC on day 1 and THC was given on days 1–8. On day 9, when peak IgG responses were obtained, mice were sacrificed and their spleen cells harvested and assayed for the production of IgG PFC. Rosette-forming cells (RFC) were detected by the technique described by Greaves and Möller (15). In these studies mice were injected for 7 days and sacrificed on day 8. On the second day of injection, animals were immunized with 0.2 ml of a 50% solution of SRBC.

Results. The susceptibility of younger mice to THC was reflected by a reduction of both splenic and body wt and a reduction in the number of spleen cells. This was either less evident or not seen in the older animals with the dosages employed (Table I).

Suppression of cellular immunity by THC appeared to depend on the age of the drug-treated animals. In 14-week-old mice, injected with either 25 or 40 mg/kg, there was little change in the responsiveness to PHA (Fig. 1). On the other hand, 4-week-old mice

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TABLE I. INFLUENCE OF AGE: EFFECTS OF Δ^9-THC ON BODY WEIGHT, SPLEEN WEIGHT AND SPLEEN CELLS.[a]

	Δ^9-THC (mg/kg)	Age at first injection (wks)	Body weight (g)[b]	Spleen weight (g)[b]	Spleen cells (mean no. × 10^6)[b]
Experiment I	25	4	16.97 ± 0.75[c]	0.063 ± 0.006[d]	55 ± 3.17[e]
	Vehicle		18.10 ± 0.79	0.085 ± 0.005	93 ± 5.55
Experiment II	25	14	27.54 ± 1.05	0.090 ± 0.005	99 ± 5.16
	Vehicle		26.06 ± 0.79	0.078 ± 0.005	98 ± 5.67

[a] Six mice were injected with Δ^9-THC, ip, for 5 days.
[b] Mean ± SE.
[c] $P \leq 0.05$.
[d] $P \leq 0.025$.
[e] $P \leq 0.005$.

possessed an impaired capacity to incorporate thymidine. The results obtained using young mice injected with 25 mg/kg were compared to those of identically-treated 14-week-old mice (Fig. 1). Similar results were obtained with 40 mg/kg.

The actions of THC on the humoral response were also influenced by age. Circulating antibody to SRBC was reduced in both 4- and 14-week-old mice following treatment with 25 mg/kg THC (Table II). However, the effect on the hemagglutination titer was more pronounced in the younger animals suggesting an increased susceptibility to the drug. IgG antibody production was compared in 7- and 14-week-old animals exposed to 25 mg/kg (Fig. 2). Again both age groups were affected, but the number of plaques in the 7-week-old drug-treated mice were reduced to 50% that of the controls, whereas the reduction in the older mice was only 29% below that of the age-matched controls. The SRBC binding capacity of splenic lymphocytes from 14-week-old drug-injected mice was unaltered. In comparison, the number of rosettes present in 4-week-old immunized mice injected with 25 mg/kg was considerably below that of the controls (Fig. 3). This reduction occurred in both T-cell and B-cell rosettes, which were delineated on the basis of numbers of attached erythrocytes (16). Specific data on this parameter was recently reported by Lefkowitz *et al.* (17).

Discussion. The dosage of THC employed in these studies represents levels reasonably comparable to those used in man when determined on the basis of body surface area and taking into account the THC dose range for an average quantity of marihuana (18, 19) and based upon the route of administration (20, 21). The selection of these levels was

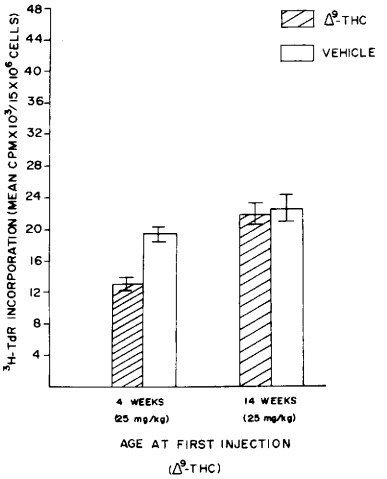

FIG. 1. PHA stimulation of spleen cells from 4- and 14-week-old BDF_1 mice after ip injection of Δ^9-THC or vehicle for 5 days. Results expressed as mean values ± SE for 4–5 replicate tubes.

TABLE II. INFLUENCE OF AGE: EFFECTS OF Δ^9-THC ON HEMAGGLUTINATION TITER TO SRBC.[a]

	Δ^9-THC (mg/kg)	Age at first injection (wks)	Hemagglutination titer[b]
Experiment I	25	4	12 ± 3[c]
	Vehicle		48 ± 17
Experiment II	25	14	38 ± 11[d]
	Vehicle		78 ± 14

[a] Five mice injected with Δ^9-THC, ip, for 4 days.
[b] Results expressed as reciprocal of highest dilution showing definite hemagglutination.
[c] $P \leq 0.05$.
[d] $P \leq 0.025$.

based on minimal toxicity with substantial immunologic effect. Concentrations as low as 10 mg/kg frequently affected the immunologic response, but the next concentration (i.e., 25 mg/kg) was invariably effective.

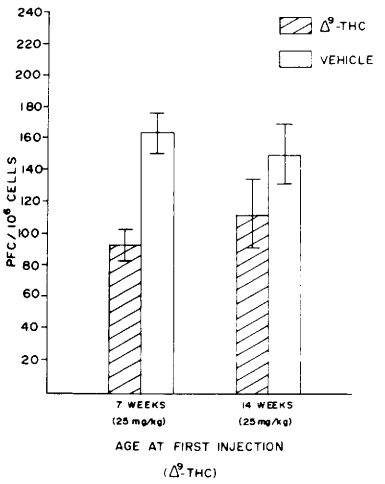

FIG. 2. Number of IgG plaque-forming cells (PFC) per 10^6 cells from 7- and 14-week-old BDF_1 mice after ip injection of Δ^9-THC or vehicle for 8 days. Mean of 5 mice ± SE.

Higher concentrations frequently resulted in low body and spleen weight with reduced splenic cellularity. Previous studies have also indicated a reduction in thymus size with higher dosages (11). Similarly, because our investigations were based on short term exposure to THC, we employed dosages higher than those which might produce equivalent effects from prolonged exposure at lower concentrations.

These studies demonstrated that the age of the animal exposed to THC seemed to determine the effects of the drug on the immune response. Cellular immunity in young drug-treated mice was diminished but remained unchanged in older mice as evidenced by PHA stimulation. Humoral immunity in both young and old mice was reduced by THC, but the suppression seen in the young mice was more pronounced.

A number of possibilities exist which may explain these age phenomena. Available evidence suggests that THC affects developing cells more than mature cells (16). Therefore, a young developing system might be more sensitive to drug exposure whereas older animals would tend to be more tolerant. The interaction of THC with the lipid phase of cell membranes is thought to play a role in many of the drug's pharmacological actions (17). Certain changes in the membrane may occur with age and alter the binding capacity of the drug, thus interfering with its immunosuppressive properties.

Recently, it has been proposed that chronic use of cannabis derivatives may lead to the development of compensatory mechanisms effective enough to overcome and restore an initially suppressed immune response (16). The younger system may be less able to develop such a compensatory mechanism so that chronic THC exposure would result in a suppression which could not be restored. In older animals, this mechanism may be fully developed and thus allows these mice to overcome the drug's immunosuppressive effects.

Another factor which is capable of influencing maturity-directed responses to THC is a change in T-cell populations observed in young mice. It is known that there is a changing of cell populations as well as alteration of function of certain T-cells in aging mice. There is a suggestion that thymic tissues from newborn mice but not mice over 3 months of age are able to repopulate lymph node T-dependent areas in mice which have been T-cell deprived (18). This would suggest a rather critical period of time during the first few months of life indicating that responses to a drug such as THC could be somewhat different from animals which are slightly more mature. These studies suggest an increased

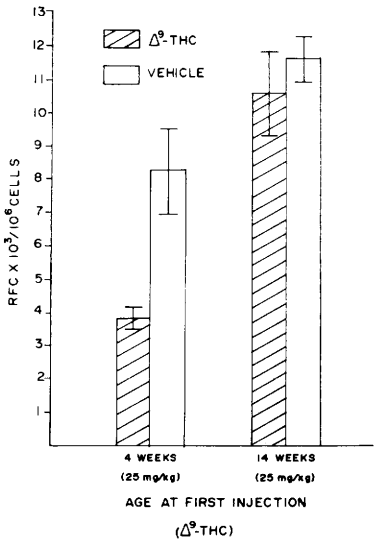

FIG. 3. Number of rosette-forming cells (RFC) per 10^6 cells from 4- and 14-week-old BDF_1 mice after ip injections of Δ^9-THC or vehicle for 7 days. Mean of 5 mice ± SE.

susceptibility of younger mice exposed to THC. Since marihuana is often used chronically by young people, the effects of the drug on the immune function in these young users must be considered.

Summary. Marihuana and its principal active ingredient Δ^9-tetrahydrocannabinol (THC) has been shown to be immunosuppressive in mice. The present study was conducted in order to determine if the immunosuppression was regulated by the maturity of the animal. Splenic lymphocytes from 4-week-old mice exposed to THC displayed a reduced capacity for mitogenic stimulation following *in vitro* exposure to phytohemagglutinin. Rosette formation, IgG plaque formation, and synthesis of circulating antibody to sheep erythrocytes (SRBC) were also inhibited after exposure of young mice to THC. Cell-mediated immunity and rosette formation in 14-week-old mice was not significantly altered by treatment with THC as evidenced by the equivalent capacity of splenic lymphocytes from both drug and control animals to respond to mitogenic stimulation and to bind SRBC. Older mice were also found to be less responsive to immunosuppression by THC in relation to IgG plaque formation and hemagglutination titer.

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1. Nahas, G. G., Suciu-Foca, N., Armand, J. P., and Morishima, A., Science **183,** 419 (1974).
2. White, S. C., Brin, S. C., and Jamiki, B. W., Science **188,** 71 (1975).
3. Lau, R. J., Lerner, C. B., Tubergren, D. G., Benowitz, N., Domino, E. F., and Jones, R. T., Science **192,** 805 (1975).
4. Rachelefsky, G. S., Opelz, G., Mickey, M. R., Lessin, P., Kiuchi, M., Silverstein, M. J., and Stiehm, E. R., J. Allergy Clin. Immunol. **58,** 483 (1976).
5. Silverstein, M. J., and Lessin, P. J., Science **186,** 740 (1974).
6. Cushman, P. and Khurana, R., Clin. Phar. Thera. **19,** 310 (1976).
7. Gupta, S., Cushman, P., Grieco, M. H., N. Engl. J. Med. **292,** 104 (1974).
8. Petersen, B. H., Lemberger, L., Graham, J., and Dalton, B., Psychopharmacol. Commun. **1,** 67 (1975).
9. Munson, A. E., Levy, J. A., Harris, L. S., and Dewey, W. L., *in* "The Pharmacology of Marihuana" (M. C. Braude and S. Szara, eds.), Vol. 1, p. 187. Raven Press, New York (1976).
10. Rosenkrantz, H., and Miller, A. J., J. Toxicol. Environ. Health **1,** 119 (1975).
11. Lefkowitz, S. S., and Chiang, C. Y., Res. Commun. Chem. Pathol. Pharmacol. **11,** 659 (1975).
12. Zimmerman, S., Zimmerman, A. M., Cameron, I. L., and Laurence, H. I., Pharmacology **15,** 10 (1977).
13. Adler, W. H., Takiguchi, T., Marsh, B., and Smith, R. T., J. Exp. Med. **131,** 1049 (1970).
14. Dresser, D. W., and Wortis, H. H., Nature (London) **208,** 859 (1965).
15. Greaves, M. F., Möller, E., Cell Immunol. **1,** 372 (1970).
16. Haskill, J. S., Elliot, B. E., Kerbel, R., Axelrad, M. A., and Eidinger, D., J. Exp. Med. **135,** 1410 (1972).
17. Lefkowitz, S. S., Klager, K., Nemeth, D., and Pruess, M., Res. Commun. Chem. Pathol. Pharmacol. (in press).
18. Rosencrantz, H., *in* "Marihuana: Chemistry, Biochemistry, and Cellular Effects" (G. G. Nahas, ed.), p. 441. Springer-Verlag, New York (1976).
19. Ho, Beng T., Fritchie, G. E., Englert, L. F., McIsaac, W. M., and Idanpaan-Heikkila, J. E., J. Pharm. Pharmacol. **23,** 309 (1971).
20. "Marihuana and Health" U. S. Department of Health, Education and Welfare, Second Annual Report to Congress. Publication No. (HSM) 72-9113, p. 1–276. Washington D. C., Government Printing Office (1972).
21. Stefanis, C. N., and Issidoudes, M. R., *in* "Marihuana: Chemistry, Biochemistry and Cellular Effects" (G. G. Nahas, ed.), p. 533. Springer-Verlag, New York (1976).
22. Gill, E. W., Ann. N. Y. Acad. Science **281,** 151 (1976).
23. Kay, M. M. B., *in* 'Basic and Clinical Immunology" (Fudenberg, H. H., Stites, D. P., Caldwell, J. L., Wells, J. V., ed.), p. 267. Lange Med. Publishers, New York, (1976).

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