Normal Skin Test Responses in Chronic Marijuana Users

Abstract. *The cell-mediated immunity of 22 chronic marijuana smokers showed no difference from that of normal controls when evaluated by in vivo skin testing with 2,4-dinitrochlorobenzene. However, a significant difference was seen between these chronic marijuana users, all of whom could be sensitized to 2,4-dinitrochlorobenzene, and age-matched cancer patients, who showed a decreased capacity to be sensitized.*

In a study of 51 subjects, Nahas (*1*) suggested that cellular immunity may be impaired by the chronic use of marijuana. His in vitro studies showed that lymphocyte blastogenesis in response to allogeneic cells (mixed lymphocyte culture) and in response to the mitogen phytohemagglutinin was decreased to levels comparable to those of patients with a known impairment of cellular immunity (cancer, uremia, and kidney transplant patients), and that this depression was statistically different from that of normal controls. However, in vivo skin testing with the foreign antigen 2,4-dinitrochlorobenzene (DNCB) and with a battery of common antigens may be a better gauge of overall immunocompetence than any currently used in vitro lymphocyte function test, because skin testing closely correlates to clinical prognosis in cancer patients (*2*). Since 96 percent of the normal population can be sensitized to DNCB (*2, 3*), we tested the capacity of chronic marijuana users to develop a delayed cutaneous hypersensitivity response to DNCB and thus grossly evaluated, in vivo, their immunologic competence.

For this study, the chronic marijuana user was defined as one who regularly smoked marijuana a minimum of three times per week for at least 6 months (*4*). Tobacco smoking and alcohol were allowed but the regular or frequent use of other drugs disqualified the subject from the study. Skin testing with DNCB was performed in a standard fashion (*2*) on 22 healthy males ranging in age from 21 to 30 years. After the skin was cleansed with acetone, a sensitizing dose of 2000 μg of DNCB dissolved in 0.1 ml of acetone was applied to the skin of the upper arm. A 100-μg dose was applied at the same time to the ipsilateral forearm to test for previous sensitization. After 14 days, the subjects were challenged on the ipsilateral forearm with 100, 50, and 25 μg of DNCB as well as with a cutaneous irritant, croton oil, as a check for an intact inflammatory response. On the same day, four common antigens in doses of 0.1 ml were injected intradermally in the contralateral forearm. The antigens were monilia (Hollister-Stier, 2 units), mumps (Lilly, 2 units), purified protein derivative (Connaught, 5 units, intermediate strength), and Varidase (Lederle, 10 units).

All positive reactions were scored as 1+ (erythema and induration of less than half the diameter of the test site), 2+ (measurable skin reaction over at least half of the test site), 3+ (reaction covering the entire test site), or 4+ (bullae formation).

All 22 subjects reacted to 50 μg of DNCB (21 were either 3+ or 4+), and 21 reacted to 25 μg of DNCB (14 were either 3+ or 4+). Seventeen subjects reacted to two or more common antigens, three subjects reacted to only one common antigen, and two subjects failed to react to any of them.

The failure of two DNCB-positive subjects to respond to any of the common antigens is probably not indicative of a defect in immunologic memory. In this age group, unresponsiveness is probably due to lack of exposure to these antigens.

The results of the DNCB skin testing are summarized in Table 1 and are compared with combined data from published evaluations of DNCB skin testing in normal subjects and cancer patients (*2, 3*). In order to obtain an age-matched control group with probable immunodepression, we reviewed the skin test records of 60 consecutive cancer patients between the ages of 21 and 30 years from the Division of Oncology, University of California, Los Angeles. These patients, who were not under drug treatment at the time of testing, were tested by the identical procedure in the same institution as the marijuana users and represent a concurrent control group. Twelve (20 percent) were DNCB-negative. Thus, this DNCB procedure can detect a defective immune response.

When our group of 22 DNCB-positive, chronic marijuana users was compared to the control group of 279 normal subjects, there was no statistical difference (*5, 6*). It appears that the chronic use of marijuana does not decrease the capacity of a subject to become sensitized and to develop delayed cutaneous hypersensitivity when challenged with the hydrocarbon hapten DNCB. However, when the marijuana smokers were compared with either cancer group [all ages (*5, 7*) or ages 21 to 30 (*5, 8*)], a statistical difference was found.

The 10 percent difference in DNCB positivity between all cancer patients and the age-matched cancer patients, although not significant (*5, 9*), may be due to the fact that many patients in the age-matched group had early, localized disease. Immunocompetence appears to decrease with increasing age and extent of neoplastic disease.

Prolonged immunosuppression has profound implications. Patients with congenital immunodeficiencies and those with iatrogenic immunosuppression (such as renal transplant patients) develop cancer at rates at least 80 times that of the general population (*10*). However, there is no clinical or epidemiologic evidence to suggest that chronic marijuana users might be more prone to the development of neoplastic or infectious processes.

In vivo skin testing has proved to be a valuable tool in the gross evaluation of the immune system because a delayed cutaneous hypersensitivity response requires the participation of a number of components of cell-mediated immunity. Since responses were normal in the chronic marijuana users we tested, it would appear that chronic

Table 1. 2,4-Dinitrochlorobenzene reactivity.

Groups	Subjects (No.)	DNCB-positive		DNCB-negative	
		Number	Percent	Number	Percent
Marijuana smokers	22	22	100	0	0
Normal controls*	279	267	96	12	4
Cancer patients (all ages)*	548	384	70	164	30
Cancer patients (ages 21 to 30)†	60	48	80	12	20

* Data from published studies (*2, 3*). † Concurrent controls.

marijuana smoking does not produce a gross cellular immune defect that can be detected by skin testing. Further study is needed to evaluate chronic marijuana use and its effect, if any, on the immune system.

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References and Notes

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4. Marijuana smoking ranged from three times per week to several times per day. No marijuana was administered by us to these subjects, so the specific dosage levels or percentage of tetrahydrocannabinol of the marijuana smoked is not known.
5. Statistical analyses performed were the Yates modification of the chi-square procedure and Fisher's exact probability test (two-tailed).
6. $\chi^2 = 0.18$, $P = .669$; Fisher's exact $P = 1.000$.
7. $\chi^2 = 7.84$, $P = .005$; Fisher's exact $P = .001$.
8. $\chi^2 = 3.68$, $P = .055$; Fisher's exact $P = .030$.
9. $\chi^2 = 2.13$, $P = .144$; Fisher's exact $P = .133$.
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11. Supported by contract HSM 42-71-89 from the National Institute on Drug Abuse, grants CA 05262 and CA 12582, and the surgical services, Veterans Administration Hospital, Sepulveda, California. We thank S. Cohen, S. Golub, D. L. Morton, C. Bodai, A. Nizze, M. Hofmann, and E. J. Shaw.

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5-Methyltetrahydrofolic Acid as a Mediator in the Formation of Pyridoindoles

Abstract. *Enzymes from chick and rat tissues catalyze the reaction of* N-*methyltryptamine with 5-methyltetrahydrofolic acid to form 2,3,4,9-tetrahydro-2-methyl-1*H-*pyrido[3,4*b*]indole.* N,N-*Dimethyltryptamine was not formed. With tryptamine as substrate the product is 2,3,4,9-tetrahydro-1*H-*pyrido[3,4*b*]indole and not* N-*methyltryptamine. These pyridoindoles were not formed when* S-*adenosylmethionine was cosubstrate.*

5-Methyltetrahydrofolic acid (MTHF) transfers its methyl group to dopamine in a reaction catalyzed by an *N*-methylating enzyme to form epinine (*1*). More recently it was reported that MTHF is a more efficient cosubstrate than *S*-adenosylmethionine in the *N*- and *O*-methylation of certain amines (*2, 3*). We observed in our studies with MTHF that the product of methylation of tryptamine by MTHF was not isographic with *N*-methyltryptamine (NMT) in some of the solvent systems used for thin-layer chromatography (TLC), and that the product of the methylation of NMT by MTHF was not *N,N*-dimethyltryptamine (DMT). We now report evidence demonstrating that MTHF reacts with tryptamine and NMT to form tricyclic compounds.

Chick hearts from 2-week-old animals were homogenized in three to five volumes of 5 m*M* sodium phosphate buffer, *p*H 7.9 (with or without 0.01 m*M* dithiothreitol); the homogenate was centrifuged at 78,000*g* for 60 minutes. The supernatant fractions were dialyzed for 18 hours at 4°C against 50 volumes of buffer, with one change. Whole brains from rats (150 to 300 g) were homogenized in distilled water, and the 78,000*g* supernatant fraction was dialyzed against 20 m*M* potassium phosphate buffer, *p*H 6.8. The dialyzed preparations (4 to 8 mg of protein per milliliter) were the enzyme sources. Incubation mixtures (0.5 ml) contained 0.03 to 0.04 ml of enzyme, 3 to 10 m*M* indoleamine substrate (Aldrich), and 0.005 to 0.05 μmole of [^{14}C]MTHF ([5-methyl-^{14}C]tetrahydrofolate) (barium salt) (specific activity, 60 mc/mmole; Amersham/Searle) or 0.004 to 0.01 μmole of *S*-adenosyl[methyl-^{14}C]methionine (specific activity, 57 mc/mmole; New England Nuclear). Mixtures were incubated for 60 to 120 minutes at 37°C in the dark, and the reactions were terminated by addition of 0.6 ml of 0.5*M* borate at *p*H 10. The samples were extracted with 6 ml of 97 percent toluene and 3 percent isoamyl alcohol containing 50 to 100 μg of nonradioactive reference standard products. After shaking and centrifugation, a 5-ml portion of the organic phase was transferred to a vial, and the solvent was evaporated to dryness at 22°C in a vacuum desiccator. The residue was taken up in methanol for TLC on silica gel GF plates (Analtech). After development, the plates were examined under ultraviolet light, and scanned with a radiochromatogram scanner. The ultraviolet absorbing and radioactive zones were scraped into counting vials for determination of radioactivity in a liquid scintillation spectrometer.

NMT was labeled (in the benzene ring) with deuterium by exchange with CH_3SO_3D (60°C for 1 hour). 2,3,4,9-Tetrahydro-2-methyl-1*H*-pyrido[3,4*b*]indole (**1**) was prepared from 4,9-dihydro-

Table 1. Formation in vitro of compounds **1** and **2** from NMT or tryptamine and MTHF or *S*-adenosylmethionine (AMe).

Enzyme source	Indoleamine substrate	^{14}C-substrate		Radioactivity (count/min) in product zone*			
		Compound	^{14}C (10^5 count/min)	DMT	**1**	NMT	**2**
Chick heart	5 m*M* NMT	MTHF	4	371†			
				2	826		
Chick heart	5 m*M* NMT	MTHF	6.5	15	2010		
		AMe	7.5	40	22		
Chick heart	5 m*M* NMT	MTHF	15	55‡	6030‡		
		AMe	14	21‡	50‡		
Rat brain	5 m*M* NMT	MTHF	9	490†			
				14			
Rat brain	10 m*M* NMT	MTHF	10		2160‡		
Chick heart	5 m*M* Tryptamine	MTHF	7			10	1110
Rat brain	10 m*M* Tryptamine	MTHF	20			70	10,600
		AMe	8			10	80
Rat brain	10 m*M* Tryptamine	MTHF	10			20	2440‡

* Unless otherwise stated the number of counts per minute are the differences in the data obtained between the complete system and incubations in which the indoleamine was omitted. When the substrate was omitted, 40 to 330 count/min were found in the product zone. † The TLC solvent system was *n*-butanol, acetic acid, water (72 : 18 : 30); in all other experiments the solvent system was methanol, 1*N* NH_4OH (5 : 1). DMT and compound **1** were isographic (R_F 0.53) in the acid solvent system. ‡ In these experiments the enzyme was heated at 90°C for 15 minutes to assess the nonenzymatic reaction; 130 to 560 count/min were found in **1** and 140 to 420 count/min in **2**. The number of counts per minute represent the difference between the complete system and incubations with heat-denatured enzyme.