

Marijuana smoking and fungal sensitization

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The possible role of marijuana (MJ) in inducing sensitization to Aspergillus organisms was studied in 28 MJ smokers by evaluating their clinical status and immune responses to microorganisms isolated from MJ. The spectrum of illnesses included one patient with systemic aspergillosis and seven patients with a history of bronchospasm after the smoking of MJ. Twenty-one smokers were asymptomatic. Fungi were identified in 13 of 14 MJ samples and included Aspergillus fumigatus, A. flavus, A. niger, Mucor, Penicillium, and thermophilic actinomycetes. Precipitins to Aspergillus antigens were found in 13 of 23 smokers and in one of 10 controls, while significant blastogenesis to Aspergillus was demonstrated in only three of 23 MJ smokers. When samples were smoked into an Andersen air sampler, A. fumigatus passed easily through contaminated MJ cigarettes. Thus the use of MJ assumes the risks of both fungal exposure and infection, as well as the possible induction of a variety of immunologic lung disorders. (J ALLERGY CLIN IMMUNOL 71:389, 1983.)

The recreational and medicinal use of MJ has reached epidemic proportions. The National Institute on Drug Abuse has documented that nearly one in 10 American high school seniors use MJ on a daily basis.¹ Furthermore, a survey of adult and pediatric oncology centers reveals that a substantial population of patients receiving cancer chemotherapy are now encouraged to use MJ as an antiemetic.²

The medicinal use of MJ, however, is not without risks. MJ may contain toxic substances such as Agent Orange, phencyclidine, or paraquat, and outbreaks of salmonellosis and hepatitis B have been traced to MJ.³⁻⁵ Similarly, *Aspergillus* has been cultured from MJ and has been considered the likely source of infection in patients who have developed invasive pulmonary and allergic bronchopulmonary aspergillosis.⁶⁻⁸ Due to the widespread use of MJ by normal and immunodeficient individuals, we thought it important to evaluate its possible role as a source of exposure and sensitization to *Aspergillus* organisms. Preliminary results of our investigations revealed that

MJ contains pathogenic, inhalable *Aspergillus* organisms that may sensitize the user.^{9, 10} This article presents additional in vitro studies and further documents the spectrum of fungal organisms present in MJ.

MATERIALS AND METHODS

Subjects

A total of 28 subjects were randomly selected to be evaluated for immunologic reactivity toward *A. fumigatus*, to which they may have been exposed while smoking MJ. Medical histories, physical examinations, cultures of their MJ, and serologic studies were performed. Ten age-matched individuals who denied ever having smoked MJ served as controls.

Cultures

Samples of MJ were plated directly onto SGA, SGA with antibiotics, TSA, and TSA with novobiocin. SGA plates were incubated at room temperature and at 37° C, while TSA plates were incubated at 55° C. Plates were observed daily for growth of organisms. Any growth appearing was subcultured, purified, and identified according to standard methods.^{11, 12}

Immunologic studies

Precipitins. Serum precipitins against *A. fumigatus*, *A. flavus*, and *A. niger*, the predominant cultured organisms, were evaluated by agar gel diffusion as previously described.^{13, 14} Serum precipitin assays were also performed with routine culture filtrate antigens from *Thermoactinomyces candidus* and *T. vulgaris*, *Mucor*, and *Penicillium* species to better assess the significance of circulating precipitins to *Aspergillus* antigens in MJ smokers.

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Abbreviations used

MJ:	Marijuana
SGA:	Sabouraud's glucose agar
TSA:	Trypticase soy agar
CPM:	Counts per minute
Con-A:	Concanavalin A
PMN:	Polymorphonuclear
THC:	Delta-9-tetrahydrocannabinol

Lymphocyte transformation. Lymphocytes were obtained from peripheral blood by Hypaque-Ficoll centrifugation and suspended at 0.25×10^6 cells/ml in 0.4 ml of RPMI tissue culture medium (Gibco, Inc., Grand Island, N. Y.), using 15% pooled human plasma, with penicillin, streptomycin, and glutamine added. The cells were cultured with or without stimulants in a humidified atmosphere containing 5% CO₂ for 5 days, at which time 1 μ Ci of ³H-thymidine was added. Twenty-four hours later the cells were harvested onto glass fiber filters. The incorporation of ³H-thymidine was counted by scintillation counting and data were expressed as either total CPM or stimulation ratios (CPM experimental/CPM control). A positive result is defined as CPM >3000 and stimulation ratios >4.0, as previously described.¹⁵ Antigens and mitogens employed included Con-A (Miles Laboratories, Inc., Elkhart, Indiana), *A. fumigatus*, *A. niger*, and *A. flavus*. The optimal final concentrations of mitogens were determined in preliminary experiments with either human or guinea pig lymphocytes (*A. fumigatus*, 5 μ g/ml; *A. niger*, 50 μ g/ml; Con-A, 10 μ g/ml).

Fungal inhalation

MJ cigarettes were obtained from patients and attached to an Andersen air sampler via rubber tubing. The cigarettes were then lit and the smoke was drawn into the sampler, deposited onto plates, and cultured. Additional unlit MJ cigarettes were similarly assessed. Control samplings of laboratory air were also obtained.

RESULTS

The results are summarized in Tables I and II.

Subjects

The study population consisted of 16 female and 12 male patients, ranging in age from 17 to 36 yr, including 18 tobacco cigarette smokers. The duration of MJ use varied from 6 mo to 14 yr, with a mean of 9 yr. The total number of MJ cigarettes smoked was estimated by multiplying the daily average by total duration expressed in days. Patient 1 had systemic aspergillosis and presented with complaints of fatigue, night sweats, and coughing episodes associated with MJ use. The chest film revealed bilateral interstitial infiltrates, and *A. niger* was cultured from sputum, nasal secretions, skin pustules, urine, and an open

lung biopsy. Hematologic studies, immunoglobulin levels, and complement components were normal, and he was later found to have a defective PMN oxidative enzyme system.

Patients 2, 3, 4, 6, 27, and 28 admitted experiencing cough and wheezing after MJ exposure. Additionally, patient 6 experienced a "chest cold" for 2 mo, which included cough, thick brown sputum, and body aches, all of which disappeared shortly after discontinuing the use of 60 to 70 MJ cigarettes weekly. The remaining 21 patients had no history of immediate or delayed respiratory symptoms with MJ use.

Cultures

Thirteen of 14 MJ samples contained potentially pathogenic fungi in various combinations. The flora consisted of *A. fumigatus*, *A. flavus*, *A. niger*, *Mucor*, *Penicillium*, and thermophilic actinomycete species in varying densities, but with *Aspergillus* predominating.

Immunologic studies

Thirteen of 23 MJ-smoking subjects had precipitins against at least one of the *Aspergillus* antigens. In the control sample of 10 MJ-nonsmoking individuals, one had a precipitin line against *A. fumigatus* and *A. niger* ($p < 0.02$). There were no differences between the MJ-smoking group and the control group with regard to precipitins to antigens other than *Aspergillus* (Table II).

Significant blast transformation to *A. niger* in the MJ-smoking group occurred in only three of 23 subjects, whereas all demonstrated significant blastogenesis to Con-A, a nonspecific mitogen.

Fungal inhalation

Fungal inhalation studies with MJ sample 25 revealed that both lit and unlit cigarettes allowed the passage of fungal spores. *A. fumigatus* in particular traveled through the MJ cigarettes unimpeded in both lit and unlit conditions. Control samplings of laboratory air were repeatedly negative for fungal growth.

DISCUSSION

MJ can now be found in nearly every high school in America, and in a growing number of medical communities. Several clinical trials employing THC and other cannabinoids present in MJ have demonstrated its potentially significant antiemetic effect.¹⁶⁻²¹ Because serum levels of THC are best attained via inhalation, it has been advocated that THC and MJ be inhaled by oncology patients shortly prior to receiving cancer chemotherapy.^{18, 22} Our studies, however,

TABLE I. Summary of immunologic findings

Patient No.	Estimated total MJ cigarette inhalation*	MJ culture	<i>Aspergillus</i> precipitins	Blastogenesis†		
				<i>A. niger</i>	<i>A. fumigatus</i>	Con-A
1	20,000	<i>A. niger</i>	+	+	+	+
2	16,380	ND	+	—	—	+
3	7,280	ND	+	+	—	+
4	1,040	<i>Aspergillus</i> spp, <i>Mucor</i>	+	—	—	+
5	3,000	No growth	+	—	—	+
6	1,200	<i>A. niger</i> , <i>A. flavus</i> , <i>Mucor</i> , <i>T. candidus</i>	+	—	—	+
7	800	<i>A. niger</i>	—	—	—	+
8	2,080	ND	+	—	—	+
9	32,760	ND	—	+	—	+
10	2,550	ND	+	—	—	+
11	1,000	<i>A. niger</i> , <i>A. flavus</i> , <i>Mucor</i> , <i>T. candidus</i>	—	±	—	+
12	6,240	<i>A. flavus</i> , <i>Mucor</i> , <i>T. vulgaris</i>	—	—	—	+
13	5,840	<i>A. flavus</i> , <i>Mucor</i> , <i>T. candidus</i>	ND	—	—	+
14	8,760	<i>A. flavus</i> , <i>Mucor</i> , <i>T. candidus</i>	+	—	—	+
15	1,000	<i>A. flavus</i> , <i>Aspergillus</i> spp	+	—	—	+
16	3,120	<i>A. flavus</i> , <i>Aspergillus</i> spp	ND	ND	ND	ND
17	9,100	<i>A. niger</i> , <i>A. flavus</i> , <i>Penicillium</i>	—	—	—	+
18	5,280	<i>A. niger</i> , <i>A. flavus</i> , <i>Penicillium</i>	—	—	—	+
19	4,580	<i>A. fumigatus</i> , <i>Mucor</i> , <i>T. vulgaris</i>	—	—	—	+
20	60	<i>A. flavus</i>	—	—	—	+
21	5,824	<i>A. flavus</i>	—	—	—	+
22	4,200	<i>A. fumigatus</i> , <i>Mucor</i> , <i>T. vulgaris</i>	+	—	—	+
23	8,000	<i>Aspergillus</i> spp, <i>Mucor</i>	ND	—	—	+
24	6,000	No growth	—	—	—	+
25	1,000	<i>A. fumigatus</i> , <i>A. niger</i> , <i>Mucor</i>	ND	ND	ND	ND
26	2,500	<i>A. fumigatus</i> , <i>A. flavus</i> , <i>Mucor</i>	ND	ND	ND	ND
27	100	<i>A. niger</i> , <i>Mucor</i>	+	ND	ND	ND
28	500	<i>A. niger</i> , <i>A. fumigatus</i>	+	ND	ND	ND

ND = not done; + = CPM >3000 and experimental/control >4.0; ± = CPM >3000 and experimental/control <4.0; — = CPM <3000 and experimental/control <4.0.

*Average daily MJ cigarette consumption times estimated total number of days used.

†The following pairs of patients submitted a single MJ cigarette sample that the two had been sharing: 4 and 23, 5 and 24, 6 and 11, 13 and 14, 15 and 16, 17 and 18, 19 and 22, 20 and 21.

have shown that illicit MJ must now be assumed to contain pathogenic inhalable fungi. As such, its use by immunosuppressed oncology patients should be discouraged.

The spectrum of fungi found in MJ included the following organisms: *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor*, *Penicillium* spp, *Thermoactinomyces candidus*, and *Thermoactinomyces vulgaris*. When inhaled, these organisms are known to cause a variety of immune lung disorders, ranging from asthma, allergic bronchopulmonary aspergillosis and hypersensitivity pneumonitis to invasive systemic fungal infections in immunoincompetent hosts.^{23–28} In addition to identifying these fungi, we have demonstrated that *A. fumigatus* may be inhaled in contaminated MJ cigarette smoke.

The presence of circulating precipitins to any given

TABLE II. Precipitins to routine antigens

	<i>T. vulgaris</i>	<i>T. candidus</i>	<i>Mucor</i>	<i>Penicillium</i> spp
MJ smokers	4/28	9/28	3/28	5/28
Controls	2/9	4/9	3/9	2/9

antigen is generally taken to mean that a significant immunologic exposure to that antigen has taken place. *Aspergillus* precipitins may thus arise from repeated antigenic inhalation, active colonization, or previous clinical or subclinical fungal infections. Of 23 MJ-smoking patients tested, 13 had precipitins to *Aspergillus* antigens. This 52% incidence is significantly greater than both our control group ($p < 0.02$) and the normal 3% to 10% incidence in populations

reported by Chmelik et al.²⁹ Furthermore, there was no correlation between the presence of precipitins and the total estimated MJ exposure. Since 13 of 14 MJ samples contained at least one *Aspergillus* species and the contaminated MJ cigarettes were shown to deliver viable organisms, it is not unreasonable to assume that our patients acquired their precipitins from smoking MJ. We were, however, unable to determine whether pulmonary infections or colonizations were present in these patients, although both occurrences were possible.

In vitro cellular immune responses to *Aspergillus* antigens in aspergillosis, in contradistinction to serum precipitins, rarely correlate with disease activity.³⁰ Substantiating this, we found no correlation between blastogenesis to *Aspergillus* antigens and the presence of serum precipitins (Table I). Of special interest was the finding that our index case (patient 1) possessed adequate cellular immune responses to *A. fumigatus* and *A. niger* antigens despite his disseminating systemic aspergillosis. Perhaps, because of his malfunctioning PMN enzyme system, he was unable to either completely metabolize *Aspergillus* antigens or sufficiently inhibit hyphal growth. The fungus would then be able to proliferate even though an active cellular immune response existed.

As illustrated by this patient, diseases induced by the inhalation of viable fungal spores depend primarily on the host's innate immune and metabolic capabilities. A defect in PMN metabolism, coexistent with fungal inhalation, may lead to the development of either systemic invasive mycoses or a fungus ball. We anticipate that future reports may continue to substantiate the already increasing incidence of systemic aspergillosis, especially if oncology patients continue to be exposed to MJ smoke.

The use of MJ thus assumes the risks of both fungal exposure and infection, as well as the possible induction of a variety of immune and infectious lung disorders. Given the extraordinary number of individuals estimated to be MJ smokers, the occurrence of these illnesses may well become more commonplace.

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