

Allergenic fungi and actinomycetes in smoking materials and their health implications

V. P. Kurup, A. Resnick, S. L. Kagen, S. H. Cohen & J. N. Fink

Research Service, Veterans Administration Medical Center, Wood (Milwaukee), WI 53193, U.S.A. and
Allergy and Immunology Section, Department of Medicine, The Medical College of Wisconsin, Milwaukee,
WI 53226, U.S.A.

Abstract

Street marijuana, commercial cigarettes and pipe tobaccos were studied for the presence of fungi and actinomycetes associated with hypersensitivity pneumonitis. *Aspergillus* species and thermophilic actinomycetes were isolated from the smoking materials. In addition, *Aspergillus fumigatus* spores were isolated from marijuana smoke, indicating the potential hazard involved in developing serious disease. Precipitin antibodies against fungi, particularly *Aspergillus*, showed a higher prevalence in marijuana smokers, whereas only very few cigarette smokers and nonsmokers demonstrated antibodies to fungi. Cigarette smokers and nonsmokers showed more or less similar prevalence of antibodies against thermophilic actinomycetes.

Introduction

A number of fungi and actinomycetes have been shown to cause hypersensitivity pneumonitis (HP) in man (3, 14). These organisms have been isolated from various working and living environments of patients (8). Inhalation of numerous spores of these organisms will sensitize the individuals and, on prolonged inhalation of heavily contaminated air, may result in HP. Hypersensitivity pneumonitis is an immunologic interstitial lung disease which may be incapacitating if not diagnosed early and controlled by avoidance of insulting antigens. The commonly recognized diseases include farmer's lung, bagassosis, mushroom worker's lung and ventilation pneumonitis are caused by inhalation of thermophilic actinomycetes and various fungi or other organic dusts.

Smoking marijuana contaminated with *Aspergillus fumigatus* has been recently attributed to several cases of aspergillosis (2, 5, 11). This led us to evaluate smoking materials such as cigarettes, pipe

tobacco and marijuana for the presence of thermophilic actinomycetes and fungi, and the possible role of these organisms in the development of immunologic lung diseases.

Materials and methods

Smoking materials. Twenty-four samples of street marijuana, 12 different brands of cigarettes (both with and without filters) and 3 samples of pipe tobacco were studied. Approximately 100 mg of the sample was suspended in 10 ml of sterile distilled water. After vigorous mixing the suspension was allowed to stand for 10 minutes. About 1 ml of the supernatant was serially diluted and cultured in appropriate media.

Culture

For the isolation of fungi, 1 ml of the suspension was plated in Sabouraud's glucose agar (SGA) and

SGA with streptomycin (40 µg/ml) and penicillin (40 units/ml) to suppress bacterial growth. One set each of the inoculated plates were incubated at 37 °C and at 25 °C. Cultures were examined regularly for up to 2 weeks and growth appearing on the plates were identified by standard procedures.

Cultures for the isolation of thermophilic actinomycetes were made in tryptic soy agar (TSA) and in TSA with novobiocin (25 µg/ml). The inoculated plates were incubated at 55 °C in plastic bags to prevent evaporation. Plates were examined daily for 10 days. Growth appearing on the plates were subcultured and identified according to procedures previously described (6).

Fungal inhalation

Marijuana cigarettes obtained from patients and commercial brands of cigarettes were attached to an Andersen air sampler (1). The cigarettes were then lit and the smoke drawn through the sampler containing SGA or TSA plates. The plates were incubated and studied as described above. Spores present in equal volume of room air were also collected on the sampler impact plates and studied for comparison.

Antigen preparations

The detailed methods of antigen extraction from *Aspergillus* and thermophilic actinomycetes were reported elsewhere (7, 9, 10). Fungi were grown in a synthetic broth for 2–3 weeks at 37 °C in stationary cultures or 5 days in shaker cultures. Following incubation the culture filtrate was separated, dialyzed and freeze-dried. These antigens were used to detect antibodies in the sera of subjects included in the study.

Thermophilic actinomycetes were grown in synthetic medium or by the double dialysis method using dialyzate of tryptic soy broth (TSB) (9). *Micropolyspora faeni* was grown in a synthetic medium incubated at 50 °C in shaker incubators for 5 days (7). Culture filtrate was separated and processed as in the case of fungal antigens. *Thermoactinomyces candidus* and *T. vulgaris* were grown in stationary cultures incubated at 55 °C for 2 weeks in dialyzate medium. The culture filtrate was processed as described above.

Agar gel double diffusion

Antibodies present in the sera against various fungi and actinomycetes were tested by agar gel double diffusion (DD) method (9, 10). Antigens were used at a concentration of 5–10 mg/ml. After filling the wells with antigen and antibody the gels were incubated in a humid chamber for 48 hours. It was then washed to remove the unreacted antigen and antibody and stained by Coomassie blue. Precipitin arcs appearing on the slides were studied in comparison with appropriate positive and negative controls run along with the test sera.

Results

Culture results

The various fungi and actinomycetes isolated from smoking materials are shown in Table 1. *Aspergillus* species were the predominant group in the marijuana samples. *Thermoactinomyces candidus*, *T. vulgaris* and *M. faeni* were also isolated. Only one sample was completely negative for fungi or actinomycetes, but it was heavily contaminated with bacteria. Quantitative cultures yielded 10^5 to 10^7 colonies of fungi per gram of marijuana sample. When lit and unlit cigarettes were studied by the Andersen sampler, they yielded *Aspergillus fumigatus* and *Mucor*.

When studied quantitatively, tobacco cigarettes yielded 1.5×10^4 to 1×10^5 colonies of thermophilic

Table 1. Fungi and actinomycetes isolated from smoking materials.

Organism	Marijuana	Cigarettes	Pipe tobacco
	(24)	(12)	(3)
<i>Aspergillus flavus</i>	12	–	–
<i>A. fumigatus</i>	7	–	–
<i>A. niger</i>	6	–	–
<i>Mucor</i> sp.	10	–	–
<i>Penicillium</i>	2	–	–
Other fungi	4	–	–
<i>Micropolyspora faeni</i>	1	–	–
<i>Thermoactinomyces</i>			
<i>candidus</i>	7	10	3
<i>T. vulgaris</i>	3	2	–
Number of negative samples	1	1	–

No. in parentheses = No. of samples analyzed.

Table 2. Precipitins against fungi and actinomycetes in the sera.

Fungi or actinomycetes	Marijuana smokers	Cigarette smokers	Normal controls*
	(24)	(19)	(14)
<i>Aspergillus fumigatus</i>	9	3	2
<i>A. niger</i>	2	—	—
<i>A. flavus</i>	1	3	—
<i>Micropolyspora faeni</i>	7	—	—
<i>Thermoactinomyces</i>			
<i>candidus</i>	13	6	5
<i>T. vulgaris</i>	9	9	6

* No smoking during the past 5 years.

No. in parentheses = No. of patients studied.

actinomycetes per gram. No fungi were isolated from the tobacco products. None of the cigarette samples studied yielded any organism when sampled through the Andersen sampler. *Thermoactinomyces candidus* was the predominant organism isolated from cigarettes and pipe tobacco. Only one cigarette was negative for any growth.

Serology

Of the 24 marijuana smokers studied, 9 showed antibodies to *A. fumigatus* (Table 2). Other fungal antigens reacted only with a few sera. However, cigarette smokers showed only low incidence of precipitins against *A. fumigatus*. All three groups, marijuana and cigarette smokers and control, demonstrated the presence of antibodies against the thermophilic actinomycetes, *T. candidus* and *T. vulgaris*, while *M. faeni* antibody was seen only in marijuana smokers.

Of the 24 marijuana smokers 18 were asymptomatic, while the remaining complained of varying degrees of respiratory symptoms. One of the smokers with proven systemic aspergillosis developed pulmonary granulomas related to a defect in his polymorphonuclear leukocyte oxidation enzyme system. The remaining 5 marijuana smokers experienced coughing and wheezing after exposure to marijuana. None of the cigarette smokers had any respiratory complaints.

Discussion

Previous studies demonstrated the presence of mesophilic and thermophilic fungi in tobacco pro-

ducts (12, 13, 15). However, in the present study, we could not demonstrate any fungi from pipe tobacco or cigarettes. This may be due to the differences involved in tobacco processing. Our study isolated thermophilic actinomycetes from cigarettes, pipe tobacco and marijuana samples. In addition, the results presented indicated that fungal spores present in the marijuana samples can be inhaled during smoking. The three species of thermophilic actinomycetes and the *Aspergillus* (Table 1) are capable of causing hypersensitivity lung diseases in man.

The distribution of antibodies against fungi was higher in the group of marijuana smokers than in the other groups, clearly indicating that the fungi present in marijuana samples led to the sensitization of smokers. On the other hand, there was no difference between the three groups with regard to the presence of antibodies against various thermophilic actinomycetes. *Thermoactinomyces candidus* and *T. vulgaris* are widely distributed in nature and may be the reason for the higher incidence of antibodies in all three groups. However, because of the universal presence of these organisms in cigarettes, cigarette smokers may inhale more thermophilic actinomycetes spores than normals.

Marijuana samples contained fungi which can cause asthma and invasive lung diseases. The respiratory complaints of marijuana smokers are most likely due to an allergic reaction elicited by the inhalation of *Aspergillus* spores. Individuals with underlying pulmonary or systemic diseases may inhale *Aspergillus* spores during smoking and may develop pulmonary or generalized aspergillosis. Both marijuana and cigarette smoking impair the function of pulmonary alveolar macrophages (4). As a result the inhaled spores may not be cleared by phagocytosis and result in colonization by the organisms and eventual invasion of the lung and other organs. Additional studies on the immunological response of smokers to the prevalent fungal and actinomycetes antigens are essential to determine the impact of smoking, if any, on the development of HP and other diseases.

Acknowledgements

This investigation was supported by Specialized Center of Research Grant (SCOR) HL 15389 from the National Heart, Lung and Blood Institute;

Grant AI-15700 from the National Institute of Allergy and Infectious Disease; and by the Veterans Administration. The technical assistance of Gertrude H. Scribner and the typing and editorial assistance of Catherine A. Walther are gratefully acknowledged.

References

1. Andersen, A. A., 1968. New sampler for the collection, sizing and enumeration of viable airborne particles. *J. Bacteriol.* 76: 471-484.
2. Chusid, M. J., Gelfand, J. A., Nutter, C. & Fauci, A. S., 1975. Pulmonary aspergillosis, inhalation of contaminated marijuana smoke, chronic granulomatous disease. *Ann. Intern. Med.* 82: 682-683.
3. Fink, J. N., Banaszak, E. J., Barboriak, J. J., Hensley, G. T., Kurup, V. P., Scanlon, G. T., Schlueter, D. P., Sosman, A. J., Thiede, W. H. & Unger, G. F., 1976. Interstitial lung disease due to contamination of forced air system. *Ann. Intern. med.* 84: 406-413.
4. Hocking, W. G. & Golde, D. W., 1979. The pulmonary alveolar macrophage. *N. Engl. J. Med.* 301: 580-587.
5. Kagan, S. L., Sohnle, P. G., Kurup, V. P. & Fink, J. N., 1981. *Aspergillus*: An inhalable contaminant of marijuana. *N. Engl. J. Med.* 304: 483-484.
6. Kurup, V. P. & Fink, J. N., 1975. A scheme for the identification of thermophilic actinomycetes associated with hypersensitivity pneumonitis. *J. Clin. Microbiol.* 2: 55-61.
7. Kurup, V. P. & Fink, J. N., 1979. Antigens of *Micropolyspora faeni* strains. *Int. Arch. Allergy Immunol.* 60: 140-147.
8. Kurup, V. P., Fink, J. N. & Bauman, D. M., 1976. Thermophilic actinomycetes from the environment. *Mycologia* 68: 662-666.
9. Kurup, V. P., Barboriak, J. J., Fink, J. N. & Scribner, G. H., 1976. Immunologic cross-reactions among thermophilic actinomycetes associated with hypersensitivity pneumonitis. *J. Allergy Clin. Immunol.* 57: 417-421.
10. Kurup, V. P., Fink, J. N., Scribner, G. H. & Falk, M. J., 1978. Antigenic variability of *Aspergillus fumigatus* strains. *Microbios* 19: 191-204.
11. Llamas, R., Hart, D. R. & Schneider, N. S., 1978. Allergic bronchopulmonary aspergillosis associated with smoking moldy marijuana. *Chest* 73: 871-872.
12. Ogundero, V. M., 1980. Thermophilic mycoflora of cigarettes and cured tobacco leaves. *Mycopathologia* 71: 9-11.
13. Papavassilion, J. G., Piperakis, G. & Marcelon-Kinti, U., 1971. Mycological flora of cigarettes. *Mycopathol. Mycol. Appl.* 44: 117-120.
14. Pepys, J., 1969. Hypersensitivity disease of the lung due to fungi and other organisms. pp. 69-111. In: *Monograph in Allergy*, No. 4. Edited by P. Kallos, M. Hasek, T. M. Inderleitzin, P. A. Miescher & B. H. Waksman. S. Karger, New York.
15. Tansey, M. R., 1975. Isolation of thermophilic fungi from snuff. *Appl. Microbiol.* 29: 128-129.