

Observations on the experimental reproduction of asthma with mold antigens

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We have previously reported that *Aspergillus* and *Penicillium* predominate among the airborne mold spores in the Puget Sound area.¹ This distribution differs from that found in other parts of the country, where *Alternaria* and *Hormodendrum* are conceded to be the most significant mold allergens.²

Since the seasonal variation in mold spore counts correlated with symptom fluctuations in selected asthmatic patients, it seemed worthwhile to confirm the role of the corresponding antigens in producing asthma under experimental conditions.

Furthermore, this created an opportunity to study the possible occurrence of acute changes in plasma histamine levels and cumulative changes in daily total eosinophil counts.

MATERIALS AND METHODS

Thirteen patients who have been followed by the authors at the University of Washington Hospital and the King County Hospital were selected for study. Twelve patients had asthma, and one had atopic dermatitis without a history

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Table I. *Patients showing a positive challenge response*

Patient	Ventilatory decrease* (%)	Plasma hist. (ug/L.)		Total eosinophils per mm. ³ of blood	
		Before challenge	After challenge	Before challenge	After challenge
C. B.	62	7.5	4.3	1,047	1,375
I. C.	57	14.2	18.3	—	—
E. H.	53	12.9	10.7	—	—
T. H.	59	12.0	7.3	329	440
G. P.	60	5.5	3.7	64	142
U. V.	13	—	—	394	375
E. W.	35	3.5	4.1	264	245

*The figures for ventilatory decrease on challenge are the maximum changes obtained (either TVC or FEV₁—see text).

All patients had positive challenge response to both *Aspergillus* and *Penicillium*, except for U.V. (*Alternaria*—see text).

Table II. *Results of direct skin tests (D) and passive transfer studies (P)*

Antigen	Patient*																			
	C. B.		I. C.		E. H.		T. H.		G. P.		U. V.		E. W.		H. K.		J. D.		M. H.†	
	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P	D	P
Aleurisma			4	2	4	3	4	2					2							
Alternaria	0	1	4	2	4	4	4	4	4	2	4	4	3	1			4	3	2	
Aspergillus	2	4	4	4	4	4	4	2	4	4	3	3	4	4	4	3			3	
Helminthosporium		2	4	2		2	4	2	4	4			2		3	4	4	3	4	0
Hormodendrum	0	1	4	4			4	0	4	2	3	1	3	4	4	0			3	
Penicillium	2	0	4	1	4	2	4	4	4	4			4	4	3		2	2	4	0
Pullularia	4	4											4	4					4	4
Scopulariopsis			4	1	4	2	4	3					0							
Verticillium			3	1	2		4	2					4	4	3	0				

Numbers indicate strength of reaction. Where no number is given, the test was not performed.

*The first seven patients are also to be found in Table I; the last three in Table III.

†Patient M. H. had positive direct and passive transfer tests for house dust and Russian thistle (see text).

of asthma. Skin reactivity to mold and other antigens was determined directly and by passive transfer. Direct skin tests were done by the intradermal method employing dilutions of 1:5,000. Each patient was admitted to a sealed, electronically filtered, air-conditioned room* in the Clinical Research Center of the University of Washington Hospital and observed for at least 24 hours until stability was established and constant base-line spirographic readings were achieved.† By means of an intermittent positive pressure apparatus, each patient was challenged for approximately three minutes at a time by aerosol inhalation of approximately 0.5 c.c. of various mold antigens and, where appropriate, other inhalant allergens.‡ Each series of antigen challenges was started at dilutions of 1:500,000, interspersed at random with

*Repeated exposure of petrolatum-covered slides and mold culture plates failed to show a yield for pollens or molds.

†Recording Vitalometer, I. E. Collins, Incorporated, 555 Huntington Avenue, Boston 15, Mass.; capacity 6 liters, drum speed 1 cm. per second.

‡All antigens were obtained from the Hollister-Stier Laboratories, 107 South Division Street, Spokane, Wash.

physiologic saline solution. All inhalant substances were "masked" with peppermint water. Recordings of the forced expirogram were measured before and periodically after each inhalation challenge. These were used to calculate changes in the total vital capacity (TVC) and the first second forced expiratory volume (FEV₁). Venous plasma histamine levels were determined before and after positive challenge responses by employing the Shore fluorometric method as modified by Noah and Brand.³ Blood total eosinophil counts were obtained at 8:00 A.M. daily.

RESULTS

Table I shows several measurements on the seven patients who had a positive challenge response. These patients had exacerbations of asthma in early spring and in the fall, and all had positive skin reactions to mold antigens. Three were sensitive only to molds. By passive transfer, all were shown to have serum skin-sensitizing antibodies to mold antigens (Table II). All patients had a significant reduction in TVC or FEV₁ (or both) which ranged from 13 per cent to 62 per cent. In most patients changes occurred in about ten minutes after challenge and reached a maximum within thirty minutes. The control values were quickly restored by brief inhalation of a 1:200 isoproterenol solution. There were no significant changes in total eosinophil counts or venous plasma histamine levels in the course of the studies on any patient.

The following three cases are representative of this group:

Patient I. C. was a 44-year-old white woman who had experienced symptoms of bronchial asthma for four years. She had positive skin test reactions to grass pollens, house dust, feathers, and molds, the ones for molds being confirmed by passive transfer tests (Table II). Her base-line spirogram is the one labeled 2c in Fig. 1. Approximately fifteen minutes subsequent to inhalation of Penicillium antigen in the 1:5,000 dilution, the patient developed

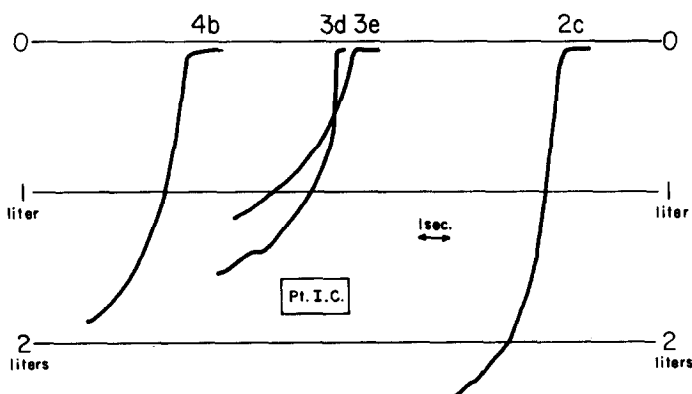


Fig. 1

Effect of antigen challenge on the forced expirogram of Patient I. C.

2c: before challenge.

3d, 3e: 15 minutes after inhaling a 1:5,000 Penicillium solution for 2 minutes.

4b: response to isoproterenol.

mild asthmatic symptoms and signs. At this time spirograms 3*d* and 3*e* (Fig. 1) were obtained. Spirogram 4*b* was taken just after inhalation of isoproterenol.

Patient T. H. was a 77-year-old Chinese man with bronchial asthma. Symptoms appeared at the age of 70, and our initial impression was that he had developed idiopathic pulmonary emphysema. However, symptoms had appeared three years after his arrival in the United States and were most pronounced from April to November, with some relief in midsummer. Skin test reactions to all inhalant antigens were negative with the exception of the positive reactions to various molds, which were confirmed by passive transfer tests (Table II). Fig. 2 shows the base-line spirograms (4*a* and 4*b*). Moderate asthmatic symptoms developed approximately two minutes subsequent to inhalation of *Aspergillus*, 1:50,000, and spirogram 5*a* (Fig. 2) was then obtained. Spirogram 6*b* (Fig. 2) was obtained shortly after administration of isoproterenol. Challenge with physiologic saline solution and Timothy pollen antigen produced no asthmatic symptoms or signs nor any spirographic changes, but a positive challenge was also obtained with *Alternaria*, 1:500, although, to our knowledge, his exposure to *Alternaria* spores in this country has been minimal.

Patient U. V. was a 33-year-old white female. Until she was 12, she lived in Minnesota and Wisconsin, where she suffered from severe seasonal allergic rhinitis and bronchial asthma, related primarily to ragweed and *Alternaria* sensitivity. She has lived in California, and more recently in Seattle, ever since, and has had only mild allergic rhinitis from May through September. By skin tests, she was sensitive to grass pollen, ragweed pollen, house dust and molds, the latter again confirmed by means of passive transfer testing (Table II). She showed positive inhalation challenge responses to *Alternaria*, ragweed, and house dust, in terms of mild asthmatic symptoms, saline controls being negative. *Alternaria*, 1:1,000, caused a 13 per cent decrease in TVC. Lack of time prevented studies with other mold antigens.

Of the six patients who gave negative responses to mold challenge (Table III), the first five were patients with asthma, and the sixth was a 17-year-old white male with long-standing, nonseasonal, atopic dermatitis, but no allergic respiratory symptoms. This last patient, M. H., gave strongly positive direct skin test reactions to molds, pollens, and house dust, some of which were confirmed by passive transfer (Table II). Four of the asthmatic subjects gave positive direct skin reactions to molds while the fifth was negative. However, only two of these four gave positive results on passive transfer. These were

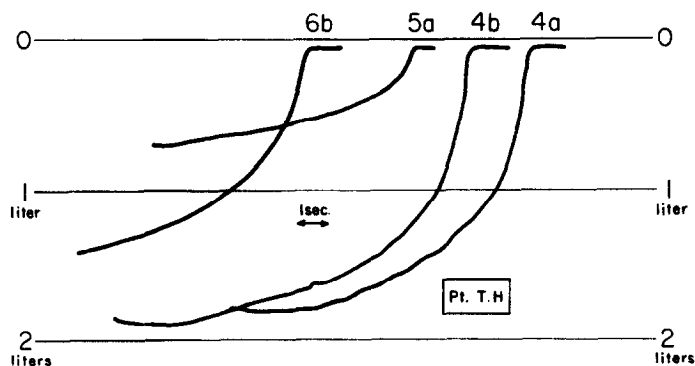


Fig. 2

Effect of antigen challenge on the forced expirogram of Patient T. H.

4*a*, 4*b*: before challenge.

5*a*: 2 minutes after inhaling a 1:50,000 *Aspergillus* solution for 2 minutes.

6*b*: response to isoproterenol.

Table III. *Patients with negative challenge response*

Patient	Direct skin tests*	P.K. tests to molds	Total eosinophils per mm. ³ of blood	
			Before challenge	After challenge
D. C.	3 + 6 (6); 2+ (1); 1+ (1)	Negative	372	397†
O. C.	Negative	Negative	681	370
J. S.	4+ (1); 3+ (1); 2+ (3)	Negative	262	240
J. D.	4+ (4); 3+ (1); 2+ (5); 1+ (1)	Positive‡	200	49
H. K.	4+ (2); 3+ (3)	Positive‡	—	—
M. H.	4+ (3); 3+ (3); 2+ (1)	Positive‡	760	712

*The figures given in parentheses represent the number of mold-antigens tested, e.g., 3+(6) = 6 antigens producing 3+ reactions.

†There was no change in total eosinophil counts after positive challenge response to other antigens by this patient. (See text.)

‡Also see Table II.

Patients J. D. and H. K., who had excellent clinical improvement with specific hyposensitization and had attained maximum dosage levels well in advance of their participation in the challenge study. Patient D. C. was a 26-year-old white male graduate student with allergic rhinitis and bronchial asthma related to the grass pollen season and to handling laboratory animals. His direct skin test reactions were strongly positive for grass pollen and guinea pig epithelium, but only mildly positive for molds; passive transfer studies for molds were negative (Table III).

None of these six patients showed a significant response to inhalation challenge with mold antigens. As a test of the clinical specificity of the experiment, Patient D. C. was challenged with mixed grass pollen, 100 PNU, and with guinea pig epithelium, 1:1,000, and showed marked responses to both. By contrast, Patient M. H. (with atopic dermatitis) was also challenged with additional antigens including cat hair, Russian thistle, and house dust without response to any of them. As in the first group, there was no significant change in daily total eosinophil counts or plasma histamine levels during challenge procedures.

DISCUSSION

Our observations of this relatively small group of patients suggest that the regionally predominant molds, *Aspergillus* and *Penicillium*, are of etiologic importance in bronchial asthma. All seven asthmatic patients who reacted to inhalation challenge had the appropriate seasonal variation in symptoms¹ and showed specific skin sensitivity confirmed by passive transfer.

Of equal interest is the group of patients who failed to respond to respiratory challenge (Table III). Even though two of those with asthma had some direct skin reactivity to molds, the clinical significance of such reactions was doubtful in the face of a negative history, negative challenge response, and negative passive transfer tests. The fourth and fifth patients, J. D. and H. K., had positive skin reactions by passive transfer as well as direct testing. The fact that they had experienced a clinical remission coincident with hyposensitization treatment for molds may explain the negligible challenge response. It

has been demonstrated in studies with ragweed pollen that patients receiving specific hyposensitization treatment show a diminished challenge response.⁴ Our studies were not designed with such a goal in mind, which would have required serial studies on each patient before and during treatment, as well as the more precise methods of study reported recently by Itkin and associates.⁵

The specificity of the negative response is best illustrated by Patient D. C., who developed unequivocal responses to other antigens which were significant by history and produced positive passive transfer reactions. The importance of "shock organ" reactivity is demonstrated by Patient M. H., who was clearly atopic, but had never developed respiratory allergic symptoms or signs. He had normal ventilatory function which did not change on challenge with antigens from molds, cat hair, Russian thistle, or house dust. This lack of response should be compared with the positive response of the two others in our study (U. V. and D. C.) with normal base-line functions, one subject having been free of asthma for several years.

In some patients with a positive challenge response to mold antigens, the changes did not develop immediately and reached a maximum only after fifteen to thirty minutes; in pollen challenge studies such responses usually occur more rapidly.⁴ This somewhat delayed response to molds has also been observed by others.⁶ Whether this type of response is mediated by mechanisms other than those responsible for immediate skin reactions, cannot be answered.

Positive challenge response occurred predominantly toward those molds common in this area, i.e., *Aspergillus* and *Penicillium*, although, admittedly, we studied them to the exclusion of others in order to gain evidence regarding their clinical importance. In certain patients, however, for example, T. H., positive challenge response was obtained with *Alternaria*, which is uncommon here.⁷ A different situation is illustrated by Patient U. V., i.e., clinical *Alternaria* sensitivity without symptoms in this area. Unfortunately, we did not have the opportunity to follow the positive *Alternaria* reaction with comparable challenge studies using *Aspergillus* or *Penicillium*. Nevertheless, her response demonstrated that lack of significant natural exposure does not necessarily diminish clinical sensitivity. Of course, this may be due to cross-sensitivity which is known to be common in mold-sensitive patients.⁸ Although Patient U. V.'s 13 per cent decrease in TVC was not as impressive as the others', the change was accompanied by the appearance of asthmatic symptoms and signs and was highly significant when compared to the constant values of the several base-line and saline control records.⁹

In the patients with positive challenge responses, some increase in blood eosinophils might be expected. Since our counts were obtained at the same time once daily, transient changes would, of course, be missed.^{10, 11} Certainly, there was no cumulative effect of repeated antigen challenges on the eosinophil counts over a period of up to four days.

The fact that the plasma histamine levels did not rise during positive challenge response is not surprising, since the rate of histamine metabolism is probably faster than the rate of antigenic histamine release (if any).¹²

All plasma samples were obtained from venous blood; arterial blood plasma samples would be more appropriate in future studies for possible detection of transient changes.* It is well known that whole blood histamine levels do not change. However, comparable studies in which plasma levels were also determined are practically nonexistent.¹³

It is worth emphasizing that we found the risk and discomfort of respiratory challenge with mold antigens to be negligible. The following precautions were sufficient: (1) initiation of the experiments with sufficiently diluted antigens (1:500,000) and (2) isoproterenol inhalation after a positive challenge response. Isoproterenol was used primarily to demonstrate that the spirographic changes were quickly reversible and was never required to give emergency relief, perhaps because all changes occurred gradually rather than explosively.

Our findings support the clinical importance of the molds *Aspergillus* and *Penicillium*. Inhalation challenge gave us more specific information than is available from skin tests for molds, which are often of questionable significance. With simple precautions inhalation challenge is a safe procedure. The extra time involved is particularly justified if the patient can be spared an unnecessary course of hyposensitization.

SUMMARY

Seven patients with predominantly seasonal asthma and positive passive transfer tests showed significant positive responses on inhalation challenges to mold antigens. Two patients who had been maximally hyposensitized showed, by contrast, a negligible response. Three patients with asthma and negative passive transfer tests showed a negative response. One patient with atopic dermatitis and positive passive transfer tests, but without any evidence of respiratory allergy, showed a negative response.

There was no change in venous plasma histamine levels or in the daily total blood eosinophil counts subsequent to positive challenge response to mold antigens.

A positive challenge response to mold antigens was associated with the triad of clinical bronchial asthma, appropriate seasonal variation, and skin sensitivity confirmed by passive transfer.

Our observations suggest that *Aspergillus* and *Penicillium*, which predominate in the Puget Sound region, are of clinical significance in the causation of bronchial asthma.

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