

Pulmonary hypersensitivity to *Alternaria* and *Aspergillus* in baker's asthma

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Summary

In two cases of baker's asthma pulmonary hypersensitivity was found to the fungi *Alternaria* and *Aspergillus*. Provocative bronchial challenge revealed a dual response; an immediate and an Arthus type hypersensitivity to *Aspergillus* in the first case. A primary binding assay revealed high titres of anti-*Aspergillus* antibody in the serum. In the second case intradermal and bronchial challenge suggested an immediate type I hypersensitivity response to *Alternaria*. The suspected organisms were present in the room air of the bakeries. It is suggested that an immunological response to these airborne fungi may have contributed to the pathogenesis of baker's asthma.

Introduction

Although baker's asthma has been described since antiquity, the pathogenesis remains obscure. Inhalation of cereal grain dust (notably, wheat) has been incriminated as the primary mechanism of disease (Ordman, 1958). Hendrick, Davies & Pepys (1976) described two cases of baker's asthma with a dual asthmatic reaction to bronchial provocation test with flour. Another recent report suggests that a type I, IgE-mediated hypersensitivity to flour is responsible (Wilbur & Ward, 1976). The grain weevil has also been implicated in baker's asthma (Franklin & Lunn, 1965). We have recently investigated two patients with histories consistent with a diagnosis of baker's asthma. The subsequent evaluation suggests that a hypersensitivity to airborne fungi may be the mechanism underlying the intermittent bronchoconstriction.

Materials and methods

Subject 1

A 49-year-old man was admitted to the Wadsworth V. A. Hospital with a 3-day history of increasing dyspnoea and sputum production. Several months earlier he had developed difficulty in breathing and consulted his private physician. A diagnosis of bronchial asthma was made and the patient was given bronchodilator medications

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without relief. He continued to experience intermittent bronchospasm and was unable to work on the day of admission. The patient had resided in Los Angeles since 1963 and had been employed for the past 7 years in a bakery. He denied an allergic history to pollens, foods or drugs, of childhood asthma or other pulmonary disease, and of familial history of atopy.

Physical examination revealed a thin, black male in no acute distress. Chest examination revealed prolonged expiratory phase with expiratory wheezes and rhonchi. Blood chemistries and other laboratory studies were within normal limits with the exception of an increase in eosinophils ranging from 3 to 61% with an absolute eosinophil count from 330 to 7500/mm³.

Ventilatory functions consistently revealed mild to moderate obstruction with a good bronchodilator response. In February 1974 when he returned with an acute severe exacerbation of asthma, the patient was advised to change occupations. He was transferred from the bakery to the meat department located in a separate building. Since that time he has not required bronchodilator medication and has remained entirely asymptomatic. He has observed that even a brief exposure to the bakery will result in acute bronchospasm.

Subject 2

A 45-year-old man presented with a history of bronchial asthma of 4 years' duration. His bronchospasm increased in severity despite bronchodilators and intermittent corticosteroid therapy. The patient had been employed as a baker for the previous 22 years. The history excluded childhood asthma or allergies and tobacco use. Subsequent severe episodes of bronchospasm most frequently occurred during working hours, requiring emergency room treatment with intravenous aminophylline and parenteral adrenalin. CBC was unremarkable except for 20–24% eosinophils. Chest film revealed hyperinflation with increased bronchovascular markings bilaterally. The patient continued to manifest hyper-reactive airways disease despite the regular use of bronchodilators and alternate-day corticosteroid therapy. He was unable to change occupations and has continued to work in the bakery. The addition of cromolyn sodium to his regimen has resulted in adequate control of his asthma.

Immunological studies

Skin testing was performed using a graded intradermal technique with commercial aqueous antigens with all pollens and environmental antigens common to southern California. In addition, specific moulds (*Aspergillus*, *Penicillium*, *Alternaria*, *Hormodendrum*, *Candida*) rye, wheat and yeast antigens were also employed. Skin tests were measured at 20 min, 6 and 24 hr. Total serum IgE was measured by the radioimmunosorbent test, RIST (Pharmacia RIA Reference Laboratory). Immunodiffusion studies were performed using standard techniques (Ouchterlony, 1962) with undiluted serum to detect antibodies against commercial (Hollister-Steir) *Aspergillus* mix, *Alternaria* mix, *Micropolyspora*, *Thermoactinomyces*, *Sitophilus granarium*, wheat, rye and yeast. Extensive immunodiffusion studies were also performed with commercial and non-commercial components (culture filtrates and mycelial preparations) from several species of *Aspergillus*. A primary binding test utilizing a modification of the Farr ammonium-sulphate precipitation technique was performed to detect anti-*Aspergillus* antibodies in the sera (Minden & Farr, 1967; Bardana *et al.*, 1972).

Bronchial provocative challenge

Bronchial challenge was performed with normal saline and individual antigen aerosols administered on separate days. Graded concentrations of antigens were put through a Devilbis nebulizer driven by a compressor at 10 l/min flow, with the subject performing deep inspiratory manoeuvres. Baseline spirometry and lung mechanics were obtained before administration of the aerosol, with selected measurements repeated at 5, 10, and 20 min intervals following administration of the saline or antigen. At the 30-min interval either an increased number of inspirations or a higher concentration of the antigen was given as indicated by the preceding measurements. These measurements included vital capacity, specific conductance, and maximal expiratory flow-volume curves. All were made in a pressure- and temperature-compensated volume displacement body plethysmograph. The flow-volume curves were monitored on an oscilloscope for immediate comparison and recorded on a multichannel magnetic tape recorder. They were played back at reduced speed and transferred to an X-Y plot for graphic analysis. The 50% value of initial control vital capacity was arbitrarily chosen as the lung volume at which flow measurements were made.

Subject 1 was challenged with normal saline, wheat, rye, and *Aspergillus* antigens; subject 2 with normal saline and *Alternaria* extract.

Fungal cultures

Sabouraud's agar plates were exposed to air in the bakeries for varying periods of time (exposure time 0–180 min). In addition the flour used in the bakeries was cultured for fungi.

Results

Subject 1

Immunological studies. Intradermal tests indicated a high reactivity to mixed moulds and moderate reactivity to feathers, cocklebur, endodust, ragweed, and rye flour; there was no significant reactivity to other pollens and environmental antigens. High reactivity was defined by a weal of at least 10 mm to 10^{-5} – 10^{-6} antigen strength, w/v; moderate reactivity a 10 mm weal to 10^{-3} – 10^{-4} antigen strength. The intradermal testing with individual moulds is illustrated in Table 1. The patient

Table 1. Results of intradermal testing with individual moulds

Mould	Antigen strength (w/v)							
	Subject 1				Subject 2			
	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
<i>Alternaria</i>	0	0	0	0	0	+++	×	×
<i>Aspergillus</i> mix	+++	×	×	×	0	0	0	0
<i>Candida albicans</i>	0	0	+	+++	0	0	0	0
<i>Hormodendrum cladosporioides</i>	+	++	+++	++++	0	0	0	0
<i>Penicillium</i> mix	++	++++	×	×	0	0	0	0
<i>Saccharomyces</i> (baker's yeast)	+	0	0	+++	0	0	0	0

0 No response; +, ++, +++, +++++, severity of response; ×, not performed.

was most reactive to *Aspergillus*. All intradermal tests were negative at the 6 and 24 hr readings.

Immunodiffusion studies for precipitating antibodies to mixed *Aspergillus*, other moulds, *Sitophilus granarium*, wheat, rye and yeast were negative. Immunodiffusion studies with individual species of *Aspergillus* revealed very weak precipitin bands to *A. nidulans*, *A. fumigatus* and no precipitins to *A. flavus*, *A. niger*, *A. fischerii* or *A. terreus*. The primary binding assay revealed anti-*Aspergillus* titre 44.1% (normal range $18.0 \pm 4\%$). Total IgE was 60 u/ml (normal range).

Bronchial challenge. Bronchial challenge revealed no response to the inhalation of rye or wheat flour antigens. Challenge with *Aspergillus* mix revealed a positive challenge after five inhalations at 10^{-3} (w/v) (Fig. 1a, b). After positive challenge the patient was given isoproterenol by inhalation and ventilatory functions returned to the normal baseline value. The patient returned to work (no longer working in the bakery) and 7–8 hr after challenge had a severe delayed reaction. This reaction consisted of acute wheezing and the patient was unable to complete his work shift. A chest X-ray

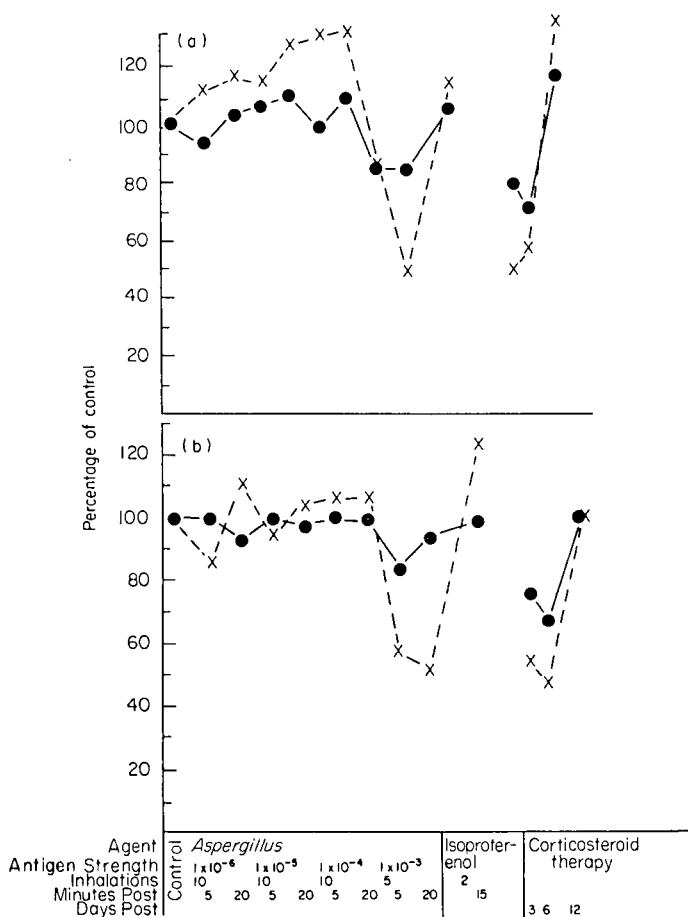


Fig. 1. Ventilatory function response of subject 1 to bronchial challenge with *Aspergillus* antigen, acute recovery with inhaled isoproterenol and later return to control level after corticosteroid therapy. (a) Response of peak flow ($\dot{V}_{max}$, ●) and flow at 50% of vital capacity ($\dot{V}_{50}$, ×). (b) Response of vital capacity (VC, ●) and specific conductance (G/V_{tg} , ×).

was normal and the patient was started on a regimen of ephedrine, aminophylline and isoproterenol every 6 hr. The patient's symptoms continued and ventilatory function studies remain impaired until corticosteroid therapy (prednisone 30 mg q.i.d.) was started on day 6 (Fig. 1). After 6 days of glucocorticoid therapy pulmonary functions returned to normal and the patient has since been entirely asymptomatic without medication.

Fungal cultures. The following fungi were identified from the room air in bakery No. 1: *Cladosporium cladosporoides*, *Aspergillus* spp., *Rhodotorula* spp., *Alternaria tenuis* and *Stemphylium* spp.

Fungal cultures from the flour at bakery No. 1 with a dilution of bakery flour of 1-10: *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Paecilomyces* spp. and *Alternaria tenuis*.

Subject 2

Immunological testing. Intradermal testing revealed high reactivity to mixed moulds, dust and cocklebur, less reactivity to ragweed, and negative for other antigens. Individual mould testing revealed reactivity only to *Alternaria* (Table 1) and no intradermal reactivity to rye, wheat or yeast antigens. All delayed skin test readings were

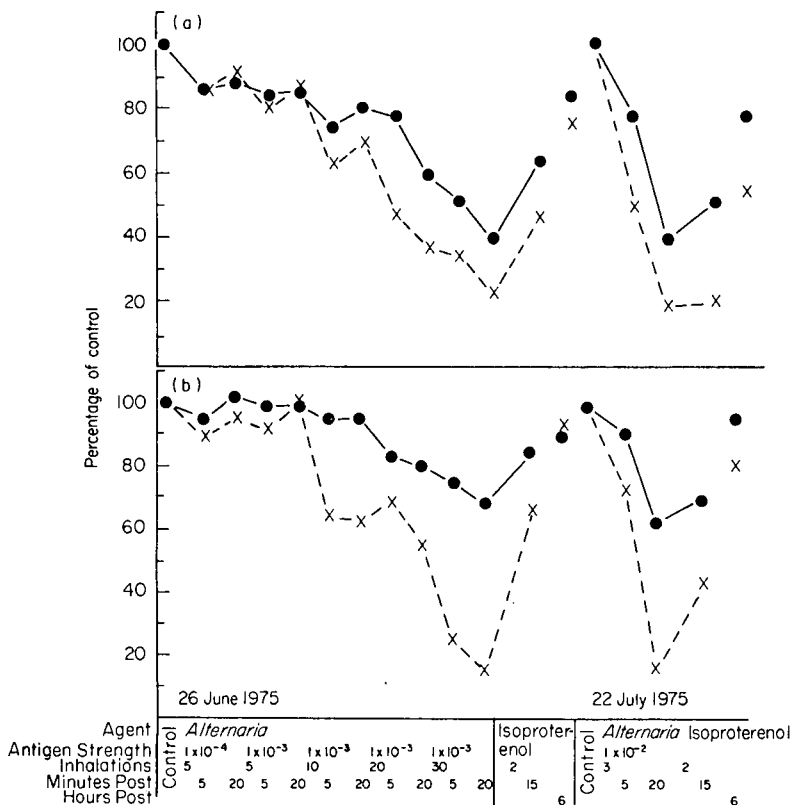


Fig. 2. Ventilatory function response of subject 2 to bronchial challenge with *Alternaria* antigen and acute recovery with inhaled isoproterenol. Positive response was noted on two separate days. (a) Response of peak flow ($\dot{V}_{max}$, ●) and flow at 50% of vital capacity ($\dot{V}_{50}$, ×). (b) Response of vital capacity and (VC, ●) and specific conductance (G/V_{t0} , ×).

negative. Immunodiffusion studies for precipitating antibodies were negative to all antigens testing including *Alternaria*. Total IgE was 110 u/ml (within normal limits).

Bronchial challenge. The patient reacted to *Alternaria* challenge at an antigen strength of 10^{-3} (Fig. 2a, b). No delayed (or Arthus type) response was noted at 7 hr. Repeat pulmonary challenge with *Alternaria* 1 month later revealed identical response (Fig. 2a, b).

Fungal cultures. Fungal cultures from room air in bakery No. 2 revealed: *Alternaria tenuis*, *Penicillium* spp., *Cladosporium cladosporoides*, *Humicola* spp., *Epicoccum* spp., *Botrytis cineria*, *Fusarium* spp., *Aspergillus* spp., *Stemphylium* spp., *Mycelia sterila*, *Trichoderma viride*.

The cultures from the flour in bakery No. 2 revealed *Aspergillus* spp., *Epicoccum* spp., and *Mucor* spp.

Discussion

Inhaled organic antigens may induce bronchospasm as well as a hypersensitivity pneumonitis (Pepys, 1973; Schlueter, 1974; Nicholson, 1972). *A. fumigatus* is an organism associated with a dual immunological response, an immediate (type I) and an Arthus (type III) hypersensitivity (Pepys, 1969; Golbert & Patterson, 1970). *Alternaria* and *Penicillium* spp. are moulds that have also been reported as causative agents in pulmonary allergic disease (Schlueter, Fink & Hensley, 1972; Sahn & Lakshminarayan, 1973). Baker's asthma has rarely been reported in the literature. The evaluation of the two patients is highly suggestive of airborne moulds as sensitizing agents. Both patients presented with a history consistent with occupational asthma and manifested significant eosinophilia. Several fungi were cultured from the air from the bakeries including *Aspergillus* spp. and *Alternaria*. Fungi were also cultured from the flour from the bakeries.

The highly reactive intradermal test and the dual immunological response on bronchial challenge to *Aspergillus* are consistent with a diagnosis of allergic aspergillosis in subject 1. The significant titre of *Aspergillus* antibody as measured by the ammonium-sulphate test may give further support to this diagnosis (Bardana *et al.*, 1975). However, it is known that antibodies that bind to fungi may be detected in sera from normal persons when one uses sensitive primary binding techniques (Minden & Farr, 1975).

The second patient demonstrated a highly reactive immediate intradermal test and a positive bronchial challenge to *Alternaria*. There was no evidence for an Arthus type response by skin testing, immunodiffusion studies or delayed response to bronchial challenge.

Bronchial challenge is a well-established technique in the investigation of the precise aetiological factors in asthma (Pepys & Hutchcroft, 1975; Spector & Farr, 1971; Aas, 1970; Bronsky & Ellis, 1969). The delayed reaction to bronchial challenge with *Aspergillus* antigen was severe and corticosteroid therapy was needed to control the patient's wheezing. In subject 1 the dual response to *Aspergillus* suggests that an immediate and Arthus type immune response has occurred. A transfer in job assignments resulted in a complete remission of his symptoms. The second patient was unable to change occupations. The evaluation was suggestive for an immediate (type I) immune response without evidence for an Arthus (type III) response. The patient was treated with cromolyn sodium and his asthma has abated.

This report demonstrates intradermal and bronchial reactivity to fungal antigens

(*Alternaria* and *Aspergillus*) in two bakers. The patients' occupational environment appears to play an aetiological role in their asthma. The presence of moulds including *Aspergillus* and *Alternaria* was documented from the room air samples obtained from the bakeries. In subject 1, sensitivity to the mould isolated from the flour was demonstrated (*Aspergillus*) but in subject 2, *Alternaria* was not cultured from the flour. It would appear that their asthma was due to the moulds rather than flour sensitivity. A cause and effect relationship between the airborne fungi and baker's asthma is suggested.

The data support the use of intradermal testing and provocative bronchial challenge in the evaluation of the specific antigens responsible for the induction of allergic asthma. The target organ response to bronchial challenge may be the most direct approach in the assessment of aetiological factors in allergic asthma. We would suggest that the fungi (*Aspergillus* and *Alternaria*) are possible allergens in the two bakers and that baker's asthma may result from a hypersensitivity to them.

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