

Bronchial asthma due to *Cannabis sativa* seed

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A 51-year-old man with asthmatic attacks due to *Cannabis sativa* seed inhalation was studied. Specific IgE against this seed was demonstrated by *in vivo* (skin and bronchial challenge tests) and *in vitro* methods (reverse enzyme immunoassay and histamine release from basophils), suggesting a Type I immunologic reaction.

Key words: bronchial asthma; *Cannabis sativa*; histamine release; reverse enzyme immunoassay.

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Occupational asthma has been defined as variable airway narrowing related to exposure in the working environment to dusts, vapors or fumes (3, 7). Several grain dusts have been implicated in occupational asthma, usually in grain handlers in whom specific IgE (*in vivo* and *in vitro* techniques) has been demonstrated (9). Recently, we have studied a male patient suffering from rhinitis and bronchial asthma because of inhalation of *Cannabis sativa* seed (CSS). *Cannabis sativa* is a plant frequently associated with byssinosis, a special kind of obstructive disease due to exposure to its bract dust (2, 3).

CASE REPORT

A 51-year-old man, grocer by profession, reported a past history of extrinsic bronchial asthma due to *Dermatophagoides pteronyssinus* sensitization which required immunotherapy. In 1988, he began to earn a living as a bird breeder. A year later, soon after exposure to hemp seeds, he developed rhinorrhea, chest tightness, dyspnea, cough and wheezing, becoming asymptomatic 1 h later, avoiding exposure. No nocturnal recrudescence was demonstrated. No constitutional symptoms, such as fever, weight loss, asthenia or headache were noted. Clinical examination revealed diffuse expiratory wheezes in both lungs on chest aus-

cultation. Complementary studies were as follows, hemogram: leukocyte count of $6,900/\text{mm}^3$, with 60 eosinophils/ mm^3 , 15.9 g/dl of hemoglobin, platelet count of $230,000/\text{mm}^3$; serum biochemical parameters: albumin, 4.4 g/dl; alkaline phosphatase, 46 U/l; AST, 26 U/l; calcium, 9.3 mg/dl; cholesterol, 263 mg/dl; creatinine, 1.3 mg/dl; glucose, 100 mg/dl; inorganic phosphorus, 2.9 mg/dl; lactate dehydrogenase, 147 U/l; and uric acid, 7 mg/dl. Electrocardiogram was normal. Chest X-rays showed no alteration. Sputum cytology exhibited inflammatory cells, 25% of which were eosinophils. Lung function tests demonstrated an important obstructive pattern: forced vital capacity, 4.3 l (99% of predicted value); 1-sec forced expiratory volume, 1.8 l; and Tiffenau test, 42% (predicted value of 78%). Arterial blood gases while breathing room air were: pO_2 , 7.41 mmHg; pCO_2 , 34.3 mmHg; CO_3H , 21 mEq/l and pH, 7.40 . Total IgE value was 249 IU/ml.

MATERIAL AND METHODS

CSS extract for in vivo and in vitro tests

After removing the grease from CSS sample using acetone several times over a 24-h period,

10 g of CSS were dissolved in 100 ml aqueous solution with 0.375 mg CO_3H_2 , 0.5 mg ClNa and 0.4 mg phenol (pH, 7.42) at laboratory temperature for 24 h. The solution was then filtered through a filter paper and then through a sterilization filter (Millipore Corp., Bedford, MA). The solution had a final concentration of 1/10 g/ml w/v. Dilutions of up to $1/10^6$ g/ml w/v were made for skin and inhalation tests. Sterilization was proved by negative cultures for aerobi, anaerobi and fungi. The sample was also examined under optical microscopy to rule out the presence of mites.

Skin prick and intradermal tests

Prick tests were carried out using the following materials: commercial reagents (Merck Diagnostics) of *Penicillium* spp, *Cladosporium*, *Aspergillus* spp and *Alternaria tenuis* (5000 units PNU/ml each), and CSS extract from 1/10 to $1/10^6$ g/ml w/v. Storage mites, such as *Tyrophagus putrescentiae*, *Glyciphagus domesticus* and *Lepidoglyphus destructor*, (Aristegui Diagnostics) were also used. The skin tests were performed with prick-lancets (1 mm) (Dome-Hollister-Stier), and intradermal tests with single 0.5 ml hubless syringe with a hypodermic needle to introduce 0.02 ml of the antigen. As a positive control, we used histamine diposphate (Lilly Ph): 0.01% for intradermal tests and 0.1% for prick tests. Concurrent tests with saline solution were done as a negative control. The criteria for measuring the reaction was the size of the papula developed after 20 min and 6 h in each case. We considered a reaction positive when papula was 3 mm > histamine control.

Specific IgE determinations

Specific IgE determinations against molds and CSS were made. For anti-mold specific IgE measurement, we used commercial RAST (Pharmacia Lab). A REIA (reverse enzyme immunoassay) and a release histamine test were done to demonstrate specific IgE against CSS.

REIA. Dynatech microELISA M 24 AR plates were paired with 0.1 ml of 1/100 dilution of monospecific antihuman IgE (Tago Lab) in phosphate-buffered saline (PBS). After several washes and following the method described by Cuevas et al. (4, 6) the antigen (CSS extract) was labeled using periodate oxidation, and the color reaction was arrested by the addition of 4 NH_2SO_4 . An optical density > 0.1 OD at 492 nm was considered positive. Three subjects were tested as controls.

Histamine release from basophils. Four five-fold PBS dilutions of CSS extract and anti-IgE were made. Twenty μl of each dilution were mixed with 200 μl of whole heparinized blood in eight different tubes. Total histamine content was calculated by adding 400 μl of 10% perchloric acid to 200 μl of blood, while basal histamine content was calculated using 200 μl of total blood alone. The 10 tubes were incubated for 30 min at 37°C when 400 μl of EDTA (0.01 M in PBS) were added to each tube, except that containing total histamine, to stop the reaction. Immediately, the tubes were centrifuged at 2000 rpm for 4 min. An automated fluorometric method was used to quantify histamine. The percentage of histamine release due to the antigen-antibody reaction was calculated and an amount > 10% was considered positive. Three control subjects were tested as well.

Bronchial challenge tests

The patient took five inhalations of the aerosolized allergen generated by a De Villbis 646 pressurized nebulizer with a fitted output of 0.3 ml/min. A phenol saline solution challenge was carried out as negative control. Baseline pulmonary function tests were performed and repeated at 10 and 30 min after challenge. The parameters employed were FEV_1 , FVC, PEF and $\text{FEV}_{25-75\%}$. A positive immediate response was defined as a 20, 10, 25 and 25% fall in the parameters marked above, respectively. Hourly peak flow measurements were recorded for 24 h following challenge. A positive late response was defined as a 25% fall in

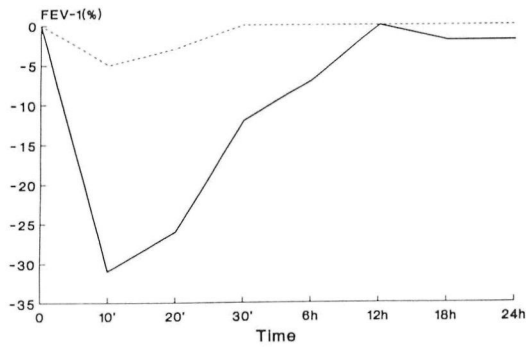


Fig. 1. Bronchial challenge test with CSS extract (1/10 w/v). Pretreatment with DSCG (dotted line) inhibits the response to the antigen.

PEF. Five asthmatic patients were challenged as controls to rule out a non-specific hyper-reactivity response.

RESULTS

Prick and intradermic tests with CSS extract $1/10^3$ and $1/10^5$ g/ml w/v, respectively, were positive (5 mm > histamine control, with pseudopods). Control patients showed no reaction to this extract. Mold and storage mite extracts did not produce a positive reaction either on prick tests or intradermic tests.

The patient showed positive CSS-REIA values (OD 492 nm = 0.8) and a positive histamine release test (52.7%). Control subjects had negative results in both tests.

Ten minutes after bronchial challenge with $1/10^6$ g/ml w/v CSS extract, all the parameters decreased (FEV₁ decreased by 31%) and returned to baseline 45 min later. No late response was observed. Preinhalation of DSCG inhibited the immediate response (Fig. 1). Control asthmatic subjects showed no alteration after bronchial challenge with CSS extract.

DISCUSSION

Cannabis sativa is a plant belonging to the family of Cannabinaceae, originating from west Asia. CSS is used for the production of vegetal oils and as feed for birds. Its stem is used as a

raw material in the textile industry and, together with cotton and flax, is involved in the etiopathogenesis of byssinosis (1, 2). Byssinosis is a significant occupational obstructive lung disease characterized by cough, dyspnea and chest tightness, typically developing 4–6 h after exposure to the dusty textile mill environment (1). The severity of these symptoms is greatest when workers return to mills after an absence of several days (1, 2).

The case reported here was noted as a type of immediate bronchial asthma after CSS exposure. It is well known that this plant is responsible for a late response when there is exposure to its stem. From an immunological point of view, we demonstrated specific IgE against CSS by *in vivo* (skin and bronchial challenge tests) and *in vitro* methods (REIA and histamine release tests). In byssinotic patients, specific IgE could not be demonstrated in every case, and it is possible that an unspecific histamine release could be the mechanism involved (5). However, seeds are frequently contaminated with molds and storage mites against which the patient could be sensitized (8). This seemed improbable in our case for two reasons: first, because we tested the patient against molds and storage mites with a negative result and, second, because sample cultures and microscopic examination were negative.

To our knowledge this is the first reported case of bronchial asthma caused by CSS. Further studies of the nature of this antigen and its possible cross-reactivity with antigens implicated in byssinotic illness may lead to a better comprehension of both entities.

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REFERENCES

1. Bouhuys A. Scheduled asthma in the textile industry. *Lung* 154, 3–16, 1976.
2. Bouhuys A, Zuskin E. Byssinosis: Occupational lung disease in textile workers. In Frazier CA, (ed.): Occupational asthma, pp. 33–52. New York, 1979.
3. Chan-Yeung M, Lam S. Occupational asthma, state of art. *Am Rev Respir Dis* 133, 686–703, 1986.

4. Cuevas M, Monco I, Ureña V, Dominguez J, Bootello A. Reverse enzyme immunoassay for the determination of *Lolium perenne* IgE antibodies. *Int Arch Allergy Appl Immunol* 72, 184-187, 1983.
5. Douglas JS, Zuskin E, Bouhuys A. Relationship between in vivo bronchospasm induced by textile dust extracts and in vitro histamine release from pig lung. *Am Rev Respir Dis* 109, 712-716, 1974.
6. Monco I, Cuevas M, Ureña V, Alcover R, Bootello A. Reverse enzyme immunoassay for the determination of *Dermatophagoides pteronyssinus* IgE antibodies. *Int Arch Allergy Appl Immunol* 71, 285-287, 1983.
7. Newman Taylor AJ. Occupational asthma. *Thorax* 35, 241-245, 1980.
8. O'Neil C, Reed MA, Aukrust L, Butcher BT. Studies on the antigenic composition of aqueous cotton dust extracts. *Int Arch Allergy Appl Immunol* 72, 294-298, 1983.
9. Warren P, Cherniack RM, Tse KS. Hypersensitivity reactions in grain dust. *J Allergy Clin Immunol* 53, 139-149, 1974.

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