

Bronchial asthma caused by *Lathyrus sativus* flour

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A 10-year-old child with asthmatic attacks related to *Lathyrus sativus* flour inhalation was studied in our department. Skin test and specific bronchial provocation challenge were positive. Specific IgE antibodies to *Lathyrus sativus* flour was demonstrated by indirect enzyme immunoassay. We suggest that our patient's allergic symptoms were due to the development of Type I allergic reactivity to *L. sativus* antigens.

Key words: bronchial asthma; bronchial provocation test; enzyme immunoassay; *Lathyrus sativus*; legumes.

Several flours have been causatively implicated in bronchial asthma, usually at work (4, 7). Cereals are the principal source of these flours, but they can also be obtained from other grains not taxonomically related to cereals, e.g. buckwheat (8, 13), legumes (3), etc.

In subjects with flour exposure symptoms the presence of specific IgE has been demonstrated by *in vivo* and *in vitro* techniques (1, 2, 13).

Recently, we had the opportunity to study a child suffering a bronchial asthmatic attack after inhaling *Lathyrus sativus* flour (LSF).

Case report

A 10-year-old child with a history of perennial rhinitis and bronchial asthma was studied in our department. He had a positive family history of atopy. For several years he had been almost asymptomatic, needing medication only during spring. In the last year, shortly after exposure to LSF in the family store, he presented with sneezing, rhinorrhea, wheezing and dyspnea attacks. When he avoided exposure to LSF the symptoms were markedly reduced and eventually became asymptomatic. The child tolerated the ingestion of foodstuffs prepared from this legume as well as exposure to other types of flours.

Clinical examination revealed diffuse expira-

tory wheezes over right lung field on chest auscultation. White blood cell count was 8,790/ml with 376 eosinophils/ml. Total IgE was 44 UI/ml. Chest X-ray was normal. A spirometry revealed a FEV₁ of 124 and FCV of 127 % predicted.

MATERIAL AND METHODS

LSF extract for in vivo and in vitro tests

Two grams of LSF were dissolved in 20 ml phosphate buffered saline (PBS) (pH 7.3) at laboratory temperature. After stirring for 60 min and passing through filter paper, the solution was filtered (Millipore Corp., Bedford, Mass.) for sterilization and had a final concentration of 1:10 w/v. Ten-fold dilutions were made for skin tests and inhalation tests.

Skin prick tests

Skin prick testing was done with LSF extract (1:10 w/v) as well as with the heated extract (75°C × 30 min). A battery of commercially available allergens including cereal flours was also tested. The same test was performed on 17 atopic subjects. Five normal persons served as controls. All skin tests were examined after 15 min and 4 to 8 h later. Our

criteria for measuring these reactions are the following:

- + = erythema and 0.5 cm wheal;
- + + = erythema and 1 cm wheal;
- + + + = erythema and 1.5 cm wheal and pseudopods;
- + + + + = erythema and 2 cm wheal and pseudopods.

Precipitin tests

The patients' sera were tested for precipitating antibodies to LSF and mold extracts by an agar-gel diffusion method (9).

LSF-specific IgE determination

Determination of specific anti-LSF IgE antibodies was performed by using an IEIA: M 24 AR microplates (Dynatech Lab) were used. 0.2 ml of a dilution (1:100 in PBS) of LSF extract was incubated in each well at 4°C for 18 h. After several washes, 0.2 ml of PBS-2 % bovine serum albumin (BAS) was added to each well. One hour later, after several washes, 0.05 ml of each patient's serum, mixed with an equal volume of PBS-25 % fetal calf serum, was added in duplicate to individual wells and left shaking for 3 h. After further washing, 0.1 ml of a dilution (1:2000 in PBS - 25 % fetal calf serum) of a conjugated antihuman IgE labelled with peroxidase (Tago Lab.) was added to each well and left shaking for 2 h. The plates were again washed as previously before adding the substrate: 1,2 phenyl diamine (2 mg/ml) citrate-phosphate buffer (0.1 M, pH 5, 0.03 % H_2O_2). After 30 min the reaction was arrested with 2N H_2SO_4 , and the plates were read on a Titertek Multiskan (Fow Lab., Irvine, Scotland) at 492 nm. Any result higher than the mean $\pm$ 2 SD of 5 control subjects was considered positive.

Bronchial provocation tests

Non-specific BPT with methacholine. Methacholine inhalation was performed according to the method of Cockcroft (5). The aerosolized particles were generated by a continuous pressurized

nebulizer model De Villbis 646, with a fitted output of 0.28 ml/min.

Specific BPT with LSF extract. The patient inhaled the aerosolized allergen in progressive concentrations for 2 min in normal breathing volume. A control challenge with PBS was carried out before antigen challenge. Baseline pulmonary function measures of FEV₁, FVC, and PEF were performed and repeated at 5, 10, 15, 30, and 60 min after each challenge. A positive immediate response was defined as $\geq$ 20 % fall in FEV₁. Hourly peak flow measurements were performed for 24 h following each challenge in order to evaluate the late response. This was considered as $\geq$ 25 % fall in PEF from the baseline. The inhibitory effect of disodium cromoglycate (DSCG) on the inhalation reaction was also studied. DSCG (40 mg) was given by inhalation using a spinhaler 30 min before the provocation test.

Three non-exposed asthmatic patients with similar non-specific reactivity were also challenged with 1:10 w/v LSF extract after obtaining their informed consent.

RESULTS

Fifteen minutes after skin testing with LSF extract (1:10 w/v), the patient exhibited a 17 $\times$ 15 mm wheal and erythema response with pseudopods (+ + +). Prick test with the heated extract was negative. Controls did not react to this extract. Prick test was also positive in our patient with pollen (+ +), and *Alternaria* (+) extracts.

Precipitins to 1:10 w/v LSF extract, but not to molds, were found in the patient's serum. The patient showed positive LSF-IEIA values (OD492 nm = 0.760). In contrast, the control subjects had negative IEIA values (OD492 nm = 0.081 $\pm$ 00) (Fig. 1).

Bronchial challenge with methacholine revealed a severe bronchial hyperreactivity (PC₂₀ = 0.95 mg/ml). After challenge with LSF extract (1:10000 w/v) the patient had an immediate response. FEV₁ decreased 28 % at 10 min and returned to baseline 1 h later. No late reaction was observed. Preinhalation of DSCG

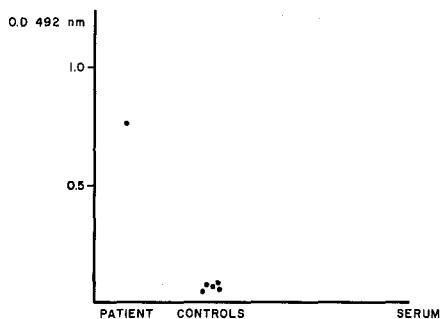


Fig. 1. Specific anti-LSF IgE antibodies by IEIA.

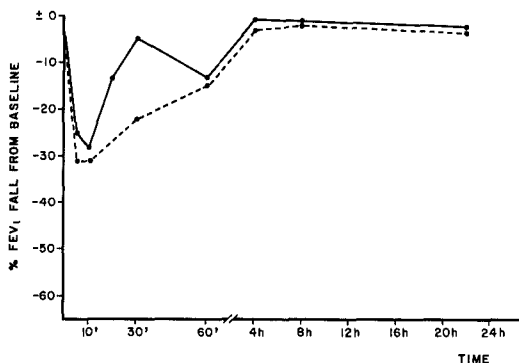


Fig. 2 Bronchial provocation test with LSF extract (1:10000 w/v). The same test performed after DSCG (40 mg inhalation).

failed to inhibit the immediate response (Fig. 2). Control subjects showed no reaction to bronchial provocation with LSF extract.

DISCUSSION

Lathyrus sativus (chickling) is a legume belonging to the genus *Vicia* and the Leguminosae family. Its flour is used for feeding animals and, in some countries, especially in times of famine, also for human consumption. The ingestion of this legume for long periods has been associated with lathyrism (6), an illness characterized by irreversible spastic paralysis of the lower limbs, spinal hyperreflexia and structural changes of the skeleton and connective tissue. It is believed

that the neuroexcitatory amino acid -N-oxalyl-L-,diaminopropionic acid obtained from the seeds of LS is the responsible neurotoxic (11).

In our patient it appears that LSF was the causative factor for the asthmatic attacks described. As cutaneous tests and specific bronchial provocation produced immediate positive, but no late, reactions, and were negative in the controls, irritant or pharmacologic mechanisms seem unlikely. Pretreatment with DSCG did not inhibit the fall in FEV₁ obtained with this antigen. However, this has been observed previously with cereal flour antigens (10, 12). It is interesting that a previous specific bronchial challenge with *Alternaria* extract in this patient had been negative.

However, specific IgE and IgG against LSF was shown by means of IEIA and agar-gel diffusion methods, respectively.

The loss of allergenic sensitivity observed in the cutaneous test with heated LSF, suggests that this might be the reason why the patient was able to ingest cooked foodstuffs prepared with this flour without reacting.

As far as we know this is the first study to be published on bronchial asthma caused by LSF. Additional studies on the nature of this antigen are at present being carried out by our group.

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