

IgE mediated hypersensitivity to pancreatic extract (PE) in parents of cystic fibrosis (CF) children

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Summary

On exposure to pancreatic extract (PE), four parents of cystic fibrosis (CF) children developed immediate hypersensitivity-like symptoms: i.e. rhinoconjunctivitis, asthma, and/or anaphylaxis. IgE to PE was demonstrated in the subjects by skin testing, leucocyte histamine release and radioallergosorbent test (RAST). No serum precipitating antibodies were found. Bronchial challenge caused an immediate asthmatic response. No delayed asthmatic response or hypersensitivity pneumonitis-like reaction occurred. The responsible antigen for PE induced asthma is unknown; trypsin failed to inhibit PE-RAST and is therefore not responsible in these subjects. Caution should be exercised in using PE for skin testing and bronchial challenge in subjects with suspected hypersensitivity to PE. Certain measures were found useful in preventing the occurrence of symptoms in the four subjects.

Introduction

A reasonable estimate of the incidence of cystic fibrosis (CF) in the United States is one in 2000 live Caucasian births (Wood, Boat & Doreshuk, 1976). After the newborn period, manifestations of pancreatic insufficiency occur in 80–90% of the CF children (Kopel, 1972). After surveying seventy families with CF children (thirty-six of which responded), four parents were identified and studied who manifested immediate hypersensitivity-like reactions on exposure to pancreatic extract (PE). Although we cannot accurately determine an incidence of hypersensitivity to PE in the populations which are exposed to PE, the incidence may be as high as 11% or as low as 5%. The magnitude of the problem of hypersensitivity to PE may be greater than previously suspected.

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Materials and methods

Subject population

A questionnaire was mailed to the parents of the seventy children who received medical care by the University of Tennessee Center for the Health Sciences Cystic Fibrosis Clinic. Thirty-six families responded. The questionnaire was designed to determine if the parents or child ever experienced any nasal or pulmonary symptoms on exposure to PE. Interviews were conducted with those who gave positive responses. Five subjects had histories which were suggestive of PE-induced immediate hypersensitivity. Four subjects agreed to participate in the investigation. Not every test was performed with each subject. Medical personnel without a history of exposure to PE were used as control subjects.

Skin testing

Skin testing and interpretation of the weal and flare response were done according to standard technique (Booth, 1972), using commercially available extracts of pork, trees, grasses, weeds, moulds and house dust (Center Laboratories, Inc., Port Washington, N.Y., U.S.A.). Prick testing was performed with extracts of 1:20 w/v with 50% glycerine added as a preservative. If prick testing was interpreted as causing no cutaneous reaction, intradermal testing was performed to all antigens (with the exception of pork) using 0.02 ml of extracts of 1:1000 w/v containing 0.4% phenol.

Prick testing was also performed with a normal saline solution of PE (Viokase, Viobin Corporation, Monticello, Illinois, U.S.A.), 0.5 mg/100 ml.

Passive transfer

Prausnitz-Küstner (P-K) transfer was performed using heated and unheated serum of a single subject in one recipient (P.L.) (Booth, 1972; Prausnitz & Küstner, 1921). P-K sites were challenged at 4 hr and 24 hr.

Radioallergosorbent test (RAST) and RAST inhibition

Direct RAST (Wide, Bennich & Johansson, 1967) tests were performed both to the PE which was used to prepare the skin testing solution and also to porcine pancreatic trypsin (crystallized, dialysed, lyophilized; Sigma Chemical Company, St. Louis, MO, U.S.A.). Both antigens were coupled to paper discs by Pharmacia Diagnostics, Piscataway, NJ, U.S.A. and RAST was performed using sera from all four subjects and controls. The results were reported using an arbitrary scale of 0 to 4.

RAST inhibition (Bernstein *et al.*, 1976) to PE using the subjects' sera were performed using trypsin as an inhibitor. A solution of porcine pancreatic trypsin was prepared using buffered saline (KH_2PO_4 0.036%, Na_2HPO_4 0.107%, NaCl 0.500%, phenol 0.400%; Greer Laboratories, Inc., Lenoir, NC, U.S.A.). This mixture was diluted to yield a trypsin concentration of 0.001 mg/ml. Sera from the four subjects and one atopic control were used. Trypsin was incubated with the subjects' sera and centrifuged at $1500 \times g$ for 15 min. The upper $\frac{2}{3}$ of the mixture was used for performing RAST to PE. The dilutional effects of the mixtures were considered in evaluating the results. Duplicate samples were run using a mixture of porcine pancreatic trypsin and soy bean trypsin inhibitor. Inhibitor was added to duplicate samples to negate any possible effects of the enzymatic activity of trypsin. In samples run with soy bean

trypsin inhibitor dilutional effects were taken into consideration and trypsin concentrations were adjusted accordingly so that the final concentration of trypsin was the same in samples containing and not containing the inhibitor.

Specific antigen-mediated leucocyte histamine release

Blood was drawn from one subject (Subject 1), an atopic control, and a non-atopic control. The white cells were separated and washed (Pruzansky & Patterson, 1966). Leucocyte histamine was determined by a fluorimetric assay (Shore, Burkhalter & Cohn, 1959) before and after exposure of the cells to varying concentrations of PE.

Precipitating antibodies

Each serum was tested for the presence of precipitating antibody to PE using a double diffusion technique (Ouchterlony, 1968).

Bronchial challenge

One subject and one atopic asthmatic control were challenged with PE using a format popularized by Pepys *et al.* (1972) by having the subject mix PE with food in a manner similar to which she prepared it at home for her child. Vital capacity, timed forced expiratory volumes, forced expiratory flow between 25 and 75% of forced vital capacity (FEF 25-75%), and total diffusing capacity of the lung (D_{LCO}) was determined by a steady state method (Bates, Woolf & Paul, 1962).

Case histories

Subject 1. Several months after delivering a CF child a 28-year-old female noted severe rhinitis, conjunctivitis, and asthma within 15 min after exposure to PE. No fever or late reactions were ever noted by the subject. The symptoms were so severe as to interfere with her ability to prepare food for the child. She frequently required anti-asthmatic medication after preparing PE and food. She was hospitalized for asthma on one occasion shortly after having prepared the child's food. Her past history and family history had no suggestion of atopic or pulmonary diseases. Physical examination of the subject revealed no abnormalities.

Subject 2. A 51-year-old female had chronic asthma since childhood but noticed exacerbations of her asthma and the onset of rhinitis shortly after exposure to PE. No fever or delayed reactions were noted by the subject, but this could not be totally excluded by history since she wheezed daily. On two separate occasions, she mistakenly ingested two pancreatic extract tablets thinking they were acetaminophen. On both occasions she developed anaphylaxis within 10 min. Her symptoms included shock, severe bronchospasm, angioedema and urticaria. On initial examination the subject had diffuse wheezing throughout both lung fields. No other abnormalities were noted.

Subject 3. A 32-year-old female noted nasal and pulmonary symptoms similar to the other cases 3 months after beginning PE therapy with her CF child. She had no fever or late reactions by history. Her past history and family history revealed no suggestion of atopic diseases. Her physical examination was normal. Her husband experienced similar symptoms but was unavailable for study.

Subject 4. A 26-year-old female noted rhinitis and asthma several months after beginning PE therapy with her CF child. She had no fever or late reactions by history. As a child she had spring and fall rhinitis but was no longer bothered by this. Her physical examination was totally normal at the time.

Results

Table 1 summarizes the tests performed on each subject and the observed results.

Skin tests

Two subjects demonstrated cutaneous reactivity to various allergens other than PE. Both subjects had histories compatible with atopic diseases. One of these two demonstrates cutaneous reactivity to pork extract. However, she ingested pork products frequently without adverse effects.

All four subjects demonstrated markedly positive prick test reactions to the PE solution. Subject 2 developed asthma 20 min after her skin test was performed. Two atopic and two non-atopic control subjects demonstrated no cutaneous reactivity to the PE solution.

Passive transfer experiment

Prausnitz-Küstner transfer of heated and unheated serum (Case 1) produced no transfer of cutaneous reactivity when challenged with PE at 4 hr and 24 hr.

Specific antigen mediated leucocyte histamine release

The only subject (1) whose leucocytes were tested demonstrated the release of large amounts of histamine upon incubation with PE. At a concentration of 5×10^{-4} micrograms PE/ml, 52% of the intracellular histamine appeared in the supernatant. Neither the atopic control nor the non-atopic control leucocytes released 50% of their cellular histamine with PE solution concentrations even 10,000 times greater (0.5 μ gPE/ml).

Table 1. Summary of test results on four subjects exhibiting respiratory symptoms on exposure to pancreatic extract (PE)

	Subject			
	1	2	3	4
Atopic history	—	+	—	+
Skin tests:				
Common inhalants	—	+	—	+
Pork	—	+	—	—
PE	+	+	+	+
PK Transfer	—	ND	ND	ND
Leukocyte histamine release	+	ND	ND	ND
RAST				
PE	+3	+4	+1	2+
Trypsin	0	0	0	0
RAST inhibition with trypsin	—	—	—	—
Precipitating antibodies	—	—	—	—
Bronchial challenge	+	ND	ND	ND

+ = Positive; — = negative; ND = not determined; PE = Pancreatic extract.

Table 2. Pulmonary function studies before and after challenge with PE in subject 1

Test	Baseline	% PN	Post challenge	% PN
Vital capacity	4.34 L	111	2.03 L	53
Forced expiratory Volumes—FEV 0.5	2.50 L	99	0.65 L	26
FEV 1.0	3.26 L	96	0.98 L	29
FEV 3.0		95		74
FEF 25–75%	2.64 L/sec	80	0.39 L/sec	12
Diffusing capacity DLCO	18.2 ml/min/mmHg		7.8 ml/min/mmHg	
PN	23.0 ml/min/mmHg			

PN = Predicted normal; PE = pancreatic extract.

RAST and RAST inhibition

RAST demonstrated specific IgE to PE in all four subjects, but not in the control subjects. Table 1 lists the arbitrary RAST values for each subject. No subject demonstrated specific IgE to trypsin (Table 1). Trypsin failed to inhibit RAST to pancreatic extract.

Precipitating antibodies

No precipitating antibodies were found in any of the subjects' sera.

Pulmonary challenge

Subject 1 was challenged with PE by asking her to perform her normal routine of mixing PE with baby food. The results are in Table 2. Fifteen min after exposure she developed rhinorrhea, sneezing, conjunctivitis and wheezing. The wheezing was so severe as to require treatment (adrenalin 0.3 ml 1:1000 intramuscularly) before obtaining postchallenge pulmonary function tests. Vital capacity, timed forced expiratory volumes, FEF 25–75%, and D_{LCO} decreased markedly. No delayed reactions were noted but the subject was treated vigorously with adrenalin, isoproterenol, and theophylline after the initial asthmatic reaction.

Challenge in an atopic asthmatic subject with no history of PE exposure produced no pulmonary function changes.

Discussion

This study confirms the existence of allergic manifestations (rhinoconjunctivitis, asthma, and/or anaphylaxis) after exposure to pancreatic extract (PE) in parents of CF children, as reported by others (Nakamura, 1972; Dolan & Meyers, 1974; Hill, 1975; Bergner & Bergner, 1975; Abernathy, 1975; Sakala, 1977; Twarog, 1977). Although we could not determine an incidence of PE hypersensitivity in exposed persons, the condition is obviously not rare. Hill (1975) studied seven atopic parents of CF children. All demonstrated positive skin tests and bronchial challenges to PE. It was not indicated if the subjects were clinically sensitive to PE or how the subjects were chosen. Our subjects and those of others (Bergner & Bergner, 1975; Abernathy,

1975; Twarog, 1977) indicate that nonatopic and atopic subjects can develop hypersensitivity to PE: this lack of requirement for atopy has been noted in other types of occupational asthma (Murphy Jr., 1976).

Specific IgE to PE was demonstrated in the affected parents by skin testing and RAST. Leucocyte histamine release results were suggestive of the presence of specific IgE in the one subject tested. Others have reported positive histamine release studies in subjects similar to ours using inactivated trypsin as the antigen (Twarog, 1977). Colten *et al.* (1975) demonstrated negative histamine release in unexposed controls using inactivated trypsin. Our data confirms the fact that the leucocytes of subjects with suspected PE hypersensitivity release histamine on exposure to solutions with low concentrations of PE.

It is unclear why we were unable to transfer cutaneous reactivity using the P-K procedure. Others have reported positive P-K reactions and passive transfer to monkeys using sera from subjects with clinical hypersensitivity to PE (Nakamura, 1972; Hill, 1975; Twarog, 1977).

Bronchial challenge was positive in the one subject tested. The asthmatic reaction was so severe that a decision was made not to perform further challenges. A positive correlation between history, skin testing and bronchial challenge in subjects with suspected hypersensitivity to PE has been demonstrated in a small number of subjects (Twarog, 1977).

Skin testing and bronchial challenge with PE should be undertaken with great caution. Subject 1 developed severe pulmonary reaction after pulmonary challenge with PE. Nakamura (1972) reported pulmonary disease induced by PE in a pharmacist. Pulmonary challenge in this same patient caused a severe asthmatic reaction; intradermal testing with PE caused anaphylaxis in this patient. Although Abernathy (1975) suggested that scratch testing with PE can be done with impunity, our subject 2 had a definitive exacerbation of her asthma shortly after prick testing was performed.

The incidence of atopic diseases in CF children is reported to be the same as that of the general population (Kulczycki *et al.*, 1961; Rachelefsky *et al.*, 1974), yet cutaneous reactivity in CF children to common aeroallergens can be as high as 75% (Allan *et al.*, 1975). No parents responding to our questionnaire noted allergic symptoms related to PE exposure in their children. Others have noted this absence of clinical hypersensitivity to PE in CF children (Twarog, 1977). In addition Twarog (1977) found negative skin tests in fifteen CF children. The reason for the lack of sensitivity to PE in CF children is unknown. The children are generally not exposed to as much PE via the respiratory route as their parents, since the food and PE are usually not mixed in the immediate proximity of the child.

PE contains a number of different proteins including trypsin, lipase, carboxypeptidase, elastase, collagenase and other enzymes. Trypsin causes pulmonary symptoms in certain industrial workers and has been shown to induce specific IgE production (Colten *et al.*, 1975).

Twarog (1977) has presented some evidence that trypsin is the offending antigen in PE hypersensitivity. The evidence included correlation between history and PE hypersensitivity and results of skin testing, leucocyte histamine release, and bronchial challenge using trypsin. Because RAST inhibition can be used to determine antigenic similarities between allergens, we attempted to inhibit PE-RAST using trypsin. Our data indicate that trypsin was not the major antigenic determinant responsible for the allergic symptoms in our four subjects.

Our patients demonstrated immediate pulmonary reactions on exposure to PE. No delayed reactions were noted. Three authors (Bergner & Bergner, 1975; Sakala, 1977) each report a single case that demonstrated a delayed pulmonary reaction in addition to an immediate reaction after bronchial challenge with PE. No mention was made in these two cases of fever, leucocytosis, rales or pulmonary infiltrates, all of which are associated with type III hypersensitivity reactions in the lung (hypersensitivity pneumonitis) (Schleuter, 1972). Although most authors reporting subjects with hypersensitivity to PE have searched for precipitating antibodies, only a single patient with precipitating antibody has been reported (Sakala, 1977). This patient had a delayed pulmonary reaction on challenge to PE. None of our subjects demonstrated precipitating antibodies. The delayed pulmonary reaction in the patient who had no precipitating antibody may have been IgE mediated (Robertson *et al.*, 1974).

Our four subjects had complete relief of PE induced respiratory symptoms by following some or all of the following suggestions:

1. Switching to granules or tablets if practical.
2. If mature enough, allowing the CF child to prepare his own food.
3. Substituting papainase, a non-animal extract of papaya fruit for PE. This does not always adequately prevent manifestations of pancreatic insufficiency in these patients. There is also the theoretical possibility of a subject becoming sensitive to papase.
4. Wearing a mask while being exposed to PE.
5. Using PE under an exhaust fan, as is found in many home kitchens.
6. Have an unaffected family member, relative or neighbour prepare the child's food.
7. If another family member is not available when the child needs food, then an unaffected member can prepare several small samples of PE and place them in tightly sealed small jars. The affected member can then use them at a later time. We have found this measure very successful in preventing pulmonary reactions.
8. Using anti-asthma medications in a prophylactic manner (isoproterenol, cromolyn sodium, aminophyllin, etc.). We have not found it necessary to use this measure in our subjects when one or more of the other measures were employed.

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