

Occupational allergy to pancreatic powder: Characterization of IgE-binding antigens in pancreatic extract by immunoblotting

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The IgE response to inhaled dust of pancreatic powder was studied with sera from two employees of a pharmacy. IgE from both sera was directed against both porcine and bovine trypsin preparations. The major IgE-binding structures had an isoelectric point in the pH 8 to 9 range. The molecular weight of the IgE-binding structures was different for both patients. In one patient, most IgE was directed against a 28 to 30 kd structure, whereas the second patient had IgE directed against components with a molecular weight of 35 and 45 kd, respectively. (J ALLERGY CLIN IMMUNOL 1991;87:650-4.)

Powdered enzymes of microbial, plant, and animal origin are well-known elicitors of occupational asthma.¹ *Bacillus subtilis* enzymes,² papain,³ bromelain,⁴ and pancreatic extract^{5,6} are able to cause IgE-mediated hypersensitivity reactions in many exposed industrial workers. Allergic reactions to pancreatic extract may also occur in parents⁷⁻⁹ and nurses¹⁰ of children with cystic fibrosis. In the present study, we describe two patients who developed symptoms of conjunctivitis and asthma on handling pancreatic extract in a pharmacy. With serum from these two patients, we analyzed the IgE-binding structures in pancreatic extract by immunoblotting.

MATERIAL AND METHODS

Patients

Patient B, 26 years old, and patient O, 28 years old, both worked in the pharmacy of a hospital. Patient B suffered from eczema and intermittent conjunctivitis and asthma. In serum from patient B, no IgE against house dust mite, grass pollen, cat dander, egg white, or bovine milk could be demonstrated. The history of patient B revealed an asso-

Abbreviations used

HRT:	Histamine-release test
IEF:	Isoelectric focusing
PACM:	Piperazine- <i>N,N'</i> -bis-2-ethanesulfonic acid, 110 mmol/L of NaCl, 5 mmol/L of KCl, 1 mmol/L of CaCl ₂ , 0.5 mmol/L of MgCl ₂ , and 0.03% HSA, pH 7.4
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

ciation between her symptoms of allergy and the handling of pancreatin (porcine pancreatic powder).

Patient O worked in the same pharmacy as patient B. She had also noted an association between symptoms of moderate asthma and the handling of pancreatic extract and decided to take part in the same allergy tests as patient B. Patient O was allergic to birch pollen, peach, and cherry.

Skin test

Pancreatin (Organon, Oss, The Netherlands) was obtained from the pharmacy in which both patients worked. For use in the skin test, 25 mg of pancreatic powder was extracted for 30 minutes under continuous rotation in 2.5 ml of PBS, 0.03% of human serum albumin (Behringwerke AG, Marburg, Germany), and 0.3% phenol. After centrifugation, the supernatant was filtered through a 0.22 μ m Millex GS filter (Millipore, Etten Leur, The Netherlands) into a sterile glass container. Tenfold dilutions of this extract were prepared in the same buffer. Intracutaneous skin tests were performed according to the method of Norman.¹¹ The reactions were graded in standardized plus signs according to the method of Norman.¹¹

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TABLE I. Results of intracutaneous skin tests with extracts of pancreatic powder

	mg of pancreas powder extracted/ml				
	0	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵
Patient B	0	±	1+	1+	2+
Patient O	0	0	1+	2+	
Control 1	0				0
Control 2	0				0

Skin test grade 2+: erythema, 21 to 30 mm in diameter; wheal, 5 to 10 mm; grade 1+: erythema, 11 to 20 mm in diameter; wheal, 5 to 10 mm; grade ±: erythema, 5 to 10 mm in diameter, wheal, ≤5 mm; grade 0: erythema, <5 mm in diameter; wheal, <4 mm.

Estimation of total IgE and allergen-specific IgE

Total IgE was determined by a competitive binding assay (IgE radioimmunoassay, Pharmacia, Woerden, the Netherlands). Allergen-specific IgE was determined by RAST with allergen disks from Pharmacia or, when this was indicated, with agarose beads as allergen support.¹² For the preparation of pancreatin-agarose, 25 mg of pancreatin (Organon) was incubated for 1 hour at room temperature with 1 to 2 ml of coupling buffer (0.1 mol/L of NaHCO₃ and 0.5 mol/L of NaCl, pH 8.5). After centrifugation, protein in the supernatant was coupled to 25 mg of CNBr-activated Sepharose 4B according to the manufacturers' (Pharmacia) instructions. Trypsin-agarose was prepared by coupling 5 mg of porcine trypsin (Sigma Chemical Co., St. Louis, Mo.) or 5 mg of bovine trypsin (BDH Chemical, Ltd., Poole, England) to 100 mg of CNBr-activated Sepharose 4B. After four cycles of acid/base washings,¹² the solid-phase allergens were suspended in RAST buffer (0.82% NaCl, 10 mmol/L of phosphate, 0.1% NaN₃, 0.2% Tween 20, and 0.3% bovine serum albumin [Sigma Chemical Co.], pH 7.4). One milligram per test of pancreatin- or trypsin-agarose was incubated overnight with 0.05 ml of patient's serum. After three washes with physiologic saline, 0.2% Tween 20, and one wash with RAST buffer, radioiodinated rabbit antihuman IgE antibodies (Pharmacia) were added. After an overnight incubation, the agarose beads were washed four times with physiologic saline, 0.2% Tween 20. Agarose-bound ¹²⁵I-labeled anti-IgE was counted in a Wallac gamma counter (LKB, Bromma, Sweden). The results of the RAST were expressed as percent binding of ¹²⁵I-labeled anti-IgE added.

Histamine release from washed leukocytes

For use in the HRT, 20 mg of pancreatin was extracted for 1 hour in PACM buffer. After centrifugation, the supernatant was used in the HRT. Incubation of washed leukocytes with pancreatic extract and assay of histamine, released into the supernatant, were performed according to the method of Siraganian and Hook.¹³ In brief, erythrocytes in ethylenediaminetetraacetic acid-anticoagulated blood were separated from the leukocytes by dextran sedimentation. Leukocytes in the upper buffycoat

layer were separated from plasma by centrifugation. After two washings, leukocytes were resuspended in PACM buffer; aliquots of this suspension were incubated with ten-fold dilutions of allergen extract in PACM. Histamine, released into the supernatant, was measured by an automated spectrofluorometric technique¹³; the amount of histamine release was expressed as percentage of the total amount of histamine present in the cell suspension.

Immunoblotting

Agarose-gel IEF was performed according to the method of Bengtsson et al.¹⁴ SDS-PAGE was performed according to the method of Laemmli.¹⁵ For analysis by electrophoresis, 100 mg of pancreatin was extracted with distilled water for 1 hour at room temperature under agitation. The protein content¹⁶ of the supernatant was 15 mg of protein per milliliter. For each electrophoretic run, 0.0075 ml of this supernatant was used. After IEF, transfer to nitrocellulose (0.45 µm, Bio-Rad, Utrecht, The Netherlands) was accomplished by the immunoprint technique.^{15, 17} After SDS-PAGE, transfer to nitrocellulose was done by electroblotting.¹⁸ After protein blotting, unoccupied binding sites on the nitrocellulose membrane were blocked by incubation of the nitrocellulose sheet in PBS, 0.1% Tween 20, and 0.02% NaN₃ (PBS-Tween) for 1 hour at 37° C; 0.5 cm wide lines were incubated overnight with a mixture of 200 µl of PBS-Tween and 200 µl of patient's serum in disposable incubation trays (Schleicher & Schuell, Kassel, Germany). After three washings with PBS-Tween, a mixture of 100 µl of ¹²⁵I-labeled anti-IgE (1.2 µCi/ml, Pharmacia) and 300 µl of PBS-Tween was added to each lane.

After 4 hours of incubation at room temperature and three washings with PBS-Tween, the lanes were air-dried and exposed for 10 days to Kodak direct exposure film, DEF-392 (Kodak, Heidelberg, Germany), as described previously.¹⁹

RESULTS

Skin test and HRT

With both patients, positive reactions were elicited in the skin test at low concentrations of pancreatic extract (Table I). No response was observed when two normal, nonallergic donors were skin tested with

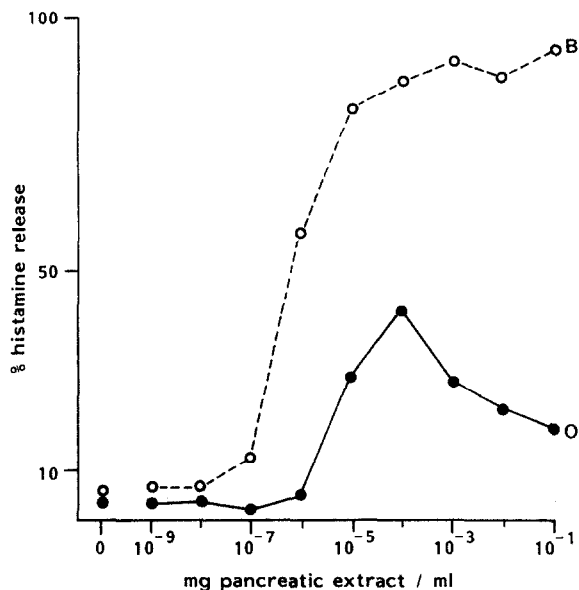


FIG. 1. Histamine release from washed leukocytes of patients B and O after challenge with pancreatic extract.

a solution of 10^{-5} mg of pancreatin extracted per milliliter (Table I). In the HRT, washed leukocytes from both patients released a substantial amount of their intracellular histamine stores after challenge with pancreatic extract (Fig. 1). Leukocytes from two normal, nonallergic donors did not release histamine after incubation with the same concentrations of pancreatic extract (data not presented).

Allergen-specific IgE antibodies

In serum from patients B and O, elevated levels of specific IgE antibodies against porcine pancreatic extract could be demonstrated (Table II). IgE from patients B and O was reactive with both bovine and porcine trypsin. After the discovery of an IgE-mediated allergy toward pancreatic extract, both patients avoided further contact with pancreatic extract. Four years later, IgE against pancreatic extract had decreased to almost undetectable levels (Table II).

IgE was directed mainly against basic proteins in the pH 8 to 9 range. Preliminary experiments indicated that the patterns of IgE binding to IEF-separated pancreatic extract for sera B and O were not significantly different; serum O, however, elicited more intense bands than serum B on the autoradiogram (data not presented). The results from serum of patient O and a control serum are illustrated in Fig. 2. Serum from patients B and O, however, demonstrated a dissimilar pattern of IgE binding to SDS-PAGE-separated pancreatic extract. IgE from patient B was mainly bound

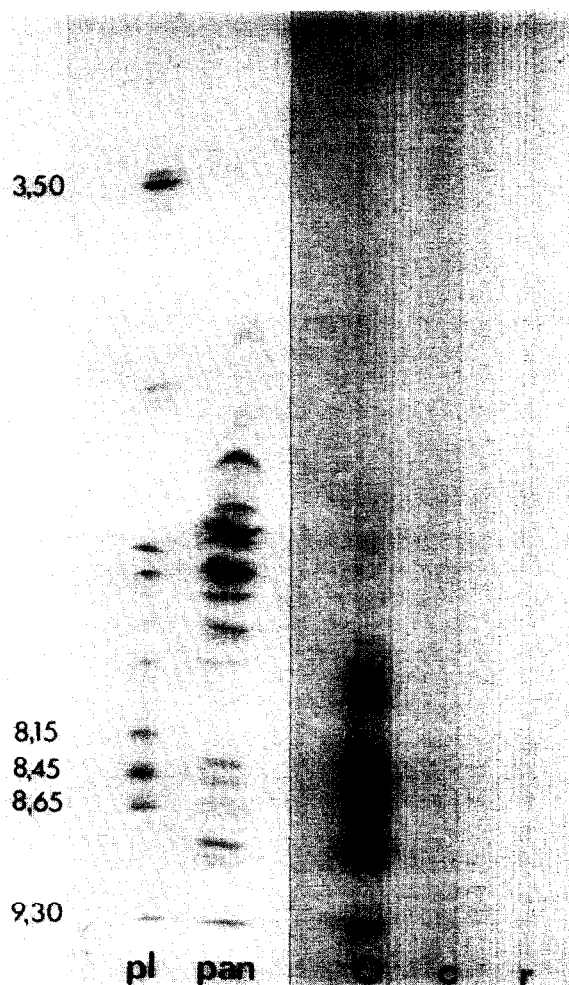


FIG. 2. Binding of ^{125} I-labeled anti-IgE to IEF-separated pancreatic extract after incubation with serum from patient O, a control serum (c, 365 IU, total IgE per milliliter), and buffer (r). The left two lanes illustrate IEF-separated pl markers and pancreatic extract stained with Coomassie brilliant blue.

to components with a molecular weight of 28 to 30 kd, whereas IgE from serum of patient O was directed at components with molecular weights of 35 and 45 kd, respectively (Fig. 3).

DISCUSSION

The strong immunogenic potency of powdered proteins to occupationally exposed individuals has been well established.¹ The present study demonstrates that workers in a pharmacy are also at risk to become sensitized toward pharmaceuticals like pancreatic powder.

The pattern of IgE binding to IEF-separated pancreatic extract (Fig. 2) was more complex than the IgE binding to SDS-PAGE-separated extract (Fig. 3).

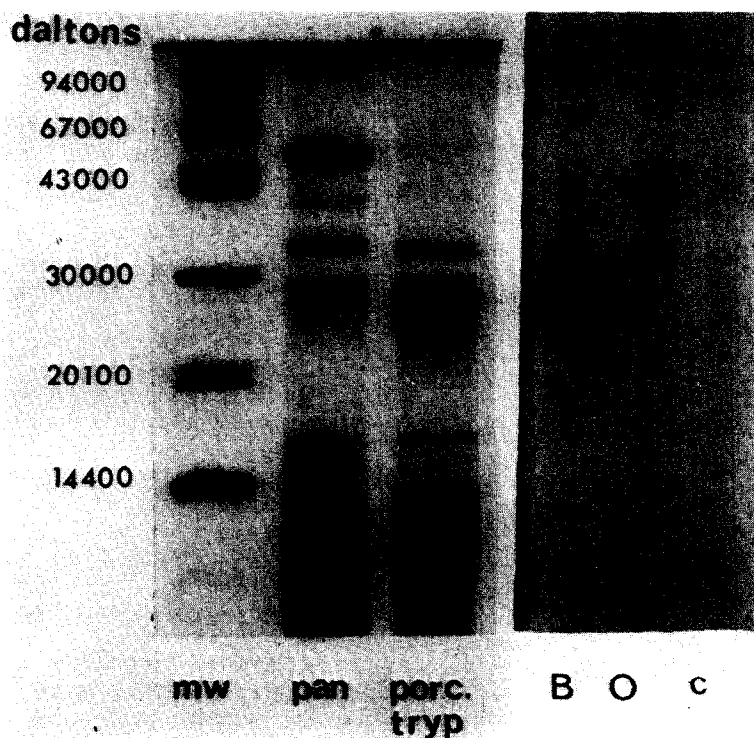


FIG. 3. IgE binding to SDS-PAGE-separated pancreatic extract after incubation with sera from patients B and O and with a control serum (*c*, 365 IU, total IgE per milliliter). The *left three lanes* illustrate SDS-PAGE-separated molecular weight markers, pancreatic extract, and porcine trypsin preparation stained with Coomassie brilliant blue.

TABLE II. Measurement of IgE against porcine pancreatic extract

	% Binding ¹²⁵ I-labeled anti-IgE			Total IgE
	pp	pt	bt	IU/ml
Patient B	20.1	29.8	23.0	160
Patient O	19.1	26.0	19.0	120
Control 1	0.7	0.2	0.2	170
Control 2	0.5	1.6	1.1	210
Control 3	0.3	0.3	0.2	120
Patient B (4)*	3.9			
Patient O (4)*	2.6			

pp, Porcine pancreatic extract; pt, porcine trypsin; bt, bovine trypsin.

*Patients B (4) and O (4) are patients from whom sera were obtained 4 years later.

Most secretory enzymes in the pancreas are present as pro-enzyme, which can be converted into an active enzyme by trypsin.^{20, 21} Multiple discrete forms are generally present for these enzymes.²⁰ Before SDS-PAGE separation, the extract was kept 5 minutes at 100° C; IEF was performed with native extract. Scheele²⁰ reported rapid autoactivation of human tryp-

sinogen during the IEF procedure. Such activation may also have occurred during IEF of porcine trypsin and may have led to further fragmentation of the proteins in the pancreatic extract, thus causing the large number of IgE-binding structures observed in Fig. 2.

In summary, this study demonstrates that employees of a pharmacy are at risk to develop occupational

asthma on handling powdered protein preparations. Furthermore, this article describes the heterogeneity of the IgE-binding structures that are present in porcine pancreatic powder.

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