

Alimentary allergy to pork. Crossreactivity among pork kidney and pork and lamb gut

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Summary

Background A patient suffered from anaphylaxis after the ingestion of pork gut and kidney, but she tolerated pork meat. Clinical symptoms were also triggered upon intake of lamb gut.

Objective To demonstrate an IgE-mediated hypersensitivity and identify the pork proteins involved. And also, to study the possible cross-allergenicity among proteins from lamb gut and pork.

Methods and results The patient had strong positive skin-prick test responses to pork kidney, gut and liver, and lamb gut and kidney. RAST technique showed specific IgE to pork kidney, gut and meat. Immunoblotting after SDS-PAGE disclosed the presence of four prominent IgE-binding polypeptides in pork kidney (200, 90, 57, and 47 kDa), two in gut (57 and 27 kDa), and three in meat (51, 40, and 28–30 kDa), apart from other weaker radiostained bands in each extract. The binding of IgE to 200 and 90 kDa allergens from pork kidney was inhibited by gut from pork and lamb in immunoblotting inhibition assays. No inhibition was produced by pork meat.

Conclusions A mechanism of IgE-mediated hypersensitivity has been demonstrated in this case of anaphylaxis provoked by pork products. Four main allergens were detected in pork kidney, two of which (200 and 90 kDa) share allergenic epitopes with proteins from pork and lamb gut. On the other hand, pork meat does not seem to have allergenic epitopes in common with pork kidney.

Keywords: food allergy, pork, anaphylaxis, meat, pork kidney, pork gut, lamb

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Introduction

Alimentary allergy is one of the causes of recurrent anaphylactic episodes. Sometimes, the aetiology is evident, with immediate appearance of symptoms, and identification by the patient of the food responsible. However, when clinical symptoms are episodic, with delayed appearance, and an urticaria of gradual evolution is superimposed to episodes of severe anaphylaxis, the identification of a food or a group of foods as the responsible agent is very difficult. Alimentary allergy is infrequent, and it may suddenly happen in adults. In previous reports on allergy to pork, the allergens were described to be localized in the meat [1].

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Subjects and methods

The patient was a 57-year-old woman, who had experienced chronic urticaria for 13 years. She complained of clinical exacerbation after ingestion of home-made sausages (stuffed using pork gut); however, the symptoms were not aggravated when she ate commercial sausages. She had suffered anaphylactic shocks between 2 and 6 h after eating pork products on three occasions, with cardiopulmonary arrest on one of them. She also experienced oral pruritus when she masticated pork kidney. She had no pets and denied any clinical symptom when exposed to cat.

Skin-prick tests showed positive responses to pork, lamb, veal and horse meat, and RAST (CAP, Pharmacia) was also positive to pork (0.85 kU/L), veal (0.8 kU/L), and lamb meat (1.02 kU/L). Skin-prick tests were negative to cat, dog, rabbit and cow epithelia and also to milk proteins.

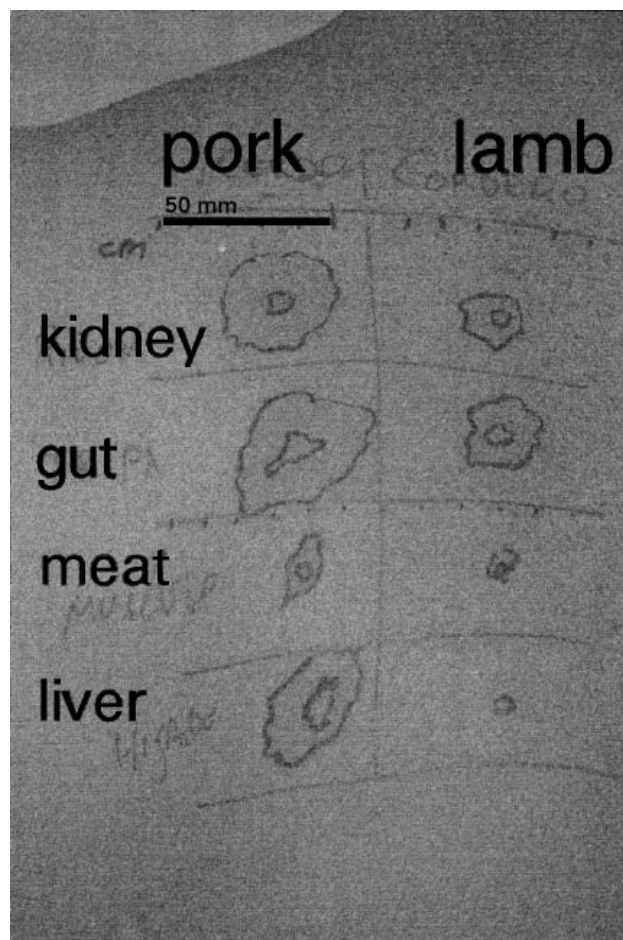


Fig. 1. Prick by prick to different pork and lamb parts.

Complement fractions (C2, C3, C4, C1 inhibitor, and CH50) and hepatic enzymes were within normal levels. Neither antinuclear, anti-DNAs, or anti-Equinecoccus antibodies, nor circulating immunocomplexes were detected.

Complete clinical remission was observed after 4 weeks of dietary regimen with restriction to chicken, turkey, and rabbit. The patient tolerated the reintroduction of lamb meat and pork ham. Occasional ingestion of lamb gut caused her facial oedema, abdominal pain and complement C2 fraction consumption.

A prick by prick test showed positive responses to pork kidney, gut and liver, and lamb kidney and gut (Fig. 1). Negative results were observed for lamb liver and pork, lamb and horse meat.

Preparation of pork and lamb extracts

Raw materials (pork meat, kidney and gut, and lamb gut) were ground and subsequently extracted by mixing with 50 mm ammonium bicarbonate buffer, pH 8.5, at a 10% w/v

ratio, and stirring for 1 h at room temperature, followed by centrifugation at 27000 *g* for 15 min. The supernatants were removed from the pellets and dialysed for 24 h against distilled water at 4 °C, using a Spectra/Por 3 membrane with a MWCO of 3500. Then, the extracts were filtered through 0.45 µm Sartorius membranes, and the protein content was determined by the Lowry [2] method, using bovine serum albumin as a standard. Pork meat extract contained 7.50 mg of protein per mL; pork kidney extract, 5.80 mg/mL; pork gut extract, 0.18 mg/mL; and lamb gut extract, 0.11 mg/mL. Finally, the extracts were lyophilized and stored at 4 °C until use.

RAST

Allergosorbent discs were prepared by coupling the extracts to cyanogen bromide activated paper discs according to Ceska *et al.* [3]. RAST was performed essentially as described by De Filippi *et al.* [4], using a calibrated *L. perenne*-specific serum as a standard curve.

SDS-PAGE and immunoblotting study

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) was performed with an 8–25% acrylamide gradient in a high molarity Tris buffer system [5]. Low range molecular weight standards (Bio-Rad) were included in each gel following the manufacturer's instructions. Electrotransfer of separated polypeptides onto PVDF membranes (Immobilon, Millipore) was carried out by the method of Towbin *et al.* [6]. Further immunodetection of IgE-binding polypeptides was carried out by sequential incubation with patient serum and a radioiodinated antihuman IgE monoclonal antibody [7], followed by autoradiography of the membrane. In the experiments of inhibition of the immunoblot, the incubation of the membrane with the patient serum was performed in the presence of the inhibitor extracts.

Results

RAST

RAST analyses demonstrated the presence of specific IgE antibodies to pork meat, gut and kidney in the serum of the patient. Thus, a RAST class 2 to kidney and gut, and a RAST class 1 to meat were found.

Allergenic components in pork extracts

Figure 2 illustrates the result of SDS-PAGE analysis of the three pork extracts. A complex pattern with protein bands with apparent molecular weights ranging from 5 to 200 kDa

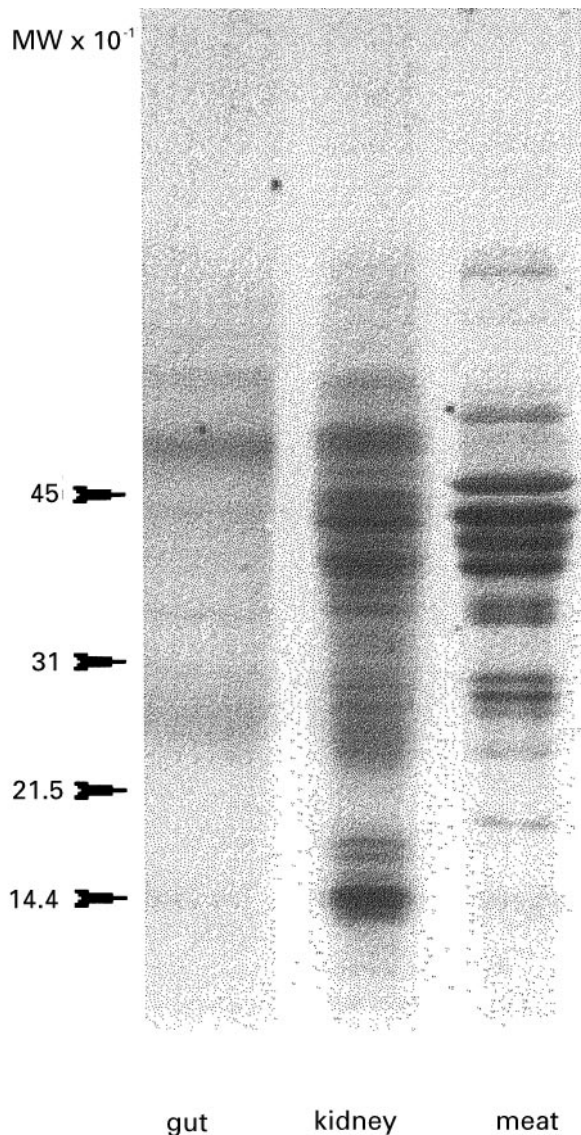


Fig. 2. Coomassie Blue stained SDS-PAGE pattern of pork extracts. The following amounts of protein were loaded per lane: 112 μ g of gut extract; 112 μ g of kidney extract; 60 μ g of meat extract.

was obtained for each extract, although gut extract displayed a number of proteins considerably smaller than the other extracts.

Results of immunoblotting after SDS-PAGE with patient serum disclosed several IgE-binding polypeptides in each extract (Fig. 3). The molecular weight values calculated for these pork allergens are gathered in Table 1. Four strong bands were observed in kidney extract ($\approx$ 200, $\approx$ 90, 57, and 47 kDa), two in gut extract (57 and 27 kDa), and three in meat extract (51, 40, and 28–30 kDa), apart from other weaker bands (Table 1). Only two bands with coincident

Table 1. Allergenic polypeptides detected by SDS-PAGE immunoblot of pork extracts (Molecular weight in kDa)

Gut	Kidney	Meat
	$\approx$ 200*	
	$\approx$ 90*	$\approx$ 90
57*	57*	65
	51	$\approx$ 51*
	47*	
	43	
		42
		40*
		38
27*		28–30*
	$\approx$ 24–29	
		19

* The more prominent bands in each extract are indicated with an asterisk.

molecular weight values in distinct extracts were observed: a 90 kDa allergen in kidney and meat extracts, and a 57 kDa allergen in gut and kidney.

Immunoblotting inhibition assay

By comparison with the SDS-PAGE-immunoblot of kidney extract (Fig. 4, lane 1), the results of the immunoblotting inhibition assay with different extracts revealed that gut extracts from both pork and lamb preferentially inhibited the specific IgE-binding to high molecular weight allergens ($\approx$ 200 and $\approx$ 90 kDa) in kidney extract (Fig. 4, lanes 3 and 5, respectively), which seems to indicate the presence of shared allergenic epitopes in kidney and gut proteins. In contrast, the meat extract did not inhibit the binding of specific IgE to any kidney allergen (Fig. 4, lane 4).

Discussion

In the case described here, the patient did not show clinical symptoms of allergy to pork meat; however, allergens from pork kidney caused her severe allergic reactions, which were also provoked by ingestion of gut from both pork and lamb. This selective sensitization to structures from certain organs, even from different species, with complete tolerance to pork meat clearly differs from other cases of allergy to pork previously reported. In the same way, this case does not seem to coincide with the allergy to a pork protein with molecular weight 67 kDa responsible for the pork–cat syndrome [8,9] (the 67 kDa protein has not been recognized

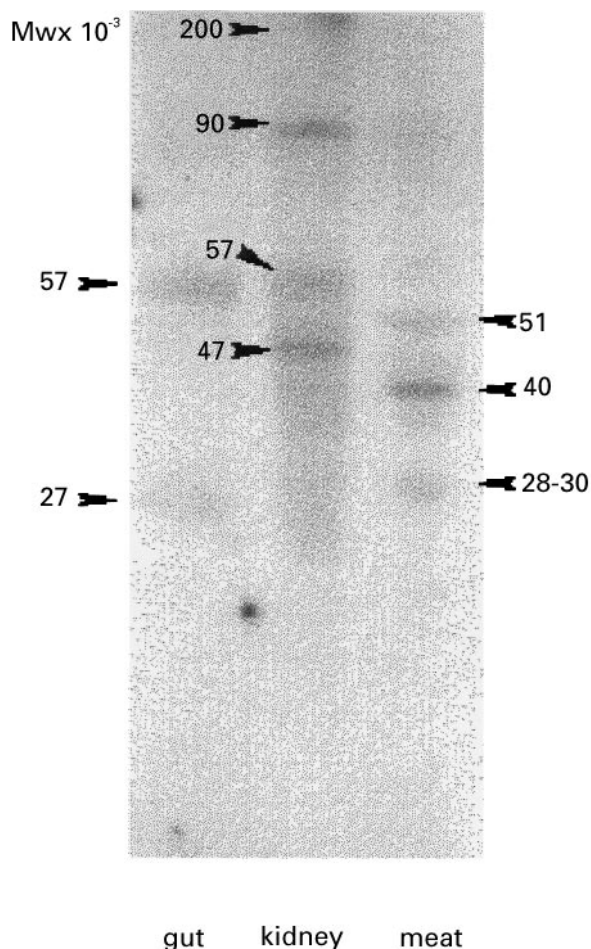


Fig. 3. Immunodetection after SDS-PAGE of pork extracts with the patient serum.

by her serum; in fact, there is no cat epithelia sensitization and clinical remission was obtained with independence of cat exposure), nor with the symptomatology found in cases of extensive allergy to meat from mammals [10].

In general, a good correlation between the clinical manifestations and the *in vitro* results was found. The fact that the patient had a positive RAST to pork meat is not in disagreement with clinical observations. Specific IgE to pork meat, as well as to meat from different species that the patient tolerates had been previously detected by CAP tests.

The results of the SDS-PAGE immunoblotting confirmed the presence of specific IgE to proteins from the distinct tissues analysed: meat, kidney and gut. Some of these proteins may be common to different tissues or, at least, share antigenic determinants, as could be inferred from the experiment of inhibition of the kidney extract immunoblot. Thus, 200 and 90 kDa kidney allergens are proteins antigenically related to proteins expressed in the intestine of

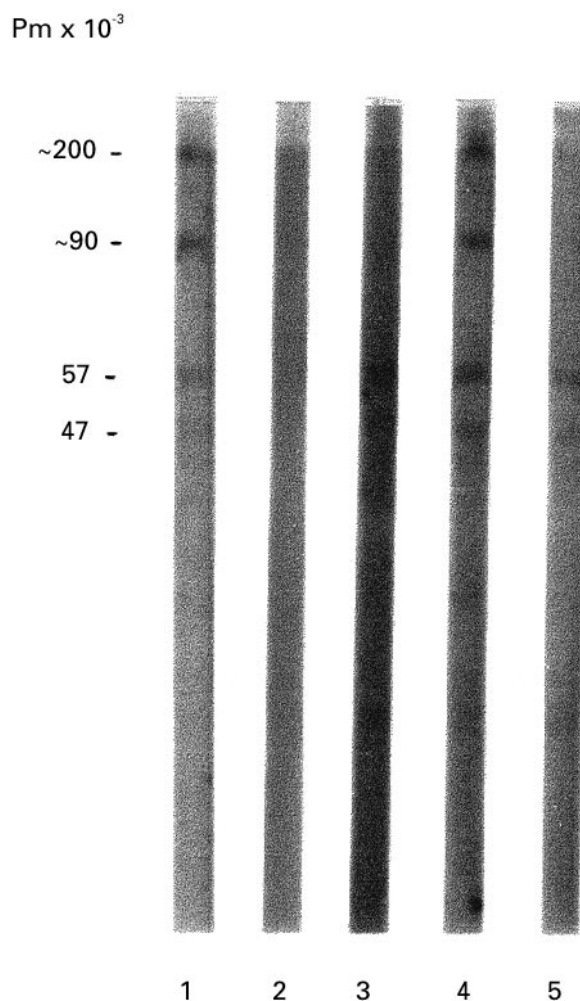


Fig. 4. Inhibition of kidney extract immunoblot. Pork kidney extract (112 µg per lane) was subjected to SDS-PAGE and the separated polypeptides were transferred to PVDF membranes that were subsequently incubated with the patient serum in the presence of the inhibitor extracts at a 4 mg/mL protein concentration: Lane 1, 1% BSA in PBS was used as a control for no inhibition; Lane 2, pork kidney extract (control for complete inhibition); Lane 3, pork gut extract; Lane 4, pork meat extract; Lane 5, lamb gut extract.

pork and lamb. In the case of pork gut and kidney, an alternative explanation for the inhibitions observed would be that the crossreacting allergens were in a low concentration in the gut extract and therefore they could not be detected in the gut extract immunoblot. Nevertheless, in the conditions used in the immunoblotting inhibition assay, the concentration of gut allergens might have been sufficiently high to favourably compete for the binding of specific IgE with the kidney allergens immobilized on the PVDF membrane. On the other hand, 57 and 47 kDa kidney allergens seem to be specific to this organ as no inhibition of the binding of IgE to them was observed, in spite of the fact

that an allergen with molecular weight 57 kDa was also detected in pork gut extract. Additional studies would be necessary to determine whether it is related to the 57 kDa kidney allergen or they are simply different proteins with the same apparent molecular weight.

Pork meat extract, on the contrary, did not appreciably inhibit the kidney extract immunoblot, which strongly suggests that the proteins responsible for the sensitization of the patient to these tissues are different. Thus, these results might represent an explanation for the fact that the patient experienced clinical manifestations upon intake of kidney and gut, and she did not when she ingested meat.

In conclusion, this study allowed us to determine the cause of an infrequent recurrent anaphylaxis that, in spite of the very specific sensitization, had not been identified for 13 years, mainly because of the torpid symptomatology and the relatively long latency periods between severe anaphylaxis. Thus, an elimination diet could be established for the patient that prevented the long-term urticaria and the risk of potentially fatal accidents.

Alimentary allergy to pork products has not been very well studied so far. To our knowledge, this is the first report in which cross-reactivity among allergens of different parts of pork has been studied and the existence of allergens either specific of each organ or common to different organs has been established. At the same time, some of these allergens share epitopes with proteins of the same organs of another mammal.

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