

IgE antibody response to vertebrate meat proteins including tropomyosin

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Background: Although meat is a main source of proteins in western diets, little information is available regarding allergy to vertebrate meats or the allergens implicated in these reactions.

Objective: To evaluate the in vitro IgE antibody response to different vertebrate meats in suspected meat-allergic subjects, as well as the possible role of tropomyosin in meat allergy and to analyze the cross-reactivity between vertebrate meats and the effect of heating on the IgE-binding to meat proteins.

Methods: Fifty-seven sera from suspected meat-allergic subjects were tested by grid blot to extracts of beef, lamb, pork, venison, chicken, and turkey and to four mammalian tropomyosins of different origins.

Results: Meat-allergic subjects have IgE antibodies to proteins in different mammalian meats (43/57 subjects); cross-reactivity with avian meat was limited: less than 50% (19/43) of meat positive sera reacted to chicken. In contrast, most of the poultry-positive sera also reacted to different mammalian meats. In general, there was stronger IgE reactivity to raw meats in comparison to cooked meats; an exception was six cases in which IgE reactivity to cooked poultry was stronger. Weak IgE reactivity to tropomyosin was detected in only 2/57 sera tested.

Conclusions: Suspected meat-allergic subjects have serum IgE directed to meat proteins. In vitro cross-reactivity among mammalian meats appears to be important, while cross-reactivity to poultry is limited indicating mammalian-specific proteins. Although cooking in general denatures meat proteins rendering them less allergenic, in some cases the process of cooking may result in the formation of new allergenic moieties. The muscle protein tropomyosin is not an important vertebrate meat allergen.

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INTRODUCTION

Meat is a main source of protein in western diets, for example, Americans eat approximately 30 kg of beef per

person and year. To date very few reports exist regarding allergic reactions to meat proteins. Some studies have used skin prick testing (SPT) to determine the prevalence of sensitization to meat in children with atopic dermatitis. This prevalence was found to be as high as 27% for pork, 23% for chicken, 26% for beef, and 11% for lamb.^{1–3} Nevertheless, the clinical relevance of these sensitizations seems to be low. In double-blind, placebo-controlled food challenges (DBPCFC) performed in subjects with positive SPT to meats, only about 4% of the challenges with pork and 14.8% with chicken were positive. Larger studies of beef demonstrated up to 12%³ or 8.9%⁴ positive food challenges among subjects with positive SPT to beef.

In adults or children without atopic dermatitis only a few reports have been published regarding IgE-mediated reactions to meat proteins. Some of these reactions were due to contaminants in the meat including streptomycin⁵ or penicillin⁶. In general, meats most frequently implicated in allergic reactions are beef^{3,4,7} and chicken,⁸ whereas turkey, lamb, pork, and venison sensitivities are rarely diagnosed.^{7,9} Of all the meat allergens, those from beef and chicken have been the most thoroughly studied. Positive skin tests to chicken, with or without clinical reactivity, are commonly seen in individuals highly allergic to egg. Chicken meat and egg yolk antigens may be cross-reactive as suggested by the high frequency of specific IgE associated to both antigens.¹⁰ Alpha-livetin, identified as a serum albumin, is the cross-reacting protein responsible for the bird-egg syndrome.¹¹

For beef allergy, particularly when associated with milk allergy, some serum and muscle proteins such as bovine serum albumin (BSA),^{4,12} bovine serum gamma globulin (BGG),^{3,13} myoglobin³ and the muscle protein actin¹⁴ have been identified as allergens.

The muscle protein tropomyosin of brown shrimp (Pen a 1) has been identified as a major allergen.¹⁵ Homologous allergenic tropomyosins Met e 1 and Pen i 1, have been shown in other shrimp species.^{16,17} Cross-reacting Pen a 1-like allergens have been detected in other crustacea species as lobster and crab.¹⁸ Tropomyosin is also an allergen in arthropods such as house dust mite *Dermatophagoides farinae*¹⁹ and also in mollusks such as squid and oyster.^{18,20} The amino acid sequence of Pen a 1 shows a high degree of homology (approximately 50% or more) with

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tropomyosins of distant species, including those of mammals.²¹ The following study was undertaken to investigate meat as a food allergen and the role of tropomyosin in meat allergy.

MATERIAL AND METHODS

Sera

Sera were collected from a total of 57 patients from Spain (Hospital del Niño Jesus and Hospital La Paz, Madrid), USA (Tulane Medical Center, New Orleans LA; Children's Hospital, Little Rock AR; Kaiser Permanente Hospital, Hollywood CA; Plasma-Lab International, Everett WA; Baton Rouge LA; Henry Ford Hospital, Detroit MI; University Suburban Health Center, Cleveland OH) and Canada (Wellesley Hospital, Toronto, Ontario). All sera donors were suspected meat-allergic patients with at least two of the following criteria: convincing history of allergy to beef, lamb, pork, rabbit, venison, chicken, or turkey; positive skin prick-test (SPT) or specific serum IgE by RAST or CAP-system (Pharmacia, Uppsala, Sweden) to any of these meats. Among the 57 subjects, 12 reported symptoms with beef, 7 with lamb, 3 with pork, 1 with rabbit, 1 with venison, and 23 with chicken. The remaining subjects had specific IgE to different meats (mainly chicken or beef), but either tolerated the meats or no history was available. The age of the 29 males and 28 females ranged from 5 months to 48 years; 42 out of the 57 were pediatric patients younger than 18 years (mean 6.3 years), and 15 were adults (mean 28.5 years).

Extracts

Extracts were prepared of raw or cooked mammalian meats: beef, lamb, pork, venison; or poultry: chicken breast, turkey leg and turkey breast according to Werfel et al³ with some modifications. Raw or cooked (20 minutes in a conventional oven, 140°C) half-inch cubes of meat were homogenized in a Waring blender, extracted in PBS (pH 7.2) for 3 hours at 4°C, centrifuged (70,000 g), the fat layer was removed and the supernatant

concentrated (Amicon, Danvers, MA; MW cutoff 1,000 D), recentrifuged (100,000 g), aliquoted, and stored at -20°C until use. Protein concentrations of the extracts were determined by the method of Bradford (modified by Pierce Lab, Rockford IL) using BSA as standard. Commercially available tropomyosins of beef, pork, rabbit, and chicken were purchased from Sigma (St. Louis, MO), aliquoted (2 mg/mL) and stored at -20°C until used.

Grid-Blot

The grid-blot is a modified version of a procedure developed by Alric et al.^{22,23} Briefly, a sheet (12.5 × 12.5 cm) of CNBr-activated nitrocellulose (NC) membrane²⁴ was incubated in TRIS-buffered saline (TBS) (0.1M Tris HCl, pH 7.4, 0.1 M NaCl, 2.5 mM MgCl₂) and positioned on the slotted portion of the acrylic plate of a Surf-blot apparatus (Idea Scientific, Minneapolis MN). The apparatus was assembled according to manufacturer's instructions. Meat extracts (100 µg of protein) and tropomyosins (10 µg) were diluted in TBS and applied separately to different channels. The Surf-blot was incubated for 90 minutes on a rocking platform to promote an even coating to the membrane. Channels were washed twice with TBS. The apparatus was disassembled and the membrane incubated in blocking solution (TBS containing 0.05% Tween and 1% non-fat dry milk) for 30 minutes, washed in TBS-Tween, and dried. For incubation with the sera, the membrane was incubated in TBS-Tween for 10 minutes and assembled with the antigen-bearing lanes rotated 90° from the original direction so that the sera would be perpendicular to the meats and tropomyosins. Seventy-five microliters of each serum, diluted 1:2 in TBS-Tween, was pipetted per channel and incubated for 90 minutes. After incubation, the channels were washed, the apparatus disassembled, and the membrane washed in TBS-Tween for 30 minutes. To visualize the IgE-binding proteins, the blot was incubated for 2 hours with alkaline phosphatase-conjugated, monoclonal

mouse anti-human IgE (Southern Biotechnology Associates, Birmingham AL) diluted 1:1000 in TBS-Tween and washed 3 times for 10 minutes in TBS-Tween. For detection of bound IgE, the membrane was washed 5 minutes in 37°C warm TBS-AP (0.1 M Tris-HCl, 0.1 M NaCl, 5 mM MgCl₂, pH 9.5), and antibody binding was visualized at 37°C using the substrate/chromogen mixture for alkaline phosphatase²⁵ containing 450 µM 5-bromo-4-chloro-indolyl-phosphate disodium salt (BCIP; Sigma) and 400 µM nitroblue tetrazolium chloride (NBT; Sigma) solubilized in TBS-AP. The reaction was stopped with deionized water and the blots were dried.

SDS-PAGE and Immunoblotting

Minigel (thickness 0.5 mm) SDS-PAGE was performed using the discontinuous buffer system of Laemmli.²⁶ A 1.5-cm high stacking gel (5% T, 1.5% C) was poured on top of a 6.5-cm high separating gel (12.5% T, 1.5% C). Of each of the 14 meat extracts (raw and cooked), 12.5 micrograms of protein per centimeter of gel in non-reducing sample buffer were boiled for 5 minutes and separated by electrophoresis. Gels were either silver-stained²⁷ or the proteins were transferred onto a CNBr-activated NC membrane (0.45 µm, BAS 45 Schneider and Schuell, Germany) at 0.8 mA/cm² for 30 minutes by semi-dry blotting.²⁸ The blots were blocked in TBS-Tween for 30 minutes, dried, and stored between filter papers until use. Seventy-five microliters of sera was diluted 1:2 in TBS-Tween, placed in each channel and incubated for 2 hours. Only sera showing strong reactivity by grid blot to the meat extracts, or those belonging to patients who reported convincing/suggestive history of meat allergy were tested by immunoblot. Detection of specific IgE binding was performed in the same way as described above.

RESULTS

SDS-PAGE

Even though both raw and cooked extracts were boiled in sample buffer, the

protein patterns of raw and cooked meat extracts differed considerably, suggesting that those differences are due to the process of cooking and not due to sample preparation. Silver staining of raw extracts revealed more than 30 bands with molecular weights between 10 and 200 kD with staining intensity being highest in the 30 to 66 kD range. The patterns were almost identical for most raw mammalian meats with only slight differences in the mobility of some bands in the raw poultry extracts. Most of the bands demonstrated in the raw extracts disappeared in the cooked meat extracts, with only some faintly staining bands persisting (a 20-kD band and some other from 31 to 66 kD) (not shown).

IgE Reactivities to the Meat Extracts and Tropomyosins by Grid-Blot

All sera ($n = 57$) were tested by grid-blot for IgE reactivity to 14 meat extracts and 4 tropomyosins (Fig 1). A reactivity was considered positive when it was stronger than the background. IgE reactivities to beef and lamb were most frequently detected and in decreasing order, to venison, pork, chicken breast, and turkey leg and breast (Table 1). Eight sera showed neither reactivity to any of the meat extracts nor to the tropomyosins tested. Only 2 out of the 57 sera had minimal IgE-binding to the tropomyosins: one to pork (sera 41) and one to chicken (sera 53) (Table 2).

Most sera (43/57) had stronger reactivity to raw meats than to cooked meats; however, there were some exceptions. This was particularly true for six patients allergic to chicken, whose sera showed strong IgE-reactivity to cooked chicken and little or no reactivity to raw chicken (subjects 7, 11, 12, 24, 30, and 39). These sera together with others from chicken-allergic patients were tested by immunoblotting (see below).

All subjects with IgE reactivity to any one mammalian meat extract (43/57) also reacted to other mammalian meat extracts, whereas less than 50% (19/43) reacted to poultry. In contrast, most of the poultry-reactive sera also reacted to several mammalian meat extracts, thus suggesting that mammalian meats contain IgE-reactive proteins that are not recognized by IgE of poultry-sensitized subjects (Table 3).

Only 12 out of 42 subjects who presented IgE reactivity by grid-blot to raw or cooked beef extract, had a convincing history of beef allergy. All other subjects tolerated this meat, although they had specific IgE to beef. Only one patient with convincing history of beef allergy had a negative grid-blot. Conversely, among the patients with a positive grid-blot to poultry extract (25), most (16/25) had symptoms after eating poultry which suggests that the grid-blot may be

more useful to identify clinically relevant chicken allergy than beef allergy.

No significant difference in IgE antibody reactivity was found between different sources of turkey. Both turkey leg and turkey breast meat showed similar results (Table 1). Additionally, a good correlation was found between IgE-reactivity to chicken and to turkey, indicating that in general both extracts share similar avian proteins. Some isolated reactivities to turkey were detected.

IgE Reactivities to Chicken Extract by Immunoblot

Since some sera showed stronger IgE-reactivity to cooked rather than to raw chicken by grid-blot, immunoblot studies were performed with sera from chicken-allergic patients to investigate possible antigenic differences between raw and cooked chicken extracts. Three sera of chicken-tolerant patients were also used as controls (Fig 2: sera 27, 28, 46).

Most sera of chicken-allergic patients showed similar IgE binding patterns to raw chicken proteins; eight bands (150, 66, 45, 31, 28, 24, 20, and 17 kD) were detected (Fig 2A) with similar although much weaker bands being recognized by the same sera in the cooked chicken extract (Fig 2B). The 150-kD and 45-kD bands disappeared almost completely after cooking. Five out of six sera that reacted strongly by grid-blot to cooked

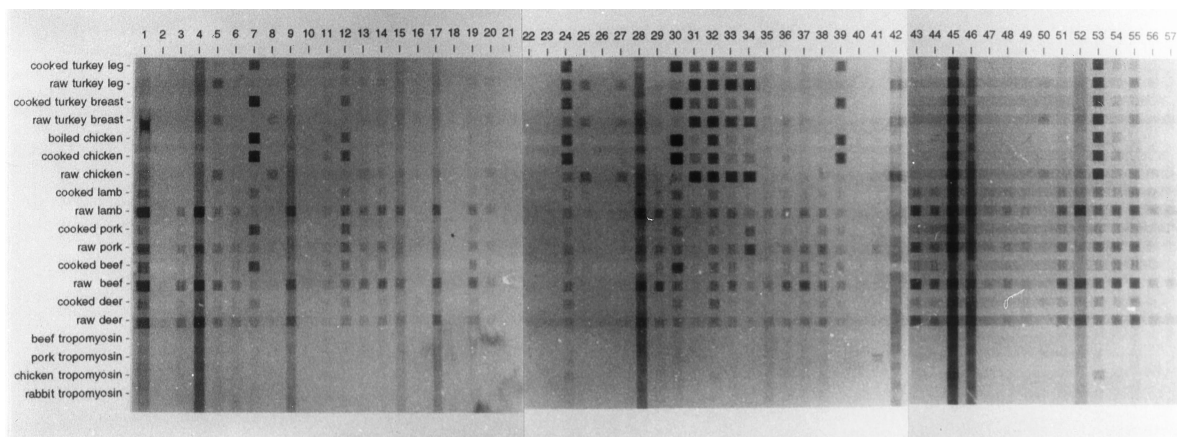


Figure 1. IgE reactivities to the meat extracts and tropomyosins by grid blot. The lanes represent the different vertebrate meats and tropomyosins tested. Columns: patient's sera 1–57. A positive result was considered when reactivity to the extract was stronger than that of the background.

Table 1. Comparison of IgE Reactivities to Raw and Cooked Meats by Grid Blot*

	Beef	Lamb	Pork	Venison	Chicken Breast	Turkey Leg	Turkey Breast
Raw only	21	28	17	22	11	9	9
Cooked only	1	1	1	1	6	6	5
Raw and cooked	20	12	15	11	7	7	8
Total	42	41	33	34	24	22	22
% of 57 sera tested	73%	71%	58%	59%	41%	38%	38%

* IgE reactivity to different meats is shown. Isolated IgE reactivity to cooked mammalian meats was rarely detected. In contrast, six sera reacted stronger to cooked than to raw poultry.

chicken extract showed by immunoblot a large number of IgE-reactivities ranging from 14 to 150 kD in the cooked extract (Fig 2B: sera 7, 24, 30, 39, and 12), whereas only very weak reactivities were detected in the raw extract.

DISCUSSION

In this study we evaluated the in vitro IgE response to different vertebrate raw and cooked meats in suspected meat-allergic subjects, as well as the possible role of tropomyosin as a meat allergen. Our results demonstrate that suspected meat-sensitive subjects have specific IgE antibodies to different vertebrate meat proteins and that tropomyosin is not an important meat allergen.

Most sera of subjects who reacted to one type of mammalian meat by grid-blot, also showed IgE reactivity to many other sources of mammalian meat tested; however, IgE-reactivity to poultry in these subjects was limited. The lack of cross-reactivity suggests that some IgE-reactive mammalian meat proteins are not recognized by IgE of poultry-sensitized subjects.

IgE antibodies to mammalian meat proteins as detected by grid-blot are present in the sera of many subjects who tolerate those meats. Only reactivity to poultry proteins has a good correlation with the clinical symptoms to poultry reported by the subjects, suggesting that a positive reactivity by grid-blot may be a good predictor of clinically relevant poultry allergy.

Although food allergens tend to be resistant to heat denaturation, this property is not universal. For example, cooked apple,²⁹ beef,³ or canned fish³⁰ may not induce clinical symptoms or

Table 2. IgE Reactivities of Sera from 57 Meat-Allergic Subjects to the Different Tropomyosins

IgE Reactivity to Tropomyosins from				No Reactivity
Beef	Pork	Rabbit	Chicken	
0/57	1/57	0/57	1/57	55/57
0%	1.7%	0%	1.7%	96.5%

Table 3. IgE Reactivity to Avian Meats and Mammalian Meats by Grid Blot*

No Reactivity	IgE reactivity to		
	Poultry Only	Mammalian Meat Only	Poultry and Mammalian Meat
8/57	5/57	24/57	19/57
14%	8.7%	42.1%	35%

* Most of the poultry-reactive sera reacted also to mammalian meats. In contrast, less than 50% (19/43) of the mammalian-reactive sera reacted also to chicken.

positive skin prick tests in subjects allergic to the same uncooked foods. Heating seems to promote protein denaturation and the loss of conformational IgE-binding epitopes for some allergenic foods. The resistance of some food allergens to heat denaturation suggests that conformational epitopes may not always be critical for IgE binding in the case of food allergens. For instance, the most potent food allergens seem to be resistant to processing; shrimp allergens are detected in shrimp meat and water following boiling,³¹ and the most potent peanut allergens are found in roasted peanuts.³² In the current study, comparison of IgE reactivities to raw and cooked meat extracts showed a higher reactivity to raw meats probably reflecting the denaturation of meat proteins by heat.³ Nevertheless, in some cases, cooking may lead to the emergence of new allergenic moieties: five chicken-reactive sera showed stronger

IgE reactivity and new bands in the range of 14 to 90 kD in cooked chicken extract. This difference was previously reported for pecan and shrimp. In a pecan-allergic subject only heated pecans induced anaphylaxis whereas fresh pecans were tolerated. Immunoblot analysis showed only IgE-binding to heated pecan proteins as well.³³ Rosen et al³⁴ described a shrimp-allergic patient with repetitively positive skin prick test to cooked shrimp but not to raw shrimp extract. Lombard et al³⁵ found stronger IgE-reactivities to extracts of boiled shrimp by prick-test and demonstrated boiled shrimp extract as the most potent RAST inhibitor as compared with raw shrimp extracts. These findings suggest that heating may enhance the allergenicity of some food proteins and that cooking possibly may produce new allergens by altering existing allergenic proteins or exposing allergenic epitopes already present.

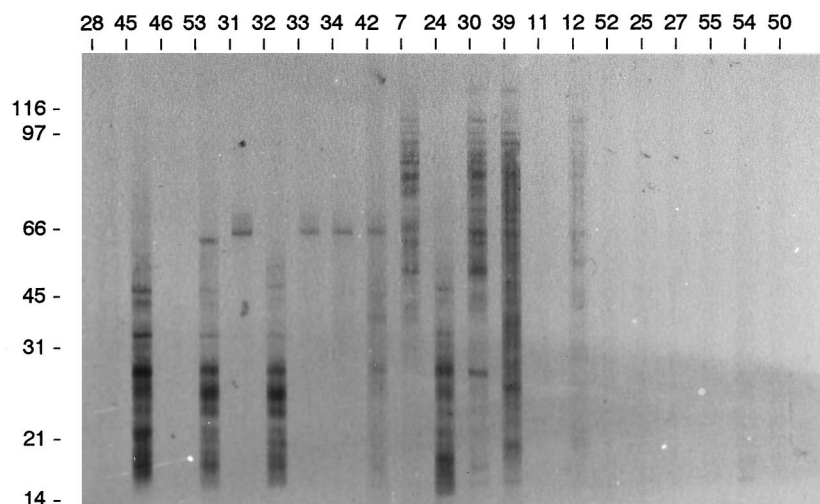
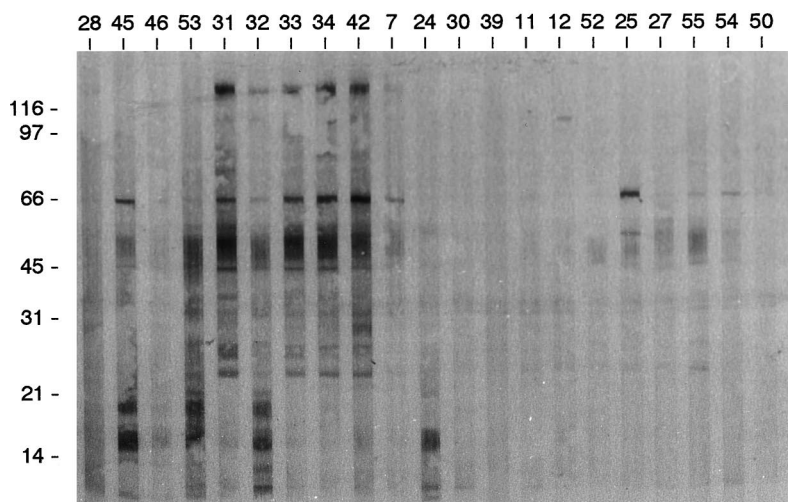


Figure 2. Immunoblotting of sera from chicken-sensitive patients against raw (A) and cooked chicken (B). Immunoblotting was performed under non-reducing conditions. In both blots, columns represent 21 different patients (all patients except number 27, 28, and 46 do not tolerate chicken). Patient's sera 7, 24, 30, 39, 11, and 12 reacted by grid blot stronger to cooked than to raw chicken.

Cross-reactivity among members of the same food group has been widely investigated and discussed. In studies performed with fish-allergic patients, positive DBPCFCs to fish of different orders have been demonstrated in

some subjects. Other individuals, however, were able to consume fish from taxonomically distinct orders without symptoms. In fact, it appears that cross-reactivity among fish exists and is of clinical relevance,^{36,37} although

the degree of cross-reactivity between different fish species varies widely among individuals. Additionally, profilins (involved in birch-pollen-fruit sensitivity) represent important cross-sensitizing allergens with other foods.³⁸ Clinically relevant cross-reactivity has been observed to a lesser degree within vegetable food groups. There was a lack of correlation between the presence of specific IgE demonstrated by SPT and a positive DBPCFC to different legumes³⁹ and grains,⁴⁰ suggesting that, in contrast to *in vitro* reactivities, *in vivo* allergic reactions to foods may be very specific; thus, provocative challenges only elicited symptoms to one or two members within vegetable food groups in less than 5% of the subjects tested. In those subjects, *in vitro* cross-reactivity among members of the same family of vegetables was not considered to be clinically relevant.

Clinically relevant cross-reactivity among meats has been demonstrated previously between beef and lamb in children by skin prick test and challenge.⁴ The same group showed *in vitro* IgE-reactivity to different mammalian serum albumins (horse, pig, rabbit, and sheep) in beef-allergic children who had been only introduced to beef.¹⁴ This points to a real cross-reactivity among meat proteins and not to a concomitant sensitization with different meats. Chicken or turkey serum albumin were seldom recognized by these patients' sera.¹⁴ Other studies have found positive SPT to beef, lamb, and pork but not to chicken in meat-allergic patients.⁴¹ Limited cross-reactivity of mammalian meats with chicken suggests the presence of mammalian-specific meat allergens.

These findings may have dietary implications since, for some patients with multiple food sensitivities, severe elimination diets with hypoallergenic foods are recommended. In these diets, beef is usually substituted by lamb meat.⁴² Certainly, in view of our results (of the 40 and 41 sera that reacted by grid-blot to raw lamb and beef respectively, only 13 sera reacted to cooked lamb, and 21 to cooked beef), lamb

may lose IgE-reactivity more readily than beef after cooking. Other studies have also analyzed the effect of enzymatic digestion with pepsin and food processing on the allergenicity of beef and lamb.⁴³ They showed a progressive reduction of reactivity of SPT and RAST to both bovine and ovine serum albumin after digestion with pepsin. This loss of reactivity occurs first and more intensively to ovine serum albumin. Also the negative SPT to freeze-dried and homogenized meats in most of the beef-allergic patients tested may be due to denaturation of the albumin. Nevertheless, even after heating or peptic digestion, some IgE-reactivity to lamb proteins remains that could potentially give rise to an allergic reaction following ingestion of lamb. In some beef-allergic subjects, allergenicity of lamb should therefore not be overlooked.

Our study indicates that tropomyosin is not a major allergen in vertebrate meats in contrast to crustaceans tropomyosin.¹⁵⁻¹⁸ The lack of in vitro IgE reactivity to vertebrate tropomyosins in meat-allergic subjects is in agreement with the low frequency of clinical symptoms to meats found in shrimp-allergic subjects. In spite of the high degree of homology between allergenic and nonallergenic tropomyosins,²¹ minor differences in their amino acid sequences result in complete loss of allergenicity in the vertebrate tropomyosins.

In conclusion, suspected meat-sensitive subjects show IgE-reactivity to meat proteins of different origins suggesting concomitant sensitization or cross-reactivity among the meats. In some cases, IgE reactivities are detected to meat proteins in some infants with no prior exposure to those meats, suggesting cross-reactivity of beef with lamb proteins that are introduced early in their diets. Nevertheless, the existence of cross-reactivity among different vertebrate meats has not been to date definitely demonstrated although clinically this has been suggested for some patients.⁴ Further studies including oral challenges are needed to demonstrate that cross-reactivity among the

different meats exists and also to characterize the meat allergens responsible for this cross-reactivity.

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