Applications of Molecular Biology in Food Allergy Allergens of Animal Origin

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INTRODUCTION

The mortality and morbidity resulting from hypersensitivity reactions to foods[1] demonstrate that food allergy can be a very serious problem. Avoidance, traditionally recommended as treatment for food allergies, may not always be possible since some food products contain components that are seemingly unrelated to their original source. Furthermore, there is concern about the safety and potential allergenicity of newly developed transgenic crops[2] since these genetically altered plants and their products may contain unknown allergens[34]. Despite renewned interest in food allergy, the immunochemical analysis of food allergens lags behind that of injected allergens such as insect venoms and inhaled aeroallergens such as pollen, dust mites, and cockroaches. Thus, it is important to identify and characterize food allergens to elucidate the structural features that separate them from nonallergens and develop better diagnosis and therapeutic regimens for food allergic subjects.

GENERAL PROPERTIES OF FOOD ALLERGENS

Although allergenic foods may contain over 10,000 different proteins, only a few (generally 10-20) elicit allergic reactions. The structural properties that are responsible for the allergenicity of a food protein are generally still poorly defined, although some broad characteristics of food allergens have been identified. These include abundance of a given protein in a particular food; physicochemical properties, such as molecular weight (10-70 kD), acidic isoelectric point, and glycosylation; and resistance to heat and digestions[5]. Although these characteristics have been associated with the allergenicity of proteins, some if not all of these properties characterize a vast number of nonallergenic proteins as well and thus are not unique to food allergens.

Food allergens frequently account for a major fraction of the total protein content within a given food. For example, the major shrimp allergen, Pen a 1, accounts for about 25%-30% of the total shrimp tail muscle protein". An exception to this rule is the major allergen of codfish Gadus callarias, Gad c I; this molecule, identified as parvalbumin, is not a dominant protein in cod muscle'. There are three aspects of molecular size that may contribute to a protein allergenicity. First, the molecule must be large enough to elicit an immune response; second, it must be a sufficient size for at least two IgE binding sites to bridge mast cell-bound IgE; third, the protein must be small enough to cross the gut mucosal membrane barrier. Most known food allergens have molecular weights between 10 and 70 kD. thus fulfilling these requirements.

Most allergens are glycoproteins with an acidic isoelectric point (pI). However, these characteristics are not unique to allergens and many nonallergenic proteins also exhibit them. Heat resistance is probably the most common feature of potent food allergens. Although heat denaturation may cause loss of the native protein's conformation, patients' IgE antibodies can still react with these denatured food proteins, suggests that the allergens epitopes are not dependent on the native conformation.

The ability of food allergen to cross the mucosal membrane of the intestinal tract is most likely an important feature. As mentioned earlier, size is one parameter in this context; another may be a resistance to digestion. The results of one study which used a gastric model of mammalian digestion to study the digestibility of food allergens point in this direction". In the study, the digestibility of allergens from egg, milk, peanut, soybean, and mustard was evaluated. Food allergens tested resisted digestion for up to 1 hour, whereas nonallergens were digested within 1 minute. However, there is still insufficient information to conclude that the resistance to digestion is a major property that characterizes food allergens since labile proteins can be allergenic and not all stable proteins are allergens.

EXAMPLES OF COMMON FOOD ALLERGEN OF ANIMAL ORIGIN

Of the eight major common allergenic foods, 4 (mills, egg, fish, and crustacea) are derived firm animal sources. An additional eight foods (abalone, beef; chicken, cuttlefish, oysters, pork, squid, and turkey) members of three major food groups (mollusks, fowl, mammalian meat) have been mentioned as less common allergenic foods'. Major allergens that have been sequenced are summarized in Table 1. The common allergenic foods of animal origin and their identified allergens are described as follows in more detail.

Cow's Milk Allergens

Cow's milk, a very complex mixture of proteins, is one of the most common food allergens. Two major groups

Table I. Identified Major Allergens of Animal Origin [5, 9-11]

Food	Allergen	Source	Molecular weight	Sequence	Comments
MILK	Bos d 8	Bos Tauris (Cow's Milk)	20-30 kDa	C	Caesins family of chemically related proteins
	Bos d 5	Bos tauris (Cow's milk)	18 kDa	C	Beta lactoglobulin Most abdundant whey protein
EGG	Gal d 1	Gallus domesticus (chicken)	22.6 kDa	C	Ovomucoid
	Gal d 2	Gallus domesticus (chicken)	42.8 kDa	C	Ovalbumin
	Gal d 3	Gallus domesticus (chicken)	77.8 kDa	C	Ovotransferrin, conalbumin
	Gal d 4	Gallus domesticus (chicken)	16.2 kDa	C	Lysozyme C
FISH	Gad c 1	Gadus callarias (Baltic Cod)	12.1 kDa	C	Parvalbumin beta
	Sal s 1	Salmo salar (Atlantic salmon)		C	Parvalbumin
CRUSTACEA	Met e 1	Metapenaeus ensis (greasyback shrimp)	34.0 kDa	C	Tropomyosin
	Pen a 1	Panaeus aztecus (brown shrimp)	36.0 kDa	C	Tropomyosin
	Pen i 1	Panaeus indicus (Indian shrimp)	34.0 kDa	P	Tropomyosin
	Hom a 1	Homarus americanus (American lobster)	32.8 kDa	C	Tropomyosin
	Pan s 1	Panulirus stimpsoni (spiny lobster)	32.8 kDa	C	Tropomyosin
	Cha f 1	Charybdis feriatus (crab)	32.8 kDa	C	Tropomyosin

of cow's milk proteins. caseins and β-lactoglobulin, have been identified as major allergens. The caseins are a family of chemically related proteins. The frequency of reactivity to different casein variants has not been systematically studied. B-lactoglobulin is a whey protein that composes approximately 20% of total milk proteins. It has a molecular weight of 18 kD, and at least six genetic variants have been identified. The whey proteins α-lactalbumin and bovine serum albumin (BSA) have been identified as minor cow's milk allergens[9].

Egg Allergens

Food allergy to proteins from egg of the domestic chicken (Gallus domesticus) is one of the most frequently implicated causes of immediate food allergic reactions of children in the United States and Europe[12]. Ovomucoid has been identified as the major egg white allergen Gal d 1. It is a glycoprotein with a molecular weight of 28 kD and an isoelectric point of 4.1. Ovalbumin has been identified as the major egg white allergen Gal d 2. It is a monomeric phosphoglycoprotein with a molecular weight between 43 and 45 kD and an isoelectric point of 4.5. Ovotransferrin or conalbumin has been identified as the major allergen from egg white Gal d 3 with a molecular weight of 77 kD and an isoelectric point of 6.0. Lysozyme (Gal d 4) from egg has a molecular weight of 14.3 kD and an isoelectric point of 10.7. In addition, a variety of other egg proteins have been described as minor allergens. These include Ovomucin, Ovoinhibitor, Ovaflavoprotein (riboflavin-binding protein), Apovitellenin I, and Apovitellenin VI.

Fish Allergens

The consumption of fish[13] is a frequent cause of IgE-mediated reactions. Fish is one among the most commonly implicated allergenic foods and has been incriminated in fatal anaphylactic reactions. Species-specific analysis of IgE reactivities has not been performed and most studies refer only to cod or generally to fish. One of the first and most comprehensive analysis of a food allergen was the purification and characterization of the major codfish allergen, Gad c 1. Gad c 1, originally designated allergen M, from Baltic cod, Gadus callarias, has been documented to be the major codfish allergen. It belongs to a group of muscle proteins called parvalbumins' and constitutes approximately 0.05% to 0.1% of the white cod muscle tissue. Minor cod fish allergens distinct from Gad c 1 were identified by CRIE but have not yet been further characterized.

Crustacea Allergens

The class Crustacea belongs to the phylum Arthropoda and includes shrimp, prawns, crabs, lobster, and crawfish. Crustacea are common causes of hypersensitivity. Like that of fish allergy, a higher incidence of crustacea allergy would be expected in geographic areas where greater amounts of shellfish are consumed on a regular basis. A 36 kD allergen, designated Pen a 1, was isolated from boiled brown shrimp, Penaeus aztecus. Sequencing of a 21 amino acid Lys-C peptide of Pen a 1 demonstrated significant homology (60%-85%) with tropomyosin from various species consistent with the conclusion that Pen a 1 is tropomyosin[6]. Pen a 1 constitutes 20% of the soluble protein and accounts for approximately 80% of pooled shrimp-allergic sera reactivity to shrimp meat extract. More than 80% of allergic subjects reacted to this allergen'. Pen a 1 was cloned and sequenced: its cDNA sequence showed 26 base pair substitutions when compared with the sequence of Met a 1: these base pair substitutions resulted in only one amino acid substitution in position 69. Homologous proteins Pen i 1, and Met e 1 from Indian shrimp Penaeus indicus and greasyback shrimp Metapenaeus ensis respectively have also been studied[14,15].

EPITOPES IN ALLERGENS OF ANIMAL ORIGIN: STUDY OF SHRIMP TROPOMYOSIN

Tropomyosin is a major muscle protein present in all living creatures. These molecules appears to be highly conserved by the substantial amino acid sequence identity of eropomyosins from unrelated species. For example, shrimp tropomyosin amino acid sequence shares homology of approximately 60% with non-allergenic tropomyosins of vertebrates. Tropomyosin has a rather unique structure in that it is composed of two polypeptide chains each in alpha helix formation coiled around one another in the coiled-coil formation". Although the structure of tropomyosin is well known, little information about its B cell epitopes (IgE-binding epitopes) and no information about the T cell epitopes was available until recently.

In order to identify areas of the tropomyosin molecule that contain important IgE binding regions, the following strategy was used by Ayuso et al[17]. Thirty-six overlapping peptides (15 amino acids long, offset 6 amino acids) were synthesized that spanned the entire sequence of Pen a 1, shrimp tropomyosin allergen. Testing the sera from 18 shrimp-allergic subjects, reactive to Pen a 1, these synthetic peptides were screened for IgE antibody reactivity. Based on the prevalence and intensity of IgE antibody binding, five major IgE binding regions were identified in shrimp tropomyosin (Pen a 1)[17]. Further efforts were directed at identification of the IgE binding epitopes (defined as the smallest sequence of amino acids that yields maximal IgE binding) within each region. The same system of overlapping synthetic peptides was employed using shorter peptide lengths with varying sizes to identify the minimal peptide that binds IgE. All peptides were synthesized and tested as described previously.

Using sera from 3 to 8 shrimp-allergic individuals who recognized a particular region. 8 IgE-binding epitopes were identified within the 5 IgE-binding regions of Pen a 1: epitope 1 in region 1, epitope 2 in region 2. epitopes 3a and 3b in region 3. epitope 4 in region 4 and epitopes 5a, 5b and 5c in region 5[18]. In some cases the same epitope was recognized with maximal intensity by all subjects showing IgE reactivity to a certain region. In other cases, a common sequence was identified (common core), recognized by all subjects tested to that particular region but the length of the whole epitope recognized presented personal variability. IgE binding sites varied from 8 to 15 amino acid long peptides, depending on the region and the subject studied.

MOLECULAR BASIS AND CLINICAL SIGNIFICANCE OF CROSS-REACTIVITIES JF FOOD ALLERGENS OF ANIMAL ORIGIN

Cross-reactivities are found among foods of related phylogenetic origin and between foods and seemingly unrelated nonfood allergens. Milk, eggs, crustacea and fishes are examples of phylogenetically related cross-reacting allergens existing in certain food families. Nonfood allergens that cross react with foods derived from animals are insects and dust mites[19]. The origin of food allergen cross-reactivity is still not ear. The clinical relevance of food allergen cross-reactivity depends on the food in question. For example, cross-reactivities among crustacea are thought to be clinically relevant. Single shrimp allergic subjects can react with crawfish. lobster and crab: however, although the reactivity to different fishes by RAST and skin test suggests cross-reactivity, the majority of .h-allergic subjects can eat other fish species or do not react trine food challenge[20,21], indicating that the in vitro cross-activity may be of limited clinical relevance.

The substantial cross-reactivity among Crustacea appears to be clinically important[22]; shrimp-allergic subjects can act to other crustaceans without additional sensitization. The use of this cross-reactivity is probably due to the major allergen tropomyosin, a highly conserved muscle protein. Allergenic tropomyosin (Pen a 1, Pen i 1, and Met a I) has been identified in three shrimp species: brown shrimp *(Penaeus aztecus)*[6], Indian shrimp (P. *indicus)* [14], and greasyback shrimp *(Metapenaeus ensis)*[15]. Pen a 1-like proteins were detected in crab. crawfish, and lobster using sera of shrimp allergic subjects and Pen a I -specific monoclonal antibodies[22]. The amino acid sequence similarity among these different shrimp tropomyosins is very high; for example, the amino acid sequences of Met e 1 and Pen a 1 only differ in one position.

Inspite of the substantial cross reactivity among plant derived allergens or among animal derived allergens as described above, there is little if any reports of cross reactivity between plant derived and animal derived allergens. Certainly from a phylogenetic and structural viewpoint, this makes sense.

Assessment of IgE antibody reactivity to foods and its relationship to specific food-allergic responses may be complicated by cross-reactivity that can occur among certain food families and between foods and seemingly unrelated allergens. Based on the fact that tropomyosin allergens have been identified in invertebrates such as cockroaches, dust mites, and shrimp, IgE antibody reactivity to the major shrimp allergen Pen a I was assessed in an unexposed population of Orthodox Jews who observe Kosher dietary laws that prohibit eating shellfish[24]. Sera from 9 subjects reporting to an allergy clinic located in a strictly orthodox town (Bnay Braq, Israel), who demonstrated positive skin tests to shrimp extract, were selected for study. Subjects were strictly observant with no prior exposure to seafood (regarded as non-Kosher). Six/9 reported symptoms of asthma, atopic dermatitis. rhinitis and/or sinusitis. All had positive skin prick tests to shrimp *(Penaeus setiferous),* and dust mite *(Dematophagoides farinae. Dematophagoides pteronissynus* or both); 2/7 subjects tested for cockroach (mix of *Blattella germanica* and *Periplaneta americana)* were found positive.

All sera were tested for IgE antibody reactivity to shrimp and the major shrimp allergen Pen a 1 by radioallergosorbent test (RAST) and immunoblot assay (IB). Cross reactivity of mite and/or cockroach with shrimp allergens was assessed by RAST and immunoblot inhibition assays. Three/9 subjects demonstrated positive IgE antibody responses to both shrimp (RAST 7.0 to 15.2%), and to Pen a 1 (6.3% to 24.1 %). Significant IgE reactivity to Pen a 1 and to mite extract was demonstrated in the 3 sera by IB. IgE binding to Pen a 1 was inhibited with either mite or cockroach extracts as demonstrated by both RAST (Table II) and IB inhibition analysis. These studies indicate that IgE antibody reactivity to a major food

Table II. Inhibition of Pen a 1 IgE reactivity

Inhibitor		% **Inhibition**	
Allergen	Concentration (Ag/ml)	Orthodox Jews	Shrimp Allergic Subjects
White Shrimp	10	105.7	102.8
(Panaeus setiferus)	1	90.3	92.8
	0.1	61.2	73.5
	0.01	53.1	48.6
Dust Mite	800	63.0	82.1
(Dermatophagoides farinae)	100	12.0	60.1
	10	0.0	31.6
American Cockroach	720	95.7	96.8
(Periplaneta americana)	100	37.8	53.8
	10	15.6	39.3
Peanut	800	2.3	0.0

allergen, shrimp, can occur in an unexposed population of individuals; subjects allergic to house dust mite and/or cockroach show substantial reactivity to the major shrimp allergen Pen a 1 (tropomyosin). Based on inhibition with cockroach and/or dust mite extracts, this reactivity appears to be due to exposure to cross-reacting tropomyosins in indoor aeroallergens. This observation suggests that individuals may become inadvertently sensitized to certain foods without prior exposure. Furthermore, it may explain the fact that many skin test positive individuals do not necessarily develop clinically significant allergic reactivity to foods.

SUMMARY AND CONCLUSIONS

Although the mortality and morbidity resulting from food allergy can be quite serious, avoidance has been traditionally the only recommended treatment. Indeed, our understanding of the immunopathogenesis of events leading to induction of a food allergic reaction, and the molecular structure of those molecules, inducing such reactions has only recently begun to be understood. Of the 8 major food types or groups that cause food induced allergic reactions, 4 are of animal origin. In addition, there are some reports of other groups of animals, namely mollusks, meat of mammals, and avian meat that also may cause allergic reactions. In spite of the lack of interest in the past concerning food allergens, recently there has been renewed efforts in better understanding the structure of these molecules in relationship to developing improved diagnostic and therapeutic regimens. The advent of molecular biology and its application to studies of food allergens has helped advance our knowledge. The major codfish allergen, Gad c 1, was the first allergen to be thoroughly investigated in the premolecular biology era[7]. The allergen structure was accurately determined, and the epitopes to which IgE antibodies bind identified. This project occurred over a period of approximately 5 years in contrast to current allergen identification and characterization studies that now take months rather than years to complete.

As an example of characterization of a major food allergen from an animal source, we reviewed our investigations of the major shrimp allergen, shrimp tropomyosin. The identification of eight IgE binding epitopes in this molecule and the fact that tropomyosin may be an important inhalant allergen in house dust mite and cockroach may explain our finding that an unexposed population of orthodox Jews has reactivity to shrimp tropomyosin. Most likely this is due to cross reactivity between invertebrate tropomyosins since these individuals are also allergic to house dust mite, and cockroach. This observation is very important since it may explain the fact that some patients who are skin test positive to a variety of foods, do not necessarily have clinical reactivity. It also suggests that perhaps we may need to further elevate the level by which allergen reactivity is measured. Could those epitopes to which patients react determine the course of their disease? It is possible that the affinity and/or special configuration of epitopes

to which IgE reacts affects the occurrence or severity of allergic diseases? The application of molecular biology to the study of food allergens of animal origin will help us further elucidate this.

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REFERENCES

1. Yunginger JW, Sweeney KG, Sturner WQ, Giannandrea LA, Teigland, JD, Bray M, Benson PA, York JA, Biedrzycki L, Squillace DL, Helm RM. Fatal food induced anaphylaxis. JAMA 1988; 260: 14501452.

2. Harlander SK. Biotechnology: a means for improving our food supply. Food Technol 1991; 45:841, 86, 91-92, 95.

3. Kessler OA, Taylor MR, Maryanski JH, Flamm EL, Kahl LS. The safety of foods developed by biotechnology. Science 1992; 256: 1747-1832.

4. Lehrer S, Reese G. Food Allergens: Implications for Biotechnology. Biotechnology and Safety Assessment, 2nd Edition. J. Thomas, ed., pp. 127-150 Taylor and Francis 1998.

5. Reese G. Lehrer SB, Food Allergens. In: Food Hypersensitivity and Adverse Reactions. Frieri/Kettelhut, ed., pp 69-97, Marcel Dekker, Inc., New York, 1999.

6. Daul CB, Slattery M, Reese G, Lehrer SB. Identification of the major brown shrimp (Penaeus aztecus) allergen as the muscle protein tropomyosin. Int Arch Allergy Immunol 1994; 105: 4955.

7. Elsayed S, Bennich H. The primary structure of allergen M from cod. Scand J Immunol 1975; 4: 203-208.

8. Fuchs RL, Astwood JD. Allergenicity assessment of foods derived from genetically modified foods. Food technol 1996; 50: 83-88.

9. Robert K. Bush and Susan L. Hefle. Food Allergens. Critical Reviews in Food Science and Nutrition 1996; 36(s): S119-S163.

10. Internet Symposium on Food Allergens, Mattias Besler, Editor, 1999-2000.

11. Seafood Allergy and Allergens: A Review. Lehrer SB, Ayaso, R, Reese, G. J. Submitted for publication.

12. Crespo JF, Pascual C, Ferrer A, Burks AW, Diaz Pena JM, Esteban MM. Egg white-specific IgE level as a tolerance marker in the IgE level as a tolerance marker in the follow-up of egg allergy. Allergy Proc 1994; 15: 73-76.

13. Elsayed S, Aas K, Slette K, Johansson SGO. Tryptic cleavage of a homogenous cod fish allergen and isolation of two active polypeptide fragments. Immunochemistry 1972; 9: 647-661.

14. Shatin KN, Martin BM, Nagpal S, Metcalfe DD, Sabba-Rao PV. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE binding epitopes. J Immunol 1993; 151: 5354-5363.

15. Leung PSC, Chu KH, Chow WK, Aftab A, Bandea CI, Kwan HS, Nagy SM, Gershwin ME. Cloning, expression and primary structure of Metapenaeus ensis tropomyosin, the major heat-stable shrimp allergen. J. Allergy Clin Immunol 1994; 92: 837-845.

16. Smillie LB. Structure and functions of tropomyosins from muscle and non-muscle sources. Trends Biochem Sci 1979; 4: 151-155.

17. Ayuso R, Reese G, Lehrer SB. Identification of continuous, allergenic regions of the major shrimp allergen Pen a 1 (tropomyosin). Int Arch Allergy Immunol 2001 (Submitted).

18. Reese G, Ayuso R, Leong-Kee, Plante M, S, Lehrer S. Protein structure and allergenicity: epitope analysis of shrimp tropomyosin (Pen a 1). 2001 (Submitted).

19. Lehrer SB and Reese G. Cross-reactivity between cockroach allergens and arthropod, nematode and mammalian allergens. Revue Francaise D'Allergologie 1998; 38: 846-850.

20. Aas K. Studies of hypersensitivity to fish: a clinical study. Int Arch Allergy Clin Immunol 1966; 29: 346-363.

21. Bernhisel-Broadbent J, Scanlon SM, Sampson HA. Fish hypersensitivity. I. In vitro and oral challenge results in fish-allergic patients. J Allergy Clin Immunol 1992; 89: 730-737.

22. Daul CB, Morgan JE, Waring NP, McCants ML, Hughes J, Lehrer SB. Immunological evaluation of shrimp-allergic individuals. J. Allergy Clin Immunol 1987; 80: 716-722.

23. Daul CB, Slattery M, Morgan JE, Lehrer SB. Common Crustacea allergens: identification of B cell epitopes with the shrimp specific monoclonal antibodies. In: Kraft D, Sehon A, eds. Molecular Biology and Immunology of Allergens. Pp 291-294.

24. Fernandes J, Reshef A, Ayuso R, Patton L, Reese G, and Lehrer S. IgE antibody reactivity to the major shrimp allergen in unexposed Orthodox Jews. J Allergy and Clinical Immunology (abstract) in press.