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Problems Of Food Allergen Extracts

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Introduction

A variety of different foods, including a number of fruits and vegetables, poultry products, meats, seafood, dairy products, grains, and nuts from a number of sources cause allergic reactions. Over 150 food extracts are commercially available for the diagnosis of food allergy (table I). Although the use of many of these reagents is based on strong clinical evidence that they cause food allergy, many food extracts that are being used to diagnose food allergy are neither well characterized with regard to allergen content nor well documented as causes of food allergy (1,2). In some cases it may be better to use the offending food rather than a questionable extract for diagnosis.

In view of the generally limited biomedical resources available for allergen characterization and standardization, the necessity for characterization and standardization of food allergens may not be deemed necessary. Indeed, some may even question the necessity of studying food allergy since food allergens are considered more easily avoided than other allergens. However, this belief is sadly mistaken. Avoidance of an offending food is clearly not always possible. In the United States, food-allergic patients die from systemic anaphylaxis due to the inadvertent exposure to allergenic food every year, even though many are well educated about their food allergies and allergen avoidance measures through organizations such as the Food Allergy Network in the United States. Since complete avoidance of offending foods is not possible, there is clearly a need to improve food allergy diagnosis and develop therapeutic reagents, based on better characterized and standardized food allergens. Increased knowledge of food allergens will aid in the elimination of any inactive or irrelevant extracts and reduce variability or instability of source materials. Characterized and standardized extracts will lead to improved, more reliable reagents with greater relevance available for use by allergists.

More recently, biotechnology has been used to modify foods; genetically modified foods expressing a variety of improved traits are now available in the marketplace (3). A major concern with these new products is the exposure of consumers to either upregulated levels of endogenous food proteins known to be allergens or the introduction of new proteins that could be allergenic. Thus, genetically modified foods must be tested for allergenicity in comparison to their wild-type parental strain. Food allergens can only be measured after such allergens are identified and standardized; however, if proper reagents are not

available, then any changes in allergen levels of genetically modified foods cannot be determined.

Finally, the issue of the modified food proteins with reduced allergenicity being developed for therapeutic use must be considered. The theory of this approach is that foods or food extracts with reduced allergenic activity (IgE binding capacity) and with preserved T-cell epitopes can be used to induce tolerance with a reduced risk of systemic anaphylaxis (4). Indeed, for certain foods, the production of hypoallergenic extracts through suppression of allergenic proteins has been proposed and tested. In one study, the synthesis of the major rice allergen has been suppressed (5); another investigation produced an altered peanut protein with reduced IgE binding activity (6). However, any new hypoallergenic foods pose unique problems for allergen standardization.

Food Allergen Characterization: Past Difficulties And Current Status

When assessing the progress of allergen characterization and standardization over the last 10 to 20 years, it is quite clear that our knowledge of food allergens and subsequently food allergen characterization and standardization has significantly lagged behind that of other allergens such as the dust mites, pollens, insect venoms and some drugs for several reasons. The variety of treatments that foods undergo, such as industrial processing, cooking and digestion makes identification of the final food component that causes such an allergic reaction more difficult, thus complicating food allergen characterization. Identification of clinically relevant allergens can be further impeded by the fact that most foods eaten are not purified materials and thus may contain a number of additional components that may cause allergic reactions. Alternatively, some foods may be so prevalent throughout the food supply such as corn or wheat that they are difficult to identify as a cause of an allergic reaction (1, 2).

Cross-reactivity of foods with inhalants, an issue that has recently been recognized, further complicates studies of food allergen identification and characterization. Cross-reactivity can occur within a given food group such as legumes and crustacea and also between foods and seemingly unrelated substances such as some pollens with fruits and vegetables (1, 2). The fact that allergen cross-reactivity occurs within a given food group is logical and suggests clinical relevance; for example shrimp-allergic individuals may have a higher propensity to react to crawfish or lobster. However, it has also been shown for other food groups such as legumes, that although significant *in vitro* cross-reactivity can be demonstrated, clinically relevant cross-sensitivity does not occur in most cases. This cross-reactivity tends to complicate the study and identification of clinically relevant food allergens. Therefore, it can be more difficult to establish clinical relevance for some food allergens as compared to other allergens in which cause and effect relationships are more obvious.

The type and treatment of source material may affect allergen content of a food and thus make standardization of food allergens more of a challenge. Different varieties of the same type of food may exist and certain varieties can present

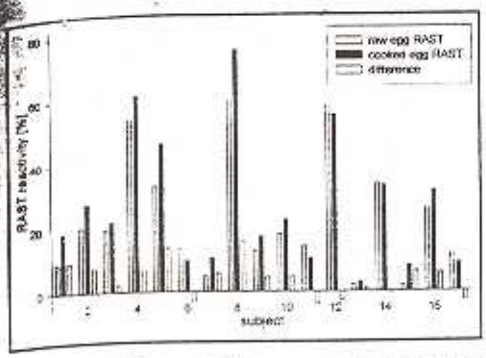

Figure 1: RAST reactivity to raw and cooked egg: IgE antibody reactivity to raw and cooked egg extracts was determined for the sera of 17 egg allergic subjects. Generally cooked egg yielded higher RAST reactivity than raw egg extract.

allergenic differences. Should the food be fresh or cooked? This can be an important issue since there is some evidence that cooking may affect certain food allergens (figure 1). The stability of food allergens is always an important issue. Some food allergens such as those present in apples are highly unstable and rapidly lose reactivity. Other food allergens appear to be very stable, such as those in peanuts, and can resist the high temperatures of the roasting process. The effects of digestion on allergen content have never been adequately resolved and require further evaluation. Finally, it is suggested that purification procedures such as dialysis can alter food allergen content.

With the exception of Gad c 1 at the beginning of the 1980s, very few food allergens were well characterized. During the last 20 years a number of food allergens have been identified and characterized in chicken, shrimp, mustard, barley, peanut, apple, and celery (tab. II). The major allergens of shrimp and peanut have been particularly well studied. Several food allergens have been cloned and expressed and the allergenic activity of the recombinant proteins demonstrated. Furthermore, epitopes studies have been performed and for the first time it is possible to study the interaction of IgE antibodies with allergens at a molecular level. Cross-reactivity may be studied by comparing the epitopes of allergens and cross-reacting homologous proteins *in vitro* and/or by protein modeling studies. Indeed, this has led to 3-dimensional molecular models for food allergens such as Ara h 1 and Ara h 2 and the shrimp allergen Pen a 1 (6, 7, 8).

Future Of Food Allergen Standardization

Although there is clearly a need to improve food allergen extracts, the future for standardization of food allergens is bright. Our current knowledge of allergen content and allergen structure for some foods is beyond that imagined, even several years ago. The methodology is clearly available for precise standardization of food allergens with monoclonal antibodies, IgE antibodies from allergic patients' sera, recombinant proteins, and epitope identification. In the near future, this higher level of food allergen characterization will provide better reagents for diagnosis and even for treatment of food-allergic patients (4).

Studies of cross-reactivity of foods and seemingly unrelated substances should provide precise reagents for specific identification of food-allergic individuals. Identification of clinically relevant protein molecules will yield more potent extracts for the diagnosis and ultimately the treatment of food allergies. Once cross-reacting allergens in foods and non-foods are identified, specific reactivity can be used to more precisely identify food-allergic reactivity in sensitized individuals. However, as problems of source material,

clinical relevance, cross-reactivity, and the effects of processing on food allergens are resolved, by progress in standardization, new challenges arise. With the advent of genetically modified foods, new allergens must be characterized and standardized. Will minor allergens become major allergens when their concentration is upregulated? How do we quantitate altered hypoallergens in the absence of IgE antibody reactivity? All of these issues can and will be resolved. As we approach the next century, more foods will be characterized and their allergens identified. There will be fewer but better reagents available with which food-allergic subjects can be more precisely diagnosed and better treated.

Table 1: Food Extracts Used in Allergy Diagnosis

Allspice
Almond
Apple, Red Delicious
Apricot Food
Arrowroot
Artichoke
Asparagus
Avocado
Banana
Barley, Whole (Grain)
Bay Leaf
Bean Kidney
Bean, Lima
Bean, Navy
Bean, Pinto-Frijole
Bean, String Mix
Beef
Beet
Black-eyed Pea
Blueberry
Brazil Nut
Broccoli
Buckwheat
Cabbage
Cantaloupe/Muskmelon
Carrot
Cashew Nut
Cauliflower
Celery
Cheese, Cheddar Garlic (American)
Cheese, Parmesan
Cheese, Swiss
Cherry, Bing
Chicken
Chicory
Chili Pepper
Chocolate/Cocoa Mix
Cinnamon
Clam
Cloves
Coconut
Codfish Mix
Coffee Mix
Cola
Corn, Whole (Grain)
Crab Mix
Cranberry
Cucumber
Curry Powder
Date
Dill Mix
Egg, White
Egg, whole
Egg, Yolk
Eggplant
Endive
Garlic
Gelatine
Ginger
Grape, Seedless
Grapefruit
Haddock
Halibut
Hazlenut
Herring
Horseradish
Lamb
Lemon
Lentil
Lettuce, Iceberg
Lime
Liver Beef (Calve)
Lobster
Mackerel
Malt
Mangoes
Maple, Syrup/Sugar Mix
Melon (see Cantaloupe)
Milk, Cow's (Casein)
Milk, Cow's (Whole)
Milk, Goat's
Mint Mix (Peppermint/Spearmint)
Mushroom
Mustard (Seed)
Nutmeg
Oat, Whole (Grain)
Okra
Olive, Black/Green
Onion, Yellow
Orange, Sweet
Oregano
Oyster Mix
Papaya
Paprika
Parsley
Parsnip
Pea, Green/English
Peach
Peanut Mix
Pear
Pecan
Pepper, Black/White Mix
Pepper, Green Bell
Perch, Lake
Pineapple
Plum
Poppy Seed
Pork
Potato, Sweet/Yam Mix
Potato, White
Pumpkin
Rabbit Meat
Radish
Raspberry
Rhubarb
Rice, Whole (Grain)
Rice, Wile
Rye, Whole (Grain)
Safflower Seed
Sage
Salmon
Scallops
Sesame Seed
Shrimp
Snapper
Sole
Soybean, Whole (Grain)
Spinach
Squash, Zucchini
Strawberry
Sugar (Beet)
Sugar (Cane)
Sunflower
Tangerine
Tapioca
Tea
Thyme
Tomato
Trout
Tuna (Thunnis spp)
Turkey
Turnip
Vanilla
Veal
Walnut, Black
Walnut, English
Watermelon
Wheat, Whole (Grain) Mix
Whitefish
Yeast Mix
Yeast, Bakers

* Bayer, Center Greer, Miles

Table II
Food Allergens Characterized

Allergen source	Allergens (systematic and original names)	Mol wt (kDa)	Sequence data[a]
Arachis hypogea (peanut)	Ara h 1	63.5	C
Bertholletia excelsa (Brazil nut)	Ber e 1; 2S albumin	12	C
Brassica juncea (oriental mustard)	Bra j 1; 2S albumin	14	C
Gadus callarias (cod)	Gad c 1; allergen M	12	C
Gallus domesticus (chicken-eggs)	Gal d 1; ovomucoid	28	C
	Gal d 2; ovalbumin	44	C
	Gal d 3; ovotransferrin	78	C
	Gal d 4; lysozyme	14	C
Glycine max (soybean)	Gly m 1	34	P
Penaeus aztecus (brown shrimp)	Pen a 1; tropomyosin	36	P
Penaeus indicus (indian shrimp)	Pen i 1; tropomyosin	34	P
Metapenaeus enis (greasyback shrimp)	Met e 1; tropomyosin	34	C
Sinapis alba (yellow mustard)	Sin a 1; 2S albumin	14	C

[a] Amino acid sequence obtained directly or deduced from cDNA sequence.

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Discussion

Chapman: I think the food situation is interesting partly because it is one of the situations where biological standardization has great limitations. And partly, because most of the highest prevalences of food allergies are in children. And in many of those children it is really not ethical to do the kind of biological skin-testing that is done for most of the allergen extracts. So to me, this represents an opportunity in terms of formulating a standardization based on the allergen content of the materials. The advantages of that in relation to foods is that we know some foods, e.g. peanut and shrimp, where the major allergens that have been identified are also the major source of the protein in those foods. So there is very good rationale for actually using either the recombinant materials or using assays for the purified food proteins to actually put together and standardize the extract. You did not really address the issue of biological standardization or how you think standardization should go ahead with foods, but I have been interested to hear what you think of that.

Lehrer: Well, I think it certainly is a real challenge – if I can use that word – to standardize foods. Particularly when you are talking about foods that affect younger children in terms of skin testing and obtaining sufficient samples of IgE-containing sera. It is difficult. Nevertheless, I think it can be done and it is being done. And I think we need to use assays that are relevant to these allergens. I mean, look at the work that Wesley Burks has done, and he is primarily using sera from children, although there are a number of adults that have profound peanut allergies.

Chapman: What about skin-testing?

Lehrer: In some cases they are doing skin-testing. But he looks at IgE-antibody responses and I think it is depending on the age of the child and if you can get a small amount of serum then you can just do *in vitro* assays. And if we have to go that route, we will. But I think either way, it will go forward in terms of identifying the allergens and even the epitopes.

Kordash: I have two questions. The first one relates to the non-standardized extracts for the major foods that you listed on your first slides. How bad do you actually think those are? It is my impression as a clinician that in general the patient is allergic to nut or shrimp or one of the major ones. The non-standardized extracts in most cases appear to be adequate. The second question is, what do you think is the possibility that allergists, at least in the U.S., would be willing to pay to cover the expenses of manufacturer to produce these standardized extracts.

Lehrer: An answer to your first question. I think that some of the major food allergens are so potent that I think in my experience the commercial extracts are alright. But alright may be that they are losing 75% of their activity. Peanut allergen is an extremely potent substance and so is shrimp. So even though they still have activity, I think that they can