

Allergens from fish and egg

Allergens from fish and egg belong to some of the most frequent causes of food allergic reactions reported in the literature. Egg allergens have been described in both white and yolk, and the egg white proteins ovomucoid, ovalbumin, ovotransferrin and lysozyme have been adopted in the allergen nomenclature as Gal d 1–d 4. The most reported allergen from egg yolk seems to be alpha-livitin. In fish, the dominating allergen is the homologues of Gad c 1 from cod, formerly described as protein M. A close cross-reactivity exists within different species of fish between this calcium-binding protein family, denominated the parvalbumins. This cross-reactivity has been indicated to be of clinical relevance for several species, since patients with a positive double-blind, placebo-controlled food challenge to cod will also react with other fish species, such as herring, plaice and mackerel. In spite of the importance of these two allergen systems, only a few studies have been performed, and the scarcity of cloned allergens from both of the systems is emphasized.

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Fish and egg together with milk and crustaceans represent the animal kingdom in the “big eight” group of food allergens, to which the majority of food allergic patients are believed to react. Fish allergy has played a prominent role in the history of allergy, it being a fish allergen with which Prausnitz & Küstner for the first time demonstrated the transferable serum factor (1) that was many years later to be identified as IgE. The major allergen from Cod, gad c 1, previously known as Cod M, was also the first allergen for which the protein sequence was described (2). Despite the importance of these allergenic materials, recently much more attention has been directed to the study of food allergens of plant origin. In the present review we shall address some basic findings related to the allergen systems of fish and egg.

Allergenic sources

Hen's egg comprises about 8–11% shell, 56–61% white and 27–32% yolk (3). While the white may be considered essentially an aqueous protein solution (10% protein and 88% water), the yolk is composed of 50% water, 34% lipid and 16% protein, giving it quite different properties. Egg white has been considered the most important source of allergens, but IgE-binding allergens have also been described in the yolk, suggesting that both common and distinct allergenic molecules are present. Thus in a study of 11 patients

with a history of egg allergy (8 DBPCFC+/3 DBPCFC–) all sera reacted positively to both white and yolk. Eight patients reacted equally or more strongly to white, and even though white and yolk could to some degree inhibit the IgE binding of the other, yolk could only be partly inhibited by white in eight sera (4).

Fish species are abundant and exist in multiple edible forms that are consumed in most countries of the world. Table 1 lists some of the species for which commercial diagnostic kits are available. Allergenic activity resides in the meat (muscle), but recently concern has been raised as to whether products such as fish gelatin, made from skin and bones, also contains allergenic activity in the form of collagens. This may present a problem since the fish gelatin is used in vaccines (5). An increasingly reported food allergic reaction is caused by the parasite *Anisakis simplex*, which may be found in fish. Since it is not a fish allergen, it will not be discussed further, but it is important to bear it in mind when allergic reactions are experienced after ingestion of fish.

Both fish and especially egg may be concealed in various forms of foods and a food allergic patient may not always know when he or she has actually been exposed to the allergens (6).

Major allergens

The most frequently reported allergens in egg are

Table 1. Egg allergens. Diagnostic preparations and defined allergens (20, 21). Allergenic molecules with a name according to the IUIS nomenclature are shown in italics

Code	Name	Organism	Allergen	MW
F1	Egg white	<i>Gallus domesticus</i>		
F233	Ovomucoid	<i>Gallus domesticus</i>	<i>Gal d 1</i>	28 kDa
F232	Ovalbumin	<i>Gallus domesticus</i>	<i>Gal d 2</i>	44 kDa
F83-III	Conalbumin (Ag22), ovotransferrin	<i>Gallus domesticus</i>	<i>Gal d 3</i>	78 kDa
F84-IV	Lysozyme	<i>Gallus domesticus</i>	<i>Gal d 4</i>	14 kDa
F75	Egg yolk (freeze-dried)	<i>Gallus domesticus</i>		
F245	Whole egg	<i>Gallus domesticus</i>		

shown in Table 1. Even though ovalbumin is probably one of the most studied antigens in immunology (a contemporary search in Medline produced about 12000 hits) it does not appear to be the most allergenic molecule in humans. Conalbumin (=ovotransferrin) and ovomucoid turned out to be the most frequent allergens when a population of 34 adults with a positive case history and/or a positive RAST to egg were tested with a battery of methods, such as western blot (reduced and unreduced), crossed radio-immuno-electrophoresis (CRIE) and dot-blot using the individual proteins. Using as the criterion the agreement between two or more of the four methods, the frequencies of reactivity were found to be 53% (ovotransferrin),

38% (ovomucoid), 32% (ovalbumin) and 15% (lysozyme) (7). Different reports have emerged on the relative importance of the various allergens in egg white. Some of the differences may be due to the studies of different populations, i.e. it is likely that the egg proteins are processed differently in the digestive system of infants and adults. It should also be mentioned, however, that a rigorous purification of the reagents may be necessary to obtain pure proteins, since commercial preparations of individual egg white proteins may be somewhat contaminated (8).

As previously mentioned, egg yolk may also act as an allergen, presumably due to α -livetins, which may be the most relevant allergen in the sensitization to egg proteins via the inhalation route (9, 10).

The dominant major allergen in codfish is gad c 1, which seems to be extremely stable to thermal and proteolytic processes (11) – a factor undoubtedly contributing to the legendary results of Prausnitz & K stner (1). Although other molecular weight species have been described as allergens in cod and other fish species, it seems that there is a quite strong correspondence between IgE reactivity to gad c 1 and clinical reactivity to cod (12) and probably other species of fish (13, 14).

Table 2. Fish allergens. Diagnostic preparations and defined allergens (20, 21). The allergenic molecule (gad c 1) with a name according to the IUIS nomenclature is shown in italics. Some species may have more than one allergen code since various commercial preparations exist

Code	Name	Organism	Allergen	MW
F3	Cod fish (muscle)	<i>Gadus morhua</i>		
F3-I		<i>Gadus callarias</i>	<i>Gad c 1 (allergen M)</i>	12
F21	Herring	<i>Clupea harengus</i>		
F205	Herring	<i>Clupea harengus</i>		
F22	Trout			
F204	Trout	<i>Oncorhynchus mykiss (Salmo gairdneri)</i>		
F40	Tuna, Yellow Fin (fish muscle)	<i>Thunnus albacares</i>		
F41	Salmon (Atlantic) (Muscle)	<i>Salmo salar</i>		
F42	Haddock			
F50	Chub mackerel (Fish muscle)	<i>Scomber japonicus</i>		
F206	Mackerel	<i>Scomber scombrus</i>		
F55	Eel (Muscle)	<i>Anguilla anguilla</i>		
F264	Eel (Muscle)	<i>Anguilla anguilla</i>		
F56	Sea perch			
F60	Jack Mackerel/scad (Muscle)			
F61	Sardine, Japanese Pilchard	<i>Sardina melanosticta</i>		
F308	Sardine, Pilchard (Muscle)	<i>Sardina pilchardus</i>		
F65	Perch			
F124	Plaice			
F254	Plaice (Muscle)	<i>Pleuronectes platessa</i>		
F129	Sole	<i>Solea solea</i>		
F142	Flounder			
F239	Cuttlefish			
F303	Halibut (Muscle)	<i>Hippoglossus hippoglossus</i>		
F307	Hake (Muscle)	<i>Merluccius merluccius</i>		
F311	Megrim/wiff (Muscle)	<i>Lepidorhombus whiffiagonis</i>		
F312	Swordfish (Muscle)	<i>Xiphias gladius</i>		
F313	Anchovy	<i>Engraulis encrasicolus</i>		
F322	Pike			
F333	Carp			

		Relative	
		Homology	
. Eukaryota	[eukaryotes]		
. . Fungi/Metazoa group	[eukaryotes]		
. . . Gnathostomata	[vertebrates]		
. . . . Euteleostomi	[vertebrates]		
. Euteleostei	[bony fishes]		
. Paracanthopterygii	[bony fishes]		
. Gadoidei	[bony fishes]		
. Gadidae	[bony fishes]		
. Gadus callarias (Baltic cod)	-----	227	[bony fishes]
. Merlangius merlangus (whiting)		131	[bony fishes]
. Merluccius bilinearis	-----	140	[bony fishes]
. Merluccius merluccius (European hake)	.	135	[bony fishes]
. Opsanus tau (oyster toadfish)	-----	133	[bony fishes]
. Electrophorus sp.	-----	141	[bony fishes]
. Cyprinus carpio (common carp)		141	[bony fishes]
. Danio rerio (zebrafish)		133	[bony fishes]
. Leuciscus cephalus (chub)		130	[bony fishes]
. Salmo salar (Atlantic salmon)		127	[bony fishes]
. Esox lucius (northern pike)		122	[bony fishes]
. Xenopus laevis (African clawed frog)	-----	127	[amphibians]
. Graptemys geographica (map turtle)		123	[turtles]
. Gallus gallus (chicken)		115	[birds]
. Rana esculenta (edible frog)		113	[amphibians]
. Boa constrictor (boa)		113	[snakes]
. Amphiuma means (two-toed amphiuma)		112	[amphibians]
. Latimeria chalumnae (coelacanth)		111	[coelacanths]
. Rattus norvegicus (Norway rat)		103	[mammals]
. Macaca fuscata fuscata (Japanese macaque)	...	103	[mammals]
. Rattus rattus (black rat)		103	[mammals]
. Homo sapiens (human)		102	[mammals]
. Mus musculus (house mouse)		102	[mammals]
. Gerbillus sp.		102	[mammals]
. Mus sp. (mice)		99	[mammals]
. Cavia porcellus (domestic guinea pig)		97	[mammals]
. Oryctolagus cuniculus (European rabbit)		97	[mammals]
. Felis catus (cat)		96	[mammals]
. Rana catesbeiana (bullfrog)		88	[amphibians]
. Triakis semifasciata	-----	91	[sharks and rays]
. Raja clavata (thornback ray)		82	[sharks and rays]
. . . Fusarium oxysporum	-----	49	[fungi] calmodulin
. . Glycine max (soybean)	-----	53	[eudicots] calmodulin
. . Arabidopsis thaliana (thale cress)		51	[eudicots] calmodulin
. . Brassica napus (rape)		51	[eudicots] calmodulin

Figure 1. Relative homology between parvalbumins (Ca^{++} binding proteins) from different species including cod fish (gad c 1).

Interspecies cross-reactivity

The substantial clinical cross-reactivity between cod and other fish species (14) is paralleled by an IgE cross-sensitization between many fish species (13–16). In a recent study by Bugajska-Schretter and coworkers, a corresponding protein to gad c1, parvalbumin from carp, has been thoroughly examined (17). The investigators found that the protein was abundantly distributed in muscle and fiber cells, and by inhibition experiments it was demonstrated that the protein could inhibit 80–90% of the total IgE binding activity to extracts of cod fish, tuna, and salmon. The parvalbumins to which the carp protein and gad c1 belongs is a family of calcium binding proteins, which play an important part in muscle physiology. As demonstrated in Fig. 1, this structure is well conserved

throughout the animal kingdom, but the homology decreases with decreasing zoological relationship. So far, no cross-reactivities have been described outside the fish species.

In terms of sensitization to egg, a single study has indicated that some patients may be allergic to eggs from duck and goose and yet be tolerant to hen's eggs (18). This lack of cross-reactivity between eggs from different birds remains to be confirmed on a larger material.

Conclusions

In spite of the well-established allergenicity of fish and egg, surprisingly few studies have been performed describing the individual allergens and, compared to

plant-derived food allergens, many allergens in various fishes still need to be described and cloned. Due to the major efforts to characterize plant-derived food allergens, new hypotheses about the relationship between

allergenicity and function have emerged (19). It will be interesting to see if the same principles can also be applied to animal-derived allergens such as fish and egg.

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