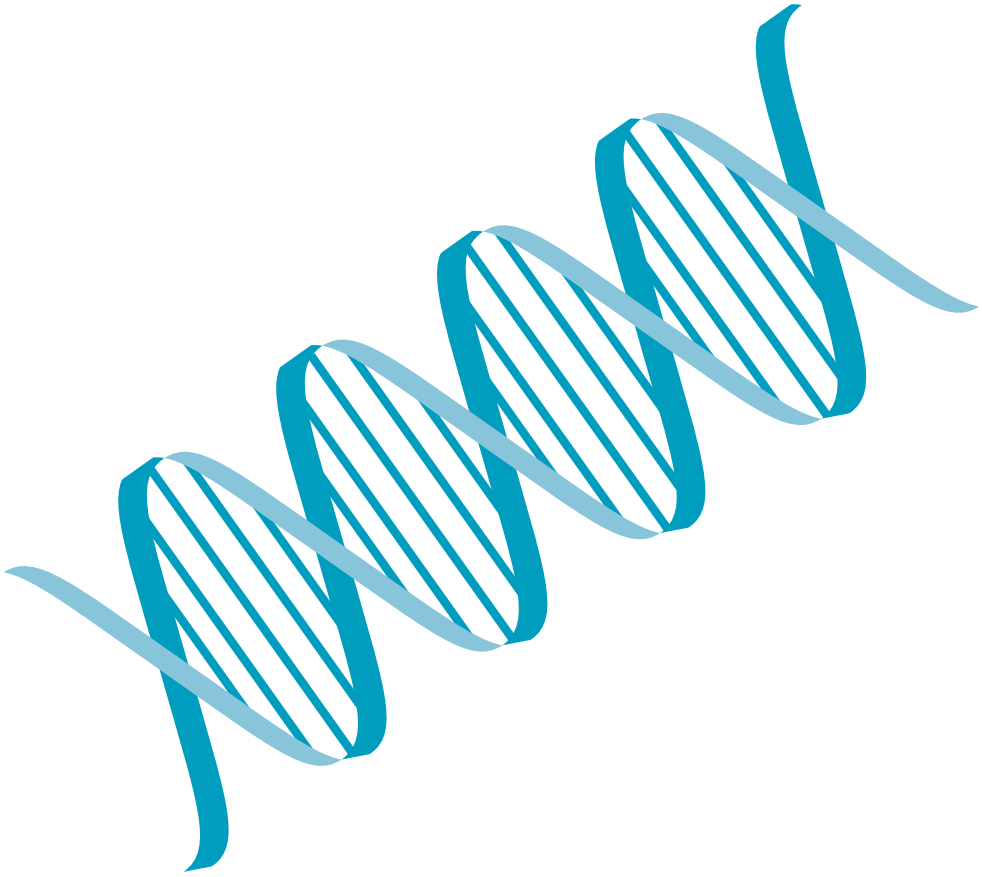
ImmunoCAP®

Native & recombinant allergen components

Allergy – Which allergens?



Thermo SCIENTIFIC

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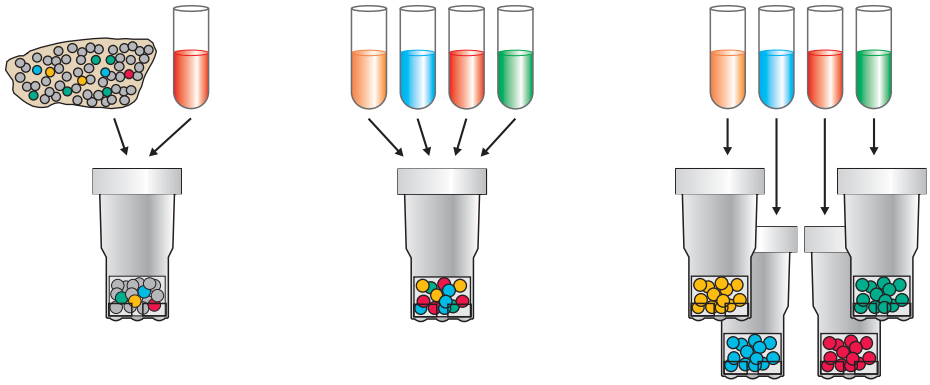
Contents

Recombinant allergen components

Recombinant allergens are biotechnology produced allergen molecules originally identified from allergen extracts. Most of the existing recombinant allergens have been expressed in *Escherichia coli* (*E. coli*) and are usually comparable with their natural templates in structural features and immunobiological properties. Other high-level expression systems have been developed to produce recombinant allergens through bacteria, yeast, and insect cells. Recombinant allergens mostly have immunoglobulin E (IgE) antibody binding comparable to that of natural allergens and generally show good reactivity in *in vitro* and *in vivo* diagnostic tests (1). To date, many different allergen components from various allergen sources have been cloned, sequenced, and expressed as recombinant proteins.



Additive to extracts

- to counteract deficiency of a particular allergen component
  - improved clinical sensitivity
  - enhanced quantitative performance

Extract replacement

- for fully defined & designed reagent composition
  - selected, "informative" molecules
  - confounding components excluded
- to ensure optimal amount of all relevant allergens

Single allergen components

- to determine individual sensitization profiles
  - helps explain cross-reactivity
  - correlation to particular clinical conditions
  - optimized patient selection for specific immunotherapy

Figure 1. Use of recombinant allergens in IgE antibody testing.

Recombinant allergens have a wide variety of uses, from the diagnosis and management of allergic patients to the development of immunotherapy to the standardisation of allergenic test products to use as tools in molecular allergology.

Many of the problems associated with using natural allergenic products for allergy diagnosis and treatment can be overcome through recombinant allergens. Currently, the diagnosis of IgE-mediated allergy is performed through natural allergen extracts, which contain a mixture of allergenic and non-allergenic molecules that are difficult to standardise. Traditional diagnosis defines the source, e.g., Birch pollen, but it does not uncover which allergenic molecule(s) elicit the sensitization. Recombinant allergens are tools to expose the allergenic molecule(s) involved. Although the diagnostic sensitivity of single allergen components may be generally lower than that of allergen extracts, the specificity is normally higher. Recombinant allergens allow more defined preparations for *in vivo* testing and *in vitro* testing.

A "component-resolved diagnostics" (CRD) is recommended for a more precise diagnosis. In this instance, the antibody reactivity profile of an allergic patient can be identified, along with the disease-eliciting allergens and potential cross-reactivity interactions. Recombinant allergens offer a highly specific way to elucidate patient- and disease-specific sensitization patterns, knowledge of which is needed for the development of patient-tailored allergen preparations to refine immunotherapy and reduce the risk of sensitising patients to other allergens.

Recombinant allergens can be produced and contain most of the epitopes present in complex allergen sources, which will facilitate innovative strategies for allergen immunotherapy (2). These include peptide-based vaccines, engineered hypoallergens with reduced reactivity to IgE antibodies, nucleotide-conjugated vaccines that promote Th1 responses, and possibly prophylactic allergen vaccines. By the destruction of the protein fold through point mutations or recombinant oligomers, low IgE-binding allergen derivatives can be created, which may reduce the risk of adverse effects in specific immunotherapy: the modified allergens have preserved immunogenicity and are still able to induce regulatory T-cells, which modulate a pathologic TH2-response into a dominating TH1-response, resulting in reduced hypersensitivity.

Recombinant allergens will also allow the standardisation of allergen products (defined mixtures of biotechnology produced allergens). Recombinant allergens have, moreover, become irreplaceable tools in molecular allergology.

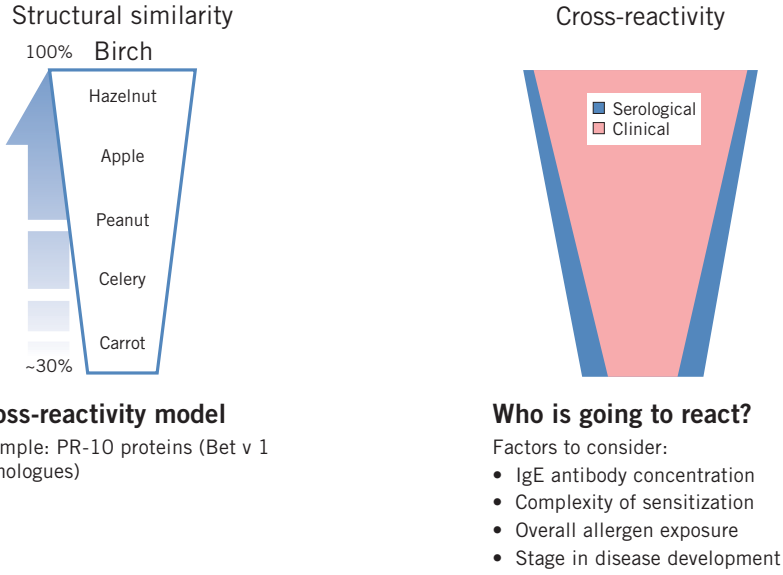


Figure 2. Component Resolved Diagnostics (CRD) helps explain clinical reactivity.

Recombinant allergens are useful tools for evaluating cross-reactivity, for better management of the patient, and for developing more efficacious immuno-therapy.

For example, the tree families *Betulaceae*, *Fagaceae*, and *Corylaceae*, belonging to the order *Fagales*, contain cross-reactive allergens. Of these allergens, birch is considered to represents the most potent and frequent allergen source; and of the birch tree allergens, Bet v 1 is a major allergen, responsible for major cross-reactivity between Bet v 1 from Birch and Bet v 1-homologous proteins in members of the *Fagales* (Alder, Hazel, Hornbeam) (3). Furthermore, as Bet v 1-related allergens are also present in a number of other trees and plants, this allergen has been said to be the cause of cross-

reactivity between birch and a number of fruits, vegetables and spices, e.g., apple, carrot, celery, cherry, and pear (4,7).

Not surprisingly, given the importance of birch pollen allergy and related allergies in the northern parts of Europe, one of the first recombinant allergens created was the cDNA coding for Bet v 1: rBet v 1. rBet v 1 has been expressed in *E. coli* as a biologically active allergen and was demonstrated to be very similar to the native allergen, allowing accurate *in vivo* and *in vitro* diagnosis of tree pollen allergy in >95% of cases (5-7). Using recombinant Bet v 1 allows improved diagnostic precision and management of not only the major sensitising allergen, but of potential cross-reactivity as well. Recombinant Bet v 1 has also made it clear that Bet v 1 is the initial sensitising allergen for many patients suffering from *Fagales* pollen allergy and Birch pollen-related plant-food allergy as exemplified by oral allergy syndrome (4,7). Other studies have confirmed that Bet v 1 may be considered a marker allergen for genuine sensitisation to *Fagales* pollen and Birch pollen-related food allergy (4,7-8).

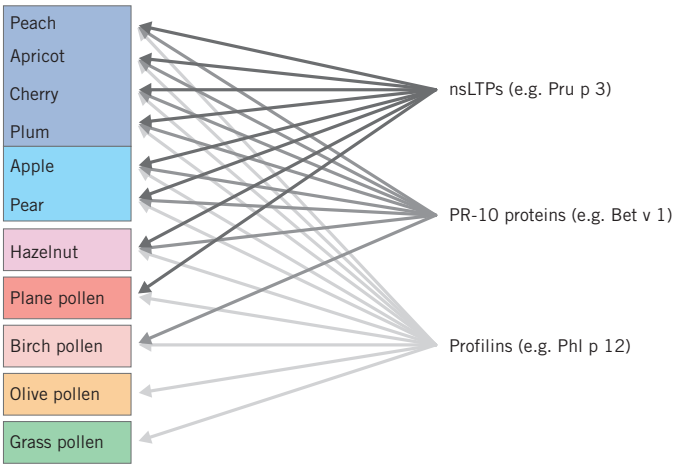


Figure 3. Distribution of some cross-reactive protein families.

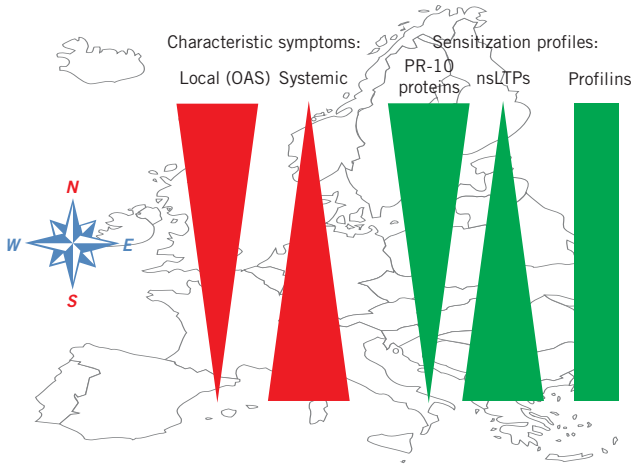


Figure 4. Geographical differences in allergy to fruits and vegetables.

Recombinant allergens have also been useful in elucidating other natural birch pollen allergens. Bet v 2 is a profilin (9-10), and Bet v 3 and Bet v 4 have been identified as 2EF-hand Ca^{2+}-binding proteins (11-13). These allergens have been shown to have a wide distribution, not only in pollen from unrelated plants (grasses, weeds, trees), but also in other plant tissues (fruits, vegetables, nuts, spices), demonstrating extensive cross-reactivity. These allergens can therefore serve as marker allergens for plant polysensitisation (14). In other words, a positive reaction to an allergen with cross-reactive potential may predict allergic reactions to all those allergen sources containing structurally related molecules.

If the common allergens from an allergen source could be identified by molecular cloning techniques and produced as recombinant allergens, these could be used for component-resolved diagnostics (CRD) of allergy, enabling the identification of the disease-eliciting allergens for each patient and thus establishing a detailed IgE reactivity profile (15). By contrast, extract-based diagnosis provides a determination of the allergen source, by telling us that a patient reacts to unspecified components in the given extract.

- PR-10 proteins (Bet v 1 homologues)
- Major grass pollen allergens
- Major epithelial/dander allergens
- Lipid Transfer Proteins (nsLTPs)
- Tropomyosins
- Nut/seed storage proteins
- 2-EF-hand, Ca^{2+}-binding proteins
- Profilins
- Cross-reactive Carbohydrate Determinants (CCDs)

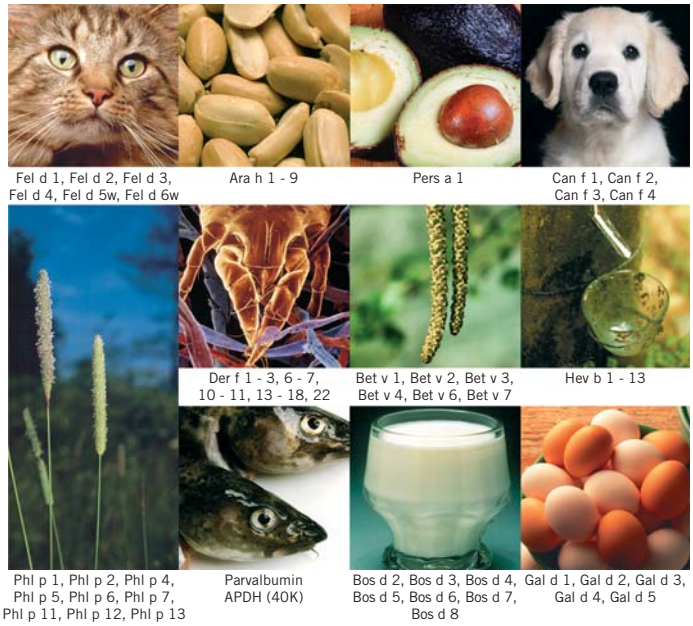


Figure 5. Examples of allergens of particular diagnostic interest.

Because immunotherapy represents a costly, time-consuming and sometimes risky treatment, the clinician must make careful decisions as to which patients are suitable for this treatment. For example, we know that clinically relevant reactions to Birch pollen can be found in patients who were not originally sensitised to Birch (16). This is a consequence of sensitisation to cross-reactive allergens from other plants (3). Recombinant allergens can distinguish patients who are genuinely sensitised to Birch pollen, as shown by their IgE reactivity to the major Birch pollen allergen Bet v 1, from patients who, as a result of IgE to cross-reactive allergens such as Bet v 2, exhibit positive skin tests to Birch pollen extracts without having been exposed to Birch (3).

The introduction of the recombinant allergens in the diagnosis of IgE-mediated allergy not only facilitates deciding whether a patient is suitable for immunotherapy, but also allows the measurement of IgE and IgG antibody responses to individual allergen components during allergen-specific immunotherapy, thereby allowing the monitoring of antibody profiles and levels during the course of immunotherapy (17-19).

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ImmunoCAP® native & recombinant allergen components available for IgE antibody testing

Origin of protein/substance	Protein family/ type of substance	Mw (kDA)	ImmunoCAP®
Alternaria alternata	Acidic glycoprotein	29-31	m229 rAlt a 1
Ananas comosus	Cross-reactive Carbohydrate Determinant (CCD) from Bromelin	1113	o214 CCD; MUXF3 (Ana c 2 derived)
Apium graveolens	PR-10 protein	16	f417 rApi g 1.01
Arachis hypogaea	Storage protein/Vicilin	65	f422 rAra h 1
Arachis hypogaea	Storage protein/Conglutin	18	f423 rAra h 2
Arachis hypogaea	Storage protein/Glycinin	57	f424 rAra h 3
Arachis hypogaea	PR-10 protein	17	f352 rAra h 8
Aspergillus fumigatus	Ribotoxin	18	m218 rAsp f 1
Aspergillus fumigatus	Fibrinogen binding protein	37	m219 rAsp f 2
Aspergillus fumigatus	Peroxisomal protein (PMP)	19	m220 r Asp f 3
Aspergillus fumigatus	Unknown	30	m221 rAsp f 4
Aspergillus fumigatus	Mn superoxide dismutase	26	m222 r Asp f 6
Bertholletia excelsa	2S Albumin	9	f354 rBer e 1
Betula verrucosa	PR-10 protein	17	t215 rBet v 1
Betula verrucosa	Profilin	15	t216 rBet v 2
Betula verrucosa	Calcium-binding protein	8	t220 rBet v 4
Betula verrucosa	Isoflavone reductase	34	t225 rBet v 6
Betula verrucosa	See above	See above	t221 rBet v 2, r Bet v 4
Bos domesticus	α-lactalbubin	14	f76 nBos d 4
Bos domesticus	β-lactoglobulin	18	f77 nBos d 5
Bos domesticus	Serum albumin (BSA)	67	e204 nBos d 6
Bos domesticus	Casein	9-25	f78 nBos d 8
Bos domesticus	Lactoferrin	76	f334 nBos d lactoferrin
Canis familiaris	Lipocalin	21-25	e101 rCan f 1
Canis familiaris	Lipocalin	19	e102 rCan f 2
Canis familiaris	Serum albumin (DSA)	69-70	e221 nCan f 3
Cyprinus carpio	Parvalbumin	12	f355 rCyp c 1
Felis domesticus	Cat-1 allergen	38	e94 rFel d 1
Felis domesticus	Serum albumin	65-69	e220 nFel d 2
Gallus domesticus	Ovomucoid	28	f233 nGal d 1
Gallus domesticus	Ovalbumin	44	f232 nGal d 2
Gallus domesticus	Conalbumin/Ovotransferrin	66-78	f323 nGal d 3
Gallus domesticus	Lysozyme	14	k208 nGal d 4
Glycine max	PR-10 protein	17	f352 rGly m 4
Hevea brasiliensis	Rubber elongation factor (REF)	15	k215 rHev b 1*
Hevea brasiliensis	Small rubber particle protein	24	k217 rHev b 3*
Hevea brasiliensis	Acidic protein	16	k218 rHev b 5
Hevea brasiliensis	Prohevein	20	k219 rHev b 6.01*
Hevea brasiliensis	Hevein	5	k220 rHev b 6.02*
Hevea brasiliensis	Profilin	14	k221 rHev b 8*
Hevea brasiliensis	Enolase	51	k222 rHev b 9*
Hevea brasiliensis	Class 1 Chitinase	32	k224 rHev b 11*

*** MBP fusion protein**

Origin of protein/substance	Protein family/ type of substance	Mw (kDA)	ImmunoCAP®
Olea europaea	Trypsin inhibitor	19-20	t224 nOle e 1
Parietaria judaica	Non-specific Lipid transfer protein (nsLTP)	14	w211 rPar j 2
Penaeus aztecus	Tropomyosin	36	f351 rPen a 1
Phleum pratense	Group 1 grass allergen	27	g205 rPhl p 1
Phleum pratense	Group 2 grass allergen	13	g206 rPhl p 2
Phleum pratense	Group 4 grass allergen	55	g208 nPhl p 4
Phleum pratense	Group 5 grass allergen	32	g215 rPhl p 5b
Phleum pratense	Group 6 grass allergen	15	g209 rPhl p 6
Phleum pratense	Calcium-binding protein	9	g210 rPhl p 7
Phleum pratense	Group 11 grass allergen	20	g211 rPhl p 11
Phleum pratense	Profilin	14	g212 rPhl p 12
Phleum pratense	See above	See above	g213 rPhl p 1, rPhl p 5b
Phleum pratense	See above	See above	g214 rPhl p 7, rPhl p 12
Prunus persica	PR-10 protein/ribonuclease	17	f419 rPru p 1
Prunus persica	Non-specific Lipid transfer protein (nsLTP)	9-10	f420 rPru p 3
Prunus persica	Profilin	14	f421 rPru p 4
Triticum aestivum	Storage protein/ω-5 gliadin	27	f416 rTri a 19: Omega-5 Gliadin

Alternaria alternata allergen components

Alternaria alternata
Available ImmunoCAP®:
m229 rAlt a 1

Summary

A. alternata (syn. *A. tenius*), growing commonly on vegetation, is a member of the imperfect fungi and is one of the most important among the allergenic fungi. Brown segmented mycelia give rise to simple or solitary conidiophores, which may produce either solitary apical spores or a string of spores. The spores produced by imperfect fungi vary in shape, size, texture, colour, number of cells, and thickness of the cell wall (1). Although other *Alternaria* species are probably also relevant clinically, most research has been directed toward *A. alternata*, in particular as a result of cross-reactivity of the species.

Alternaria is one of the main allergens affecting children. In temperate climates, airborne *Alternaria* spores are detectable from May to November, with peaks in late summer and autumn (2). Dispersion of *Alternaria* spores occurs during dry periods. These feature higher wind velocity and lower relative humidity, which result in peak dispersion during sunny afternoon periods (3).

Despite the large spore size, spore dispersal may occur for hundreds of miles from the source. Counts of *Alternaria* on dry, windy days can be in the range of 500 to 1,000 spores per cubic metre in grass- or grain-growing areas. Outdoor spore counts of up to 7,500 spores per cubic metre of air were associated with indoor spore counts between 0 and 280 per cubic metre (4). Significant concentrations of *Alternaria* allergens, between 3.0 and 1,000 U/g of dust, have been found in house dust of allergic children, supporting the hypothesis that fungal allergen exposure is an important component in the pathogenesis of asthma (5-6). Recently, *Alternaria* has been found in house dust samples in the absence of outdoor environmental mould spores (7).

Allergens from *Alternaria alternata* listed by IUIS*

Alt a 1	Alt a 3	Alt a 4
Alt a 5	Alt a 6	Alt a 7
Alt a 8	Alt a 10	Alt a 12
Alt a 13		

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

Sensitivity to *Alternaria*, a potent allergen, has been increasingly recognised as a risk factor for the development, persistence, and exacerbation of asthma (8-13).

Mould allergy diagnoses are performed with fungal extracts consisting of a complex mixture of proteins, glycoproteins, polysaccharides, and other substances; these extracts show a considerable variability as a result of inter-strain genomic differences, different culture conditions, and variable extraction procedures (14-15). It is almost impossible to grow 2 consecutive cultures with similar antigenic profiles (16). Thus, the number of allergens in *A. alternata* extracts may range from 10 to 30, and few allergens are present in nearly all extracts studied (17). The presence of specific allergens, including the major allergens, depends very much on the growth conditions, and may vary during the growth cycle, being greater one day than another (18-19). Furthermore, the major allergens are secreted proteins, whereas the other allergens are intracellular proteins, and the latter are presented to the immune system

in the spores of this mould, which are too large to reach the alveoli of the lung (18). Furthermore, germination of spores significantly increases allergen release (but not all spores release allergens). For example, Alt a 1, the major allergen, may be a minor contributor to the total amount of allergens released from spores, except when spores have germinated (20). How the phenomena revealed in these results reflect the allergen content of spores in the air that we breathe has, however, not been fully elucidated. Nevertheless, advances in molecular biology have led to a better understanding of these allergens and their relationship to allergic disease (21).

Although it is clear from a number of epidemiologic studies that sensitisation to indoor allergens and to the spores of *Alternaria* are risk factors for the development of asthma in both children and adults (22), detailed investigation is problematic. Many studies have utilised skin test and IgE antibody determination, but, as already discussed, there are inherent difficulties in the manufacturing and standardising of fungal extracts (23), and variability in epidemiologic studies inevitably results (8). Non-standardised mould extracts may also result in poor outcomes in specific immunotherapy (24).

Therefore, diagnostic and therapeutic procedures with purified allergens may be of benefit. Recombinant mould allergens of suitable purity and consistency can be produced. They have become standardised diagnostic material and may be of benefit in component-resolved diagnosis.

The following allergens have been characterised:

Alt a 1, a 29.2-31 kDa major allergen, a heat-stable protein (18,25-42).

Alt a 2, a 25 kDa protein, a major allergen, an aldehyde dehydrogenase (25,38-43).

Alt a 3, a heat shock protein (18,25,38-39,41).

Alt a 4 (18,25,38-40).

Alt a 5, a 47 kDa protein, an enolase (formerly Alt a 11) (25,38,39,43,48).

Alt a 6, a 11 kDa protein, an acid ribosomal protein P2 (17,25,38-39,41,49).

Alt a 7, a 22 kDa protein, a YCP4 Protein (25,38-39,41).

Alt a 8 (38-40).

Alt a 9 (38-40).

Alt a 10, a 53 kDa protein, an aldehyde dehydrogenase (25,38-39,41,50).

Alt a 11, now reclassified as Alt a 5.

Alt a 12, a 12, an acid ribosomal protein P1 (25,39).

Alt a 13, a glutathione-S-transferase (51).

Alt a 70kD, a 70 kDa protein (52-53).

Alt a NTF2, a nuclear transport factor 2 protein (54).

m229 rAlt a 1

ImmunoCAP®: m229 rAlt a 1

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Alternaria alternata* allergen Alt a 1

Common name:	Alt-1
Biological function:	Unknown
Mw:	29-31 kDa

Allergen description

More than 9 allergens have been described in *A. alternata* extracts, although only 2 of them are major allergens. Alt a 1 is a dimer of 29 kDa that dissociates into 14.5 and 16 kDa subunits under reducing conditions (29). Studies indicate that this allergen may have several conformational or structural isoforms of this protein, which may be responsible for the allergen appearing to have a number of different molecular sizes (31,36).

Alt a 1 is recognised by approximately 80% to 100% of all *Alternaria*-allergic patients (29,55). rAlt a 1 has been shown to be similar to natural Alt a 1. In a study of *A. alternata*-sensitised individuals, 85.7% to >90% were shown to be sensitised to to rAlt a 1 (25,29). Similarly, in a study of patients with *A. alternata* allergy, sensitisation could be detected by means of skin test. No false-positive results were obtained with control patients, even at the highest concentration (14). Evaluation of recombinant Alt a 1 using skin test was positive in 6 of 7 individuals allergic to *Alternaria*. In contrast, in a study using commercially available *A. alternata* extracts, researchers failed to correctly diagnose *Alternaria*-allergic patients in 2/10 cases (25).

In a study of 42 patients allergic to *A. alternata*, 10 atopic patients were found to have no skin-reactivity for the *A. alternata* extract; commercial extracts were used for testing. However, all patients were shown to have skin-reactivity to *A. alternata* when purified allergens were used for testing. No false-positive reactions were detected. Analysis showed no IgE-binding differences between nAlt a 1 and rAlt a 1. Specific IgE levels to nAlt a 1 or rAlt a 1 showed significant correlation and similar sensitivity and specificity (14).

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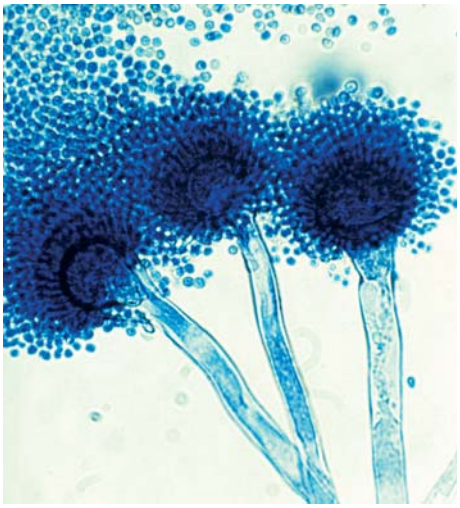
Aspergillus fumigatus allergen components

Aspergillus fumigatus

Available ImmunoCAP®:

m218 rAsp f 1
m219 rAsp f 2
m220 rAsp f 3
m221 rAsp f 4
m222 rAsp f 6



Summary

Recombinant components from *Aspergillus fumigatus* (*A. fumigatus*) are available for allergen-specific IgE antibody testing.

Available fungal extracts used either for serological detection of IgE antibodies or for skin testing, although very effective, may not be as effective as a single recombinant or a mix of recombinant allergens. This is mainly because of the lack of recognised standardisation procedures, resulting in large batch-to-batch variation of the allergen content in the extracts used (1). *A. fumigatus* is able to produce more than 40 IgE-binding proteins, and this makes standardisation of extracts a difficult task, the growth phases of the fungus complicating it further. Molecular cloning production and characterisation of *A. fumigatus* allergens has contributed to improving the precision of the diagnosis of sensitisation to *A. fumigatus* (2).

Recombinant allergens, which are biotechnology produced molecules originally identified from allergen extracts, have IgE antibody binding usually comparable to that of natural allergens and generally show good reactivity in *in vitro* and *in vivo* diagnostic tests (3). To date, many different recombinant allergens have been cloned, sequenced, and expressed.

Recombinant allergens have a wide variety of uses, from the diagnosis and management of allergic patients to the development of immunotherapy to the standardisation of allergenic test products as tools in molecular allergology.

Inhalation of the conidia and the mycelium of *A. fumigatus* is responsible for many allergic respiratory diseases, the most notable of which – due to its severity – is allergic bronchopulmonary aspergillosis (ABPA). The complexity of the antigenic structure of *A. fumigatus* and the varying host immune responses determine the severity, on a wide spectrum, of the clinical conditions seen, which include allergic asthma, extrinsic allergic alveolitis (hypersensitivity pneumonitis), Farmer's Lung, invasive aspergillosis, and aspergilloma. It is reported that 15 to 20% of allergic asthmatics suffer from *Aspergillus*-induced allergies.

The allergenic proteins are derived from 2 function categories: secreted and cytoplasmic proteins. Secreted allergens are recognised by serum IgE antibodies of *A. fumigatus*-sensitised individuals with or without ABPA, whereas nonsecreted allergens are exclusively recognised by serum

Allergens from *Aspergillus fumigatus* listed by IUIS*

Asp f 1	Asp f 2	Asp f 3
Asp f 4	Asp f 5	Asp f 6
Asp f 7	Asp f 8	Asp f 9
Asp f 10	Asp f 11	Asp f 12
Asp f 13	Asp f 15	Asp f 16
Asp f 17	Asp f 18	Asp f 22W
Asp f 23	Asp f 27	Asp f 28
Asp f 29	Asp f 34	

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

IgE antibodies of ABPA patients (4). The use of recombinant allergens therefore may be of great value in assessing individuals affected by this mould.

ABPA is an immunologically complex disorder. Various allergens and antigens of *A. fumigatus* induce IgE-mediated but also other hypersensitivity reactions in ABPA patients. Elevated levels of total IgE, allergen-specific IgE and IgG antibodies in sera are important immunodiagnostic criteria for ABPA (5). High levels of IgE and IgG antibodies in these patients are of diagnostic value (6). However, the large differences reported in the incidence of ABPA in asthmatics sensitised to *A. fumigatus*, ranging from 7% to 22%, and from 0.1% to 12% in patients with cystic fibrosis, may partially be explained by the lack of reliable *A. fumigatus* extracts (7).

Through molecular characterisation, several allergenic components have been identified: complex carbohydrate moieties, heat-shock proteins, and enzymes such as elastase, protease, catalase, dismutase, and cytotoxic ribonuclease. Some have a multifunctional nature, which may play an important role in the pathogenesis of the disease (6).

Studies have evaluated different combinations of recombinant allergens for diagnostic use in *Aspergillus* allergy.

In one study of serum IgE antibodies to the recombinant *A. fumigatus* allergens rAsp f 1, 3, 4 and 6 in 74 patients suffering from cystic fibrosis (CF) with and without ABPA, 40 were found to be sensitised to A. fumigatus, of which 23 had ABPA. Of the 23 ABPA patients, 11 expressed the full clinical ABPA picture, and 12 had positive serology indicating ABPA but did not show sufficient clinical signs of the disease. The 23 ABPA patients had 16-18 times higher serum levels of allergen-specific IgE to rAsp f 4 and/or rAsp f 6. The combination of increased total serum IgE (>1000 IU/l) and increased IgE antibodies to rAsp f 4 and/or rAsp f 6 was associated with symptomatic ABPA with 100% specificity and 64% sensitivity, and with a high positive predictive value (100%) and a high negative predictive value (94%) (2).

It has also been suggested that the measurement of IgE antibodies for *A. fumigatus* with purified recombinant allergens may differentiate ABPA from atopic cystic fibrosis (CF). In a study evaluating serum IgE reactivity to 7 recombinant purified allergens and to a crude extract of *A. fumigatus* in 15 ABPA patients, in 23 CF patients with skin reactivity to *A. fumigatus*, and in 19 CF patients with no skin reactivity to *A. fumigatus*, ABPA patients had significantly increased IgE reactivity to rAsp f 2, f 3, f 4, f 6, and f 16, compared with the 2 other groups of patients. In the ABPA patients studied before and after developing ABPA, IgE reactivity also increased to rAsp f 2, f 3, f 4, and f 6, and to the crude extract. In ABPA CF patients, IgE reactivity to rAsp f 1, f 2, f 3, and f 6 significantly increased during periods of ABPA flares, compared with periods of remission. IgE antibodies to rAsp f 3 and rAsp f 4 gave the best sensitivity and specificity and were more useful than IgE reactivity to a crude extract of *Aspergillus*. Furthermore, in ABPA patients studied during periods of remission, the IgE reactivity to Asp f 3 and f 4 remained significantly elevated compared with the other groups. The conclusion reached was that allergen-specific IgE reactivity to a panel of purified *Aspergillus* allergens, especially to Asp f 3 and f 4, differentiates ABPA patients from atopic *Aspergillus* skin-specific positive and non-ABPA CF patients. In particular, serial evaluation of IgE reactivity to individual purified *Aspergillus* antigens, especially Asp f 3, showed that increases in IgE reactivity may provide improved distinction between stages of flares and remission, compared with changes in IgE reactivity to a crude *Aspergillus* extract (8).

Recombinant *Aspergillus* allergens Asp f 1, f 2, f 3, f 4, and f 6 were studied for their specific binding to IgE antibodies in the sera of ABPA patients and *A. fumigatus* skin-specific IgE-positive asthmatics from the USA and Switzerland. Serum IgE to all the recombinant allergens was detected in sera from patients with ABPA, whereas only a few asthmatics had serum IgE antibodies detected to these allergens. Asp f 2, f 4, and f 6 were reported to be effective in the

serodiagnosis of ABPA, with allergen-specific IgE being detected to these 3 recombinant allergens together in all the ABPA patients studied. IgE antibodies to Asp f 1 and f 3 were not specific (9).

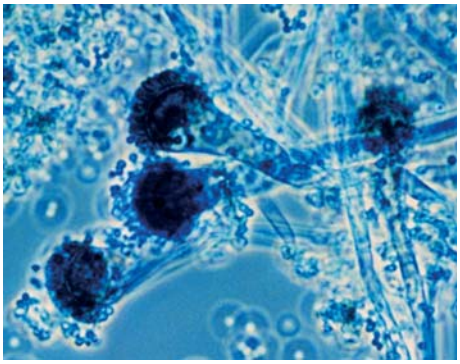
Another study suggested that a smaller panel of recombinant *Aspergillus* allergens might allow reliable diagnosis of ABPA. Fifty patients with CF were evaluated using skin-specific IgE tests: 12 had ABPA, and 21 had allergy to *A. fumigatus*. All patients with ABPA reacted to at least 1 of the 2 allergens rAsp f 4 and rAsp f 6. IgE antibodies in skin prick test were negative or only marginally positive in the patients with allergy to *A. fumigatus*, and completely negative in the CF control patients. The authors concluded that rAsp f 4 and rAsp f 6 can be considered specific markers for ABPA, and that early diagnosis of the disease, allowing optimal management, might help to prevent irreversible lung damage and minimise possible steroid-mediated side-effects (10).

Similarly, in a study examining the differential IgE antibody responses to the allergens in *A. fumigatus*-sensitised CF patients with or without ABPA and in CF controls without sensitisation to *A. fumigatus*, rAsp f 1 and rAsp f 3 were recognised by sera from *A. fumigatus*-sensitised CF-patients with or without ABPA. rAsp f 6 and rAsp f 4 were recognised exclusively by IgE antibodies from sera of CF patients with ABPA. The study concluded that Asp f 4 and Asp f 6 were specific markers for ABPA and allowed a sensitive, fully specific diagnosis of the disease (11). Specific sensitisation found to nonsecreted *Aspergillus* proteins in ABPA suggests substantial differences in the pathways of exposure to and immunologic recognition of this mould and a specific disease (7), supporting the use of recombinant single allergens for diagnosis in these situations.

It has been reported that *A. fumigatus* is an important causative agent in allergic fungal sinusitis (AFS) in the southeastern United States, that most confirmed AFS patients have *A. fumigatus*-specific IgE, and that many have specific IgE antibodies to rAsps (12).

The recombinant allergens, Asp f 1, Asp f 3, Asp f 4 and Asp f 6 have also been tested in large-scale skin test studies in patients with asthma or cystic fibrosis and coexisting sensitisation to *A. fumigatus*, and have been demonstrated to be reliable diagnostic reagents (7,13-15). The dissection of the causes of IgE-mediated immune responses down to single *A. fumigatus* allergens will allow the clinician to discriminate between the various clinical manifestations attributed to the same mould with high specificity and sensitivity (4).

m218 rAsp f 1



ImmunoCAP®: m218 rAsp f 1
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Aspergillus fumigatus* allergen Asp f 1

Common name:	Ribotoxin
Biological function:	Ribonuclease
Mw:	18 kDa

Allergen description

Asp f 1 (2,4,7-9,11-12,14-23) is a major allergen produced by the mycelia of *Aspergillus fumigatus* (*A. fumigatus*). It is not present in spores and can be used as a specific marker for the detection of germination of this fungus (24). Asp f 1 is a species-specific allergen, in contrast to other IgE-binding proteins of *A. fumigatus* that are highly cross-reactive with related proteins from phylogenetically distant species (25). Asp f 1 is a ribotoxin; ribotoxins are potent inhibitors of eukaryotic protein synthesis (4).

Early studies have shown that 85% of *A. fumigatus*-allergic patients with allergic bonchopulmonary aspergillosis (ABPA) have IgE antibodies to Asp f 1, and that there is an absence of homologous proteins in other fungi (26-27). Significant levels of Asp f 1-specific antibodies are present in a majority of ABPA patients in the early stages of the disease (26).

rAsp f 1 has been shown to have similar characteristics to native Asp f 1, and can therefore be used as a standardised antigen/allergen for serologic and clinical diagnosis of *A. fumigatus*-associated diseases (17).

A study evaluated the diagnostic value of rAsp f 1 in 55 patients with cystic fibrosis (CF); based on clinical presentation and laboratory data, 10 of these CF patients had ABPA, 27 had *Aspergillus* allergy, and 18 were not allergic to *A. fumigatus* (CF control group). Serologic assays showed a 10-fold increase in rAsp f 1-specific IgE antibodies, a 5-fold increase in rAsp f 1 -specific IgG_1, and a 4-fold increase in rAsp f 1-specific IgG_4 antibodies in ABPA patients, compared with the *Aspergillus* allergy and CF control groups. The study concluded that rAsp f 1-specific serology is a highly sensitive and specific test that can be used to identify ABPA reliably in CF patients (21).

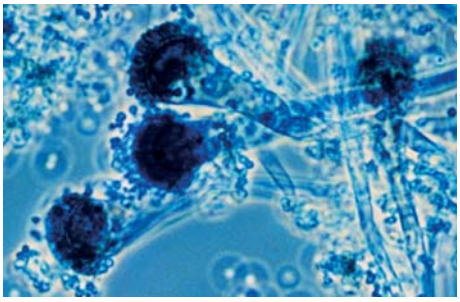
In an early study of sera from 147 CF patients, IgE antibodies to *A. fumigatus* and 5 common inhalant allergens were measured. Thirty (20%) of the patients had allergen-specific IgE antibodies to *A. fumigatus*, and 22 (15%) of these patients had developed total IgE levels higher than 400 kU/L, suggesting a diagnosis of ABPA. Eighty-four percent of the CF sera contained IgG antibodies to Asp f 1, compared with 6% of control patients and 20% of sera from allergic children with asthma (n = 25), only one of whom had IgE antibodies to *A. fumigatus* (28).

m219 rAsp f 2

ImmunoCAP®: m219 rAsp f 2

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Aspergillus fumigatus* allergen Asp f 2

Biological function:	Fibrinogen binding protein
Mw:	37 kDa

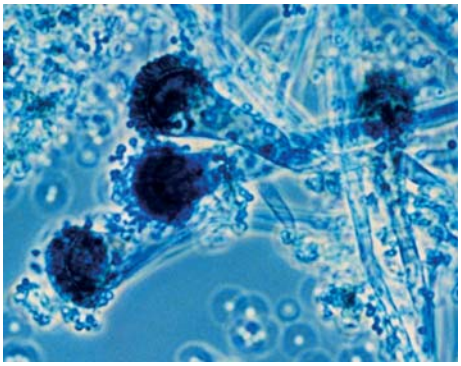


Allergen description

Asp f 2 (8-9,12,29-31) is a major allergen from the fungus *Aspergillus fumigatus* (*A. fumigatus*), and >90% of *A. fumigatus*-sensitised individuals have IgE antibodies to Asp f 2 (32).

In a study of 25 patients with allergic bronchopulmonary aspergillosis (ABPA), 96% had IgE antibodies directed against rAsp f 2, as did none of the subjects with allergic asthma, nor any of the normal control subjects (33).

m220 rAsp f 3



ImmunoCAP®: m220 rAsp f 3
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Aspergillus fumigatus* allergen Asp f 3

Common names:	Peroxisomal protein, PMP
Biological function:	Peroxisomal protein
Mw:	19 kDa

Allergen description

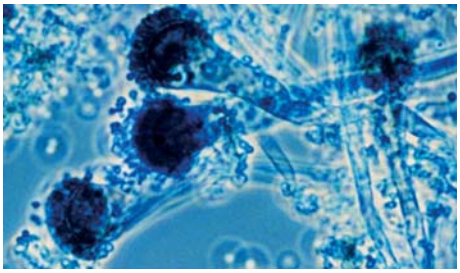
rAsp f 3 (2,4,8-9,11-12,19,34), a peroxisomal protein, was evaluated using skin and serum tests on 11 patients with allergic bronchopulmonary aspergillosis (ABPA) and 8 patients with allergic asthma with sensitisation to *Aspergillus fumigatus* (*A. fumigatus*). All 11 patients with ABPA and 5 of 8 (84%) *A. fumigatus*-sensitised asthmatics without ABPA exhibited an IgE-mediated skin test. Serum rAsp f 3 IgE antibodies were found in all rAsp f 3 skin positive subjects and none without skin positive tests to rAsp f 3. rAsp f 3-specific IgE APBA patients had significantly higher serum levels of IgG, IgG_1, IgG_4 and IgE, compared with *A. fumigatus*-sensitised asthmatics and healthy controls. The authors concluded that serological tests with recombinant allergens are of great use in diagnosing sensitisation to *A. fumigatus* (35).

rAsp f 3 has been demonstrated to have a 36% identity and a 58% similarity to 2 peroxisomal membrane proteins of *Candida boidinii*. Serum IgE antibodies to rAsp f 3 were found in 72% of 89 individuals sensitised to *A. fumigatus*, indicating that this protein represents a major allergen of this mould (34). Cross-reactivity is likely between Pen c 3 (*Penicillium citrinum*) and Asp f 3, given an 82.6% identity between these proteins (36). A study of the allergens of *Malassezia furfur* reported significant homology between Mal f 3 and Asp f 3 (37).

m221 rAsp f 4

ImmunoCAP®: m221 rAsp f 4
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Aspergillus fumigatus* allergen Asp f 4

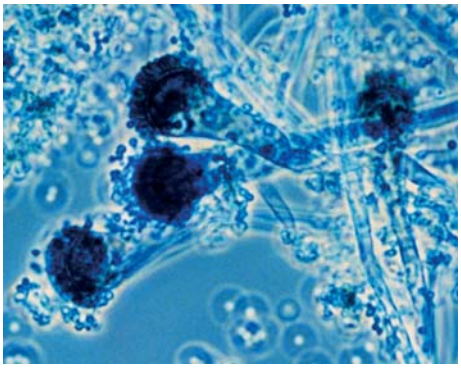
Biological function:	Unknown
Mw:	30 kDa



Allergen description

The biological function of the allergen Asp f 4 (2,4,8-13,19) has not been determined yet. As a clear distinction between allergic sensitisation to *Aspergillus fumigatus* (*A. fumigatus*) and allergic bronchopulmonary aspergillosis (ABPA) is essential for therapy, to prevent deterioration of pulmonary function in subjects with ABPA, research constantly evaluates the significance of individual allergens as predictive indicators. One study demonstrated that rAsp f 4 and rAsp f 6 were able to provoke immediate skin reactions exclusively in patients with ABPA; i.e., these allergens are highly specific for ABPA. The reactions were elicited by a few nanograms of the allergens and therefore allowed a sensitive and highly specific diagnosis of ABPA (13). The rAsp f 4- and rAsp f 6-based serological diagnosis of ABPA has a specificity of 100% and reaches a sensitivity of 90% in asthmatic patients sensitised to *A. fumigatus* (38), whereas the serological discrimination between *A. fumigatus* sensitisation and ABPA in patients suffering from cystic fibrosis reached 100% (11). Specific sensitisation to nonsecreted *Aspergillus* proteins in ABPA suggests substantial differences in the pathways of exposure to and the immunologic recognition of this mould and a specific disease (7), supporting the use of recombinant single allergens for diagnosis in these situations.

m222 rAsp f 6



ImmunoCAP®: m222 rAsp f 6
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Aspergillus fumigatus* allergen Asp f 6

Common names:	Mn-SOD, SOD
Biological function:	Mn superoxide dismutase
Mw:	26 kDa

Allergen description

Asp f 6 (2,4,8-13,18-19,39-41) is an allergen belonging to the manganese superoxide dismutase (MnSOD) protein family.

As a clear distinction between allergic sensitisation to *A. fumigatus* and allergic bronchopulmonary aspergillosis (ABPA) is essential for therapy, to prevent deterioration of pulmonary function in subjects with ABPA, research constantly evaluates the significance of individual allergens as predictive indicators. One study demonstrated that rAsp f 4 and rAsp f 6 were able to provoke immediate skin reactions exclusively in patients with ABPA; i.e., these allergens are highly specific for ABPA. The reactions were elicited by a few nanograms of the allergens and therefore allowed a sensitive and highly specific diagnosis of ABPA (13). The rAsp f 4- and rAsp f 6-based serological diagnosis of ABPA has a specificity of 100% and reaches a sensitivity of 90% in asthmatic patients sensitised to *A. fumigatus* (38), whereas the serological discrimination between *A. fumigatus* sensitisation and ABPA in patients suffering from cystic fibrosis reached 100% (11). Specific sensitisation to nonsecreted *Aspergillus* proteins in ABPA suggests substantial differences in the pathways of exposure to and the immunologic recognition of this mould and a specific disease (7), supporting the use of recombinant single allergens for diagnosis in these situations.

MnSODs from other organisms, including humans, are recognised by IgE antibodies from individuals sensitised to *A. fumigatus* MnSOD (40). The MnSOD from *A. fumigatus* has been reported to have homology with *Drosophila melanogaster*, *Saccharomyces cerevisiae*, and human MnSOD; cross-reactivity was shown between the MnSOD at B and T cell level; moreover, the different MnSODs can induce proliferative responses in peripheral blood mononuclear cells of sensitised individuals (39). Cross-reaction with human MnSOD suggests that human proteins can act as autoallergens *in vivo* (7). A cloned allergen from the yeast *Malassezia sympodialis* has also been reported to have a sequence similarity with MnSODs (42). A cloned *Hevea brasiliensis* (Latex) MnSOD protein, Hev b 10, was shown to have IgE binding in Latex- as well as *A. fumigatus*-allergic patients (43).

Based on the cross-reactivity of rAsp f 6 and MSODs' potential to act as panallergens, rAsp f 6 is useful for assessing other cross-reactive allergens to which an individual may be sensitive.

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Birch allergen components

Betula verrucosa

Available ImmunoCAP®:

t215 rBet v 1
t216 rBet v 2; profilin
t220 rBet v 4
t225 rBet v 6
t221 rBet v 2, rBet v 4

Summary

Recombinant allergen components from pollen of birch are available for allergen-specific IgE antibody testing.

Recombinant allergens, which are biotechnology produced protein molecules originally identified from allergen extracts, have immunoglobulin E (IgE) antibody binding comparable to that of natural allergens and generally show excellent reactivity in in vitro and in vivo diagnostic tests (1). To date, many different recombinant allergens have been cloned, sequenced and expressed.

Recombinant allergens have a wide variety of uses, from the diagnosis and management of allergic patients to the development of immunotherapy to the standardisation of allergenic test products as tools in molecular allergology.

Recombinant allergens are particularly useful for further investigations in allergies manifesting wide cross-reactivity, such as allergy to birch pollen, which frequently involves cross-reactivity among pollens of trees belonging to the order *Fagales* (e.g., *Fagaceae*, *Corylaceae*, and *Betulaceae*) (2). Birch pollen is considered to be the most powerful allergen in this complex (3).

Allergens from *Betula verrucosa* listed by IUIS*

Bet v 1	Bet v 2	Bet v 3
Bet v 4	Bet v 6	Bet v 7

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

t215 rBet v 1

ImmunoCAP®: t215 rBet v 1

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Betula verrucosa* allergen Bet v 1

Common name:	PR-10 protein
Biological function:	Pathogenesis-related protein, ribonuclease (23)
Mw:	17 kDa

Other allergens isolated:

rBet v 1a and rBet v 1d isoforms. These isoforms differ in their ability to bind IgE but are similar in their immunogenicity for T cells (24-25)

Allergen description

The major allergen of birch tree pollen is Bet v 1 (4-22). Recombinant Bet v 1 was among the first allergen-encoding cDNAs isolated and has significant sequence homology to a group of pathogenesis-related plant proteins and has been classified as a PR-10 protein. Recombinant Bet v 1 has been shown to bind IgE in most birch-pollen allergic patients (20-22). In one study, the accuracy of *in vivo* and *in vitro* diagnosis of birch pollen allergy by means of rBet v 1 was > 95% (49/51) (22). Nevertheless, differences in IgE antibody reactivity to rBet v 1 and rBet v 2 were demonstrated among allergic patients from 6 countries. The complexity of reactivity tended to be greater in individuals from the central and southern parts of Europe than from Sweden and Finland (26).

Several cross-reactive Bet v 1-homologoues are major allergens of *Fagales* pollen (Alder, Hazel, Hornbeam) and taxonomically related fruits, vegetables, and spices (e.g., Carrot, Celery, Apple, Apricot, Cherry, and Pear). This seems to be clearly related to the clinical pollen/food cross-reactivity found in oral allergy syndrome (OAS) (22,27). Studies have suggested that Bet v 1 is the initial sensitising allergen in many cases of *Fagales* pollen allergy and Birch pollen-related plant-food allergy (OAS) at least in areas where birch trees are common, as in Northern Europe (22,27). Population studies affirm that Bet v 1 is a marker allergen for genuine sensitisation to *Fagales* pollen- and Birch pollen-related food allergy (22,27-28).

Bet v 1 is recognised by IgE antibodies from about 95%of Birch-allergic patients Bet v 2 and Bet v 3 from10% and Bet v 6 by approximately 32% 29. The sensitisation profiles to Bet v 1 and Bet v 2 differ among geographical areas. Bet v 2, a profilin and a minor allergen, has also been shown to be involved in cross-reactivity to certain foods.

It has been suggested that Bet v 1 can be a diagnostic marker allergen for identifying patients with genuine sensitisation to birch-pollen (30), whereas more highly cross-reactive allergens, such as Bet v 2 and Bet v 4, may serve as marker allergens for syndromes involving cross-reactivity with unrelated plants/plant products (3,30). Accordingly,

patients who exhibit positive skin tests to birch pollen extracts but have never been exposed to Birch might be considered to have IgE antibodies to cross-reactive allergens such as profilin (3). Therefore, the use of rBet v 1 to identify patients with genuine birch pollen sensitisation and to confirm the diagnosis of birch pollen allergy before initiating immunotherapy with birch pollen extract has been recommended (3).

One example of a diagnostic application of recombinant birch allergens is found in a study examining allergen-specific serum IgE antibodies using the recombinant allergens Bet v 1, Bet v 2 and Bet v 4, as examined in birch-sensitive patients from the province of Cuneo, in northwestern Italy. It was reported that of 372 patients, 215 (58%) had serum IgE antibodies to Bet v 1, 166 (45%) to Bet v 2, and 35 (9%) to Bet v 4. Mono-sensitisation to Bet v 1 occurred in 146 (39%) of patients; in 96 (26%) to Bet v 2; and in only 4 (1%) to Bet v 4. Thirty-nine sera (11%) did not contain allergen-specific IgE antibodies to any of these three individual birch pollen allergens. All 372 sera (100%) had IgE antibodies against natural Birch pollen extract; 162 (44%) contained IgE antibodies reacting with Apple extract (75% of Bet v 1 positive sera). The study concluded that the 3 recombinant birch pollen allergens alone could identify 90% of birch pollen-sensitive patients (7).

t216 rBet v 2

ImmunoCAP®: t216 rBet v 2
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Betula verrucosa* allergen Bet v 1

Common name:	Profilin
Biological function:	Actin-binding protein
Mw:	15 kDa

Allergen description

Bet v 2 (5,7,14,31-38), a well-described minor allergen from birch pollen, belongs to the family of profilins, a group of common actin-binding proteins (37,39). Profilins can be found as cross-reactive allergens not only in pollen from unrelated plants (trees, grasses, weeds) but also in other plant tissues (of fruits, vegetables, nuts, spices, and latex) (40).

Bet v 2 and Bet v 3 are recognised by IgE from about 10% of Birch-allergic patients, Bet v 6 by approximately 32%, and Bet v 1 by 95% (29). The sensitisation patterns to Bet v 1 and Bet v 2, differ geographically; among Swedish and Finish patients, for example, approximately 5-7% were shown to be sensitised to Birch-profilin, compared to 20-38% of patients in Central and Southern Europe. Also, differences in IgE reactivity to rBet v 1 and rBet v 2 were demonstrated among allergic patients from six countries. The complexity of reactivity tended to be greater in individuals from the central and southern parts of Europe, as compared to Sweden and Finland (26,40).

The profilin Bet v 2, has also been shown to be involved in cross-reactivity to certain foods in tree pollen-sensitive patients.

It has been suggested that Bet v 1 can be a diagnostic marker allergen for identifying patients with genuine sensitisation to Birch-pollen (30), as opposed to patients reacting to highly cross-reactive allergens, such as Bet v 2 and Bet v 4; these may be considered marker allergens for syndromes involving cross-reactivity with numerous unrelated plants and plant products (3,30). In other words, patients who exhibit positive skin tests to Birch pollen extracts, but have not been exposed to Birch, might have IgE to highly cross-reactive allergens such as Bet v 2 (3).

One study gives an example of the diagnostic application of recombinant Birch pollen allergens. Specific serum IgE antibodies to recombinant allergens Bet v 1, Bet v 2 and Bet v 4 were examined in Birch-sensitive patients from the province of Cuneo, in northwest Italy. The study concluded that the 3 recombinant Birch pollen allergens alone could identify 90% of Birch pollen-sensitive patients (7).

t220 rBet v 4

ImmunoCAP®: t220 rBet v 4

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Betula verrucosa allergen* Bet v 1

Common names:	2 -EF-hand, Ca^{2+}-binding protein, CBP, Polcalcin
Biological function:	Calcium-binding protein
Mw:	8 kDa

Allergen description

Bet v 4 (7,41-43), a calcium-binding protein (44-46), is a minor allergen in birch pollen that reacts with IgE antibodies from approximately 10-20% of pollen-sensitised subjects (29,41,44). Bet v 4 is a 9 kDa calcium-binding protein of 2-EF-hand type, which is represented in pollen of a wide range of plant species. It is 67-90% identical in amino acid sequence to homologous pollen proteins from *Phleum pratense*, *Cynodon dactylon*, *Brassica rapa*, *Brassica napus*, *Olea europea* and *Alnus glutinosa* (43,47), and because of this extensive cross-reactivity can serve as a marker allergen for plant polysensitisation (48).

Bet v 3 and Bet v 4 have both been identified as EF-hand calcium-binding proteins primarily expressed in mature pollen (41,43-44). Unlike Bet v 4, which contains only 2 calcium-binding domains, Bet v 3 is a 23.7 kDa protein containing 3 typical calcium-binding motifs (48).

It has been suggested that Bet v 1 can be a diagnostic marker allergen for identifying patients with genuine sensitisation to Birch-pollen (30), as opposed to patients reacting to highly cross-reactive allergens, such as Bet v 2 and Bet v 4; these may be considered marker allergens for syndromes involving cross-reactivity with numerous unrelated plants and plant products (3).

A study gives an example of the diagnostic application of recombinant birch pollen allergens. Serum IgE antibodies to Bet v 1, Bet v 2 and Bet v 4 were examined in birch-sensitive patients from the province of Cuneo, in northwest Italy. The study concluded that the 3 recombinant birch pollen allergens alone could identify 90% of Birch pollen-sensitive patients (7).

t225 rBet v 6

ImmunoCAP®: t225 rBet v 6
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Betula verrucosa* allergen Bet v 6

Common name:	IFR, Isoflavone reductase, (PCBER)
Biological function:	Isoflavone reductase (29)
Mw:	34 kDa

Allergen description

Bet v 6, a minor Birch pollen allergen, is an isoflavone reductase (IFR). IFRs have been found in apple, pear, orange, mango, lychee, carrot, banana, pea and chickpea (29). An IFR-like protein has also been isolated from maize and tobacco (29,49). IFR has been demonstrated in legumes and alfalfa, and a IFR-like protein had been documented in maize (250-51). The laboratory and clinical relevance of IFRs in other plants has yet to be determined.

Birch IFR has a sequence identity of 56% to 80% to IFR homologues proteins from various plants (29). The IFRs are plant defense proteins and appear to be induced by plant stress. A gene that is selectively induced both in roots and shoots in response to sulfur starvation has been demonstrated (50). The role of plant stress in the induction of IFRs is demonstrated by grapefruit, which when treated to induce resistance against the mould decay, produced an isoflavone reductase-like protein which had a high homology to other isoflavone reductase-like proteins present in non-legume plants (52).

Isoflavone reductase (IFR) belongs to a family of plant proteins collectively termed as phenylcoumaran benzylic ether reductases (PCBERs) based on demonstrated catalytic activity. Bet v 6 displays a high degree of sequence identity of up to 81% with isoflavone reductase-homologous proteins (IFRH) and phenylcoumaran benzylic ether reductase (PCBER) as well as lower identities of 60% and 51% with isoflavone reductases (IFR) and pinoresinol-lariciresinol reductase (PLR), respectively. These reductases (IFR, IFRH, PCBER and PLR) all appear to be evolutionary derived from a common ancestor and each catalyzes a rather similar conversion in the isoflavonoid and lignan pathways.

Although the precise biochemical products so formed differ in each case, products of each reductase appear to be employed in plant defense. However, antibodies raised against PCBER do not cross-react with PLR (53-56). A characteristic difference between PCBER and isoflavone reductases (IFR) appears to be a 10 amino-acid insertion that is not present in PCBER, PLR and IFRH (55).

Bet v 6 has been found to share an 80% amino-acid sequence identity with Pyr c 5, a Bet v 6-related food allergen from pear. Assays with recombinant Pyr c 5 from Pear and Bet v 6 showed PCBER catalytic activity for both recombinant allergens, and both allergens had similar IgE binding characteristics and bound IgE from sera of birch-pollen-allergic and pear-allergic subjects. Inhibition experiments with Pyr c 5 suggested that homologous allergens may be present in many vegetable foods such as apple, peach, orange, lychee fruit, strawberry, persimmon, zucchini (courgette), and carrot. Laboratory tests of the recombinant Pyr c 5 using sera of a pear-allergic subject suggested that Pyr c 5 had the potential to elicit type I allergic reactions. This study's data was reported to indicate that PCBER and IFR may represent a new family of pollen-related food allergens that occur not only in typical birch-pollen-related foods, but also in rarely allergenic fruits and vegetables such as orange, strawberry, persimmon, or zucchini (55,57)

Bet v 6 may be responsible for pollen-related oral allergy to specific foods in a minority of patients with birch pollen allergy (29). Bet v 6 is recognized by IgE from approximately 32% of Birch pollen allergic individuals. Recombinant Bet v 6 bound IgE from 32% of 28 sera from patients allergic to birch pollen with a ImmunoCAP® class of at least 3 compared to Bet v 1 binding in 89% of these patients (29).

Japanese cedar pollen, a major cause of seasonal pollinosis in Japan where more than 10% of Japanese people are affected, was shown to contain an isoflavone reductase-like protein. In contrast to Bet v 6 being reported as a minor allergen, this recombinant protein exhibited an IgE binding frequency of 76% (19/25) in Japanese cedar pollen allergic patients (58).

Allergy to Sharon fruit (persimon) has been only rarely reported. Cross-reactivity with pollen (profilin, Bet v 1 and Bet v 6) appears to be involved. In a study of two patients with allergic reactions on first exposure to Sharon fruit, as well as 7 patients with birch-pollen-related apple allergy, found that an open challenge with Sharon fruit in 7 patients allergic to Birch pollen and Apple, who had not eaten Sharon fruit previously, was positive in 6/7 cases. The study concluded that Birch-pollen-related allergy to Sharon fruit is mediated by the known cross-reactive pollen allergens including Bet v 1 (59).

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Brazil nut allergen components

Bertholletia excelsa

Available ImmunoCAP®:
f354 rBer e 1

Summary

The Brazil nut is actually the seed of a giant tree that grows wild in South America's Amazon jungle. The seeds, about 6 cm long, come in clusters of 8 to 25 inside a large, hard, thick-walled globular pod that resembles a coconut and weighs up to 2 kg. Brazil nut may be eaten raw or roasted, and may be a "hidden" allergen in cookies, etc. The oil extracted from the nuts is commonly used in Peru and other South American countries to manufacture soap, and for lighting, and the empty pods are used as implements and burned to repel insects.

Allergy to Brazil nut is common. It frequently has an onset in the first few years of life, generally persists, and accounts for severe and potentially fatal allergic reactions. The ubiquity of this food in the modern diet makes avoidance difficult, and accidental ingestions, with reactions, common (1-7).

Allergens from *Bertholletia excelsa* listed by IUIS*

Ber e 1	Ber e 2

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

A number of allergenic proteins has been isolated from Brazil nut. These range in size from 4 kDa to 58 kDa (8). A 9 kDa allergen corresponding to 2S albumin of Brazil nut has been identified as a major allergen. A number of other minor allergens have been detected: of 18 kDa, 25 kDa, 33 kDa, 45 kDa and 58 kDa, including a 12S globulin protein, a legumin-like storage globulin (8-9).

The following allergens have been characterised:

Ber e 1, a 9 kDa protein, a 2S albumin, resistant to digestion by pepsin, and a major allergen (10-12).

Ber e 2, an 11S globulin-like protein (13).

f354 rBer e 1

ImmunoCAP®: f354 rBer e 1
An *E. coli* strain carrying a cloned cDNA-encoding *Bertholletia excelsa* allergen, Ber e 1

Common names:	2S Albumin
Biological function:	rBer e 1 is a 2S Albumin storage protein
Mw:	9 kDa

Allergen description

Ber e 1 is a 2S albumin, a common major storage protein found in a number of edible seeds and nuts and recognised as a panallergen. The 2S albumins are the major storage proteins in Walnut, Mustard, Sesame, Brazil nut, Peanut, Cottonseed, Sunflower and Castor bean (12,15-16).

Typical 2S albumins are small globular proteins that undergo proteolytic processing in the vacuoles of the plant cells, the full-length precursor protein is usually cloven into large and small subunits that stay associated through 2 disulphide bonds (9). For example, in Brazil nuts the precursor protein is 14-15 kDa in size, but the mature 2S albumin obtained from the nut extract consists of a large 10-12 kDa and a small 5 kDa subunit (13). S albumins are significantly resistant to proteolytic digestion, and to thermal and chemical denaturation (14). In a study evaluating Brazil nut 2S albumin, after 2 h of gastric digestion, approximately 25% of Ber e 1 remained intact. During duodenal digestion, residual intact 2S albumin disappeared quickly, but a modified form of the "large fragment" remained, even after 2 h of digestion. The main immunoglobulin E epitope region of 2S albumin allergens was found to be largely intact following gastric digestion. There were also previously identified putative T-cell epitopes (17). This was similarly demonstrated with the 2S albumin in Sesame seed and Sunflower seed. Such properties are thought to be crucial for a protein both to sensitise the mucosal immune system and to provoke an allergic reaction in a sensitised individual (14,18). rBer e 1, the recombinant Brazil nut 2S albumin, is likewise resistant to digestion by pepsin (10).

The Brazil nut 2S albumin has been recognised as a methionine-rich protein that could be used to increase the nutritional value of certain foods through genetic engineering techniques. However, the 2S albumin of the Brazil nut is also the major allergen of Brazil nuts (Ber e 1) and shows IgE-reactivity with more than 80% of the sera from Brazil nut-allergic subjects. This was also demonstrated in transgenic Soybean: the newly expressed protein in transgenic Soy retained its allergenicity (19-20).

A strong correlation between IgE-binding to 2S albumins and food-induced anaphylaxis has been demonstrated for Brazil nut and Sesame seeds (12). The 2S albumins may be very important in food-induced anaphylaxis, whereas minor Brazil nut allergens have been thought not to be relevant (12). However, a 15-year-old boy who experienced 2 distinct episodes of generalised urticaria about 30 minutes after eating Brazil nut had positive skin- and serum-specific IgE tests to Brazil nut but negative serum-specific IgE for Mustard, Poppy seed, Sesame seed and Sunflower seed, suggesting no sensitisation to the major 2S albumin allergen (9).

Brazil nut contains a 2S albumin storage protein, a protein common to many seeds, which displays similarity to the 2S albumin of Cotton, Cocoa bean, Sunflower seed, Rape seed, Castor bean, English Walnut (Jug r 1), Mustard seed (Sin a 1) and Sesame seed (Ses i 2). Comparison of the amino acid sequence shows a high degree of similarity, from 34% between Sunflower seed and Brazil nut, to >52% similarity and >38% identity between Brazil nut and many other plant 2S albumins (21-25). The English walnut allergen (Jug r 1) exhibits a 46.1% identity with the Brazil nut 2S albumin seed storage protein Ber e 1 (25-26). A 2s albumin has also been detected in Buckwheat (27).

Cross-reactivity observed between Peanut or Walnut and Brazil nut presumably depends on other ubiquitous seed storage protein allergens, namely the vicilins. However, the major IgE-binding epitope identified on the molecular surface of the Walnut Jug r 1 allergen shared a pronounced structural homology with the corresponding region of the Pecan nut Car i 1 allergen. With the exception of Peanut, 2S albumins could thus account for the IgE-binding cross-reactivity observed between some other dietary nuts, e.g. Walnut and Pecan nut (28).

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Carp allergen components

Cyprinus carpio

Available ImmunoCAP®:

f355 rCyp c 1

Summary

There are no allergens from *Cyprinus carpio* listed by International Union of Immunological Societies (IUIS) January 2008.

The Carp is a native of Asia, but extensive introductions have helped to make it the world's most widely distributed freshwater fish.

The Carp is recognised by its small eyes, thick lips with two barbels at each corner of the mouth, large scales and strongly serrated spines in the dorsal and anal fins. The colour is variable, but often olive green to silvery grey dorsally, fading to silvery yellow on the belly. Small Carp could be confused with Goldfish, *Carassius auratus*. The latter, however, have no barbels on the corners of the mouth.

Carp are reported to grow to over a metre in length and 60 kg in weight, but 4-5 kg is more usual. They are omnivorous, sucking and straining mud from the bottom, and insects and plants from the surface.

The common Carp has been introduced as a food and ornamental fish into temperate freshwaters throughout the world.

The following allergen has been characterised:

Cyp c 1, a parvalbumin (1).

f355 rCyp c 1

ImmunoCAP®: f355 rCyp c 1
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Cyprinus carpio* allergen Cyp c 1

Common names:	Parvalbumin
Biological function:	Calcium binding and muscle functioning protein
Mw:	12 kDa

Allergen description

Cyp c 1 is a major fish protein, a parvalbumin (1-3). Parvalbumins are small, acidic calcium-binding buffer proteins found in fast muscle of lower and higher vertebrates. They are thought to be involved in the relaxation process in fast-twitch muscle. They have been identified as the major fish allergens (3). This is the basis for the use of recombinant Carp parvalbumin as a tool for *in vitro* and *in vivo* diagnosis of fish allergy. Carp parvalbumin is a 3 EF-hand calcium-binding protein. It has remarkable stability, which explains why, despite cooking and exposure to the gastrointestinal tract, it (along with other paralbumins) can sensitise patients.

Recombinant Carp parvalbumin was found to contain 70 % of the IgE epitopes present in natural extract of Cod, Tuna and Salmon. This suggested that the substance would make a valid tool in the diagnosis of patients with fish allergy (4). In a study aimed at characterising cross-reactive IgE-binding components in 6 different fish species (Cod, Tuna, Salmon, Perch, Carp, and Eel), sera from 30 patients allergic to fish found IgE reactivity to a common allergen in all. This allergen was identified as a parvalbumin and shown to have cross-reactive IgE epitopes (5).

Two Carp parvalbumin isoforms (Cyp c 1.01 and Cyp c 1.02) with comparable IgE binding capacities have been isolated. rCyp c 1.01 reacted with IgE antibodies from all 60 fish-allergic patients tested, induced specific and dose-dependent basophil histamine release, and contained most of the IgE epitopes (70%) present in natural allergen extracts from Cod, Tuna, and Salmon, thus demonstrating the value of recombinant Carp parvalbumin as a diagnostic tool (2).

Parvalbumin has been shown to be a major allergen in other fish. Parvalbumin from Alaska Pollack, a globally important commercial fish species belonging to the *Gadidae* family (which includes Atlantic cod), was shown to be as potent in antibody binding as Cod Gad c 1 (which has been thoroughly studied and considered as a reference for sensitisation in fish allergy) (6).

Purified Carp parvalbumin has been shown to react with IgE antibodies of more than 95% of individuals allergic to fish, and to contain around 83% of the IgE epitopes present in other fish species(1). Although cross-reactivity may extend to other fish containing parvalbumin, the degree of cross-reactivity would depend on the degree of homology. For instance, a study used sera from 10 patients allergic to fish to evaluate the cross-reactivity among 9 commonly edible fish: Cod, Salmon, Pollack, Mackerel, Tuna, Herring, Wolffish, Halibut, and Flounder: Cod (Gad c 1), Salmon (Sal s 1), Pollack (The c 1), Herring, and Wolffish

were shown to share antigenic and allergenic determinants, whereas Halibut, Flounder, Tuna, and Mackerel displayed the lowest level of cross-reactivity. The highest mean IgE ELISA inhibition was obtained by Gad c 1, followed by The c 1, Herring, Sal s 1, Wolffish, Halibut, Flounder, Tuna, and finally Mackerel with the least. Nine of the 10 patients showed positive skin test to Cod, Salmon, and Pollack; 8 patients reacted to recombinant Sal s 1. Positive skin-reactivity to rGad c 1 and rThe c 1 was demonstrated in 1 patient (7).

Parvalbumin has also been shown to be a major allergen in 3 species of Mackerel (*Scomber japonicus*, *S. australasicus* and *S. scombrus*) that are widely consumed and considered to be most frequently involved in incidents of IgE-mediated fish allergy in Japan. In a study in which parvalbumin was purified from the white muscle of 3 species of Mackerel, 4 of 5 sera from fish-allergic patients reacted to all the purified parvalbumins, demonstrating that parvalbumin is the major allergen in common among the various Mackerels (8).

Cod parvalbumin has been shown to also share IgE binding epitopes with frog parvalbumin. In a study investigating whether IgE antibodies of fish allergic persons cross-react with frog parvalbumin, sera of 15 fish-allergic patients and 1 fish- and frog-allergic patient were tested by IgE-immunoblotting against recombinant parvalbumin alpha and beta from frog muscle extract. Fourteen of the sera tested had IgE antibodies recognising low-molecular-weight components in frog muscle extract. Tested against recombinant parvalbumins, 3 of 13 sera reacted with alpha parvalbumin and 11 of 12 reacted with beta parvalbumin from frog. Skin prick tests performed in selected patients with recombinant frog parvalbumin were positive in fish-allergic patients. Inhibition studies showed that a fish- and frog-allergic patient was primarily sensitised to fish parvalbumin (9).

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Cat allergen components

Felis domesticus

Available ImmunoCAP®:

e94 rFel d 1
e220 nFel d 2

Summary

The Cat is a small feline carnivorous mammal of the subspecies *Felis domesticus*. The Domestic cat is now considered a subspecies of the Wild cat. There are dozens of breeds of Cats.

Several studies have reported that Cat allergen is a risk factor for childhood asthma (1-3). Recent studies have also reported the contrary, that sensitisation to Cat allergen protects against allergic disease (4-5).

The majority of studies, however, have reported that Cat allergens are associated with increased allergic disease. Allergy has been reported to Cat dander, Cat saliva, Cat urine and Cat serum. But Cat serum and Cat urine collected by bladder puncture have no detectable levels of Fel d 1 (1).

Different prevalences of sensitization to cat allergens in atopic patients have been reported from many countries all over the world and figures range from 1-75 % (6-12).

Cat allergen is ubiquitous and may be found in many environments, transferred even by an individual's hair. This kind of phenomenon results in Cat allergen being found even in environments with strict allergen avoidance measures (13). Even upholstered seats in workplaces may constitute significant reservoirs of Cat allergens (14).

Individuals may be sensitised to a range of Cat allergens and, by extension, to sources for these allergens. For example, analyses of sera from 43 individuals with a history of Cat allergy showed that 39.5% were positive to Cat pelt, 37.5% to Cat saliva, and 12% each to Cat urine and serum. The Cat pelt and saliva extracts contained Fel d 1, but Cat serum and Cat urine collected by bladder puncture had no detectable levels of this allergen (1). The pelt allergens were mainly of salivary origin (15). Early studies reported that allergens from different Cat breeds appear to be closely related (16).

Allergens from *Felis domesticus* listed by IUIS*

Fel d 1	Fel d 2	Fel d 3
Fel d 4	Fel d 5w	Fel d 6w

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

At least 13 serum-related and 8 dander-related antigens have been identified in Cat dandruff and hair (17), but Fel d 1 is the most important (18). Sizes range from 10 to 66 kDa (19-20).

The following allergens have been characterised.

Fel d 1, a uteroglobin-like protein (21).

Fel d 2, serum albumin (22-23).

Fel d 3, cystatin, a cysteine protease inhibitor (24).

Fel d 4, a Lipocalin (25).

Fel d 5 (26).

Fel d 6 (26).

Fel d 7 (26).

See Cat dander e1 and Cat serum albumin e220 for further details.

e94 rFel d 1

ImmunoCAP®: e94 rFel d 1

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Felis domesticus* allergen Fel d 1

Common names:	Cat I, Ag4, Fel d 1-related protein
Biological function:	Uteroglobin-like protein
Mw:	38 kDa

The allergen is a 38 kDa dimer composed of two 19 kDa subunits. Each 19 kDa subunit comprises 2 disulfide-linked polypeptide chains, a light alpha-chain and a heavy beta-chain containing an N-linked oligosaccharide (39)

Allergen description

Although more than 12 allergens have been identified in Cat, Fel d 1 (27-38) is the most important, eliciting IgE responses in 80% to 90% of patients with Cat allergy and accounting for 60% to 90% of the total allergenic activity of Cat extracts (21,40-41).

Fel d 1 is found in Cat hair, dander and saliva. Fel d 1 was found to be significantly higher at the base than the tip of the hair (42). A study that tried to demonstrate that Fel d 1 can accumulate on Cat skin without licking, i.e., that Fel d 1 originates from skin, found that Fel d 1 is in fact produced by Cat skin (43-44). Fel d 1 levels on the skin are dramatically higher on the facial area than on the chest of the Cat. Washing reduces levels of this major allergen on Cat skin and fur, but the accumulation on skin is restored within 2 days (45).

The allergen is produced primarily in Cat sebaceous glands and, to a lesser extent, by basal squamous epithelial cells, from which it is secreted onto the skin and fur (46). It is also produced, though to an lesser extent, in salivary glands and excreted into the saliva (18,47). The sublingual salivary glands and the anal glands are also involved in production of the allergen. The allergen is thought to be under hormonal control; male Cats produce more Fel d 1 than female Cats, castration reduces its production, and testosterone injections into castrated Cats allow recovery of production (48-49). The presence of Fel d 1 has also been demonstrated in the serous cells of the lacrimal gland (50).

Long- and short-haired Cats produce this allergen. The allergen is carried on particles ranging from less than a micrometre to greater than 20 micrometres in mean aerodynamic diameter. At least 15% of this allergen is carried on particles less than 5 micrometres in diameter (51-52).

Cat allergy is unique among allergies to mammals in that the major allergen Fel d 1 is an uteroglobin-like protein and not a lipocalin. Its function is not known, but researchers have proposed that it is involved in protecting dry epithelia, which would be a function parallel to uteroglobin protecting wet epithelia. Since Cats lick themselves and each other extensively, coating their pelts with this protein may be part of this or another essential biological function (53).

Fel d 1 is secreted in copious amounts and accumulates in house dust. Fel d 1 levels in domestic living rooms are not related to Cat colour or hair length (54). In some women with a Cat at home, the human hair constitutes a significant reservoir of Fel d 1. The amounts of Cat allergen involved might contribute to allergic sensitisation when released in Cat-free environments (55). Transfer through human hair may help explain why Cat allergen is found even in environments with strict allergen avoidance measures. Hair may be an important means of transfer and deposition of Cat allergen in schools (13). The concentrations of Cat (Fel d 1) and Dog (Can f 1) allergens may even be higher in dust collected in schools than in homes (56). Similarly, the highest levels of Fel d 1 have been found in homes with a Cat, but high levels have also been found in homes of allergy patients who did not have a Cat but visited others with Cats (57). Upholstered seats in workplaces have also been reported to constitute a significant reservoir of Cat allergens (and also of House dust mites) (14).

Recombinant Fel d 1 shows biologic activity similar to that of the native form (58). Recombinant Fel d 1, consisting of chain 2 and chain 1 fused together without an additional linker, seem to have immunological properties indistinguishable from the natural heterodimeric protein (30). In a study of 258 Cat-allergic individuals, excellent quantitative correlation between IgE and IgG antibody binding to rFel d 1 and nFel d 1 was demonstrated (33).

In a study of sera of 509 Cat-allergic individuals, selected on the basis of the presence of Cat serum-specific IgE and tested by RAST for IgE reactivity to purified Fel d 1, Cat albumin (CA), or both, natural and recombinant Fel d 1 exhibited similar results: 94.1% and 96.1% positive test results, respectively. The addition of determining Cat albumin (16.7% positive sera) resulted in a decrease in the number of discrepancies between purified allergens and whole extract to 2.8%. In 2% of all sera, sensitisation to Cat was largely explained by IgE reactivity to Cat albumin. The authors concluded that natural and recombinant Fel d 1 are good candidates for replacing Cat dander extracts in diagnostics for Cat allergy (34).

Recombinant hypoallergenic Fel d 1 with reduced IgE binding capacities and retained T cell reactivity may therefore be of value in the assessment of Cat allergy and in immunotherapy (59).

e220 nFel d 2

ImmunoCAP®: e220 nFel d 2
Native cat serum albumin purified from *Felis domesticus*

Biological function:	Serum albumin
Mw:	65-69 kDa

Allergen description

Cat albumin (Fel d 2), a 65-69 kDa protein, is found in serum, dander and saliva (22-23). About 15%-25% of Cat-allergic individuals are sensitive to Cat albumin, and for a few patients this may be the predominant allergen (17,20,60-63).

Albumins from Cat, Dog and Horse share some epitopes that account for the cross-reactivity observed in around a third of patients sensitised to Cat, Dog and Horse, but more than 50% of specific IgE that cross-reacts among these 3 animals is directed to allergens other than albumin (64-65). Significant cross-reactivity has been reported between Cat hair and Dog dander in specific IgE inhibition studies, whereas saliva and urine were more species-specific (66). Although a high degree of sequence homology exists among different animal albumins, a remarkable variability of IgE cross-reactivities has been observed, indicating that some patients are sensitised preferentially against certain albumins. Most of the patients allergic to albumins, however, reacted to Dog, Cat, and Horse albumin, which also bound a high percentage of albumin-specific IgE (67).

Some Cat-allergic individuals are likely to experience allergic symptoms following the consumption of Pork. Inhibition experiments showed that the spectrum of IgE reactivity to Cat serum albumin completely contained IgE reactivity to Porcine serum albumin. Sensitisation to Cat appears to be the primary event. Sensitisation to Cat serum albumin should be considered a useful marker of possible cross-sensitisation not only to Porcine serum albumin but also to other mammalian serum albumins (24).

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Apium graveolens

Available ImmunoCAP®:
f417 rApi g 1.01

Summary

Celery is an herbaceous edible biennial plant in the family *Apiaceae*, native to the coasts of western and northern Europe, and to the Middle East. It was used by the ancient Greeks and Romans as a flavouring. The ancient Chinese used it as a medicinal plant.

The wild form of Celery is known as smallage. The stalks are furrowed and more stringy, the leaves are wedge-shaped, and the taste is rank and bitter. The most common commercial variety now sold is the Pascal variety, although gardeners can grow a range of cultivars under two classes, white and red.

Celery grows to 1 m tall, with pinnate to bipinnate leaves and rhombic leaflets 3-6 cm long and 2-4 cm broad. The edible Celery stalk is not a plant stem but a petiole, which is part of a leaf.

Celery stalks are not only consumed raw as fresh salad but also as a cooked vegetable and as a constituent of sauces and soups.

Celery seed is dried and used as a spice. When it is combined with salt, the resulting blend is called Celery salt. The furano-coumarin bergaptene, found in the seeds, is a potent photosensitiser and may cause photo-dermatitis, particularly in gardeners and field workers.

Celeriac (*Apium graveolens rapaceum*) is a species variety, forming a greatly enlarged, solid, globular body just below the soil surface. It is not used raw, but is especially suited for soups and stews.

The first case of allergic reaction to Celery root was reported in 1926 (1). Since then, a number of studies from across the world, and in particular from European countries, have documented the high prevalence of allergy to Celery, especially in association with cross-allergy to pollen (2-15). IgE antibodies to Celery may occasionally be present in an individual's sera without clinical sensitisation occurring (3).

Allergens from *Apium graveolens* listed by IUIS*

Api g 1	Api g 3	Api g 4
Api g 5		

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

In Switzerland, about 40% of patients with food allergy are sensitised to Celery, some experiencing severe anaphylactic reactions (13-14). Other studies have reported an even higher prevalence of allergy to Celery; in one study this was 42% (23); among the 69% of a group of 32 patients with a history of Celery allergy, DBPCFC resulted in systemic reactions in 50% (11/22) (4). In a study from 1978 to 1982, 173 cases of food allergy were diagnosed in patients (predominantly adults) attending the University of Zurich. The most frequent food allergens were found to be Celery in 40.5%, Carrots (20%), Green beans (6%), Hen's egg (21%), Cow's milk and other dairy products (20%) and fish (12%) (17).

In France, 30% of 580 patients with food allergy were sensitised to Celery, as determined by IgE antibodies. Sixty presented with severe, near-fatal reactions, in which the most common food implicated

was Celery: 30% of severe anaphylactic reactions to food were thought to be due to Celery, according to patient histories (15).

In Germany, of 167 patients with a pollen-related food allergy, 70% were sensitised to Celery, as shown by skin test or allergen-specific IgE antibodies, and 14% reported clinical allergy to Celery (18).

Celery can cause oral symptoms (aphthae, stomatitis, swelling of the lips or tongue, pharyngitis, hoarseness and laryngeal oedema) and often also induces acute generalised symptoms, such as severe laryngeal oedema, bronchial asthma, urticaria and allergic shock (19). Oral allergy syndrome has been documented (20-21), and the symptoms have been reported to be more marked in severity compared to reactions to other vegetables (22).

Early allergen studies indicated the presence of IgE antibody binding to Celery proteins of molecular weight of around 14 kDa, 15 kDa, 16 kDa, and 17 kDa (2,23). Celery was also shown to contain at least 3 distinct cross-reacting allergens: a homologue of Bet v 1, a homologue of Birch profilin (Bet v 2), and a group of proteins with a molecular-weight range of 46 to 60 kDa (3). These allergens cross-reacted not only with Birch and Mugwort pollen, but also with a number of other fruits and vegetables (28). Early studies did not necessarily differentiate between Root celery (Celeriac) and Stick Celery, possibly presuming the allergens to be similar.

A number of allergens have been identified and characterised:

Api g 1, the major allergen, a 16 kDa protein and a Group 1 *Fagales*-related protein (a Bet v 1 homologue) (4,20,24-27,29-35).

Api g 1.0101 and Api g 1.0201, the isoforms of Api g 1 (27,36).

Api g 3, a chlorophyll Ab-binding Protein.

Api g 4, a 14.3 kDa protein, a profilin, a minor allergen (18,31-32,37-43).

Api g 5, isolated from the tuber, is a 60 kDa protein, a glycoprotein with homology to FAD-containing oxidases (44-45). This allergen carries carbohydrate determinants with cross-reactive structures (CCD); and importantly, convincing evidence that IgE directed to CCD is capable of eliciting allergic reactions in vivo has been reported (44-45).

A lipid transfer protein has also been detected (46-47).

The presence of CCDs (cross-reactive carbohydrate determinants) has been reported (36). Celery-allergic individuals have been shown to be monosensitised to CCDs, with exclusively CCD-specific IgE (37). A report stated that IgE specific for CCDs is common in Celery-allergic patients and can represent the major proportion of IgE against this food. Alpha 1, 3-fucose was shown to be an essential part of the IgE epitope, and immunoblotting inhibition indicated the presence of this carbohydrate determinant on multiple glycoproteins in Celery extract (5). Similarly, other studies have concluded that ubiquitous CCDs are important in allergy to Celery (and Zucchini) (4); and that, depending on the structure of the CCD-containing glycoproteins, CCDs can indeed be important epitopes for IgE; they may be clinically relevant allergens in certain patients and irrelevant in others (37).

One major allergen of Celery, possibly the lipid transfer protein, has been shown to be heat-stable. Heating Celery tuber for 30 minutes at 100 degrees C did not deplete the immunoreactivity of the major allergens (48). Other studies have concurred (49); Celery remained allergenic even after extended thermal treatment (430.5 min/100 °C), indicating that Celery spice (dried and powdered Celery) is allergenic for patients with an allergy to raw Celery (50). All patients undergoing DBPCFC with Celery spice reported reactions comparable to symptoms observed with raw Celery challenges (50).

f417 Api g 1.01

ImmunoCAP®: f417 Api g 1.01
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Apium graveolens* allergen Api g 1.01

Common name:	Bet v 1-homologous allergen, Group 1 *Fagales*-related protein, PR-10 protein
Biological function:	Ribonuclease
Mw:	16 kDa

Allergen description

Api g 1 is a major Celery allergen and a Bet v 1-homologous protein (a *Fagales*-related protein) (35). Api g 1 has been shown to be a heat-labile protein, but stable upon exposure to high voltage, high pressure, gamma rays, drying and powdering, and therefore having allergenicity potential as a spice (18).

Api g 1 has had 2 isoforms characterised: Api g 1.0101 and Api g 1.0201, which share only a 52% sequence identity between each other and have approximately 40% identity with Bet v 1 (35). Compared with Api g 1.0201, Api g 1.0101 lacks Leu, and the negatively charged Glu is substituted for by the positively charged Lys (51).

In studies examining the prevalence of IgE antibodies against Api g 1, results varied from 59% of 22 patients who had positive DBPCFC to Celery (37), to 80% of 30 patients with pollen allergy reporting immediate allergy after ingestion of raw Celery (52), to 74% of a group of 23 patients with IgE mediated Celery allergy (3).

The Birch pollen allergen Bet v 1 plays a significant role in the cross-reactivity described. Celery Api g 1 has a 40% identity with (60% similarity to) the major allergen of Birch pollen, Bet v 1 (20), and Birch pollen-allergic individuals frequently develop IgE mediated reactions to Celery (53-54). A number of studies have demonstrated that cross-reactions among Birch pollen, Celery, Carrot, and various fruits and vegetables are based on allergens related to Bet v 1 and Art v 1, the major allergens of Birch and Mugwort pollen, respectively (26,32,54-55).

Considering that Api g 1, the major Celery allergen, is a homologue of the major Birch pollen allergen Bet v 1 (35), cross-reactivity with homologous proteins in Apples, stone fruits, Carrot, nuts, Soybean, Hazelnuts and pollens of several tree species can be expected to varying degrees (3, 56). Approximately 70% of patients who are allergic to Birch pollen may experience symptoms after consumption of foods from these groups (57).

The patterns may appear complex. For example, among sera of 61 patients with IgE antibodies to Mugwort pollen, 36 were positive for Celery and 23 had IgE antibodies to Birch pollen (23). Similarly, of 196 Birch pollen-hypersensitive patients with oral allergy syndrome (OAS), 195 had Apple and/or Hazelnut allergy, and 103 had *Apiaceae* sensitivity; only 1 patient had *Apiaceae* (Carrot, Celery, and Fennel) allergy alone. The study suggested that most *Apiaceae* determinants cross-react with Apple or Hazelnut determinants, whereas only some Apple or Hazelnut determinants cross-react with *Apiaceae*-allergenic determinants (58). Similarly, cross-reactivity has been reported between Celery and Zucchini, and it is stated that a specific association with Birch pollen allergy exists in allergy to Celery (mediated by Api g 1), but not in Zucchini allergy (4).

Nevertheless, epitope differences between Bet v 1-related food allergens exist, indicating different degrees of cross-reactivity among these allergens (59).

Similar results with other allergens have been reported: concurrent sensitisation to Mugwort and Birch pollen and to Camomile may occur, and binding was inhibited to varying degrees by extracts from Celery and Anise, and by pollen from Mugwort, Birch and Timothy grass. Profilins were not detected in the Camomile extracts (60).

In a study of the IgE antibody binding of 50 Bet v 1-positive patients' sera to different food allergens, reactions with homologous Bet v 1 allergens were in the following proportions: 99% with Mal d 1 (Apple), 93% with Cor a 1 (Hazelnut), 59% with Api g 1 (Celery) and 38% with Dau c 1 (Carrot). Vice versa, patients with Birch pollen-related food allergy were predominantly sensitised to Bet v 1 homologues and less frequently recognised other allergens contained in both sources, e.g., profilins (56).

Individuals may be allergic to Celery without allergy or sensitisation to Birch tree pollen; 8% of Swiss patients allergic to Celery were not sensitised to rBet v 1 or rBet v 2 (61). Similarly, in a study of sera from 4 patients showing strong immediate systemic reactions after contact or ingestion of raw Carrot, all the patients had significant levels of IgE antibodies to Carrot allergen, Dau c 1, a Bet v 1 homologue, but no IgE antibodies to Birch pollen was detected in any. The sera contained a single band of around 18 kDa in raw Carrot and in Celery (with a weaker reaction), but no reactive band was found with Birch pollen. The Carrot IgE-binding protein's N-terminal sequence was homologous to that of Bet v 1 and to allergens previously described in Celery and other foods. The 4 patients studied were not sensitised to Birch pollen, and 3 of them tolerated fruit ingestion. The study indicated that a sensitisation to Dau c 1 can induce IgE antibodies that do not cross-react with Birch pollen allergens (62).

Research has focused on the T cell response and epitope involvement influencing cross-reactivity between Birch pollen and Celery. In a study evaluating the T cell response to the major allergen Api g 1 in Celery, along with the cellular cross-reactivity with its homologous major allergen in Birch pollen, Bet v 1, the latter allergen was identified as the most important T cell epitope for cross-reactivity with Api g 1. The study concluded that the activation of Bet v 1-specific Th2 cells by Api g 1, in particular outside the pollen season, may have consequences for Birch pollen-allergic individuals (63). A study investigating the IgE-binding capacity of 2 cross-reactive allergens, Apg1.0101 from Celery and Pru av 1 from Cherry, showed that the IgE-binding epitopes are highly patient-specific (51, 64).

The influence of stronger IgE binding, and of the dissimilar sequence identity of rApi g 1.0101 compared to rApi g 1.0201, on clinical expression and cross-reactivity may be clarified in future studies using the 2 recombinant allergens.

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Cow's milk allergen components

Bos domesticus

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f76 α-lactalbumin (nBos d 4)
f77 β-lactoglobulin (nBos d 5)
e204 Bovine serum albumin, BSA (nBos d 6)
f78 Casein (nBos d 8)
f334 Bovine lactoferrin (nBos d lactoferrin)

Allergens from *Bos domesticus* listed by IUIS*

Bos d 2	Bos d 3	Bos d 4
Bos d 5	Bos d 6	Bos d 7
Bos d 8		

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

Summary

Milk contains more than 40 proteins, and all of them may act as human species antigens.

Milk of ruminant species other than Cow (e.g., buffalo, Sheep, Goat, human, and many other species) is constituted from the same or very homologous proteins, which share the same structural, functional, and biological properties. However, human milk does not contain β-lactoglobulin (beta-lactoglobulin)(BLG) (2). Human and Bovine milk differ substantially in the ratio of Whey to Casein protein (approximately 60:40 in human milk and approximately 20:80 in Bovine milk) and in the proportions of specific proteins (3).

Milk composition changes during processing. Cow's milk contains approximately 30 to 35 g/L (3-3.5%) of Cow's milk proteins (CMPs), which can be divided into 2 main classes: Caseins (80%) and Whey proteins (20%) (4). Caseins are precipitated out by chymosin (rennin) or the acidification of the Milk to pH 4.6, forming the coagulum (curd). The Whey or Lactoserum remains soluble in the Milk serum. Lactoserum constitutes approximately 20% of the CMPs, and coagulum approximately 80% of the CMPs. Caseins and Whey proteins show very different physico-chemical properties.

Coagulum contains the Casein fraction, comprising 4 proteins: α_{S1}-, α_{S2}-, β -, and κ-caseins (alpha$_{S1}$-, alpha$_{S2}$-, beta -, and kappa-caseins). Lactoserum contains mainly globular proteins, β-lactoglobulin (beta-lactoglobulin)(BLG) and α-lactalbumin (alpha-lactalbumin)(ALA), followed by minor constituents such as Bovine serum albumin (BSA), Lactoferrin (LF), immunoglobulins (Ig) and proteosepeptone. BLG and ALA are the major ones and are synthesised in the mammary gland. Others, such as BSA, Lactoferrin, and immunoglobulins, come from the blood. Proteosepeptone is derived from Milk proteins through the action of indigenous enzymes, the most significant of which are the hydrolases, such as the lipoprotein lipase, plasmin, and alkaline phosphatise (5). In addition to the above-mentioned proteins, proteolytic fragments of Casein and fat globule membrane proteins have been reported to occur in this fraction (6).

Cow's milk allergen components

The main characteristics of the major Milk proteins are presented in Table 1 (7-8).

Table 1. Main Characteristics of the Major Bovine Milk Proteins (1).

Milk Proteins	Concentration in Milk (g/L)	Molecular weight (kDa)
20% Whey (approximately 5 g/L)		
10% BLG (Bos d 5)	3-4	18.3
5% ALA (Bos d 4)	1-1.5	14.2
3% Immunoglobulins Bos d 7	0.6-1.0	150
1% BSA (Bos d 6)	0.1-0.4	66.3
Traces of Lactoferrin	0.09	80
80% Whole Casein (Bos d 8) (approximately 30 g/L)		
32% αS1-casein	12-15	23.6
10% αS2-casein	3-4	25.2
28% β-casein	9-11	24.0
10% κ-casein	3-4	19.0

Abbreviations: ALA; α-lactalbumin, BLG; β-lactoglobulin, BSA; Bovine serum albumin.

It was classically accepted that the major allergen in Cow's milk allergy was beta-lactoglobulin, but subsequent research has proved that sensitivity to the various Cow's milk proteins is widely distributed (3,9,10).

There is a great variability in human IgE response to Cow's milk, and no single allergen or particular structure can account for a major part of Milk allergenicity (2).

Studies of large populations of allergic patients show that most of the patients are sensitised to several proteins, including BLG (Bos d 5), Casein (Bos d 8), ALA (Bos d 4), BSA (Bos d 6), Lactoferrin, and immunoglobulins (Bos d 7). A great variability is observed in IgE antibody response. Both Casein and BLG, as well as ALA, are major allergens. However, proteins present in very low quantities, such as BSA, immunoglobulins, and especially Lactoferrin, also appear to be important, since 35% to 50% of patients are sensitised to those proteins and sometimes to those proteins only (2).

Lactoserum (Whey)

β-lactoglobulin (BLG)

BLG is the most abundant protein in Whey, accounting for 50% of total protein in the Lactoserum fraction. It has no homologous counterpart in human milk.

α-lactalbumin (ALA)

ALA is a monomeric globular calcium-binding protein representing about 25% of Lactoserum (Whey) proteins. It is a regulatory component of the enzymatic system of galactosyl transferase responsible in mammary secretory cells for the synthesis of lactose.

Bovine serum albumin (BSA)

BSA accounts for around 5% of the total Whey proteins. BSA is physically and immunologically very similar to human blood serum albumin. Its main role is the transport, metabolism and distribution of ligands and the protection from free radicals (7).

Lactoferrin (LF)

LF is a protein of mammary origin and is a Milk-specific iron-binding glycoprotein of the Transferrin family. It can be found in the Milk of most species at levels lower than 1%. LF is present in much higher concentrations in human breast milk, and particularly in colostrum, as compared to Bovine milk. Although it is present in very low concentrations in Cow's milk, it has been shown to be an important allergen.

Immunoglobulins

The Immunoglobulin (Ig) fraction, which includes IgG and IgE, accounts for about 1% of total Milk protein and 6% of Whey protein. The basic structures of Ig in Bos species are very similar to those in humans, possessing a basic "Y- shaped" unit composed of 4 polypeptide chains linked through intra- and intermolecular disulfide bonds (4). Three IgG classes in Cattle have been recognised as IgG1, IgG2 and IgG3 (11). Data on the potential allergenicity of Bovine immunoglobulins are very limited. However, some studies propose IgG as another Milk allergen due to the observation that IgE from CMA patients specifically binds Bovine IgG (23). Bovine IgG has been reported to be a major Beef allergen (12).

Proteose-peptone

The proteose-peptone fraction represents about 1.1% of the total Milk protein. it is a heat-stable and acid-soluble protein fraction of Milk with important functional properties. This Milk component is derived mainly from the proteolysis of Beta-casein, and the enzymatic activity of plasmin can over time increase its concentration in Milk (4).

Coagulum

Casein

The coagulum consists of the whole Casein fraction (i.e., the solid fraction of proteins obtained after coagulation of Milk). It is subdivided into a number of families, of which the most important are α_{S1} -, α_{S2} -, β-, κ-, γ-caseins (alpha$_{S1}$ -, alpha$_{S2}$ -, beta-, kappa-, gamma-caseins) (4). Each individual Casein represents a well-defined chemical compound, but they cross-link to form ordered aggregates (nanoclusters) (i.e., micelles) that assemble into larger structures, forming Casein micelles characterised by a central hydrophobic part and a peripheral hydrophilic layer in suspension in Lactoserum (Whey) (13-14). Their proportion in the micelles is relatively constant at approximately 37%, 13%, 37%, and 13%, respectively.

The main characteristics that should be emphasised are the multiplicity and diversity of proteins that are involved in Cow's milk allergy (CMA). Polysensitisation to several proteins occurs most often, and all Milk proteins appear to be potential allergens (1). A great variability is observed in the affinity, specificity and magnitude of IgE responses in patients' sera (15). Most Milk-allergic patients are sensitised to several proteins, including BLG (Bos d 5), Casein (Bos d 8), ALA (Bos d 4), BSA (Bos d 6), Lactoferrin, and Immunoglobulins (Bos d 7) (10,14,16-22). A great variability is observed in IgE response (1).

Casein and BLG, as well as ALA, are major allergens. However, proteins present in very low quantities, such as BSA, immunoglobulins, and especially lactoferrin, also appear to be important since 35% to 50% of patients are sensitized to those proteins and sometimes to those proteins only (19). In the last few years, sensitivity to Casein seems to have increased in terms of both frequency and intensity of IgE response (1). Sensitizations to Casein, BLG, and ALA are closely linked. In contrast, sensitivity to BSA appears to be completely independent, with 50% of the patients being sensitized to BSA regardless of their sensitivity to other Milk allergens (1).

The role of various Cow's milk proteins (CMPs) in the pathogenesis of CMA is still controversial. Sera from 20 Milk-allergic subjects have been used for Cow's milk major allergen identification. The prevalence of CMP allergens has been measured as the following: 55% Alpha(s1)-casein, 90% Alpha(s2)-casein, 15% Beta-casein, 50% Kappa-casein, 45% Beta-lactoglobulin, 45% BSA, 95% IgG-heavy chain, 50% Lactoferrin, and 0% Alpha-lactalbumin (23).

f76 nBos d 4

ImmunoCAP®: f76 nBos d 4
Native protein purified from cow's milk (*Bos domesticus*)

Biological function:	α-lactalbumin
Mw:	14 kDa

Allergen description

α-lactalbumin (Alpha-lactalbumin) (ALA) is one of the major allergens in Cow's milk and represents about 25% of Lactoserum (Whey) proteins and approximately 5% of Cow's milk protein. (See Table 1, page 52.)

Human and Bovine milk differ substantially in the ratio of Whey to Casein protein (approximately 60:40 in human milk, and approximately 20:80 in Bovine milk) and in the proportions of specific proteins. Although current infant formulas closely mimic the ratio of total Whey to Casein in human milk, the concentration of ALA (the dominant whey protein in human milk) is relatively low in formula, whereas Beta-lactoglobulin, a protein not found in human milk, is the most dominant Whey protein in formula (3). During ALA's digestion, peptides appear to be transiently formed that have antibacterial and immunostimulatory properties, thereby possibly aiding in the protection against infection. A novel folding variant ("molten globule state") of multimeric ALA has recently been discovered that has anti-infective activity and enhances apoptosis, thus possibly affecting mucosal cell turnover and proliferation. Cow's milk also contains ALA, albeit less than human milk (2-5% of total protein in Bovine milk), and protein fractions enriched with ALA may now be added to infant formula to provide some of the benefits of human ALA (3). Recently, Whey sources with elevated concentrations of ALA have become available, which has permitted the development of formulas with increased concentrations of this protein and decreased concentrations of Beta-lactoglobulin (3).

Bos d 4, Alpha-Lactalbumin, is a 14.2 kDa protein (4,28,37,86-101).

An isoform, Bos d 4.0101, has been characterized.

ALA is a monomeric globular calcium binding protein with a molecular weight of about 14 kDa and 4 disulfide bridges, representing about 25% of Lactoserum (Whey) proteins. The protein is stabilised by 4 disulfide bonds and contains 2 structural domains. One of these domains (the alpha-domain) is rich in alpha-helix. The other domain (the beta-domain) is rich in beta-sheet, has 2 disulfide bonds, and includes 1 calcium binding site (102).

ALA plays a central biochemical role in the mammary gland as the regulatory subunit of lactose synthase, and also plays a nutritional role for the rapidly growing neonate as the protein in highest concentration in human milk (103). It is a regulatory component of the enzymatic system of galactosyl transferase, responsible for the synthesis of lactose in mammary secretory cells. It interacts with the enzyme beta-1,4-galactosyltransferase to form the lactose synthase complex. ALA modifies the substrate specificity of beta-1,4-galactosyl-transferase, allowing the formation of lactose from glucose and UDP-galactose (5). In its role in the production of lactose, this protein plays a major role in regulating physiological functions in the mammary gland (104).

ALA possesses a high-affinity binding site for calcium, and this bond stabilises its secondary structure. The complete amino acid sequence of Bovine ALA shows extensive homology with Hen's egg white lysozyme but also with human ALA (87-90). Some forms of ALA can induce apoptosis in tumour cells (105).

ALA is a simple model Ca^{2+} binding protein, which does not belong to the EF-hand proteins. It is a classical example of molten globule state. It has a strong Ca^{2+} binding site, which binds Mg^{2+}, Mn^{2+}, Na^{+}, and K^{+}, and several distinct Zn^{2+} binding sites. The binding of cations to the Ca^{2+} site increases protein stability against heat and various denaturing agents, while the binding of Zn^{2+} to the Ca^{2+}-loaded protein decreases its stability. Some folding variants of Alpha-LA demonstrate bactericidal activity (106).

ALA is characterised by 4 disulfide bridges and is present in 2 variants. As Bovine ALA shows a 72% sequence identity to human ALA, it makes an ideal protein for the nutrition of human infants. Conformational epitopes are important for the allergenicity of the protein. However, in some patients reduced peptides exhibited a similar or even higher IgE-binding capacity than the native corresponding fragment, suggesting that linear epitopes also exist, located in hydrophobic regions, and are exposed as a consequence of protein denaturation (7,28). The significance of this was demonstrated in an investigation of IgE antibody binding capacity of native Bovine ALA and tryptic peptides, utilising sera of 19 patients with CMA; 58% reacted exclusively with intact ALA, while 42% also presented an allergen-specific IgE response to different tryptic peptides derived from ALA (28).

IgE binding to native ALA and to large peptides confirms the importance of conformational epitope(s). However, in some sera, peptides of reduced size, e.g., 59-94 kDa, exhibited a similar or a higher IgE-binding capacity than did the native corresponding fragments, suggesting the existence of sequential epitope(s) exposed through protein denaturation (4,28). Moreover, IgE-binding sequences were also located in hydrophobic regions of the ALA molecule, where antigenicity is very unlikely to be predicted, and/or within parts of the molecule having a very high sequence homology with human ALA (2).

Cross-reactivity between Bovine ALA and ALA from other animal sources is possible but has not been fully elucidated, and unexpected results may be possible. For example, in a report of Mare's milk allergy in a 51-year-old woman who was able to tolerate Cow's milk, skin test and serum IgE antibodies for Cow's milk was negative but was positive for Mare's milk. Further investigation demonstrated 2 allergen bands most likely representing ALA and Beta-lactoglobulin (106). Allergy to Mare's milk is rare.

Antibodies to Beta-lactoglobulin show 10% cross-reactivity with Bovine ALA, both in its native and in its denatured form, which has been attributed to a continuous stretch of 4 amino acids common to ALA and Beta-lactoglobulin. Cross-reactivity between this antibody and Bovine serum albumin was negligible. No cross-reaction was seen with antibodies to ALA and to serum albumin (107).

As the deduced amino acid sequence of buffalo ALA differs at 1 position from the Bovine ALA sequence, cross-reactivity between these 2 is possible but has not been clinically investigated (108).

f77 nBos d 5

ImmunoCAP®: f77 nBos d 5
Native protein purified from cow's milk (*Bos domesticus*)

Biological function:	β-lactoglobulin
Mw:	18 kDa

Allergen description

β-lactoglobulin (Beta-lactoglobulin) (BLG) is one of the major allergens in Cow's milk. BLG is the most abundant protein in Whey, accounting for 50% of total protein in the lactoserum fraction and approximately 10% of Cow's milk. (See Table 1, page 52)

Although current infant formulae closely mimic the ratio of total Whey to Casein in human milk, the concentration of Alpha-lactalbumin is relatively low in formula, whereas BLG, a protein not found in human milk, is the dominant Whey protein in formula. Whey sources with elevated concentrations of Alpha-lactalbumin have been developed, which has permitted the provision of formulae with increased concentrations of this protein and decreased concentrations of BLG (3). BLG was measured in 7 different infant Cow's milk protein Whey or Casein hydrolysated formulae. BLG levels in these formulae were 1/100 to 1/4,800,000 lower than in Cow's milk. There was a great difference in the BLG levels between the partly and the extensively hydrolysed formulae; the amount of BLG was 40,000-fold higher in the partially hydrolysated *vs*. the extensively hydrolysated formulae. Nontheless, residual BLG or peptides (see below) may still be responsible for allergic reactions described in some children with Cow's milk allergy who are receiving these formulae (109).

Bos d 5, Bovine beta-lactoglobulin, is a 18.3 kDa protein (4,70,86,95,97,100-101,110-115).

rBos d 5

Beta-lactoglobulin is the most abundant protein in Whey, accounting for 50% of total protein in the Lactoserum (Whey) fraction. BLG occurs naturally in the form of a 36 kDa dimer possessing 2 disulfide bridges and 1 free cysteine. This structure is responsible for the main physicochemical properties and also for interaction with Casein during heat treatments. It has no homologous counterpart in human milk; i.e., human milk does not contain BLG (2). The relative resistance of BLG to acid hydrolysis and gut proteases allows part of the protein to be absorbed intact through the intestinal mucosa. By resisting digestion in the stomach, BLG is believed to act as a transporter of vitamin A and retinol to the intestines (116).

BLG belongs to the lipocalin superfamily and is one of the best characterised lipid-binding proteins. As such, it is capable of binding a wide range of molecules, including retinol, beta-carotene, saturated and unsaturated fatty acids, and aliphatic hydrocarbons (117-118). Lipocalins have a high allergenic potential, and several allergens of animal origin belong to this family. They share well-conserved sequence homologies in their N-terminus moiety (119-126). Other lipocalin protein family members include several allergens of animal origin such as the major Mouse (and Rat) urinary proteins (mMUP), the major Horse allergen Equ c 1, and the major Cockroach allergen BIa g 4 (125).

The molecule possesses 2 disulfide bridges and 1 free cysteine. This structure is responsible for the relative resistance of BLG to acid hydrolysis, as well as to proteases, which allows some of the protein to remain intact after digestion and increases the probability that intact BLG as well as digested fragments will be absorbed as antigens (116). The 2 intramolecular disulfide bonds may be responsible for the allergic effects (19). BLG is present in several variants. There are 2 main isoforms of BLG, genetic variants A and B, which differ only by 2 point mutations on residues 64 and 118; these are aspartic acid and valine in BLG A, and glycine and alaninc in BLG B. Variant C is found only in the Jersey breed (127). BLG occurs naturally as a mixture of monomers and dimers, but the proportion of monomers increases after heating to 70 °C (128). It has been demonstrated that there are many allergenic epitopes spread over the BLG structure.

Although the structure of the 2 variants A and B is very similar, in animal models the intensity and duration of the IgE response varies (7). Cleavage of the intra-chain disulfide bonds within the BLG molecule, and consequently the loss of the conformation of the molecule, had little if any effect on its immunoreactivity, suggesting that linear epitopes are implicated (7).

Chemical and immunological studies of BLG have identified a continuous epitope recognised by human IgE (71). However, sensitisation involves many epitopes that are widely spread all along the BLG molecule. Some have short linear sequences, while other immunoreactive structures corresponded to quite large fragments that might encompass conformational epitopes or parts of epitopes. In a study aimed at mapping the major allergenic epitopes on BLG by using specific IgE from sera of 46 Milk-allergic patients, several peptides capable of specifically binding human IgE were identified. Three fragments appeared to be major epitopes recognised by 92, 97 and 89% of sera, while a second group with 2 fragments was recognised by 58 and 72% of the population. A third group of peptides was detected by more than 40% of sera. Thus, 3 peptides were identified as major epitopes, recognised by a large majority of human IgE antibodies. The authors concluded that numerous other epitopes are scattered all along the BLG sequence (27).

A number of the BLG epitopes were mentioned as markers for persistent CMA. In addition to B cell epitopes, T cell epitopes of BLG have also been described (129). The monitoring of BLG IgE concentrations and the calculation of a ratio of IgE to IgG antibodies could be useful in predicting which patients will ultimately lose clinical reactivity (52).

Heating of Beta-lactoglobulin results in changes in the degree of allergenicity of the allergen, but this is dependent on the extent of heating: a slight but significant decreased IgE binding was seen between unheated Beta-lactoglobulin solution and Beta-lactoglobulin solution heat-treated at 74 degrees C. A more pronounced decrease was found at 90 °C. The inhibition of IgE binding of Milk after heat treatment at 90 °C was also significantly decreased. However, at all heat treatments, a similar total amount of IgE antibodies could be inhibited at a sufficiently high concentration of Beta-lactoglobulin (130). BLG also resists pasteurisation (131). Furthermore, heat-denatured proteins can also present new antigenic sites, uncovered by the unfolding process or created by new chemical reactions with other molecules present in the food. Heat-denatured BLG has been reported to have at least 1 new epitope, not found in the native state (132). Similarly in a study evaluating the specificity of serum IgE to different fragments of BLG in a group of 19 individuals allergic to Cow's milk, a large number of epitopes were shown to be recognised by allergen-specific IgE of human allergic sera, and there were differences in the specific determinants recognised, depending on the serum (31).

The IgE binding of Beta-lactoglobulin appears to also be significantly impaired in some fermented, acidified Milk products such as yogurt, as compared to nonfermented Milk (130).

f77 nBos d 5

BLG chemical hydrolysates appeared to retain most of the immunoreactivity of the native protein. IgE antibodies from 10 patients with CMA recognised enzymatic digestion products of BLG from pepsin or pepsin + trypsin (10 patients out of 10); the recognition of peptides was even better than that of the intact molecule in 4 of 10 patients. Researchers concluded that the digestive processes unmask new allergenic epitopes (40). It has been confirmed that cleavage may allow the presentation of determinants that, on the whole native protein, were not accessible to the antibodies (27).

BLG may be found in house dust. In a study of house dust, the amount of BLG ranged from < 16 to 71 ng/g dust, compared with Ovomucoid which ranged from 170 to 6,300 ng/g dust (133).

Bovine BLG seems to share structures with corresponding Milk proteins from other species.

Anti-bovine BLG antibodies show 10% cross-reactivity with Bovine alpha-lactalbumin, both in its native and in its denatured form, which appears to be a result of a continuous stretch of 4 amino acids common to Alpha-lactalbumin and BLG (107).

Crossreactivity between Cow's milk and Mare's milk has previously been demonstrated in inhibition studies, but this is contradicted by an earlier study in which an individual allergic to Mare's milk was not allergic to Cow's milk. Two allergenic proteins of 16 and 18 kDa were detected, and were thought to most likely represent Alpha-lactalbumin and BLG, but it was suggested that these 2 are not cross-reactive with Bovine equivalents (106).

Cross-reactivity has been suggested between Reindeer BLG and Bovine BLG. In a study of Reindeer milk-allergic patients, the patterns of Bovine BLG-specific IgE to Reindeer BLG varied among patients, suggesting only partial cross-reactivity (134).

e204 nBos d 6

ImmunoCAP®: e204 nBos d 6
Native protein purified from cow's milk (*Bos domesticus*)

Common name:	BSA
Biological function:	Serum albumin
Mw:	67 kDa

Allergen description

Serum albumin is the main protein in mammalian blood tissue. It plays a very important role in the transport of nutritional substances into the system by virtue of its ability to bind with a large number of molecules. Beef also contains bovine serum albumin (BSA) and gamma globulin. These are heat-labile proteins found also in Cow's milk. BSA is a distinct Milk allergen comprising approximately 1% of the total Milk protein.

BSA may be obtained from Bovine plasma collected in slaughterhouses, which is then highly purified and used in biochemistry, immuno-chemistry, haematology and microbiology, in all countries where these sciences are practiced. It is most often employed in the production of diagnostic test systems, as a growth medium for bacteria, and as a cell culture.

It is used in the manufacture of antiwrinkle skin-tightener and is a basic protein for biological reactants. It may be used as a medium for *in vitro* fertilisation techniques.

BSA, a 67 kDa, heat-labile protein, is a major allergen in Beef and a minor allergen in Milk (2,37,101,167-174).

In Cow's milk, BSA accounts for around 5% of the total Whey proteins. BSA is physically and immunologically very similar to human blood serum albumin (HSA). Its main role is the transport, metabolism and distribution of ligands and the protection from free radicals (127). Its tertiary structure is quite stable, even under denaturing conditions. A reduction of disulfide bonds results in a complete abolishment of binding with anti-BSA antibodies. IgE antibodies specific for BSA from sera of allergic children were shown to be able to cross-react with albumins from Sheep and Pig, but they did not recognise those of Horse, Rabbit and Chicken (41).

Heating reduces sensitisation to Beef and to Bovine serum albumin but does not abolish reactivity to BSA under home conditions. However, industrially heat-treated and sterilised homogenised Beef and freeze-dried Beef may not be allergenic (169). Heat treatment and chemical denaturation are not able to decrease BSA's capacity to bind BSA-specific IgE antibodies (175). Directly heated UHT Milks suffer less heat damage than indirectly heated Milk. During storage, BSA in directly heat-treated Milks decreased significantly, unlike Alpha-lactalbumin and Beta-lactoglobulin, in which changes were not statistically significant (176). Pepsin incubation at pH 4.0 was shown to result in a decreased hydrolysis and enhanced residual antigenicity of BSA (177). Research indicates that serum albumin antigenicity is only partially correlated to its native 3-dimensional structure (175).

e204 nBos d 6

There is a great variability in human IgE response to Cow's milk, and no single allergen or particular structure can account for a major part of Milk allergenicity (2).

Studies of large populations of allergic patients show that most of the patients are sensitised to several proteins, including BLG (Bos d 5), Casein (Bos d 8), ALA (Bos d 4), BSA (Bos d 6), Lactoferrin, and immunoglobulins (Bos d 7). A great variability is observed in IgE antibody response. Both Casein and BLG, as well as ALA, are major allergens. However, proteins present in very low quantities, such as BSA, immunoglobulins, and especially Lactoferrin, also appear to be important, since 35% to 50% of patients are sensitised to those proteins and sometimes to those proteins only (2). Bovine BLG is a major Cow's whey allergen, which together with a-lactalbumin is regarded as a major allergen in Cow's milk. It is the main Whey protein, without any counterpart in human Milk.

Bovine serum albumin occurs as a major allergen in Beef, and a minor allergen in Cow's milk. Beef-allergic individuals are at risk of being allergic to Cow's milk and vice versa (36).

In a study evaluating the cross-reactivity between Lamb and Beef and the role of BSA and Ovine serum albumin (OSA) as allergens in Beef-allergic children, it was found that BSA and OSA are important Beef and Lamb allergens. They have similar amino acid sequences and allergenic properties (178-180). Considering that the major Beef allergen is BSA and that Beef-sensitive children are also sensitised to Ovine serum albumin, as well as to other serum albumins, the use of alternative meats in Beef-allergic children must be carefully evaluated on an individual basis (168).

There is a high degree of homology between the primary structures of human Milk protein serum albumin and the corresponding Bovine serum albumin (identity 76.6%), which has resulted in the hypothesis that there may be cross-reactivity between the bovine and the human albumin, since IgE antibodies from Birch profilin-allergic individuals have been reported to cross-react with human profilin where the identity between the 2 proteins is only 34% (159).

Previous reports have suggested that allergy to animal epithelia, possibly even sub-clinical allergy, may predispose towards sensitisation to mammalian meat as a result of sensitisation to BSA (159,180). And patients with persistent Milk allergy and specific IgE antibodies to BSA have a greater risk of rhinoconjunctivitis and asthma because of animal dander (181).

The aim of a study was to prove the cross-reactivity between serum albumin of different mammals in Milk, meat, and epithelia, and to determine whether heat treatment of meats decreases the allergenicity of albumins. All the patients' sera, with the exception of 1, recognised serum albumin in different meats (Beef, Lamb, Deer, and Pork), epithelia (Dog, Cat, and Cow), and Cow's milk. Some patients were sensitised only to serum albumin in meat and epithelia. Patients with allergy only to dander were sensitised to other proteins in epithelia but not to serum albumin. No patients reacted to serum albumin from heated meat extracts. Therefore, serum albumin appears to be an important allergen involved in Milk, meat, and epithelia allergy. The authors suggest that sensitisation first occurred to BSA in Cow's milk and thereafter was developed to epithelial serum albumin, even though no direct contact with animals had been made; and that patients with both BSA and Cow's milk allergy must avoid raw meats and furry pets (182).

Thiomucase (a mucopolysaccharidase obtained from Ovine tissues that is used mainly to facilitate the diffusion of local anaesthetics and in the treatment of cellulitis) is partially cross-reactive with BSA, Cat dander and Sheep dander (182).

f78 nBos d 8

ImmunoCAP®: f78 nBos d 8
Native protein purified from cow's milk (*Bos domesticus*)

Biological function:	Casein
Mw:	19-25 kDa

Allergen description

Casein is a major allergen in Milk (19) and the main protein constituent of cheese. Casein makes up about 75-80% of all Milk protein and is heat-stable. (See Table 1, page 52).

Casein is found in Milk and dairy products, especially cheese, and in other foods containing Milk. Even highly hydrolysed Milk-derived infant formulas may contain allergenic Casein residues (32,69,135-136).

Casein may occur in "Milk-free" products as undegraded residual Milk proteins or as contamination from previous productions of food containing Milk. Casein may be a cause of allergic reactions in patients eating so-called "non-dairy" products (48).

Casein and caseinates are used as extenders and tenderisers in sausages, loaves, soups and stews. They are often used to nutritionally fortify foods and as supplements because of the large amount of high-quality protein they contain, their low level of lactose, and their bland flavour. Such nutritionally fortified foods include high-protein beverage powders, fortified cereals, infant formula and nutrition bars. Casein is often an ingredient in coffee whiteners, sauces, ice cream, salad dressing, formulated meats, bakery glazes, and whipped toppings.

Bos d 8, Casein consists of a range of proteins varying in size from 19 to 25 kDa (4,29,37,70,95,97,99,11,137-140).

The coagulum consists of the whole Casein fraction (i.e., the solid fraction of proteins obtained after coagulation of Milk). It is subdivided into a number of families, of which the most important are α_{S1} -, α_{S2} -, β-, κ-, and γ-caseins (α = alpha, β = beta, κ = kappa, γ = gamma) (5).

Each individual Casein among the types α_{S1} -, α_{S2} -, β-, κ- represents a well-defined chemical compound, but they cross-link to form ordered aggregates (nanoclusters or micelles) that assemble into larger structures, forming Casein micelles characterised by a central hydrophobic part and a peripheral hydrophilic layer in suspension in Lactoserum (Whey) (2,13-14). Their proportion in the micelles is relatively constant at approximately 37%, 13%, 37%, and 13%, respectively.

Their distribution is not uniform within these micelles, which comprise a central hydrophobic part and a peripheral hydrophilic layer, where major sites of phosphorylation that contain phosphoserine residues are presented in relation to the calcium-binding and transfer properties of Caseins (2). α_{S1}-, β-, α_{S2}-, and κ-casein have little primary structure homology. Their functional properties also differ, since 3 of them, α_{S1}-, α_{S2}-, and β-casein, appear to be calcium-sensitive, whereas κ-casein is not. However, the 4 Caseins display common features that are unusual, which means that they differ greatly from other Milk proteins. They are phosphorylated proteins (2). Casein is rapidly and extensively degraded by proteolytic enzyme during digestion. Caseins are not significantly affected by severe heat treatments but are very susceptible to all proteinases and exopeptidases. Multisensitisations to the different Caseins occur most often in patients sensitised to the whole Casein fraction (2,138).

Casein is thermostable, whereas BLG is thermolabile, but it may be protected through interaction with Casein. Thermostability of Cow's milk proteins depends not only on temperature and time spent heated but also on interactions within the food matrix. Heat denaturation, which leads to the loss of organised protein structures, does not always result in a decreased allergenic potential: formation of aggregates may increase the allergenicity of the heated product. When the treatment results in a decrease in the allergenicity, the decrease is always limited. Boiling of Milk for a few minutes (2, 5, or 10 minutes) results either in no difference or in a reduction of approximately 50% to 66% in positive reactions, compared to reactions to raw Milk; similar observations have been reported with raw *vs*. pasteurised or homogenised and pasteurised Milk (2). The Caseins are heat-stable, and even high pasteurisation (121 °C for 20 minutes) only reduces and does not eliminate the allergenicity of the Caseins (141).

α_{S1}-casein represents up to 40% of the Casein fraction in Cow's milk. α_{S1}-casein consists of major and minor components; both are single-chain polypeptides with the same amino-acid sequence, differing only in their degree of phosphorylation (5,142). Variants A, B, C, D, F, G, H have been identified as characteristic of different cattle breeds.

The α_{S2}-casein family accounts for 12.5% of the Casein fraction in Cow's milk and comprises the most hydrophilic of all Caseins. α_{S2}-casein consists of 2 major and several minor components. A post-translational modification occurring in this protein is the formation of disulfide bonds that do not participate in the interaction with other Caseins (5).

Further studies have confirmed that a-casein largely lacks a tertiary structure and therefore also lacks conformational epitopes (29,143). Indeed, Casein appears to preferentially have linear epitopes (35). The sequential epitopes are exposed even in denatured Casein, resulting in an apparent stability of the allergen to denaturing conditions, e.g., heat. In fact, some of the major epitopes already characterised on alpha-S-caseins are continuous epitopes that have also been located in hydrophobic regions of the molecule, where they are not accessible to antibodies unless the Casein is denatured or degraded, such as for instance, during digestion (144). This may explain the apparent difference in epitope recognition among patients with different natural histories of CMA (144).

The β-casein family accounts for 35% of the Casein fraction and is quite complex because of the action of the native Milk protease plasmin. Plasmin cleaves the β-casein, generating γ1-, γ2-, and γ3-casein fragments. β-casein is the most hydrophobic component of the total Casein fraction. There are 10 genetic variants (5).

κ-Casein accounts for 12.5% of the total Casein fraction. κ-Casein consists of a major carbohydrate-free component and a minimum of 6 minor components. It is isolated from Milk as a mixture of disulfide-bonded polymers ranging from dimers to octamers. There are 2 common and 9 other genetic variants. The κ-casein group plays an important role in the stability and coagulation properties of Milk. Hydrolysis by chymosin in rennet produces para-κ-casein and a caseinomacropeptide that is important for the first stage of the cheesemaking process (145).

Anionic regions contain clusters of α_{S1}-, α_{S2}- and β-casein, and these clusters are able to chelate Ca^{2+} and other metal ions, including Zn^{2+} and Fe^{3+} (5).

Caseins, although less ordered in structure and more flexible than the typical globular Whey proteins, have significant amounts of secondary and, probably, tertiary structure (146). However, Caseins appear to preferentially have linear epitopes. A study carried out on sera of 15 Milk-allergic children showed that 6 major and 3 minor IgE-binding epitopes as well as 8 major and 1 minor IgG-binding regions were identified on β-casein, while 2 major and 2 minor IgG-binding epitopes were found for κ-casein. In another study, overlapping synthetic peptides were used to identify major IgE-

and IgG-binding regions of α_{S1}-casein in patients with CMA. Six major and 3 minor IgE-binding regions, and 5 major and 1 minor IgG-binding epitopes were identified (35). The implication of linear epitopes is that denaturing does not affect this protein to the same extent as those in which conformational epitopes are relevant.

A number of studies have demonstrated that most patients allergic to Casein are sensitised to each of the 4 major Caseins, and that there is great variability in the specificity and intensity of IgE response to these Casein fractions, which indicates, among other things, the presence of distinct epitopes on the individual Casein molecules (138,147). The intensity of the IgE responses appears to be closely related to the proportion of the 4 Caseins in Milk, and sensitisation probably occurs after the disruption of the Casein micelles during the digestive process (126,138). However, cross-sensitisation mechanisms also occurred through common or closely related epitopes. Importantly, Casein "allergenic epitopes" may be present in Whey (138). Therefore, polysensitisaton appears to be due both to cross-sensitisation and to common or closely related epitopes.

Partial hydrolysis of a fraction of the Casein, e.g., Beta-casein, occurring naturally due to endogenous enzymes such as plasmin, which are normally present in Milk, gives rise to Gamma-caseins, and to smaller fragments called proteoses-peptones, corresponding to the N-terminal part of the Beta-casein molecule. These peptides are soluble and remain in the Lactoserum (2). Similarly, the limited proteolysis due to the action of chymosin during clotting of Milk splits κ-casein into 2 peptides: hydrophobic para-κ-casein and a highly polar caseino-macro-peptide, which is soluble and remains in the Whey. Some proteoses-peptones are still allergenic, as is the caseino-macro-peptide, which explains why reactions may be observed after ingestion of Whey protein; hydrolysates in babies have serum-specific IgE to Casein but negative to Whey proteins (4).

Furthermore, the balance between Caseins and Whey proteins appears to play an important role in the sensitisation capacity of Cow's milk (148). Also, a reduced Casein content and poorer renneting properties of Milk may occur in late summer, which may result in differences in the frequencies of sensitisation to Cow's milk proteins (149). The formation of Casein monomers into a high-molecular-mass fraction to which CMA individuals display reactivity has been described (19).

It has been supposed that the majority of linear IgE epitopes in Caseins could contribute to persistent allergy (150). Milk-allergic children with persistent symptoms have significantly higher levels of specific IgE antibodies to linear epitopes from α_{S1}-(AA69-78) Casein and β-Casein than children who have achieved tolerance (151). Five IgE-binding discriminative epitopes (2 on α_{S1}-casein, 1 on α_{S2}-casein, and 2 on κ-casein) have been shown to be exclusively recognised by patients with persistent CMA (152).

A high degree of cross-reactivity occurs between Cow's, Sheep and Goat's milk as a result of the high sequence homology between their Caseins. Goat and Sheep Milk allergy may involve the Casein fraction and not Whey proteins (38,42,153). The high degree of cross-reactivity between these three Caseins appears to be as a result of alpha-caseins which share more than 85% identical amino acids homology (38).

Furthermore, multi-sensitisation to the different Caseins most often occurs in patients sensitised to the whole Casein fraction. It has been suggested that conserved regions shared by both Bovine and human Beta-caseins, and particularly those comprising clusters of phosphorylated seryl residues, are responsible for IgE cross-reactivity (138).

Twenty patients allergic to Cow's milk proteins and with high levels of IgE antibodies directed against Bovine whole Casein were selected to evaluate the reactivity of their IgE antibodies to human Beta-casein. Seven sera contained IgE directed against human Beta-casein.

Inhibition studies using native human and Bovine beta-caseins as well as Bovine beta-casein-derived peptides demonstrated that, depending on the sera, 1 or several common epitopes located in different parts of the molecule were shared by the 2 homologous proteins (154).

In a study evaluating the Alpha-caseins from Bovine, Ovine, and Goat's milk sharing more than 85% identical amino acids, sera from 17 children with immediate-type allergy to Cow's milk were compared with sera from non-CMA-allergic individuals. The sera of Cow's milk-allergic children showed a significantly higher IgE and IgG binding to Alpha-caseins from all 3 species than did the sera of the other groups. All groups showed an increased antibody binding to Bovine alpha-casein, as compared to the Sheep and Goat proteins, but the differences were significant only in the groups of atopic children and of healthy controls. Inhibition of the IgE binding to Bovine alpha-casein with Alpha-casein from Cow, Goat, and Sheep revealed that the Alpha-caseins from these species are highly cross-reactive, on the basis of the small differences in their primary structures (38).

Structural homologies in Caseins of different species can share common epitopes for IgE of CMA patients, suggesting that prevention of Cow's milk allergy cannot be achieved by using Milk from other species as substitutes. In A study of sera from 58 CMA individuals to determine the specificity of their IgE response to the whole Casein fraction of Milk from different ruminant and nonruminant species (e.g. Cow, Sheep, Goat, Rabbit and Rat), co-and/or cross-sensitisations to Caseins of the different species occurred extensively, though IgE responses to Sheep and Caprine casein appeared to be lower than those obtained with Casein from Cow, and in terms of specificity and intensity, the IgE response to Caseins demonstrated a great variability (155).

However, although many children who are allergic to Cow's Milk cannot tolerate Goat's or Sheep's milk either, there are instances of patients who are allergic to Sheep and/or Goat's milk and not to Cow's milk Caseins (42,153). In a report on Goat and Sheep milk-allergic children who were not allergic to Cow's milk, IgE specificity and affinity was high to Goat and Sheep milk, and lower to Cow's milk caseins despite their marked sequence homology (42). It has also been shown that Sheep casein shows a high degree of cross-reactivity with Goat casein but not with Cow casein (153,156). These results may indicate sensitisation to Casein per se but not to the alpha-Casein fraction, which may contribute mostly to the cross-reactivity usually seen.

Adverse reactions have been reported in Milk-allergic patients fed Soy-based formulae as Cow's milk substitutes. A 30-kDa, glycinin-like protein from Soybean that cross-reacts with Cow's milk casein has been isolated and partially sequenced. The results of this study indicate that Soy-based formula, that contains the A5-B3 glycinin molecule could be involved in allergic reactions observed in Cow's milk-allergic patients exposed to Soy-containing foods (44).

f334 nBos d lactoferrin

ImmunoCAP®: f334 nBos d lactoferrin
Native protein purified from cow's milk (*Bos domesticus*)

Biological function:	Bovine lactoferrin
Mw:	76 kDa

Allergen description

Lactoferrin is a major allergen in Milk. Lactoferrin is an allergen of the whey fraction of Milk and can be found in the Milk of most species at levels lower than 1%. (See Table 1, page 52)

Lactoferrin is a non-heme-iron-binding globular multifunctional glycoprotein with antimicrobial activity, produced during lactation and by epithelial cells at mucosal surfaces. The protein is a prominent component of the first line of mammalian host defence, and its expression is up-regulated in response to inflammatory stimuli. Lactoferrin may act as a potent anti-inflammatory protein at local sites of inflammation, including the respiratory and gastrointestinal tracts (157). Human colostrum has the highest concentration, followed by human milk, then Cow's milk.

Lactoferrin appears to play several biological roles. Owing to its iron-binding properties, Lactoferrin is thought to play a role in iron uptake by the intestinal mucosa of the neonate.

Besides in Cow's milk, the topic of this review, Lactoferrin is found in many mucosal secretions such as tears, saliva, bile, pancreatic juice, and genital and nasal secretions. Lactoferrin is released from neutrophil granules during inflammation and is also secreted by some acinar cells.

As Bovine milk-derived Lactoferrin is known to be an effective natural antimicrobial, it is used as a spray, applied electrostatically to raw Beef carcasses to detach bacteria adhering to the surface, in order to reduce microbial contamination. It is used only on Beef carcasses (not on subprimals or finished cuts) at a level not to exceed 0.20 ml of formulation per kg of Beef. An assessment of its use found that its application to Beef carcasses is in the range of existing background exposures of Lactoferrin, because Lactoferrin is found naturally in Beef, and that this potentially small incremental increase in Lactoferrin is safe (i.e., there is no reasonable expectation that the substance will become an allergen under the conditions of its intended use) (158).

Bos d Lactoferrin, a 76.1 kDa protein, has been characterized (8,97,127,159).

Lactoferrin (LF) is an allergen of the whey fraction of Cow's milk (21). It is a protein of mammary origin and is a Milk-specific iron-binding glycoprotein of the transferrin family. It can be found in the Milk of most species at levels lower than 1% (160). LF is present in much higher concentrations in human breast milk (*ie*, 1 g/l), particularly in colostrum (2). Although it is present in very low concentrations in Cow's milk, it has been shown to be an important allergen (8).

LF consists of a single polypeptide chain folded into 2 globular lobes. The molecular weight of this protein varies depending on the extent of its glycosylation. The LF content is species-dependent, with significantly higher levels in human milk and colostrums compared to Bovine milk, whereas the sequence homology and structure are very similar, with human and Bovine lactoferrin having an amino acid sequence homology of 69%, and structural similarity (2,5). Lactoferrin is partially heat-stable and relatively stable to enzymatic degradation by gut proteases and remains partly unchanged during digestion (2).

f334 nBos d lactoferrin

Lactoferrin is a multifunctional member of the transferrin family of nonheme, iron-binding glycoproteins. Lactoferrin is found at the mucosal surface, where it functions as a prominent component of the first line of host defence against infection and inflammation (161-162).

Its main role is to defend the organism against infections and inflammations through its ability to sequester iron from the environment and thereby remove this essential nutrient for bacterial growth, and to act as an antioxidant and a scavenger for free radicals, thus providing protection against oxidative stress (5,161). It also has antibacterial properties and has been shown to stimulate cellular immune defence of the organism against infections (2). Lactoferrin is also an abundant component of the specific granules of neutrophils and can be released into the serum upon neutrophil degranulation (161). Neutrophil lactoferrin has also been shown to inhibit tryptase released from mast cells (163). While the iron-binding properties were originally believed to be solely responsible for the host defence properties ascribed to this protein, it is now known that other mechanisms contribute to the broad-spectrum anti-infective and anti-inflammatory roles of this protein. Lactoferrin appears to function, collectively, as a key component of mammalian host defence at the mucosal surface (161). Recently, human lactoferrin was shown to be implicated in the pathophysiology of an asthma attack (164).

Bovine lactoferrin is able to form non-covalent complexes with Beta-lactoglobulin or Albumin, with Lactoferrin-protein molar ratios of 2:1 and 1:1 respectively. No association was detected with Alpha-lactalbumin (165).

Milk from related animals is important. Lactoferrin is present in human and Bovine milk, the proteins from the 2 species having about 70% homology. It is thus likely that the 2 proteins share common or similar epitopes, and it is thus possible that exposure and immunological reaction to Bovine lactoferrin during ingestion of Cow's milk or Milk products in infancy could prime the immune system to subsequently react against human lactoferrin. A study testing this hypothesis concluded that there is evidence that development of anti-Lactoferrin autoantibodies in patients with anti-neutrophil cytoplasmic antibodies may be result, at least in part, from prior stimulation from Bovine lactoferrin in Cow's milk (166).

Further support for potential cross-reactivity between Bovine and human lactoferrin is the evidence of a dose-dependent inhibition of serum IgE to Cow's milk protein binding to human milk proteins, shown by denatured Bovine whey proteins, and vice versa, which suggests the presence of common epitopes (cross-reactivity) between Bovine and human milk proteins. A study investigating this hypothesis argued that lack of inhibition by native Bovine and human whey proteins suggested that such epitopes are probably linear (continuous) and should lie in the internal part of the molecules. The authors suggest that the rather high degree of homology between the primary structures of human milk proteins and the corresponding Bovine proteins (Serum albumin, identity 76.6%; Alpha-lactalbumin, identity 73.9%; Lactoferrin, identity 69.5%; Beta-casein, identity 56.5%) is congruent with cross-reactivity towards specific antibodies. In a similar way, IgE antibodies from Birch profilin-allergic individuals have been reported to cross-react with human profilin where the identity between the 2 proteins is only 34% (159).

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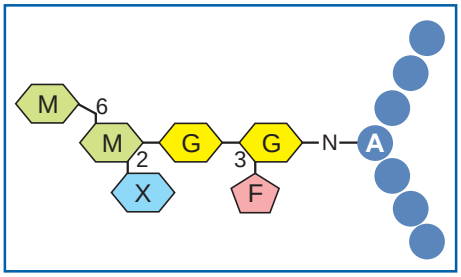
Cross-reactive Carbohydrate Determinant (CCD)

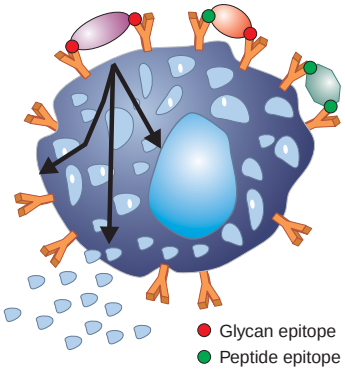
Available ImmunoCAP®:
o214 CCD; MUXF3 from bromelain



Summary

Many allergens are glycoproteins, i.e. they contain one or several complex oligosaccharide chains linked to the peptide structure of the protein. Studying the structure of allergens and their IgE antibody binding epitopes several research groups have searched for a role of the carbohydrate moieties of allergenic molecules. Since glyco-epitopes can share significant structural homologies beyond the limits of protein families they are prone to extensive cross-reactivity and they have been called Cross-reactive Carbohydrate Determinants or CCDs.

Whether or not IgE antibodies against carbohydrate epitopes on glycoproteins have a clinical role is debated, but data supporting a clinical effect are emerging. As long as the demonstration of a clear *in vivo* effect remains to be confirmed, we must consider the sometimes confusing role of these epitopes in serum-based IgE antibody assays.



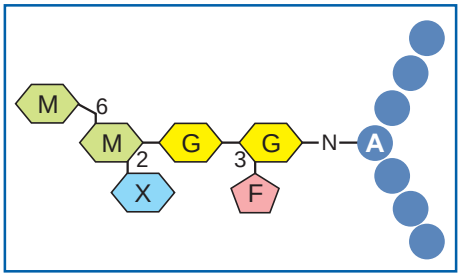
Degranulation of mast cells require the binding of at least two epitopes to two adjacent IgE antibody molecules. This cross-linking may be achieved by two peptide epitopes, by one glycan and one peptide epitope, but also by two glycan epitopes.

Testing for CCD-specific IgE reactivity

A CCD test could be useful when *in vitro* results do not match the clinical picture (symptoms, skin tests), especially when numerous results are found positive without obvious clinical symtoms to all these allergens. Checking the possible presence of anti-CCD IgE is advisable in three types of situations:

- Sensitization to foods of plant origin, mainly vegetables and fruits, but could also be useful with seeds such as peanuts.
- Sensitization to *Hevea* latex in a pollen allergic patient without occupational risk factors.
- In subjects tested positive both for honeybee and for wasp venoms, or in subjects allergic to these venoms and tested positive for pollen.

o214 CCD; MUXF3 from bromelain



ImmunoCAP®:	o214 CCD; MUXF3 from bromelain
Common name:	MUXF3, carbohydrates, CCD, glycans
Biological function:	Glycosylation of proteins brings better hydrophilicity and stronger resistance to thermic shocks

Allergen description

Bromelain (Ana c 1) is a glycoprotein extracted from pineapple, *Ananas comosus*. Bromelain has widely been used for checking the cross-reactivity between a glycan and other glycoproteins since its MUXF3 carbohydrate chain is found in many plant proteins. True allergy to bromelin is also very rare.

ImmunoCAP® Allergen, CCD; MUXF3 from Bromelain, is a pure CCD reagent containing only the MUXF3 carbohydrate epitope, thus avoiding IgE antibody binding to other bromelain epitopes. The MUXF3 carbohydrate epitope is purified from digested bromelain.

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Dog allergen components

Canis familiaris

Available ImmunoCAP®:

e101 rCan f 1
e102 rCan f 2
e221 nCan f 3

Allergens from *Canis familiaris* listed by IUIS*

Can f 1	Can f 2	Can f 3
Can f 4		

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

Summary

The Dog is a relative of the Wolf, the Jackal, and the Fox, all belonging to the family *Canidae*. Two characteristics distinguish the Dog from other canids: its worldwide distribution in close association with humans, and its huge variety as a result of adaptation and breeding for specific purposes. Dogs through the centuries have acquired the body types and dispositions to pursue and retrieve game, and to be draught animals, guides (e.g., for the blind), guards, companions, and so on.

Dogs are found in almost every human environment. Some Dogs are feral, but not in such large numbers as Cats.

As with Cat, major Dog allergens can be found in hair, dander, pelt, saliva and serum, and are considered epithelial allergens; unlike with Cat, however, Dog urine and faeces do not have any significant allergenic activity (1-5). The concentration of allergens varies within breeds and among them (2,6). Although allergen differences occur according to the origin of the allergen (e.g., epithelium or saliva), no breed-specific allergens occur (7-8. This is contrary to reports of much earlier studies (9-10.

Dog allergens are ubiquitous in the environment. They may be found, for example, on automobile seats in concentrations well above the thresholds for both sensitisation and symptoms, regardless of the presence of a pet in the home (11). Dog allergens are also prevalent on walls, smooth floors, and finished furniture in homes with and without pets (12), as well as on furnishings and textiles in classrooms (13-14). The concentration of Dog (Can f 1) allergen may even be higher in dust collected in schools than in homes (15). High Dog allergen levels can be found in households without a pet if the former occupants had a pet or if Dogs often visit the building (16).

Upholstered chairs in hospitals constitute a significant reservoir of Cat and Dog allergens, and inhalation of airborne allergen by patients attending their hospital appointments may exacerbate asthma in those highly allergic to Cats or Dogs (17).

The association between pet exposure and asthma or allergic sensitisation can be very confusing, and many conflicting findings have been published (18). Recent studies can be used to support nearly any viewpoint on the issue: Dog exposure decreases (19-20) or has no effect (21) on the risk of sensitisation; asthma is negatively (21) or positively (22) associated with Dog exposure. What makes certainly impossible is that Dog (and Cat) allergen is ubiquitous in human society and may affect sensitisation in predisposed individuals regardless of pet ownership (18,23,24).

Dog allergen components

Nevertheless, Dog dander clearly represents an important source of inhalant allergens, and many studies report that Dog may frequently induce symptoms in sensitised individuals (1, 25-27). Symptoms include asthma, allergic rhinitis and allergic conjunctivitis. Thirty percent to 35% of atopic individuals display type I allergic symptoms on exposure to Cat and/or Dog allergens (28-30). Furthermore, occupational allergy to Dog allergens may occur in animal workers, animal pelt workers, and laboratory workers (31).

Early studies reported that over 28 Dog antigens were detected, 11 of which were found in Dog serum. IgE antibody in the sera of Dog-sensitive patients was reported to bind to 21 of these antigens to varying degrees (3-4).

The following allergens have been characterised:

Can f 1, a lipocalin (2,32-34).

Can f 2, a lipocalin (2,32,33) .

Can f 3, Dog serum albumin (28,35).

Can f 4 (36).

Two serum proteins, alpha-1-antitrypsin and IgG, have been identified as minor allergens (8).

Can f 1 was originally named Ag13 and was found to be identical to Ag8 (2). Can f 1 is a 22 - 25 kDa protein found in hair, dander and saliva but not serum, and is a lipocalin family member (32).

The amount of Dog allergens produced appears to have wide variability among Dog breeds. Hair length or hormonal status does not influence the production of Can f 1 (except that males produce more than females), whereas seborrhoea strongly influences the presence of Can f 1 on hair (2). Older animals produce more dander than younger ones, because their skin is drier. Also, epidermal turnover is more rapid in Dog breeds that are prone to the various forms of dry and oily seborrhoea. Instead of the normal 21-day cycle, the epidermal turnover time of seborrhoeic Dogs is 3 to 4 days.

Can f 2, a 19 kDa protein found in dander and saliva, previously known as Can d 2, is a lipocalin family member and has homology with Mouse urinary protein (MUP) (32, 37). In the majority of studies, it is shown to be a minor allergen.

Can f 3, Dog serum albumin, a 69 kDa protein, is found in dander, epithelia, saliva, and serum (35). It has also been found in salivary glands (parotid and submandibular) and liver (38). Dog albumin represents an important allergen for up to 35% of patients who are allergic to Dogs (28).

Can f 4 is an allergen found in Dog dander.

Shared IgE epitopes of the major Cat and Dog allergens may provide an explanation for the clinical observation that allergies to Cats and Dogs are frequently associated (39). However, several studies report that actual common allergens are responsible for the cross-reactivity, and that these allergens appear to be serum albumin and lipocalin. Furthermore, in a study of 36 cat-allergic patients, in 25% of Fel d 1-reactive patients, more than 50% inhibition of IgE reactivity to Dog allergens was achieved with recombinant Fel d 1. A Fel d 1 cross-reactive 20 kDa allergen was detected in dander extracts of several different Dog breeds, which may be responsible for double positivity to Cat and Dog in serology. However, the clinical relevance of this cross-sensitisation was not clinically evaluated (40).

Importantly, Dog-allergic individuals are sensitised to a heterogenous range of Dog allergens. For example, in a study of such individuals, 52% were shown to be sensitised to Can f 1, about 33% to Can f 2, 60% to an 18 kDa protein, 44% to a 40 kDa protein, and 48% to a 70 kDa protein (probably serum albumin, now known as Can f 3 (33).

e101 rCan f 1

ImmunoCAP®: e101 rCan f 1
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Canis familiaris* allergen Can f 1

Common name:	Ag 13
Biological function:	Lipocalin
Mw:	21-25 kDa

Allergen description

rCan f 1 (32-33,41-43), originally designated Can d 1, is a lipocalin. Can f 1 is a major allergen and the most important Dog allergen, and Dog dander and saliva have a high content of it (but serum has none). The protein is produced in the canine Von Ebner's glands, which are small salivary glands opening in the lingual epithelium. This protein ranges in size from 21 kDa to 25 kDa (44-45). Can f 1 has demonstrated greater heat resistance than Mite allergens after 60 minutes at 140 °C (16). The protein is also relatively stable in house dust (26).

Major respiratory allergens of Dogs, Mice, Rats, Horses and Cows belong to the lipocalin group of proteins. The amino acid sequence identity among lipocalins is often less than 20%, but they contain between 1 and 3 structurally conserved regions, and their 3-dimensional structures are similar. Lipocalins share certain biological functions, predominantly related to the transport of small hydrophobic molecules such as vitamins and pheromones. Immune reactivity to lipocalin allergens is not well understood. In Bos d 5, the IgE-binding epitopes are spread along the molecule, whereas in Bos d 2, the C terminus appears to contain the human B cell epitopes. Bos d 5 contains several murine T cell epitopes. To explain these observations, it has been proposed that the allergenicity of lipocalins may be a consequence of molecular mimicry between lipocalin allergens and endogenous lipocalins at the T cell level (45).

Can d 1 and Can d 2 are found in sera of approximately 74% of Dog-allergic individuals (38). More than 90% of Dog-allergic patients have been shown to have specific IgE antibodies directed to Can f 1 alone (2, 32, 46-47). In another study, sera from 96% of patients with Dog allergy demonstrated allergen-specific IgE to Can f 1 and Can f 2. Can f 1 was preferentially detected in dander and saliva, but not in skin, salivary gland, serum and liver extracts. Can f 2 was strongly expressed in skin, but not in dander, serum and liver (1,25). However, not all studies have found a high prevalence of IgE reactivity in Dog-allergic patients; one study reported that, according to ELISA determination, 52% of Dog-allergic patients recognised recombinant Can f 1 (33). The authors postulated that this may have been due to their selection of patients, but whether certain populations are less frequently sensitised to Can f 1 has not been determined.

Recombinant Can f 1 and Can f 2 are immunologically concordant with natural Can f 1 in skin prick test and IgE antibody analysis. The concordance is slightly lower with recombinant Can f 2. Fifty-two percent of Dog-allergic patients reacted against Can f 1, and about a third of the patients reacted to Can f 2 (33).

As the amount of important allergens in commercial Dog extracts can vary extensively, and as natural preparations may be contaminated with Mite allergens, potentially causing false-positive skin test results, recombinant Can f 1 and recombinant Can f 2 have a role to play in assessing allergy to Dog (48).

Can f 1 and 2 are two important and useful tools identified so far, but further components are needed for diagnosing Dog allergy (33).

e102 rCan f 2

ImmunoCAP®: e102 rCan f 2

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Canis familiaris* allergen Can f 2

Biological function:	Lipocalin
Mw:	19 kDa or 27 kDa

Allergen description

Can f 2 (32-33,42-43), previously known as Can d 2, is a protein with a molecular weight of 19 kDa (38) or 27 kDa (4). It is a lipocalin and has homology with Mouse urinary protein (MUP) (2). It was found to react with IgE antibodies of 66% of Dog-allergic patients, and to bind 23% of the IgE antibodies directed against Dog dander extract, both of which findings confirm its role as a minor allergen (2). Can f 1 and Can f 2 share epitopes (44).

A study evaluated the recombinant Dog allergens Can f 1 and Can f 2 in clinically diagnosed Dog-allergic patients' and healthy non-atopic Dog owners. These allergens were compared to commercial Dog epithelial extract, and it was found that patients' IgE reactivity to natural Can f 1 and to the recombinant allergen were perfectly concordant, but the concordance was slightly lower with recombinant Can f 2. About one-third of the patients reacted to Can f 2. The study concluded that the recombinant allergens can be used reliably to identify Can f 1 and Can f 2-sensitised individuals, but that on their own the 2 allergens were insufficient as reagents for diagnosing Dog allergy (33).

e221 nCan f 3

ImmunoCAP®: e221 nCan f 3
Native serum albumin purified from Dog (*Canis familiaris*)

Common name:	DSA
Biological function:	Serum albumin
Mw:	69-70 kDa

Allergen description

Can f 3 (3,8,10,28,35-36,48-52), also known as Dog Serum Albumin (DSA), is a protein with a molecular weight of 69-70 kDa. It is a serum albumin. It is found in Dog serum, saliva, dander, hair and epithelia, and it is also synthesised in the Dog salivary gland and Dog liver (35). Dog serum albumin has been reported to be particularly abundant allergen in Dog epithelia extracts (50). Dog and Cat serum albumins are also very common allergens present in house dust (38). A recombinant Can f 3 has been produced (35).

Sensitisation to Dog serum albumin has been previously documented as varying from around 35-48% although early studies reported even lower frequencies (5,28,33,53). The importance and frequency of sensitisation to DSA also varies among different populations (3).

In a study, 51 patients with a clinical history of Dog allergy were evaluated for skin reactivity for 8 individual standardised Dog breed allergen preparations, and for 1 mixed-breed allergen preparation, Dog serum albumin, and histamine hydrochloride. The sensitivity rate shown by skin prick test was 67% to 88% for the various Dog breed allergen preparations, but only 18% for DSA (10).

The deduced amino acid sequence of DSA was shown to be highly homologous to the sequences of albumins from both other animals and humans, which explained the perceived extensive cross-reactivity among albumins, and was corroborated by the demonstration of the presence of similar epitopes on Dog, Cat, and human albumin (35). In immunoblot inhibition studies and histamine release tests, it was demonstrated that patients who react to Dog albumin exhibit IgE reactivity with purified albumins from Cat, Mouse, Chicken, and Rat. The deduced amino acid sequence of DSA was found to have significant sequence homology with albumins from human (82.6%), Pig (81.8%), Beef (77.3%), Sheep (78.8%), Mouse (75.8%), and Rat (76.2%) (28).

Cross-reactivity between DSA and albumins from other animals was demonstrated in other studies. In a study aimed at assessing the importance of albumin as a cross-reactive allergen in patients sensitised to Cat, Dog and Horse, 117 patients sensitised to Cat were tested for the presence of skin reactivity allergen specific IgE. Twenty-two percent of patients were found to have IgE antibodies to Cat albumin, and 41% of these patients were also sensitised to Dog and Horse. Of this group, 21% had IgE to all 3 albumins and 17% to 2. However, inhibition studies demonstrated variable degrees of inhibition, suggesting that albumins from these 3 animals share some epitopes that account for the cross-reactivity observed in around a third of patients sensitised to Cat, Dog and Horse, but that more than 50% of allergen-specific IgE that cross-reacts among these 3 animals is directed to allergens other than albumin (54).

Similarly, in a study evaluating the degree and significance of IgE-cross-reactivity to various albumins in 200 patients allergic to animals, it was found that approximately 30% of those allergic to animal hair/dander extracts reacted to albumins from various animals. Although a high degree of sequence homology existed among different animal albumins, a remarkable variability of IgE cross-reactivity was observed, indicating that some patients were sensitised preferentially against certain albumins. Most of the patients allergic to albumins reacted to Dog, Cat, and Horse albumin, which also bound a high percentage of albumin-specific IgE. Recombinant dog albumin fragment, representing 265 amino acids of the mature protein, bound IgE from all 15 patients allergic to albumin tested (55).

An association between allergy to epithelia and allergy to mammalian meat has also been reported, and most authors ascribed this to serum albumin as the responsible cross-reacting allergen. For example, a 28-year-old asthmatic male cook sensitised to Dog epithelium who developed wheezing and contact urticaria when handling raw Beef in an occupational setting was reported. Skin reactivity was found to raw and cooked Beef and raw Lamb, and to Cat and Dog. Dog-specific IgE was positive. The secondary cross-reactivity was attributed to Bovine Serum Albumin (BSA) (56).

Furthermore, patients with persistent Milk allergy and IgE antibodies to BSA were reported to be at greater risk of rhinoconjunctivitis and asthma because of cross-reactivity with serum albumin present in animal dander. In a study evaluating the cross-reactivity among serum albumin of various mammals in milk, meat, and epithelia, sera from all but 1 patient recognised serum albumin in Cow's milk, in meat from Beef, Lamb, Deer, and Pork, and in epithelia from Dog, Cat, and Cow. Some patients were sensitised only to serum albumin in meat and epithelia. Patients allergic only to dander recognised other proteins in epithelia but not serum albumin. The authors concluded that serum albumin is an important allergen in Cow's milk, meat, and epithelia allergy. The authors proposed that sensitisation first occurs with contact with serum albumin in Cow's milk and that patients develop sensitisation to serum albumin present on animal epithelia even without direct contact with animals. The authors cautioned that patients with both BSA and Cow's milk allergy must avoid raw meats and furry pets (57).

Although studies have demonstrated a high degree of homology among serum albumins from various mammals, epitope diversity results in different clinical expressions of cross-reactivity. This is particularly well described in Pork-Cat cross-reactivity, where cross-reactivity has been demonstrated between Pork meat and Cat epithelia as a result of serum albumin, but not to Dog (58-59).

Dog serum albumin may be used for diagnostic purposes to identify patients who are cross-sensitised to many animal species, and perhaps may be used for specific immunotherapy of sensitised individuals (35).

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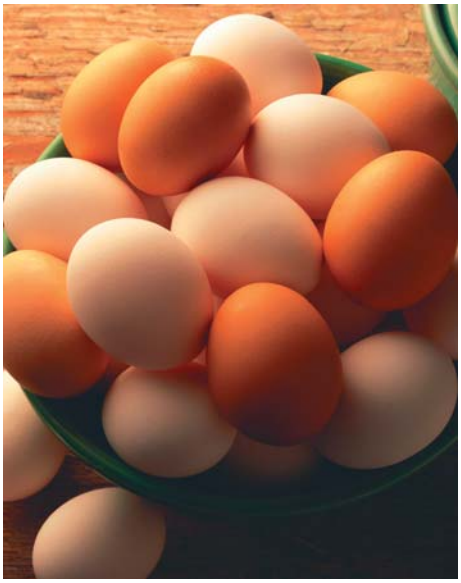
Egg allergen components

Gallus domesticus

Available ImmunoCAP®:

f233 Ovomucoid (nGal d 1)
f232 Ovalbumin (nGal d 2)
f323 Conalbumin (nGal d 3)
k208 Lysozyme (nGal d 4)



Summary

Hen's egg comprises about 8-11% shell, 56-61% white and 27-32% yolk. The white is essentially an aqueous protein solution (10% protein and 88% water), and the yolk is composed of 50% water, 34% lipid and 16% protein, giving it quite different properties (1-2).

Egg white

Egg white is the common name for the clear liquid (also called the albumen) contained within an Egg. It is the cytoplasm of the Egg, which until fertilisation is a single cell (including the yolk). Egg white is approximately 88% water and 10% protein. Its primary natural purpose is to protect the Egg yolk and provide additional nutrition for the growth of the embryo, as it is rich in proteins and is of high nutritional value. Unlike the Egg yolk, it contains a negligible amount of fat. Fifty-four percent of Egg white protein is composed of the major protein albumin (Ovalbumin). Other major proteins here are Conalbumin (Ovotransferrin) (12%), Ovomucoid (11%), Ovomucin (3.5%) and Lysozyme (3.4%). Other proteins have also been identified in Egg white: ovoinhibitor, avidin (0.5%), ovomacroblobulin, G2 and G3 globulins, and cystatin. Ovoflavoprotein is found in Egg white and yolk (3-4).

Allergens from *Gallus domesticus* listed by IUIS*

Gal d 1	Gal d 2	Gal d 3
Gal d 4	Gal d 5	

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

Egg yolk

The Egg yolk serves as the food source for the developing embryo inside. Prior to fertilisation, the yolk together with the germinal disc is a single cell. The Egg yolk is suspended in the Egg white (known more formally as albumen or Ovalbumin) by 1 or 2 spiral bands of tissue called the chalazae. Yolk contains all of the Egg's fat and cholesterol, and almost half of the protein. Egg yolk contains approximately 50% water, 16% protein and 32-35% lipid (4). Egg yolk can be separated into 2 fractions. The granule fraction contains 60% protein and 35% lipid, whereas the clear supernatant (the plasma fraction) contains 18% protein and 80% lipid. The granule fraction contains lipovitelin, phosvitin (16%) and lipoprotein (different from the lipoprotein found in Egg white). Phosvitin is the iron-carrying molecule of the yolk (4).

The total number of Egg proteins is not known, but more than 40 have been suggested for Egg white alone (5), and up to 24 different antigenic protein fractions have been isolated.

Egg allergen components

Characteristics of the Major Egg Proteins are presented in Table 1.

Table 1. Characteristics of the Major Egg Proteins.

Egg white proteins	Heat sensitivity	Molecular weight (kDa)
11% Ovomucoid (Gal d 1)	stable	28
54% Ovalbumin (Gal d 2)	stable?	44
12% Conalbumin* (Gal d 3)	labile	66-78
3.5% Lysozyme (Gal d 4)	labile	14

*also known as Ovotransferrin

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. This was demonstrated in a study of 11 patients with a history of Egg allergy, in all of whom 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 each inhibit the IgE binding of the other, yolk could be only partly inhibited by white in 8 sera (6).

Even though Ovalbumin is probably one of the most studied antigens in immunology, it does not appear to be the most allergenic molecule in humans. In a study of 34 adults with confirmed Egg allergy, Conalbumin (Ovotransferrin) and Ovomucoid were demonstrated to be the most prevalent allergens. Using the agreement between 2 or more of 4 laboratory methods as a criterion for evidence of sensitisation, the frequency of reactivity was found to be 53% (Conalbumin), 38% (Ovomucoid), 32% (Ovalbumin), and 15% (Lysozyme) (7).

However, 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. For example, it is likely that the Egg proteins are processed differently in the digestive system of infants and adults. 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).

The main allergens in Egg are found in the Egg white, but Egg yolk also contains a large portion of specific IgE-binding allergens (9). Gal d 1, Gal d 2, Gal d 3 and Gal d 4 are the most important allergens in Egg white. All are glycoproteins. Ovomucoid makes up approximately 10% of Egg white and is often regarded the major allergen (10-11).

Cross-reactivity has been shown among Conalbumin (Ovotransferrin), Ovomucoid and Lysozyme, and between Ovalbumin and the Yolk protein apovitellenin I. The cross-reactions among all of these proteins may signify that there is a number of common allergenic determinants on these Egg proteins, which gives a molecular basis for the phenomenon of cross-reactivity (4).

ImmunoCAP®: f233 nGal d 1
Native protein purified from Egg white (*Gallus domesticus*)

Biological function:	Ovomucoid
Mw:	28 kDa

Allergen description

Ovomucoid, or Gal d 1, previously known as Gal d I or Gal d III, is a major allergen of Hen's egg (1,8,10-11,14-15,23,25,33,45-53).

Ovomucoid is, together with Conalbumin (Ovotransferrin), the major allergenic protein in Hen's egg. The highest concentration is found in Egg white. Ovomucoid is a unique Egg protein and is the dominant allergen in Hen's egg, even though Ovomucoid comprises only 10% of total Egg white protein and Ovalbumin comprises >50% (25,52).

Ovomucoid is a highly glycosylated 28 kDa protein comprising 186 amino acids arranged in 3 tandem domains (Gal d 1.1, 1.2, and 1.3) (52). The amino acid sequences of the first 2 domains, Gal d 1.1 and 1.2, are 50% homologous, whereas <30% of Gal d 1.3 is homologous to Gal d 1.1 and 1.2 (54). The epitopes are conformational rather than linear, and the carbohydrate moieties have only a minor effect, if any, on allergenicity. Ovomucoid is highly homologous to pancreatic secretory trypsin inhibitor, although its trypsin inhibitory activity is confined to the second domain (55).

The significance of the 3 domains remains to be fully elucidated. In a study using serum samples from 45 patients with elevated serum-specific IgE to Hen's egg (IgE >20 kU_A/l), and with Egg allergy confirmed by DBPCFC conducted with direct ELISA to determine the percentage of patient Ovomucoid-specific IgE reactive with each of the 3 Ovomucoid domains, 42 patients had IgE antibodies specific to all 3 domains; 3 patients had no detectable IgE antibodies to Gal d 1.1. Although most patients had IgE antibodies to all 3 domains, the percentage of IgE antibodies directed to Gal d 1.2 was significantly greater ($p < 0.05$) than that to either Gal d 1.1 or 1.3 (52).

Ovomucoid is heat-stable (e.g., 100 °C for 1 h) and is not denatured by urea. It is resistant to protease digestion (56-57). The allergenic potential of Ovomucoid is thought to depend on its stability to heat treatment and digestion. When the digestion of Ovomucoid in simulated gastric fluid was kinetically analysed, 21% of the examined patients retained their IgE-binding capacity to the small 4.5 kDa fragment. Patients with a positive reaction to this small peptide fragment were thought to be unlikely to outgrow their Egg white allergy (45).

It has been suggested that Ovomucoid is the immunodominant protein fraction in Egg white and that the use of commercially purified Ovalbumin has led to an overestimation of the dominance of Ovalbumin as a major Egg allergen and antigen in humans (25).

As the stomach in newborn infants has little secretary pepsin and an out-of-optimum pH of peptic activity, Ovalbumin and Ovomucoid in raw and heat-coagulated Egg white are said to be poorly digestible at pH over 3.0, and this is purported to be responsible for their allergenicity and for the delayed outgrowth from Hen's egg allergy in patients with delayed maturation of stomach functions. In a study of the peptic digestibility of raw and heat-coagulated Hen's egg white proteins in the acidic pH range, Ovalbumin in raw Egg white was slightly digested by pepsin at pH 1.5 and pH 2.0, and was almost resistant to the

enzyme at pH 2.5 and over. This was altered in heat-coagulated Egg white at the pH range from 1.5 to 2.5, where the protein was well digestive against the enzyme, whereas peptic digestibility of Ovomucoid in raw Egg white was good at the pH range from 1.5 to 2.5, but almost non-existent at pH 3.0 and over, where improvement of the digestibility of the protein was not found even in heat-coagulated Egg white (40).

Studies have elucidated the contribution of individual Hen's egg components, e.g., Ovomucoid, to adverse clinical effects. Sensitisation and elicitisation of symptoms to Ovomucoid may occur through ingestion, inhalation or skin contact. The possibility of sensitisation due to Ovomucoid in house dust has been suggested (42), and Ovomucoid has been shown to be present in human breast milk (43).

In a study designed to determine the importance of Ovomucoid in the development of allergies to Egg white, a double-blind, placebo-controlled food challenge in subjects with high levels of IgE antibodies for Egg white was conducted to compare the allergenicities of heated and Ovomucoid-depleted Egg white, freeze-dried Egg white, and heated Egg white. Twenty-one of 38 subjects with positive challenge responses to freeze-dried Egg white had negative challenge responses to heated Egg white, whereas 16 of 17 subjects (94.1%) with positive responses to heated Egg white did not respond to the heated and Ovomucoid-depleted Egg white challenge. The subjects with positive challenge responses to freeze-dried Egg white tended to have higher IgE antibody values to Ovomucoid than did those with negative responses. IgE antibody levels to Ovomucoid were significantly higher in subjects with positive responses to a challenge with heated Egg white than in those with no response. The authors concluded that Ovomucoid has a more important role in the pathogenesis of allergic reactions to Egg white than other proteins in Egg white (33).

Subjects are often encountered without overt symptoms despite high IgE antibodies to Egg white and its components. The measurements of these antibodies are not necessarily efficient for the diagnosis or the prediction of the outcome of Egg allergy in children. A study measured specific IgE antibodies to Egg white and its components, including Ovomucoid, Ovalbumin, (Ovotransferrin), Conalbumin and Lysozyme, by direct RAST assays and by inhibition studies in 30 subjects who were divided into 2 groups with positive (n=18) and negative (n=12) oral challenge tests with Egg white antigens. The individuals with positive results to the first challenge tests were given the second provocation tests at mean intervals of 32 months. IgE-binding activity of the sera collected on the first challenge to these Ovomucoid fragments was compared between subjects with positive and negative reactions to the follow-up challenge tests. There were no significant differences in IgE antibody titers to Egg white and its components between the positive and negative groups at the first and the second challenge tests. IgE-binding activity to Ovomucoid digests after treatments with pepsin and trypsin, except chymotrypsin, were significantly higher in subjects with positive challenge tests than in those with negative results. The study concluded that IgE-binding activity to pepsin-digested Ovomucoid was of diagnostic value for distinguishing the challenge-positive subjects from the negative subjects, and that subjects with high IgE-binding activity to pepsin-treated Ovomucoid are unlikely to outgrow Egg white allergy (49).

Approximately two-thirds of Egg-allergic infants become tolerant within the first 5 years of life. A study sought to compare the recognition of sequential (linear) and conformational binding sites of Ovomucoid, Ovalbumin and Ovotransferrin (Conalbumin) by IgE antibodies of children with persistent and transient Egg allergy, to identify immunodominant IgE- and IgG-binding epitopes of Ovomucoid, and to compare epitope specificity of IgE antibodies between patients with differing histories of Egg allergy. Patients with long-lasting Egg allergy had higher concentrations of IgE antibodies against sequential and native Ovomucoid and Ovalbumin than did the children who subsequently gained tolerance ($p < 0.01$). Four major IgE-binding epitopes were identified in Ovomucoid. IgE antibodies of all 7 patients with persistent Egg allergy recognised these epitopes, whereas the antibodies in none of the 11 children who outgrew their Egg allergy did so. The study concluded that patients with persistent Egg allergy develop IgE antibodies against more-sequential and conformational epitopes of Ovomucoid and Ovalbumin, and that the presence of serum IgE antibodies to specific sequential epitopes of Ovomucoid may be used as a screening instrument for persistent Egg allergy (58).

f232 nGal d 2

ImmunoCAP®: f232 nGal d 2
Native protein purified from Egg white (*Gallus domesticus*)

Common name:	Albumin
Biological function:	Ovalbumin
Mw:	44 kDa

Allergen description

Gal d 2, also known as Ovalbumin and Albumin, is a 44 kDa phosphoglycoprotein (8,11-30).

Gal d 2 was previously known as Gal d I and Gal d II. An isoform, Gal d 2.0101, has been characterised.

Ovalbumin is a major allergen of Hen's egg white and is the most abundant of Egg white proteins, comprising 54% of the total proteins and a fivefold greater quantity than Ovomucoid. It has 4 cysteine residues and a single cystine disulphide bridge. When Egg white proteins are separated by electrophoresis, 3 Ovalbumin bands appear, corresponding to the dephosphorylated, mono- and di-phosphorylated forms (12).

Ovalbumin was previously considered to be the most important allergen of Egg white. But its importance was over-estimated due to frequent contamination of commercial preparations with Ovomucoid (25). In spite of a difference in the molecular weights of Ovomucoid and Ovalbumin, they cannot be completely separated by some processes, which has lead to the erroneous assumption of cross-reactions.

Ovalbumin has homology with a group of proteinase inhibitors known as serpins. However, Ovalbumin does not have proteinase inhibitory activities (12).

Ovalbumin is also susceptible to proteolysis when treated with subtilisin. However, the cleaved product does not show a conformational change or a difference in heat stability (12). However, it easily aggregates and becomes difficult to extract by heating (31). Ovalbumin digestion in both simulated gastric fluid and simulated intestinal fluid has been demonstrated to be markedly decreased (32).

Although Ovalbumin is heat-stable, in a study the heated and Ovomucoid-depleted Egg white preparation was less allergenic than heated or freeze-dried preparations. Ovomucoid must have a more important role in the pathogenesis of allergic reactions to Egg white than do other proteins in Egg white (33). A more recent study indicated that heated and Ovomucoid-depleted Egg white was less allergenic than heated Egg white (34). It has been reported that Ovalbumin allergenicity could be effectively reduced by the combination of heat and gamma irradiation treatment (22). A study of Egg white proteins in an animal model suggested that over-cooking of proteins may affect their intestinal antigen processing and thus prevent the induction of oral tolerance (35).

Ovalbumin has the ability to cross the placenta in a dose-dependent and molecular-weight-dependent manner in full-term and premature babies, with clear accentuation in preterm placentas, and may provide the foetus with the necessary stimulus for T cell priming or potential sensitisation (36-38).

Ovalbumin has also been shown to cross into human breast milk and may result in sensitisation that elicits symptoms in the infant. In a study to determine whether the concentration of Ovalbumin in human milk is directly related to the quantity and form of Egg consumed by breastfeeding mothers, 41 breastfeeding women were randomly allocated to receive a test breakfast, identical except for the Egg content (no Egg, 1 raw Egg, half a cooked Egg or 1 cooked Egg). There was a response directly dose-dependent on the amount of cooked Egg ingested and the peak Ovalbumin concentration (no Egg, 0.05 ng/ml; half a cooked Egg, 2.24 ng/ml; 1 cooked Egg, 3.16 ng/ml), as well as on the total Ovalbumin excretion (no Egg, 0.18 ng/ml/h; half a cooked Egg, 4.93 ng/ml/h; 1 cooked Egg, 9.14 ng/ml/h). There was no detectable OVA in the breast milk of 24% of the women (10/41) up to 8 hour after any Egg challenge (39).

As the stomach in newborn infants contains little secretary pepsin and has an out-of-optimum pH of peptic activity, there is low digestibility of Ovalbumin and Ovomucoid in raw and heat-coagulated Egg white at over pH 3.0, and this is supposed to be responsible for their allergenicity and for the delayed outgrowth from Hen's egg allergy in patients with delayed maturation of stomach functions. In a study of the peptic digestibility of raw and heat-coagulated Hen's egg white proteins at the acidic pH range, Ovalbumin in raw Egg white was slightly digested by pepsin at pH 1.5 and pH 2.0, and was almost resistant to the enzyme at pH 2.5 and over. This was altered in heat-coagulated Egg white at the pH range from 1.5 to 2.5, where the protein was well digestive against the enzyme, whereas peptic digestibility of Ovomucoid in raw Egg white was good at the pH range from 1.5 to 2.5, but almost non-existent at pH 3.0 and over, where the improvement of the digestibility of the protein was not found even in heat-coagulated Egg white (40).

Studies have elucidated the contribution of individual Hen's egg components, e.g., Ovalbumin, to adverse clinical effects. Sensitisation and elicitisation of symptoms to Ovalbumin may occur through ingestion, inhalation or skin contact. Adverse effects have been documented to the ingestion of as little as 10 mg of Ovalbumin (41). As sensitisation due to Ovomucoid in house dust has been suggested (42), and as Ovomucoid has been shown to be present in human breast milk (43), both may also be true for Ovalbumin, although neither has been evaluated yet.

Egg-allergic children may occasionally develop contact urticaria to Hen's egg and yet have no overt symptoms on ingestion. In a study to investigate possible mechanisms, 21 subjects with positive reactions to patch tests with Egg white allergens were divided into subgroups with positive (n = 10) and negative (n = 11) results to oral challenge tests with the same allergens. There were no significant differences in serum-specific IgE levels to Egg white (positive vs. negative: 30.3% *vs*. 15.3%), Ovomucoid (21.5% *vs*. 10.2%), Ovotransferrin (Conalbumin) (9.9% *vs*. 3.7%), and Lysozyme (3.4% *vs*. 2.9%). But in the case of Ovalbumin (16.8% *vs*. 5.6%), there was a difference between the positive and negative subjects in the provocation tests. The study suggested that IgE antibodies from subjects with contact urticaria to Hen's egg but tolerance to ingestion of Egg white recognise the epitope(s) unstable to digestive enzymes (44).

In a study suggesting that Egg contributes to the development of atopic dermatitis in younger infants by inducing the production of IL-5 but not IL-4, the results demonstrated that Ovalbumin-induced IL-5 production fluctuates with age in a different manner than IL-4 or Egg white IgE (24).

f323 nGal d 3

ImmunoCAP®: f323 nGal d 3
Native protein purified from Egg white (*Gallus domesticus*)

Common name:	Ovotransferrin, Ag22
Biological function:	Conalbumin
Mw:	66-78 kDa

Allergen description

Gal d 3, also known as Conalbumin, Ovotransferrin, and previously as Ag22, is a 66-78 kDa protein (8,11-12,14-16,35,59-64).

Conalbumin is a glycoprotein which is present in Egg white, Egg yolk, and plasma. The proteins from all 3 sources have the same amino acid sequence, but there are slight differences in the glycosylation. The protein is made up of 2 domains with a short linking region. Each domain has a very strong binding site for iron. There is about 40% homology in the sequences of the 2 domains. The function of Conalbumin is generally accepted as being iron transport. It binds 2 atoms of iron, 1 in each domain (12). Conalbumin has complex disulfide and bilobal structures, which are derived from the same gene as Chicken serum transferring (59).

In a RAST inhibition study with heat-treated Egg white allergens (100 °C, 5, 10, and 30 minutes) performed on 13 serum samples from subjects with immediate hypersensitive reactions and 9 serum samples from subjects without immediate hypersensitive reactions, it was demonstrated that heat treatment decreased the IgE-binding activity of Egg white. When the individual allergens were assessed, IgE-binding activities to Egg-White components, including Ovalbumin, Conalbumin, and Lysozyme but not Ovomucoid, were significantly decreased with heat treatment (30). Although heat denaturation of proteins can minimise allergenicity, a study suggested that over-cooking of proteins may affect their intestinal antigen processing and thus prevent the induction of oral tolerance (35).

Only partial cross-reactivity has been demonstrated between Chicken serum albumin and Conalbumin (65).

Transferrins are an important class of iron-binding proteins widely distributed in the physiological fluids of vertebrates and invertebrates. In vertebrates they are present mostly in serum, as serotransferrins. In birds and reptiles transferrins are also found in Eggs as Conalbumins. A study demonstrated significant homology of Conalbumin among red-eared turtle, African ostrich and Turkey, but allergenic potential was not investigated (66).

k208 nGal d 4

ImmunoCAP®: k208 nGal d 4
Native protein purified from Egg white (*Gallus domesticus*)

Biological function:	Lysozyme
Mw:	14 kDa

Allergen description

Lysozyme is an enzyme that consists of 129 amino acids cross-linked by 4 disulfide bridges. Lysozymes are small globular proteins found in animal tissues, organs and serum as well as in tears, milk, saliva, nasal secretions and cervical mucus. Lysozymes differ from species to species. Lysozyme also occurs naturally in many organisms such as viruses, plants, insects, birds, reptiles and mammals.

Egg lysozyme is also known as Gal d 4 (see below) and is a potent allergen (28).

The major source of commercial Lysozyme, in particular for pharmaceutical use (where it is known as Lysozyme chloride or Lysozyme hydrochloride), is extraction and purification of Hen egg albumen. Lysozyme concentration in Egg albumen is about 0.5% (67). Lysozyme chloride is usually extracted from fresh Egg white by means of a biotechnological process. One method involves a food-grade inert material (a polymer resin) being mixed with the Egg white, where it binds specifically with the Lysozyme. The resin carrying the Lysozyme is then stripped off, concentrated, purified and dried. The dried, purified protein is almost 100% Lysozyme chloride. The substance is heat-stable (80 °C for 2 minutes). It is inactivated at lower temperatures with increased pH. The optimum temperature for activity is 55 to 60 °C (67).

Gal d 4, Lysozyme, is a 14.3 kDa protein (8,11,14-16,68-70).

Lysozymes are small globular proteins and may be found in many other animal tissues, and in tears and saliva. They differ from species to species (28). Lysozyme has also been described as a defence-related protein found in Latex and a number of fruits. Latex and Fruit lysozyme is enzymatically very similar and has been demonstrated to be allergenic. However, this Lysozyme is not the same as Egg lysozyme (71).

Lysozyme is an allergen for some patients (9). Additionally, Lysozyme per se may be used as an additive and through this route may uncommonly induce symptoms of food allergy in sensitised individuals. However, Lysozyme has been reported to be a more common allergen in occupational settings, resulting in adverse effects following skin contact or inhalation.

Early studies reported that, due to its protein nature, Lysozyme has immunogenic properties and can provoke anaphylactic reactions (72-73). But its potency was regarded as moderate and considerably lower than that of other proteins such as Albumen and Ovalbumin. Hen's egg lysozyme was initially thought to be a minor problem: patients who experienced adverse reactions after consumption of Eggs most frequently showed IgE antibodies to one of the many protein components of Egg white, but very rarely to Egg white lysozyme (28).

However, a more recent study found that, with 31% of food-allergic children and 8% of food-allergic adults being allergic to Egg, out of 52 patients clinically allergic to Egg, 35% had anti-Lysozyme IgE. The authors concluded that, because of this high incidence of Lysozyme sensitisation, the presence of Lysozyme as an additive should be indicated on food labels (74).

Allergy to Lysozyme may be more frequent than has been previously documented. Over a period of more than 10 years (1990-2002), 171 cases of adverse reaction to food were registered by the Swedish authorities; 5 of 21 cases of allergic reaction to Egg were attributed to Lysozyme as an additive to cheese (75).

Lysozyme has often been used as a preservative in the pharmaceutical industry, and drug allergy to Lysozyme preparations has been reported (76). Hen's egg white Lysozyme has also been commonly used in some countries to treat diseases of the respiratory tract. In a study examining Egg-specific IgE in patients with Egg allergy, and in patients with allergies other than to Egg, high levels of allergen-specific IgE to Lysozyme were found in 30 out of the 39 patients allergic to Egg. The study also described a patient with anaphylaxis following exposure to Lysozyme, who had a level of 1.0 (PRU/ml) of IgE antibodies to Lysozyme. The authors cautioned against treating allergic patients with Hen's egg white lysozyme (77).

A pharmaceutical industry worker developed occupational asthma and rhinitis from both serratial peptidase and Lysozyme chloride. Skin prick tests were strongly positive to peptidase and Lysozyme extracts, and bronchoprovocation tests showed an immediate and delayed asthmatic response to peptidase, and an immediate asthmatic response to Lysozyme. Allergen-specific IgE antibodies to peptidase and Lysozyme were detected (78).

Initially, Lysozyme was considered of little significance as an allergen because of its thermolability (14), but the presence of IgE antibodies to Lysozyme was found to be common in Egg-processing workers (79-80).

Occupational asthma resulting from the inhalation of Egg lysozyme was described in a 26-year-old man employed in the manufacture of Hen's egg white-derived Lysozyme for use in the pharmaceutical industry. He began to experience immediate-onset asthmatic symptoms 2 months after starting to work with Egg lysozyme powder. Skin prick test was positive to Egg lysozyme and other Egg proteins, but negative to whole Egg white and Egg yolk. Serum-specific IgE to Egg lysozyme was found. A specific bronchoprovocation challenge to Lysozyme powder was positive, resulting in an immediate asthmatic response (81).

Inhaled allergens are a serious problem in the bakery and confectionery industries. Sensitisation to Wheat flour and enzymes such as α-amylase is a frequent cause of occupational asthma (82). Bakers are often exposed to aerosolised Egg allergens. In a study of 4 bakery workers who had developed work-related allergic respiratory symptoms upon exposure to Egg aerosols, skin-reactivity to Egg white extract and to Lysozyme was detected in all the workers, to Ovalbumin in 2, to Ovomucoid in 1, and to Egg yolk in 2. They were additionally sensitised to Wheat, Rye and Barley flour.

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Hevea brasiliensis

Available ImmunoCAP®:

k215 rHev b 1*
k217 rHev b 3*
k218 rHev b 5
k219 rHev b 6.01*
k220 rHev b 6.02*
k221 rHev b 8*
k222 rHev b 9*
k224 rHev b 11*
* MBP fusion protein

Summary

Natural latex is a milky sap produced by over 2,000 plants, whereas the Latex that is used industrially is derived almost exclusively from the rubber tree *Hevea brasiliensis*. Ammonia is added to the sap as a preservative at the time of harvesting. Processing of Natural latex results in Natural rubber latex (cis-1,4-polyisoprene). Many chemicals are added to Natural latex before, during and after processing, including antioxidants, emulsifiers, stabilisers and accelerators. Processing converts the sap into liquid Latex concentrate or solid dry rubber. Latex concentrate is used to make items such as gloves, condoms, balloons, catheters, baby pacifiers, and dental dams. Dry rubber is the essential ingredient in tires, tubing, hoses, footwear, automotive components, engineering parts, and adhesives. Latex concentrate contains about 1% total protein, of which a small fraction remains in the manufactured product. This protein is responsible for IgE-mediated reactions. Dry rubber, however, contains very little protein and therefore is much less immunogenic. Besides Latex protein, additives from the manufacturing process such as mercaptobenzothiazoles, carbamates, and thiurams may form haptens and act as allergens.

True Latex allergy develops from plant protein in the Latex sap itself, whereas concomitant allergies may also develop from the chemicals added during processing. An individual may have concomitant allergies, such as an IgE-mediated allergy to Latex proteins and a lymfocyte-mediated hypersensitivity to carbamates. Carbamates may be found in some non-Latex substitutes recommended for Latex-sensitive individuals. A third type of reaction is an irritant contact dermatitis, which is often associated with Latex but not caused by Latex itself, and may result, for example, from the alkaline pH found in many powdered gloves.

Natural rubber products must be distinguished from items manufactured with synthetic rubber, such as butyl rubber and neoprene (polymers of 2-chlorobutadiene), which pose no risk to persons sensitised to natural rubber proteins.

In general, the Latex serum obtained by centrifugation may be quite variable in its protein content, depending on the treatment of the Latex after collection from the rubber tree as well as on the considerable batch-to-batch variation in the protein content of the rubber tree sap due to genetic or environmental factors (1). Latex allergen content may vary widely even in the same product, with a variance of 3,000-fold having been documented for Latex gloves obtained from 10 separate manufacturers (2).

Latex allergen components

Rubber latex contains more than 200 proteins. Ultra-centrifugation of the fresh Latex sap results in as many as 9 fractions, of which 3 are most easily discerned: the rubber particle proteins, the C-serum and the bottom fraction (B-serum). Rubber particle proteins are water-insoluble. Most of the C-serum and B-serum proteins are water-soluble (1).

Rubber particle proteins

The rubber particle proteins comprise the rubber particles and 2 main insoluble proteins, which are extractable from the surface of the rubber particles. Two allergens have been identified, Hev b 1 and Hev b 3, both major allergens and strongly associated with Latex allergy in spina bifida (SB) patients (1).

C-serum proteins

Latex C-serum contains various proteins (more than 200 polypeptides), of which some are enzymes associated with rubber biosynthesis. Four characterised Latex allergens, Hev b 5, Hev b 7.02, Hev b 8, and Hev b 9, belong to the group of C-serum proteins, which are present in the cytosol fraction of the Latex. The most important allergen of this subgroup is Hev b 5, a heat-stable protein (1).

B-serum proteins

B-serum contains a smaller number of proteins, among which hevein is the most prominent and makes up more than 50% of the total soluble B-serum proteins. B-serum currently includes a group of 9 characterised Latex allergens (Hev b 2, Hev b 4, Hev b 6.01, Hev b 6.02, Hev b 6.03, Hev b 7.01, Hev b 10, Hev b 11, Hev b 13), which are extra-cytosolic proteins. With the exception of Hev b 7.01, all belong to the group of plant defense proteins. A tenth allergen belonging to this group is Hev b 12, a lipid transfer protein (1).

Natural rubber latex-allergenic proteins include those involved in the biosynthesis of polyisoprene and the coagulation of Latex rubber elongation factor, small rubber particle protein, prohevein, and patatin. Structural and pathogenesis-related proteins include beta-1,3-glucanases, endochitinases (chitinase), hevamine, microhelix protein complex, proline-rich protein, profilins, enolases, and manganese superoxide dismutase (3). Other proteins isolated include proteasome subunit C5, malate dehydrogenase, and triosephosphate isomerase (4-5). Recently, a number of other proteins with allergenic activity have been isolated: Hev b Thioredoxin h, Hev b UDPGP (a UDP-glucose Pyrophosphorylase), Hev b Citrate-binding protein, Hev b Hevamine (a chitinase), Hev b IFR (an isoflavone reductase), and Hev b Rotamase (a cyclophilin) (6).

Latex allergy occurs more frequently among individuals heavily exposed to natural rubber latex (NRL) products, including healthcare workers (HCW), laboratory workers, food handlers, hairdressers, cleaning staff and rubber industry workers. Children with neural tube defects such as SB have a particularly high prevalence of Latex allergy. Latex-sensitive persons with spina bifida have been shown to react preferentially to Hev b 1 and Hev b 3 proteins, whereas Latex-sensitive healthcare workers are more apt to be sensitised to Hev b 5 and Hev b 6.

Latex allergy is perhaps more complex than many other allergies in that it stems not from a single protein, but from no fewer than 13 known Latex allergens, with no single allergen deemed to be dominant. Sources of NRL are of varying quality and difficult to standardise for diagnostic purposes. As most Latex-allergic patients are sensitised to more than one Latex allergen, a blend of a number of allergens allows the identification of a greater number of Latex-allergic patients (7). Recombinant allergens may therefore be of great value in composing an appropriate blend of for more exact diagnosis.

Hev b 1 and the homologous Hev b 3 are associated mainly with young SB patients, whereas Hev b 5, Hev b 6 and Hev b 7 are linked more to adult Latex-allergic patients (8). A recent review study reported that native Hev b 2, recombinant Hev b 5, native

or recombinant Hev b 6, native Hev b 13, and possibly native Hev b 4 are the major allergens relevant to Latex-sensitised adults (7).

There has been a number of epidemiological studies of varying subject sizes, many attempting to determine which proteins behave as major allergens in different risk groups.

In a study evaluating sensitisation to Latex allergens in HCW with histories of Latex allergy, Hev b 2, Hev b 5, Hev b 6.01, and Hev b 13 produced positive skin reactions in more than 60% of subjects, with Hev b 1, 3, 4, and 7.01 eliciting reactions in less than 50%. Specificity of 7 Hev b allergens was 100% in identifying workers with confirmed NRL allergy, and 98% for Hev b 13 (9).

A study population of 38 Latex-allergic and 15 SB Latex-sensitised children showed that natural Hev b 1 was recognised by 82% and natural Hev b 3 by 79% of the SB Latex-allergic children. Fifteen (39.5%) of 38 Latex-allergic and 2 (13%) of 5 SB Latex-sensitised children demonstrated IgE binding to natural Hev b 7. Further studies including rHev b 7 demonstrated that Hev b 7 was a third SB-associated Latex allergen (10).

The relative propensities for IgE binding to individual Latex allergens, compared using sera from Latex-allergic patients, found that IgE antibody binding to Hev b 4, Hev b 7b, Hev b 5 and Hev b 2 occurred in 75, 61, 31 and 28% of the study group, respectively. Multiple allergen sensitisation was common: of the 31 sensitised patients, 23 (74%) had specific IgE directed against at least 2 Latex allergens, while 12 (39%) had IgE antibodies for at least 3 allergens. The data suggested that many patients might have acquired sensitivity to Hev b 2, Hev b 4 and Hev b 7b from Latex products. Sensitivity to Hev b 5 and to Hev b 7c were interrelated and thought to have been acquired from sources other than Latex products, i.e., from certain foods (11).

Using purified Latex allergens, Hev b 1, 2, 3, 4, 6 and 7, allergen-specific IgE was demonstrated in 32-65% of HCW and 54-100% of SB patients with Latex allergy. Using a combination of Hev b 2 and Hev b 7, 80% of HCW and 92% of SB patients with Latex allergy were identified by ELISA technique, but the combination gave lower positive rates when IgE antibody tests were used. The addition of Hev b 3 allowed the detection of allergen-specific IgE in all SB-Latex allergic patients (12).

A study comparing skin reactivity of 6 recombinant Latex allergens with NRL proteins in 31 Latex-allergic individuals found that rHev b 2, 3, 5, 6, 7, 8 were positive in at least one Latex-allergic patient. Sensitisation to the various recombinant allergens was similar to that shown by previous studies using the native proteins. The use of a combination of recombinant Latex allergens, Hev b 5, 6 and 7, diagnosed Latex allergy with 93% sensitivity and 100% specificity (13).

The IgE antibody pattern has also been shown to differ between children with Latex allergy who have not undergone surgery and those with a history of multiple operations. The major allergens in children with no history of surgery appear to be Hev b 6.01 and Hev b 6.02 and not Hev b 1, a finding similar to that reported for HCW with allergy to Latex (14).

Therefore, one or a combination of Latex recombinant allergens may be used to easily determine allergen sensitisation profiles in different groups of Latex-allergic patients. Natural and recombinant allergens may also be used for assessing the allergenic potential of glove samples. A study detected all 6 Latex allergens tested for in at least some of the glove samples; Hev b 5 and Hev b 13 were identified as the marker allergens that combined best to explain the variation in the glove allergenicity. The study concluded that the overall allergenic potential of Latex gloves could be estimated by using Hev b 5 and Hev b 13 as indicator allergens. The correlation between glove allergenicity and the level of these allergens was maintained for low-protein gloves (<200 µg/g) (15).

Latex allergen components

Immunological and clinical properties of characterised Latex allergens. Listed by IUIS*

Latex allergen	Significance as Latex allergen	Significance of cross-reactivity	IgE-binding prevalence of the allergen	Documented on k82 Latex ImmunoCAP®
Hev b 1	**High** (especially in spina bifida patients)	Not observed yet	HCW: 55/105 (52%) SB: 56/69 (81%)	++
Hev b 2	**Medium**	Medium	HCW: 20/31 (65%) SB: 7/13 (54%)	++
Hev b 3	**High** (especially in spina bifida patients)	Not observed yet	HCW: 13-20% SB: 76-78%	++
Hev b 4	**Not determined**	Not observed yet	No clear results	nt
Hev b 5	**High** in all risk groups: HCW, spina bifida, atopics	Not observed yet (structural homology with a Kiwi fruit protein)	HCW: 68-92% SB: 33-66%	++
Hev b 6.01	**High** in all risk groups: HCW, spina bifida, atopics	High (especially with Banana, Kiwi, Avocado)	LAP: 15/20 (75%) LAP: 24/29 (83%)	++
Hev b 6.02	**High** in all risk groups: HCW, spina bifida, atopics	High (especially with Banana, Kiwi, Avocado, etc.; main IgE-binding epitope)	LAP: 24/43 (56%) HCW: 48/64 (75%) SB: 3/11 (27%)	++
Hev b 6.03	**High** in context with Hev b 6.01	High (structural homology to plant stress proteins)	LAP: 3/20(15%) LAP: 11/52(21%)	nt
Hev b 7.01	**Low-Medium**	Unclear (structural homology to proteins from Potato and Tomato, but no cross-reactivity with Banana and Avocado	LAP: 4/36 (11%) LAP: 17/35 (49%)	++
Hev b 7.02	**Medium only in SB**	Unclear – see Hev b 7.01	SB: 15/30 (39.5%)	++
Hev b 8	**Low** (profilin is a ubiquitous pan-allergen)	Medium	LAP: 2/19 (11%) HCW: 20-24% SB: 6-12%	++
Hev b 9	**Low**	Medium cross-reactivity with moulds	LAP: 15/110 (15%)	+
Hev b 10	**Low**	Medium cross-reactivity with moulds	HCW: 0/20, SB: 2/20 LAP: 4/15 (27%)	++
Hev b 11	**Low**	High cross-reactivity with fruit allergens, especially hevein-like sequences	LAP: 10/57 (19%) LAP (53 ??? HCW (5SB): 17/58 (29%)	++
Hev b 12	**Low**	Medium pan-allergen; cross-reactivity with fruits	LAP: 9/37 (24%)	nt
Hev b 13	**High**	Not determined yet	HCWs by SPT: 39/62 (63%)	nt

LAP = Latex-allergic patients
HCW = healthcare workers
SB = spina bifida patients

++ Satisfactory amounts on k82 Latex ImmunoCAP™
\+ Acceptable but low amounts on k82 Latex ImmunoCAP™
nt Not tested/not available
* International Union of Immunological Societies (www.allergen.org) Jan. 2008.

From: Rihs H-P, Raulf-Heimsoth M. *Natural rubber latex allergens: Characterization and evaluation of their allergenic capacity*. New Horizons, Phadia AB 2003; No 3.

ImmunoCAP®: k215 rHev b 1
Recombinant non-glycosylated MBP-fusion protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 1

Common name:	Rubber elongation factor (REF)
Biological function:	Involved in biosynthesis of polyisoprene
Mw:	15 kDa

Allergen description

Hev b 1 (16-18), also known as rubber elongation factor (REF), is one of the most important Latex allergens and is a leading cause of Latex IgE-mediated allergy in children with spina bifida (SB) (19). Hev b 1 has also been shown to be an important allergen in healthcare workers (HCW) (20). It is a Latex-specific allergen without relevant homology to other plant proteins. Hev b 1 makes up 10% to 60% of the total protein found in Latex (3). Although Hev b 1 is not constantly found in Latex sap, it is the main protein found in Latex glove extract (21). In a laboratory where gloves are worn for protection, the use of Latex gloves resulted in a 26-fold increase in inhaled Latex allergen over background levels measured where vinyl gloves were worn as controls (22).

Hev b 1 is closely related to Hev b 3 (23). Both Hev b 1 and Hev b 3 are major water-insoluble proteins located on the surface of rubber particles in *H. brasiliensis* Latex. Hev b 1 is found mainly on large rubber particles, and Hev b 3 mainly on small rubber particles. Both allergens bind IgE from patients with SB and Latex allergy (17).

Studies have reported a wide range of allergen-specific IgE binding to Hev b 1, with 54-100% of SB patients with Latex allergy reacting to Hev b 1, whereas a frequency of only 13-32% was observed in HCW (1).

In a study of serum from 140 SB patients as well as from 105 HCW allergic to Latex evaluated for sensitisation to highly purified Hev b 1, 81% with SB and allergic to Latex had IgE antibodies directed to Hev b 1, whereas antibodies to Hev b 1 were found in 52.3% of HCWs allergic to Latex (24).

Similarly, other studies have reported that Hev b 1 is a more prevalent allergen in SB Latex-allergic individuals than HCW Latex-allergic individuals. For example, 4 of 6 SB Latex-allergic children exhibited IgE antibodies against Hev b 1, compared to only 1 of 30 Latex-allergic patients (25).

SB patients have been reported to display a unique pattern of sensitisation: IgE reactivity is preferentially directed against Hev b 3 and Hev b 1, the 2 Latex allergens with high sequence similarity (26). In a study of 35 Latex-allergic patients with SB, 29 showed IgE binding to rHev b 3, as did 4 of 15 of the Latex-sensitised group. Hev b 3 is related to Hev b 1 by a sequence identity of 47%. Although cross-reactivity between these 2 Latex allergens was illustrated by the large extent of inhibition of IgE binding to nHev b 1 by rHev b 3 (27), no cross-reactivity between Hev b 3 and Hev b 1 has been shown at the T cell level (26).

k215 rHev b 1

Studies performed with recombinant Hev b 1 (rHev b 1) showed that 16 out of 71 Latex-allergic HCW (23%) had IgE antibodies to rHev b 1. This confirmed the results of studies performed with the native counterpart (18).

Although no major cross-reactivity has been reported to Hev b 1, in a study of cross-reactivity of IgE antibodies recognising epitopes of Latex allergens and papain in sera of 36 Latex-exposed subjects and 22 papain workers, it was reported that 8 of 24 Latex-sensitised individuals showed low or moderate levels of allergen-specific IgE to papain, and 6 of the 12 sensitised papain workers had serum IgE to Latex allergen(s). Comparison between the primary sequences of Hev b 1 and papain suggested that the cross-reactivity might be due to several identical trimers and tetramers (28). Further studies may clarify this relationship.

In summary, Hev b 1 is one of the major Latex allergens in SB patients and is of intermediate relevance in the risk group HCW.

k217 rHev b 3

ImmunoCAP®: k217 rHev b 3
Recombinant non-glycosylated MBP-fusion protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 3

Common name:	Small rubber particle protein, SRPP
Biological function:	Involved in the biosynthesis of polyisoprene
Mw:	24 kDa

Allergen description

Hev b 3 (13,16,29) forms an integrated part of the "small rubber particles" (23). It has a significant role in rubber synthesis because of its ability to synthesise long-chain polyisoprene (3).

Hev b 1 is closely related to Hev b 3 (23). Both Hev b 1 and Hev b 3 are major water-insoluble proteins located on the surface of rubber particles in *H. brasiliensis* Latex. Hev b 1 is found mainly on large rubber particles, and Hev b 3 mainly on small rubber particles. Both allergens bind IgE antibodies from patients with spina bifida (SB) and Latex allergy (17).

Several studies with sera of Latex-sensitised SB patients showed IgE reactivity frequencies of 67-83% (1). The reason for these observed high frequencies might be due to stretches of high sequence homology between Hev b 3 and Hev b 1 (1).

Hev b 3 has been found to be an important allergen in SB patients, but in contrast, the reactivity to Hev b 3 is less frequent among health care workers (HCW) (30). In immunoblots 29/35 SB patients were shown to have allergen-specific IgE binding to rHev b 3, whereas this was only shown in 4 of 15 of the Latex-sensitised group. IgE epitopes on rHev b 3 were shown to abolish all IgE binding to nHev b 3. Hev b 3 is related to Hev b 1 by a sequence identity of 47%. Cross-reactivity between these 2 Latex allergens was illustrated by the large extent of inhibition of IgE binding to nHev b 1 by rHev b 3 (27). However, no cross-reactivity between Hev b 3 and Hev b 1 has been demonstrated at the T cell level (26).

rHev b 3 coupled to ImmunoCAP™ (Rk217) revealed a comparable frequency of 12.5% in 40 Latex allergic HCW tested (1).

k218 rHev b 5

ImmunoCAP®: k218 rHev b 5

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 5

Common name:	Acidic protein
Biological function:	Unknown, a structural protein
Mw:	16 kDa

Allergen description

Hev b 5 (11,13,31-34) is a potent Latex allergen and is heat-stable (35). Its physiological function is unknown. Hev b 5 exists as multiple isoforms, but only small amounts are present in the non-ammoniated Latex preparations, such as those used for diagnostic tests, and this may help to explain the relatively poor sensitivity of some *in vitro* tests (36). Therefore, most of the research has been performed with the recombinant form, rHev b 5. In serological tests, 92% of Latex-allergic adult health care workers (HCW) and 56% of the spina bifida (SB) Latex-allergic patients showed Hev b 5-specific IgE antibodies in their sera (32).

It has also been shown that rHev b 5 could be used as a complement reagent to enhance the quantitative performance of Latex ImmunoCAP™ for allergen-specific IgE measurement (37). A significant number (16%) of serum samples became more strongly positive to the improved k82 (spiked with rHev b 5) than to the regular k82 Latex ImmunoCAP™, and a rather small number of previously negative serum samples became positive (37). Hev b 5 may be the missing allergen to fill the diagnostic gap for some allergic patients with clear clinical Latex allergy but with negative serological reactivity (1).

Furthermore, Hev b 5-specific monoclonal antibodies and human IgE from Latex-allergic HCW demonstrate the greater content of Hev b 5 in high-protein powdered glove extracts. This may explain the observed higher frequency of sensitisation to this allergen in HCW (33).

The nucleotide and deduced protein sequences or rHev b 5 have significant homology to sequences from Kiwi and Potato, which are known to cause allergic reactions in some Latex-allergic patients (32). The sequence homology (47% sequence identity) between these 2 acidic proteins suggests a molecular explanation for the high frequency of fruit hypersensitivity in Latex-allergic patients (38). A novel gene has been isolated from a Sugar beet cDNA library that resembles members of the Latex allergen Hev b 5 family (39). However, the clinical significance has not been established.

Hev b 5 has been identified as a potential candidate for immunotherapy. A recombinant Hev b 5 protein with significantly reduced IgE-binding activity has been described, and this may prove to be a valuable reagent for immunotherapy (31).

k219 rHev b 6.01

ImmunoCAP®: k219 rHev b 6.01
Recombinant non-glycosylated MBP-fusion protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 6.01

Common name:	Prohevein (hevein precursor)
Biological function:	Class I endochitinase containing a hevein domain
Mw:	20 kDa

Allergen description

Hev b 6.01 (40-41), prohevein, is one of the most important Latex allergens in health care worker (HCW) Latex allergy. Prohevein, Hev b 6.01, is processed to yield 2 allergenic fragments, the N-terminal hevein, Heb b 6.02, and the C-terminal portion, Hev b 6.03 (3,42). All 3 allergens exist in the plant, although the ratio between Hev b 6.01 and Hev b 6.03 is about 30:1 (43). All 3 components act as independent allergens (41). Hevein comprises the most important part of IgE-binding epitopes in the prohevein molecule.

Hev b 6.01 was reported to be recognised by 88.9% of 54 Latex-allergic patients (21). Other studies have reported a prevalence of between 70-86% sensitisation to this allergen in Latex-allergic individuals (40, 44).

In a study of Latex-allergic patients, prohevein bound IgE from sera of 15 of 20 (75%) patients, and the prohevein C-domain bound 3 of 20 (15%) Latex-allergic patient sera. In ELISA, 36 of 52 (69%) patient sera showed IgE binding to prohevein, whereas 11 of 52 (21%) sera had IgE antibodies to the prohevein C-domain. Purified hevein inhibited 72% of IgE binding from pooled sera of Latex-allergic patients to solid phase glove extract and 45% of IgE binding to solid phase Natural rubber latex (NRL) (45).

The recombinant allergen has allergenic activity very similar to that of native Hev b 6.01. Seventeen of 18 (94%) serum samples from Latex-allergic HCW showed increased levels of allergen-specific IgE to rHev b 6.01, 16 (89%) to rHev b 6.02, and 13 (72%) to rHev b 6.03 in a study evaluating recombinant Hev b 6 allergens. In the Hev b 6.01 precursor, the regions responsible for IgE binding and those for inducing the T-cell proliferation responses are settled in different parts of the protein. The Hev b 6.02 domain is responsible for IgE binding and carries discontinuous B-cell epitopes, whereas Hev b 6.03 is a better inducer of a proliferation response and contains HLA-DR4-binding motifs (41).

Individuals with NRL allergy often have immediate reactions to plant-derived foods and fresh fruits, such as Avocado and Banana. More than 50% of subjects having IgE-mediated NRL allergy are reported to be sensitised to Avocado, as demonstrated by allergen-specific IgE. About 10-20% report hypersensitivity reactions after ingesting Avocado. The conserved hevein domain of the major Latex allergen

k219 rHev b 6.01

prohevein (Hev b 6.01) is a ubiquitous chitin-binding protein structure that can be found in several plant proteins and may be responsible for the observed cross-reactivity between Latex and Avocado. Sensitisation to endochitinase class I containing a hevein domain is the main underlying pathomechanism in Latex-mediated Avocado allergy (46). In a study evaluating skin testing against purified proteins in 15 patients with NRL allergy, 11 (73%) patients were found to have reactivity to isolated hevein-like domains of Avocado and Banana, but only 1 (7%) patient reacted to their corresponding endochitinases. Proteins from Avocado and Banana inhibited binding of IgE antibodies to prohevein (Hev b 6.01) in 59% and 38% of patients, respectively (47). Other studies have also identified this as the panallergen responsible (48). In immuno-blotting studies, sera of 9 of 15 patients allergic to NRL with IgE antibodies to hevein also demonstrated specific IgE binding to 32- and 33-kDa Banana proteins (49). Similarly, in a patient who experienced an anaphylactic reaction to Apple juice containing acerola, cross-reactivity with Latex due to prohevein was demonstrated (50).

k220 rHev b 6.02

ImmunoCAP®: k220 rHev b 6.02
Recombinant non-glycosylated MBP-fusion protein produced in an E. coli strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 6.02

Common name:	Hevein
Mw:	5 kDa

Allergen description

Hev b 6.02 (41,51-52), hevein, is a small protein, which has been identified as the most common allergen for healthcare workers (HCW) allergic to Latex. About 75% of these workers allergic to Latex had hevein-specific antibodies (53). Hevein is not only a major IgE-binding allergen in Natural rubber latex (NRL) but also in other Latex manufactured products (42,54). Prohevein, Hev b 6.01, is cleaved naturally to yield 2 allergenic fragments, the N-terminal hevein, Heb b 6.02, and the C-terminal portion, Hev b 6.03 (3,42). All 3 allergens exist in the plant, although the ratio between Hev b 6.01 and Hev b 6.03 is about 30:1 (43). All 3 components act as independent allergens (41).

In a study, serum-specific IgE to hevein was detected by ELISA in 48 of 64 (75%) sera from HCW allergic to Latex, and in 3 of 11 (27%) sera from patients with spina bifida (SB) and hypersensitivity reactions to Latex. Skin-positive tests hevein was found in 17 of 21 (81%) patients with Latex allergy (55).

Hevein (Hev b 6.02) is the main allergen cross-reacting with Avocado in subjects with Latex allergy. Results of immunoblots and immunoblot inhibition with 11 serum samples confirmed that a 30-kDa protein in Avocado was the major IgE-binding component; the IgE-binding reactivity to this protein could be inhibited by hevein in all sera tested. Sixty-seven of 91 (73%) subjects from the HCW group and all 19 subjects in the SB group with positive IgE antibodies to hevein also had elevated IgE values to Avocado (53).

k221 rHev b 8

ImmunoCAP®: k221 rHev b 8
Recombinant non-glycosylated MBP-fusion protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 8

Common name:	Profilin
Biological function:	Actin-binding protein
Mw:	14 kDa

Allergen description

Hev b 8 (13,56-58,60) is a profilin. Plant profilins are important panallergens. They are responsible for a significant percentage of pollen-related allergies. The observed frequencies of Hev b 8-specific IgE antibodies in sera of Latex-allergic patients in different risk groups range between 6 and 24% (1,57-58,60).

rHev b 8 has a sequence identity of 75% with Birch profilin (Bet v 2) (57). Recombinant isoforms of Hev b 8 with marginal differences in the amino acid sequence were reported to have no influence on the IgE-binding properties of the rHev b 8 isoforms. In a study evaluating the prevalence of serum IgE antibodies to rHev b 8, among 17 SB patients, IgE antibodies to rHev b 8 were found in 2, and in 5 of 25 sera (20%) from HCW. Further studies demonstrated the presence of IgE-binding epitopes on the Hev b 8 molecule which did not cross-react with Birch profilin. The study concluded that Latex profilin represents a minor allergen in Natural rubber latex (NRL) and may have IgE-binding epitopes different from Bet v 2 (58).

These factors may explain the variability in the prevalence of allergen-specific IgE binding to Latex profilin in studies. For example, skin tests and allergen-specific IgE antibodies to natural and recombinant purified Hev b 8 were positive in 15 of 17 spina bifida (SB) children and all 14 adults allergic to Latex. However, only 42% of the Latex-allergic patients had allergen-specific IgE levels of 0.35 kU_A/L or higher, and only 39% of them exhibited IgE binding with any natural or recombinant Hev b 8 forms (56).

Between 30% and 50% of individuals who are allergic to Latex products are also allergic to specific plant foods, and this is aptly described as Latex-fruit syndrome. However, the roles of the Latex chitinase, Latex profilin and Latex beta-1,3-glucanase need to be clarified. This is well illustrated in a study, which reviewed simultaneous sensitisation to Latex and Bell pepper, sensitisation that had previously been reported. In sera of 4 patients with allergy to Latex and Bell pepper, 3 were shown to have IgE antibodies to profilin from Bell pepper and Latex. Two patients also had IgE antibodies to Hev b 2 (a beta-1,3-glucanase) and a homologous protein in Bell pepper. One patient was shown to have allergen-specific IgE to an L-ascorbate peroxidase, and another patient to a 38 kDa protein. The study concluded that Hev b 2 (beta-1,3-glucanase) and the Bell pepper L-ascorbate peroxidase were also cross-reactive allergens, and that profilin was responsible for some of the IgE cross-reactivity (59).

Similarly, other studies have demonstrated the variable responsibility of profilin in cross-reactivity between Latex profilin and other plant profilins. In a study of sera of 36 individuals containing IgE antibodies to Ragweed profilin, 35 reacted with profilin from Latex, indicating structural homologies between profilins from Latex and Ragweed. Fifty-nine percent of these sera were found to be positive for Latex-specific IgE. As profilin is also present in Banana, it was proposed that Latex profilin would likely be involved in cross-reactivity between Banana and Latex. However, among 19 individuals allergic to Latex, only 2 had anti-profilin IgE antibodies. The authors suggested that IgE antibodies to Latex profilin might be a questionable factor in sensitisation of occupationally exposed patients, but that sensitisation to profilin should be taken into account when interpreting the results of Latex-specific IgE investigation (60).

Recombinant profilin from Banana and Pineapple has a high sequence identity (71-84%) to known allergenic pollen and food profilin. In a study demonstrating IgE binding in sera to recombinant profilin, in 7/16 (44%) subjects with suspected Banana allergy, and in 8/19 (42%) subjects with suspected Pineapple allergy, high cross-reactivity to Birch pollen profilin Bet v 2 and Latex profilin Hev b 8 was demonstrated. Profilin was therefore shown to be an important mediator of IgE cross-reactivity between pollen and exotic fruits (61-62).

In a study using rHev b 8 to screen sera from Latex-allergic HCW with well-documented histories of food and pollen allergy and Latex-allergic SB patients, 12 of the 50 HCW and 2 of the 34 SB patients were sensitised to Hev b 8. All Hev b 8-sensitised patients showed allergic symptoms to pollen or plant foods. Cross-reactivity among profilins of Latex, pollen and plant food was demonstrated by their ability to inhibit IgE binding to rHev b 8. The authors concluded that primary sensitisation to Latex profilin in the majority of cases took place via pollen or food profilin, and that pollen- and food-allergic patients with profilin-specific IgE antibodies could be at risk of developing Latex allergy (57).

Other studies have also demonstrated the relevance of Latex profilin cross-reactivity, for example between Chenopodium profilin and Latex (65), and between 2 Rice profilin cDNAs (highly homologous to each other) and profilin from Maize, Bermuda grass, Timothy grass and Latex (63).

k222 rHev b 9

ImmunoCAP®: k222 rHev b 9
Recombinant non-glycosylated MBP-fusion protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 9

Common name:	Enolase
Biological function:	Enolase
Mw:	51 kDa

Allergen description

Hev b 9 (64) is an enolase, an ubiquitous enzyme involved in the carbohydrate catabolism pathway. A sequence identity of about 60% was reported between the enolase of *H. brasiliensis* and the enolases of moulds, and, in particular, cross-reactivity was demonstrated with the enolases from *Cladosporium herbarum* and *Alternaria alternata* (64). With the use of recombinant Hev b 9 (rHev b 9), IgE-binding reactivity was observed in 16 out of 110 Latex-allergic adults (14.5%).

In a recent study, only 1 out of 40 health care workers tested, and no patient with spina bifida, was reported to have Hev b 9-specifc IgE antibodies (1).

k224 rHev b 11

ImmunoCAP®: k224 rHev b 11
Recombinant non-glycosylated MBP-fusion protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Hevea brasiliensis* allergen Hev b 11

Common name:	Class 1 Chitinase
Biological function:	Chitinase plant defence
Mw:	32 kDa

Allergen description

Hev b 11 (65-66) is a class 1 chitinase with an N-terminal chitin-binding domain with homology to hevein (3). Heb v 11 shows greater than 65% identity with several other plant endochitinases (7). Chitinases are abundant proteins found in a wide variety of seed-producing plants. Most chitinases hydrolytically degrade chitin which is a major structural component of the cell wall of many fungi and the exoskeleton of many insects (3).

rHev b 11.0102 has been reported to have a 56% homology to hevein. rHev b 11.0102-specific IgE antibodies were found in 17 of 58 sera (29%) of IgE-mediated Latex-allergic subjects. Due to its IgE-reactivity, rHev b 11.0102 was reported to represent an allergen of intermediate prevalence in Natural rubber Latex (NRL), and it was stated that its cross-reactive potential with certain fruit makes it an important supplement in the diagnostic panel of recombinant NRL allergens (65).

Class I chitinases from Chestnut, Avocado and Banana have been identified as relevant allergens. The chitin binding (hevein) domain from these class I chitinases is thought to contain the important IgE binding epitopes. The *H. brasiliensis* chitinase, Hev b 11, was shown to have a 70% identity with the endochitinase from Avocado, and the identity was 58% between its hevein domain and Hev b 6.02 (hevein). rHev b 11 bound IgE antibodies in Latex- and fruit-allergic patients in 19% of 57 patients.

The study concluded that Hev b 11, although having a chitin-binding domain, displays a different IgE binding capacity compared with hevein (66).

Similarly, a study of class I chitinases, evaluated for their potential role as cross-reactive allergens in Latex-food allergy, found polyclonal antibodies to chitinases in sera from patients with Latex-fruit allergy; the antibodies were in response to chitinases of Chestnut, Cherimoya, Passion fruit, Kiwi, Papaya, Mango, Tomato, and Wheat flour extracts. Prs a 1, the major allergen and class I chitinase from Avocado, was shown to strongly or fully inhibited the IgE binding of Latex chitinase. The study concluded that putative class I chitinases appear to be relevant cross-reactive components in foods associated with Latex-fruit syndrome, but do not play a specific role in allergy to Latex without a concomitant allergy to fruit (67).

Cross-reactivity has been described between Obeche wood dust and Latex. The Obeche allergen, Trip s 1, a class I chitinase, was homologous to Latex hevein (68).

Japanese cedar (*Cryptomeria japonica*) pollen allergy is one of the most prevalent allergic diseases in Japan. The cDNA high-frequency IgE-binding protein (CJP-4) cloned from *C. japonica* pollen was reported to have significant sequence homology to class IV chitinases and was able to bind IgE antibodies from all 31 patients tested by ELISA. Pre-incubation with latex C-serum completely inhibited the reaction to purified CJP-4 of pooled serum IgE antibodies from patients with *C. japonica* pollinosis and/or Latex allergy (69).

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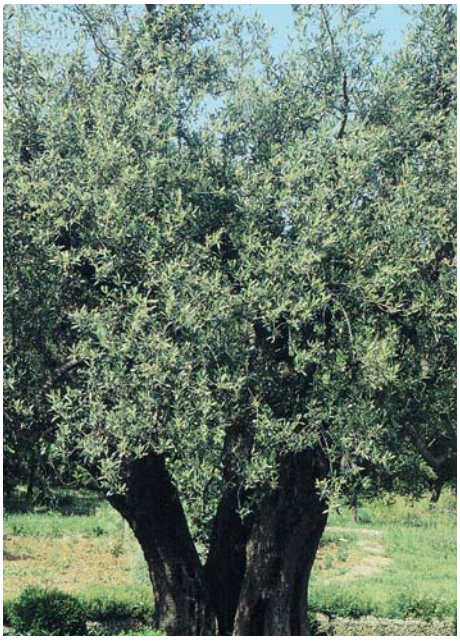
Olive allergen components

Olea europaea

Available ImmunoCAP®:
t224 nOle e 1



Summary

Olea europaea, the Olive tree, is one of the most important causes of seasonal respiratory allergy in the Mediterranean area (1) and also in other parts of the world where this tree is now grown. Olive tree is a member of the *Oleaceae* family, which has 4 important genera: Olive (*Olea*), Ash (*Fraxinus*), Lilac (*Syringa*), and Privet (*Ligustrum*).

Olive tree probably originated in Asia Minor, spread to the Mediterranean region, and was then introduced into North America (especially California and Arizona), South America (Chile), Australia and South Africa. Although in North America Olive trees are found only in the Southwest, Ash and Privet are widespread, a circumstance of relevance to cross-reactivity (2). Countries and regions have distinct varieties of Olive. In Italy, individual varieties of *Olea europaea*, which differ between the northern and southern parts of the country, may induce different IgE-mediated reactions (3).

The Olive tree is an evergreen growing to 10 m, with a broad, round crown and a thick and knotty trunk. The flowers are hermaphrodite (have both male and female organs). The plant is self-fertilising. Pollination is by insects but also by wind when pollen is in abundance. The pollination period varies: it typically occurs in the spring, but in Europe may start as early as January, depending on the region (1). In southern Italy it lasts from early April to late June, and as one moves north, lasts until July (3).

Olive pollens can induce asthma, allergic rhinitis and allergic conjunctivitis in sensitised individuals (4-11).

Allergens from *Olea europaea* listed by IUIS*

Ole e 1	Ole e 10

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

The frequency of sensitisation to Olive tree pollen varies in the Mediterranean region from ~10% of atopic individuals in Sicily to ~40% in Greece (1,12). In Greece, one study found that more than 37% of atopic individuals were sensitised to *Oleaceae* (13). Fifteen percent of atopic patients in southern France were found to be skin-prick positive to *Oleaceae* (14). In Italy, atopic sensitisation varied from 12% in Sicily to 30% in Apulia (15-19). In Naples, of 4,142 patients examined consecutively over a two-year period, 13.5% of adults and 8.5% of children of all skin prick test-positive patients were positive to *Olea* pollen allergens on skin-prick testing (20). Less than 1.4% of children and 2.3% of adults were found to be monosensitised to Olive pollen (20). In another study on 507 asthmatic atopic children in the Chieti-Pescara area of Italy, skin-prick tests found that 21% were sensitised to Olive tree pollen (21).

Olive allergen components

Sensitisation to Olive pollen has also been reported in Israel (22-23). Positive skin reactions to Olive pollen, among atopic patients of the Jewish population, was shown to be high where Olive trees are abundant (66%), and lower (29%) where the trees are scarce (24-25). In Spain, a study demonstrated that the frequency of sensitisation could vary greatly within the same country (26-27). The daily pollen concentration in the atmosphere showed pollen from the Olive tree to be one of the most common pollen grains (28).

Olive tree pollen has also been shown to result in sensitisation in Japan as well as in Israel; in the Japan 16% of pollinosis patients were positive to this allergen (25,29). Skin-prick tests for sensitisation to Olive tree pollen in the southern part of Switzerland (Canton Ticino) showed a high sensitisation rate of 54% (30).

The majority of studies demonstrate a higher prevalence of rhinoconjunctivitis than of asthma (1). Patients are more likely to be polysensitised than monosensitised to Olive tree pollen. Monosensitised individuals, children and adults, may have symptoms throughout the year without an apparent increase during the Olive pollination season (11,31).

The following allergens have been characterised.

Ole e 1 (32-36).

Ole e 2, a profilin (37).

Ole e 3, a calcium-binding protein (38).

Ole e 4 (39-40).

Ole e 5, a superoxide dismutase (39-40).

Ole e 6 (41).

Ole e 7, a lipid-transfer protein (42).

Ole e 8, a calcium-binding protein (41).

Ole e 9, a 1,3-beta-glucanase protein (43).

Ole e 10 (44).

t224 nOle e 1

ImmunoCAP®: t224 nOle e 1

Recombinant non-glycosylated protein produced in an *E. coli* strain strain carrying a cloned cDNA encoding *Olea europaea* allergen Ole e 1

Common name:	Common olive group 5, Group 1 *Oleaceae*
Biological function:	Trypsin inhibitor
Mw:	19 and 20 kDa

Allergen description

Ole e 1 (45) exhibits a high degree of polymorphism (46) and is present in Olive tree pollen in 2 main forms, glycosylated and nonglycosylated, with apparent molecular masses of 20 and 18.5 kDa, respectively (47). nOle e 1 is actually a mixture of polypeptides with different glycosylation patterns (46).

Of the many allergens isolated and characterised from Olive pollen, Ole e 1 is the most frequent sensitising agent, affecting more than 70% of patients with sensitisation to Olive pollen, although other allergens, such as Ole e 4 and Ole e 7, have also been shown to be major allergens. The prevalence of many Olive pollen allergens is dependent on geographical location (41).

Not all allergens are found in every Olive tree cultivar. In a study examining the various IgE-binding proteins of the pollen extracts of the various Olive tree cultivars, 6 predominant IgE-binding bands, some of which appear in all the cultivars, were found. Ole e 1 appeared in only 8 of the cultivars, but not in the 9 others (48).

Current standard diagnostic methods utilise crude pollen extracts that contain a complex mixture of allergenic and non-allergenic proteins. Furthermore, Ole e 1 concentration has been shown to have a 25-fold variation in pollen extracts (49). Therefore, using a well-defined allergen such as nOle e 1 allows for improved diagnosis and therapy.

A high degree of cross-reactivity has been demonstrated among Olive tree (*Olea europaea*), Ash (*Fraxinus exselsior*), Privet (*Ligustrum vulgare*) and *Phillyrea angustifolia* (a bush usually confined to certain areas of the Mediterranean) (2). All are members of the *Oleaceae* family, although there is no total identity among these 4 pollen species (50). The major pollen allergens from Ash (Fra e1) Privet (Lig v 1) and Lilac or Syringa vulgaris (Syr v 1), another member of the *Oleaceae* family, are proteins homologous to Ole e 1 (4,36,51-54). Ole e 1 has been reported to be a marker allergen for the diagnosis of Olive and European ash pollen allergy (55).

Therefore, nOle e 1 may be of diagnostic benefit in particular in areas where no Olive trees exist but other Ole e 1-cross-reactive pollens are found. For example, in northern and central Europe, where there are no Olive trees, 2 commonly occurring genera of the *Oleaceae* family, *Fraxinus* and *Ligustrum*, are present; but these have a low frequency of allergic sensitisation compared to *Olea*. The importance of cross-reactivity is demonstrated by a study in Michigan, USA, where in 103 atopic subjects, cross-reactivity among Olive tree, *Fraxinus*, Privet and Russian olive tree pollens was demonstrated, even though the Olive tree does not grow in that area. Nineteen subjects were skin prick-positive to this allergen, confirming the effect of cross-reactivity (2).

Cross-reactivity between extracts of *Oleaceae* and some species of the *Poaceae* family has also been shown (56-57). The major allergen of *Plantago lanceolata* (English plantain) pollen, Pla l 1, has been shown to have significant sequence homology with the major Olive pollen allergen Ole e 1 (58).

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Peach allergen components

Prunus persica

Available ImmunoCAP®:

f419 rPru p 1
f420 rPru p 3
f421 rPru p 4

Summary

Peach is the fruit of a small deciduous tree growing to 10 m tall, belonging to the subfamily *Prunoideae* of the family *Rosaceae*. It is classified with the Almond in the subgenus *Amygdalus* within the genus *Prunus*, distinguished from the other subgenera by the corrugated seed shell. Cultivated Peaches are divided into "freestone" and "clingstone" cultivars, depending on whether the flesh sticks to the stone or not. These two classes merge in different varieties, and even the same variety of tree may yield freestone and clingstone fruit in different seasons. Both kinds can have either white or yellow flesh. At least 300 varieties of Peach are grown throughout the world, each with distinct physical characteristics and a distinct ripening season.

The nectarine is a cultivar of Peach that has a smooth skin without fuzz (hair). Nectarines can be white, yellow, clingstone, or freestone. Regular Peach trees occasionally produce a few nectarines, and vice versa. Peaches and nectarines look very similar, but they can be told apart by their skin texture: Peaches are fuzzy and dull, while nectarines are smooth and shiny.

Peach is a well-documented and common cause of allergy in children and adults, resulting in oral allergy and systemic reactions such as urticaria, asthma and anaphylactic shock following the ingestion of fresh or processed fruit. This is particularly notable in the Mediterranean area, where Peach is regarded as a major allergen (1-14). Peach has also been described as the primary food causing anaphylaxis in Israel (12).

Several Peach allergens of major importance have been detected, including a lipid transfer protein, a profilin, and many larger proteins (15-16).

Allergens from *Prunus persica* listed by IUIS*

Pru p 1	Pru p 3	Pru p 4

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

The following allergens have been characterised:

Pru p 1, a Group 1 *Fagales*-related Protein, PR-10 protein.

Pru p 3, a non-specific lipid transfer protein (1, 15-31).

Pru p 4, a Profilin (16-17,20,32).

Pru p glucanase, a 1,3-beta-glucanase (33-34).

The allergen that was known as Pru p 1 has been renamed Pru p 3 and Pru p 1 is now the name for a PR10 protein, the Group 1 *Fagales*-related Bet v 1 homologue.

Peach-allergic individuals in the Mediterranean area are in most cases not allergic to Birch tree pollen, and the main reactions are not directed to Bet v 1 homologues or profilin but to non-specific lipid transfer proteins (nsLTPs) (35). Allergic symptoms involving nsLTPs are more likely to be systemic and severe, in addition to causing oral allergy syndrome. In contrast, sensitisation to the lipid transfer protein Pru p 3 is rare among Central and Northern European populations (17). Morever, allergy to Peach and other *Rosaceae* fruits in patients with a related pollen allergy, like most patients in these populations, is a milder clinical entity, and profilin- and Bet v 1-related structures are involved (36).

f419 rPru p 1

ImmunoCAP®: f419 rPru p 1
Recombinant non-glycosylated protein produced in an *E. coli* strain strain carrying a cloned cDNA encoding *Prunus persica* allergen Pru p 1

Common name:	Bet v 1-homologous allergen, Group 1 *Fagales*-related protein, PR-10 protein
Biological function:	Ribonuclease
Mw:	17 kDa

Allergen description

Pru p 1 is a 17 kDa peach protein belonging to the PR-10 protein family. The major birch pollen allergen Bet v 1 is the most prominent member of this family, with which Pru p 1 shares 59% amino acid sequence identity (40). In some allergen sources, PR-10 like proteins have been shown to be encoded by multiple genes, giving raise to arrays of closely related isoforms. Further, PR-10 proteins are produced intracellularly in a tissuedependent manner during plant development and their expression is subject to regulation by factors such as environmental stress or pathogen attack (41). The threedimensional structure of several PR-10 protein has been determined and found to contain a solvent-exposed cavity in which ligands such as fatty acids, brassicosteroids or phospholipids may bind (42-43).

Pru p 1 is heat labile (44-45) and most subjects suffering from birch pollen induced peach allergy may therefore tolerate food items containing cooked peaches.

The concentration of Pru p 1 in peach fruit is low (46). In addition Pru p 1 is easily degraded and/or chemically modified during extraction procedures and may thus be inadequately represented in natural peach extracts (47).

PR-10 proteins have been identified in many plant foods as well as in pollen of *Fagales* species (e.g. birch, hazel, alder, oak, hornbeam, beech). Despite relatively modest levels of sequence identity, homologues from more distantly related plant species, such as Pru av 1 from cherry and Api g 1 from celery, are structurally similar (48-49) which explains the observed cross-reactivity patterns within the protein family. Pru p 1 cross-reacts extensively with Bet v 1 homologous from *Prunus* pecies (e.g. cherry, apricot, plum) and other *Roseaceae* fruits such as apple and also, although to a lower degree, with PR-10 proteins from foods like carrot, celery, soy and peanut.

While Pru p 1 is the vastly predominant allergen in birch pollen-related peach allergy, IgE reactivity to Pru p 1 is less common among peach allergic subject in birch-free areas such as many Mediterranean regions.

Sensitization to Pru p 1 is not necessarily manifested as clinical reactions to peach but is a good marker for the birch-fruit syndrome.

Ingestion of peach and other related foods may elicit local reactions such as the oral allergy syndrome (OAS) and rhino-conjuctivitis but also, in rare cases, more severe systemic reactions (37-39).

f420 rPru p 3

ImmunoCAP®: f420 rPru p 3
Recombinant non-glycosylated protein produced in an *E. coli* strain strain carrying a cloned cDNA encoding *Prunus persica* allergen Pru p 3

Common name:	nsLTP 2
Biological function:	Non-specific lipid transfer protein
Mw:	9-10 kDa

Allergen description

Pru p 3 is a non-specific lipid transfer protein (nsLTP). nsLTPs are panallergens that have a ubiquitous distribution in tissues of many plant species, resulting in variable degrees of cross-reactivity, and in particularly relevant cross-reactivity in fruits and vegetables (23).

Lipid transfer proteins are small molecules of approximately 9 -10 kDa that demonstrate great stability and are very resistant to pepsin and heat treatment (50). Lipid transfer proteins facilitate the transport of phospholipids and galactolipids across membranes. Non-specific lipid transfer proteins belong to the PR 14 family of pathogenesis-related proteins.

Lipid transfer proteins are highly conserved and widely distributed throughout the plant kingdom. They have been identified as allergens in the *Rosaceae* subfamilies of the *Prunoideae* (Peach, Apricot, Plum) and of the *Pomoideae* (Apple). They belong to a family of structurally highly conserved proteins that are also present in non-*Rosaceae* vegetable foods. They have been linked to severe and systemic symptoms and induce sensitisation by the oral route in fruit-allergic patients who do not have associated pollen allergy. This is probably due to extreme pepsin stability; the allergens probably reach the intestinal tract in an almost unmodified form.

The lipid transfer proteins essentially concentrate in the skin of *Rosaceae* fruits as cell surface-exposed allergens (15, 28). LTP is found in Peach peel in amounts approximately 7 times greater than in pulp (26). It may be absent from chemically peeled fruit, and levels of LTP vary in different cultivars and at different stages of the ripening process, showing a progressive increment during ripening (51). A study was made to evaluate the hypothesis that Peach may lose its allergenicity and therefore its primary role as a sensitiser to LTP as a consequence of processing preceding marketing in Northern Europe: Peach surface fuzz reactivity in Peach-allergic individuals was shown to be stronger than reactivity to peel. Pre-absorption of one serum with Peach LTP caused an 87% reduction of IgE antibodies reactivity to Peach fuzz extract (35).

Allergy to lipid transfer protein is quite common in the Mediterranean countries but almost absent in Northern Europe (35). Lipid transfer protein is usually associated with more severe systemic reactions than oral allergy syndrome. Peach LTP (Pru p 3) is a minor allergen in Northern European countries but a major allergen in the South, affecting over 60% of patients allergic to Peach in the Spanish population (1). In Peach-allergic patients who have experienced systemic reactions to Peach, up to 100% may be sensitised to LPT (17).

f420 rPru p 3

Pru p 3, possibly along with other larger proteins, is involved in allergenic relationships with other fruits from the family *Rosaceae*, particularly Apricot, Cherry, and Plum (15-16,52). A high level of cross-reactivity occurs among fruits and vegetables containing lipid transfer proteins, which include Sweet chestnut (53), Cabbage (with 50% identity to Peach LTP) (54), Walnut (55), Lettuce (56), and Hazelnut (57). Grape and wine may contain lipid transfer protein homologous to and cross-reactive with Peach LTP (58). A report was made on a 19-year-old boy with a history of oral allergy syndrome after eating Peach, who presented with several episodes of generalised urticaria and angioedema approximately 15-20 minutes after drinking beer. It was found that the responsible allergen was a lipid transfer protein from Barley that was present in beer (59). Lipid transfer protein cross-reactivity is often accompanied by clinical food allergy, frequently including systemic reactions (22).

In a study examining the relationship between Peach LTP-specific IgE antibodies levels and cross-reactivity to several non-*Rosaceae* plant-derived foods, patients with negative skin reactivity for non-Rosaceae foods showed significantly lower levels of IgE antibodies to Peach LTP than did patients showing skin reactivity to one or more non-*Rosaceae* foods. Increasing levels of IgE antibodies to Peach LTP were associated with skin reactivity to nuts (29/40 [72%]), Peanut (27/40 [67%]), Maize (16/39 [41%]), Rice (14/39 [36%]), Onion (13/37 [35%]), Orange (9/32 [28%]), Celery (11/40 [27%]), and Tomato (8/39 [20%]). The study suggested that all allergenic determinants in LTP from vegetable foods other than Peach cross-react with Peach LTP determinants, whereas only some Peach LTP epitopes cross-react with allergenic determinants on botanically unrelated plant-derived foods. The high levels of IgE antibodies to Peach LTP suggested the presence of IgE antibodies that targeted common allergenic determinants of LTP, causing cross-reactivity to botanically unrelated vegetable foods. The authors concluded that in LTP-allergic patients, increasing levels of IgE antibodies to Peach LTP are paralleled by an increasing number of foods other than *Rosaceae* that are positive on skin test and cause clinical symptoms (60).

Allergenic LTPs from Peach fruit and Mugwort (*Artemisia vulgaris*) pollen are responsible for clinical symptoms in Mediterranean patients as a result of cross-reactivity (53, 61). In a study assessing the pattern of sensitisation to an array of Mugwort allergens in a Mediterranean population and the cross-reactivity of Art v 3 (Mugwort) with Pru p 3 and Par j 1, relevant lipid transfer proteins (LTP) allergens in the area, the 3 *Artemisia* allergens elicited a positive skin reactivity in 70 to 80% of the patients. Seven patients were clearly sensitised to Par j 1 and 11 to Pru p 3. There was no correlation between Par j 1 and Pru p 3 sensitisation, but a highly significant correlation was found between Peach and Art v 3 with regard to skin reactivity. No IgE antibodies cross-reactivity was observed between Art v 3 and Par j 1, or between Pru p 3 and Par j 1. In contrast, Art v 3 significantly inhibited the binding to Pru p 3 of IgE antibodies from 3 patients' sera out of 6 studied, but Pru p 3 was not able to inhibit IgE antibodies binding to Art v 3. The study concludes that Art v 3 is a major Mugwort allergen, and that in some patients with IgE antibodies to both Art v 3 and Pru p 3, Art v 3 behaves as the primary sensitising agent (62).

Therefore, hypersensitivity to Mugwort in patients with Peach allergy is due to a common lipid transfer protein allergen and is often without clinical expression (63). This is illustrated by a study of 47 patients allergic to Peach and 20 patients sensitised to Mugwort pollen who had no clinical food allergies; the rate of positive skin test for Peach, Apple, Chestnut and Mugwort LTPs were, respectively, 91, 77, 23, and 36% in the Peach group, and 30, 5, 15 and 40% in the *Artemisia* group. In Peach-allergic patients, the most frequent pattern of cross-reactivity to LTPs appears to be the combination Peach-Apple (45%), followed by Peach-Apple-Mugwort-Sweet chestnut (21%). Significant correlation was found between Peach and Apple LTPs, and between Mugwort and Sweet Chestnut LTPs (4).

It has been suggested that the primary sensitiser to LTP is Peach (35). Cross-reactivity to non-*Rosaceae* vegetable foods is strongly dependent on the level of IgE antibodies to Peach LTP (35,60).

In a study, immunodetection and immunoblot inhibition assays were carried out with sera from Peach-allergic patients and demonstrated that both the recombinant and natural forms of Pru p 3 displayed similar IgE antibodies-binding capacity (25).

In a study of 10 patients with allergy to Peach, all having experienced systemic reactions to Peach, all 10 patients had positive skin responses to nPru p 3, and 9 of 10 patients had positive FAST and CAST responses both with nPru p 3 and rPru p 3. Histamine release test responses were considered positive in 5 and 7 patients for nPru p 3 and rPru p 3, respectively. The study concluded that recombinant Pru p 3 showed a strong immunologic activity equivalent to that of its natural counterpart (17).

f421 rPru p 4

ImmunoCAP®: f421 rPru p 4
Recombinant non-glycosylated protein produced in an *E. coli* strain strain carrying a cloned cDNA encoding *Prunus persica* allergen Pru p 4

Biological function:	Profilin
Mw:	14 kDa

Allergen description

Pru p 4, a Peach profilin and an actinbinding protein, is a member of the profilin plant family. Profilins are panallergens that are recognised by IgE antibodies of about 20% of patients allergic to Birch pollen and plant foods (64). However, sensitisation to profilin can be expected in different populations at levels varying between 5 and 40%, depending on exposure to various profilin-containing allergen sources (65). They are heat- and digestion-labile and are therefore more often associated with less severe allergic reactions and oral allergy syndrome.

Profilins are small eukaryotic proteins, 14 - 17 kDa in size, involved in modulating the assembly of actin microfilaments in the cytoplasm. Profilins are ubiquitous in all eukaryotic organisms. They are able to bind both phosphatidylinositol-4,5-bisphosphate and poly-L-proline (PLP) and thus play a critical role in signaling pathways. Plant profilins are of particular interest because immunological cross-reactivity between pollen and human profilin may be the cause of hay fever and broad allergies to pollens (66). IgE antibodies reactivity to profilin appears to strongly depend on the highly conserved conformational structure, rather than on a high degree of amino acid sequence identity or even linear epitopes identitied, as demonstrated in a study evaluating Melon profiling (67).

Profilins can be isolated from tree pollens, e.g., Birch tree (*Betula verrucosa*), from pollens of grasses, e.g., Timothy grass (*Phleum pratense*), and from pollens of weeds, e.g., Mugwort (*Artemisia vulgaris*) (67).

Peach contains 2 profilin isoforms, Pru p 4.01 and Pru p 4.02, which show 80% amino acid sequence identity and are very similar (>70% identity) to allergenic profilins from plant foods and pollens. A complete correlation between reactivity to rPru p 4 and to rBet v 2 has been found in sera from Peach-allergic patients. In a study evaluating recombinant Peach profilin isoform reactivity, using sera of 29 patients with Peach allergy (as proved by DBPCFC), Pru p 4.01 was recognised by all sera (15 of 15) with IgE antibodies to Bet v 2, whereas no sera (0 of 14) without IgE antibodies to Birch allergen reacted with rPru p 4.01 (2). In the Spanish population, where Peach LTP is a major allergen, sensitisation to profilin is observed to be connected to pollen allergy but does not appear to be related to clinical reactivity to Peach (1). This may also be observed in other countries, in particular in Southern Europe, where Peach LPT is the dominant allergen.

IgE antibodies antibodies to profilin seem to be responsible for at least part of the observed allergenic relationship between Peach and grass and Olive tree pollen in the Mediterranean area, where *Betulaceae* pollens in the air are rare or absent (11,32, 68-70). Melon profilin has been shown to have substantial cross-reactivity with the Peach, Tomato, Grape and Bermuda grass (*Cynodon dactylon*) pollen profilins (67).

Some studies suggest partial or even absent IgE antibodies cross-reactivity among certain profilins. A study reports that the large amount of cross-reactivity among plant profilins justifies using a single profilin for diagnosis. However, it should be kept in mind that the fine specificity of IgE antibodies directed to variable epitopes may influence the clinical manifestation of profilin sensitisation (71).

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Peanut allergen components

Arachis hypogaea

Available ImmunoCAP®:

f422 rAra h 1
f423 rAra h 2
f424 rAra h 3
f352 rAra h 8

Summary

Peanuts are the seeds of an annual legume, which grows close to the ground and produces its fruit below the soil surface. This is in contrast to tree nuts like Walnuts and Almonds. Peanut is a member of the *Fabaceae* or legume family, whereas tree nuts are not.

Multiple Peanut varieties are grown, with more than 40% of the American Peanut crop consumed as Peanut butter (1). Runners have become the dominant Peanut type grown in the U.S. due to the spectacular increase in yield that they allow; they are a very important source of Peanut butter. Virginias have the largest kernels and account for most of the Peanuts roasted and sold in their shells. Spanish peanuts have smaller kernels covered with a reddish-brown skin. Valencias are small, very sweet Peanuts usually roasted and sold in the shell, or boiled, but seldom used in processed foods.

The difference in the methods of preparing Peanut as practiced in China compared with that widely used in the United States and Western countries may help explain the difference in prevalence of Peanut allergy observed (28). Roasting of Peanut uses higher temperatures (150-170 °C) than boiling (100 °C) or frying (120 °C), and roasting has been shown to increase the allergenic property of Peanut proteins (2).

However, part of the difference in allergenicity may not be as a result of the heat-treatment per se but as a result of other factors. Some authors suggest that the decrease in allergenicity of boiled Peanuts results mainly from a transfer of low-molecular-weight allergens into the water during cooking (3). Allergen content may vary depending on the Peanut variety and may explain the differences in the prevalence of sensitisation between different population studies (4).

Allergens from *Arachis hypogaea* listed by IUIS*

Ara h 1	Ara h 2	Ara h 3
Ara h 4	Ara h 5	Ara h 6
Ara h 7	Ara h 8	Ara h 9

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

The major Peanut allergens are homologous to the seed storage proteins of the conglutin, vicilin, and glycinin families (5).

Peanut proteins were originally classified as albumins (water-soluble) or globulins (saline-soluble); the globulins were in turn subdivided into arachin and conarachin fractions (the major storage proteins). Components of the albumin fraction of Peanuts are agglutinins, lectin-reactive glycoproteins, protease inhibitors, alpha-amylase inhibitors and phospholipases (6).

Peanut contains, among other, storage and non-storage proteins. The allergens, Ara h 1, Ara h 2, Ara h 3, Ara h 4, Ara h 6, Ara h 7 are seed storage proteins. The major Peanut allergen, Ara h 1, is a heat stable 7S vicilin-like globulin, Ara h 2 is a conglutin (functioning as a trypsin inhibitor), and Ara h 3 is a glycinin. A 59% sequence identity exists between Ara h 2 and Ara h 6, and 35% between Ara h 2 and Ara h 7 (7).

Peanut contains up to 32 different proteins, of which at least 18 have been identified as being capable of binding

allergen-specific IgE antibodies (8-9). Varieties of Peanuts from different parts of the world contain similar proteins, including Ara h 1 and Ara h 2, and the IgE-binding properties have also been reported to be similar to a great extent (10).

Allergens characterised to date include:

Ara h 1, a 7S vicilin-like globulin (11).

Ara h 2, a 2S albumin, a conglutin, a trypsin inhibitor (12).

Ara h 3, an 11S globulin, a glycinin, a trypsin inhibitor (13).

Ara h 4, an 11S globulin, a glycinin (14).

Ara h 5, a profilin (14).

Ara h 6, a 2S albumin, a conglutin (15).

Ara h 7, a 2S albumin, a conglutin (14).

Ara h 8, a Bet v 1-homologous allergen, PR-10 protein (16).

Ara h Agglutinin (17).

Ara h LTP, a lipid transfer protein (18).

Ara h Oleosin (9).

Ara h 3 and Ara h 4 are regarded as isoforms of each other, i.e., Ara h 4 and Ara h 3 are considered to be the same allergen (13,20).

Ara h 1 comprises 12% to 16% of the total protein in Peanut in population studies, sensitisation to Ara h 1 was found in 95% of Peanut-allergic patients from North America (6,21-23), but in fewer Peanut-allergic patients of 3 European populations, varying from 35% to 70% (1,15,24-25). These differences were not reported for Ara h 2, even though Peanuts from different varieties and from different parts of the world contain similar proteins and the IgE binding properties are similar (10). Unidentified Peanut proteins with molecular weights somewhat lower than 15 kDa may be important allergens as well (26). Ara h 3 is recognised by serum IgE from 45% - 50% of patients with Peanut sensitivity (27). Ara h 5 shows up to 80% amino acid sequence identity with the panallergen profilin, but is present only in low amounts in Peanut extracts. 13% to 16% of Peanut-allergic individuals are sensitised to Peanut profilin (28). Nonetheless, a number of peanut allergens are involved in the sensitisation process.

Sensitisation to Peanut occurs with a high degree of heterogeneity to a number of Peanut allergens. Mono-sensitisation to a single Peanut allergen is relatively rare (29). Although sensitisation to Ara h 1 and Ara h 2 occurs in the great majority of Peanut-allergic individuals, the wide range of allergens present in whole Peanut protein extract appears to be most appropriate to consider when testing for Peanut allergy (23).

For example, in a British study, evaluating sera of 40 Peanut-allergic individuals, of 18 allergens identified, 8 were bound by >50% of patients. The study concluded that promiscuity of IgE binding appears more important than the recognition of individual proteins (30).

Furthermore, some Peanut-allergic subjects fail to bind to either Ara h 1 or 2 suggesting that whole Peanut, rather than Ara h 1 or 2, or the use of individual Peanut allergens would be more appropriate for measuring allergen-specific-IgE responses. This also illustrates that the relative contribution of all Peanut allergens needs to be investigated (23).

In a recent Dutch study examining the IgE reactivity to major Peanut allergens in 20 Peanut-allergic children at two subsequent time-points, before DBPCFC, all 20 Peanut-allergic children were shown to have IgE antibodies to Ara h 2, 16 to Ara h 6, and 10 to both Ara h 1 and Ara h 3. After 20 months, Peanut-specific IgE levels and the individual recognition of major allergens were comparable with the levels and recognition before challenge. Skin reactivity was detected to Ara h 2 and Ara h 6 in most children, whereas for Ara h 1 and Ara h 3 in approximately 50% of the children. No parameters could be related to the severity of Peanut allergy (31).

The availability of recombinant Peanut allergens has resulted in a greater ability to assess the sensitisation and clinical profiles of individual Peanut allergens in different population groups. This is illustrated by a number of studies.

In an evaluation of recombinant allergens, Ara h 1, Ara h 2, and Ara h 3, using sera of 77 American Peanut-allergic patients, seven different patterns of sensitisation were identified. The majority of patients (97%) had IgE antibodies to at least one of the recombinant allergens (Ara h 1, Ara h 2, and Ara h 3), and 77%, 75% and 77% recognized rAra h1, rAra h 2 and rAra h 3 respectively. High epitope diversity was found in patients with a history of more severe allergic reactions (32).

A European study evaluating sera from 40 patients for sensitisation to six recombinant Peanut allergens, showed 14 individual recognition patterns. Of the sera, Ara h 1 was recognized by 65%, Ara h 2 by 85%, Ara h 4 by 53%, Ara h 5 by 13%, Ara h 6 by 38% and Ara h 7 by 43% (14).

Similarly, a French and American study aimed at evaluating the diagnostic value of the 3 major recombinant Peanut allergens utilizing skin test and serum IgE antibody determination in 30 Peanut-allergic patients. All patients with Peanut allergy demonstrated skin reactivity to rAra h 2; 40% reacted with rAra h 1 and 27% with rAra h 3. Monosensitisation to rAra h 2 was observed in 53% of patients. Levels of allergen-specific IgE did not correlate with the disease severity. However, patients with monosensitisation to rAra h 2 had a significantly lower severity score than polysensitised subjects and a lower level of allergen-specific IgE against Peanut extract and rAra h 2. Cosensitisation to rAra h 2 and rAra h 1 and/or rAra h 3 appeared to be predictive of more severe reactions (33).

A recent Dutch study investigated whether a sensitisation to individual allergens Ara h 1, Ara h 2, Ara h 3 and Ara h 6 could be correlated with clinical severity. Purified Peanut allergens were utilized for skin test and IgE antibody evaluation in 30 patients. The majority of patients were found to have allergen-specific IgE to Ara h 2 (25/30, 83%) and Ara h 6 (26/30, 87%). Sixteen patients (53%) were sensitised to Ara h 1 and 15 patients (50%) to Ara h 3. All patients with skin reactivity for Ara h 1 and/or Ara h 3 were also sensitised to Ara h 2 and/or Ara h 6. Patients with severe symptoms had a higher skin response to Ara h 2 and Ara h 6 at low concentrations (0.1 mug/ml) and to Ara h 1 and Ara h 3 at higher concentrations (100 mug/ml) compared with patients with mild symptoms. Patients with more severe symptoms also recognized a greater number of allergens and showed a higher cumulative skin response than with patients with mild symptoms. Ara h 2 and Ara h 6 appeared to be more potent than Ara h 1 and Ara h 3. Both skin reactivity to low concentrations of Ara h 2 and Ara h 6 and to higher concentrations of Ara h 1 and Ara h 3 were shown to be indicative of severe symptoms (34).

Recombinant Peanut allergens have been evaluated for their ability to predict the outcome of tolerance in Peanut-allergic individuals. An American study was performed using sera from 15 patients with symptomatic Peanut allergy and 16 patients who were sensitized but tolerant (of which 10 of these 16 patients had "outgrown" their allergy) investigated 8 peptides representing the immunodominant sequential epitopes on Ara h 1, 2, and 3. It was found that regardless of their Peanut-specific IgE levels, most patients with symptomatic Peanut allergy showed IgE binding to the 3 immunodominant epitopes on Ara h 2. In contrast, each of these epitopes was recognized by < 10% of the tolerant patients. Tolerant patients did not recognize 2 immunodominant epitopes on Ara h 1. At least 93% of symptomatic, but only 12.5% of tolerant patients, recognized 1 of these "predictive" epitopes on Ara h 1 or Ara h 2. With up to 50% of patients with Peanut-specific IgE levels below suggested diagnostic decision levels still being clinically reactive, oral food challenges could be avoided in approximately 90% of these patients through the determination of peptide-specific IgE. This study analyzed only selected allergen epitopes rather than whole proteins (35).

f422 rAra h 1

ImmunoCAP®: f422 rAra h 1
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Arachis hypogaea* allergen Ara h 1

Common name:	Conarachin, Vicilin
Biological function:	7S Vicilin-like globulin
Mw:	65 kDa

Allergen description

Ara h 1 is a vicilin, a member of the 7S vicilin-like globulin family (11,14,25,33-34, 36-40,41-54). It is also known as Conarachin. Ara h 1 is a 65 kDa protein that comprises 12% to 16% of the total protein in Peanut extracts (25) and causes sensitization from 35% to 95% of patients with Peanut allergy, depending on the population group studied (14,21,23-24,26-27,33,44,55). Ara h 1 has been reported to form a stable trimeric protein (21) but upon purification of native Ara h 1 from Peanuts using only size exclusion chromatography, the allergen appeared to exist in an oligomeric structure rather than as a trimeric structure (49).

Seed storage proteins commonly comprise various groups of multiple isoforms encoded by different gene families. Arachin (11S globulin), conarachin (7S globulin) and conglutin (albumin) are the three major storage proteins in Peanut. Ara h 1 has high sequence similarity with other plant vicilins (36).

Studies have demonstrated the changes that may occur to Ara h 1 during heat processing that may play a role in the allergenicity of Ara h 1 (52, 56). Oven-roasted Peanut (177 °C for 5-30 min) resulted in a level of Ara h 1 that were up to 22-fold higher than in raw Peanut (820 *vs.* 37 mug/ml) (34).

In vitro gastric digestion was reported to result in rapid degradation of Ara h 1 into small fragments. However, gastric digestion did not affect the ability of Ara h 1 to stimulate cellular proliferation and histamine release of basophils from Peanut allergic individuals was induced to the same extent by native Ara h 1 and its digestion products. Therefore gastro-duodenal digestion fragments of Ara h 1 retain T cell stimulatory and IgE-binding and cross-linking properties of the intact protein (57). This finding is supported by an earlier study that indicated that although at least twenty-three different linear IgE-binding epitopes had been located throughout the length of the Ara h 1 protein that some epitopes are major binding sites resulting in significant Peanut-antibody binding even if Ara h 1 were cleaved into peptides. The cleaving-off of an N-terminal peptide from Ara h 1, which contains three allergenic epitopes of which two are major, found that Peanut-specific IgE-antigen binding occurred as a result of the epitopes that are contained in the cleaved-off peptide, implying that the peptide, or part of it, is still present in Peanuts that are consumed (11).

Other factors may play a role in heat or digestion of Ara h 1. For instance, Ara h 1 has been shown to resist proteolysis when in a trimeric configuration, a property that may contribute to its allergenicity (47). Ara h 1 and Ara h 2 were also reported to bind higher levels of IgE and were more resistant to heat and digestion by gastrointestinal enzymes once they had undergone the Maillard reaction (58). Roasted Peanut from two different sources bound IgE from patients with Peanut allergy

at approximately 90-fold higher levels than the raw Peanut from the same Peanut cultivars (57).

Between 35%-95% of Peanut-allergic individuals are sensitized to Ara h 1 (14,21,23-24,26-27,33,36-41,44). The prevalence of sensitization to a specific Peanut allergen varies between population groups (33).

Sensitization and clinical effects of maternal peanut intake may occur soon after birth in breast-fed infants. Both major Peanut allergens Ara h 1 and Ara h 2 were detected (40).

Peanut is a very potent allergen and exposure to this allergen through saliva via kissing and utensils may cause local and systemic allergic reactions and saliva has been shown to contain up to 1110 mg/ml Ara h 1 (41). Another study concluded that patients with Peanut allergy require counseling regarding risks of kissing or sharing utensils, even if partners have brushed teeth or chewed gum (40).

Recombinant allergens may also play a role in the evaluation of cross-reactivity between plant families. Ara h 1 is a vicilin, a member of the 7S vicilin-like globulin family, and therefore cross-reactivity between Ara h 1 and other vicilins is likely (39). For example, the vicilin allergen Ara h 1 accounts for the IgE-binding cross-reactivity commonly observed between the vicilin allergens from edible legume seeds such as Lentil (Len c 1) and Pea (Pis s 1) (50). An additional study confirmed that clinically relevant cross-reactivity between Pea and Peanut occurs and as a result of vicilin homologues (59).

Assessment of isoforms of the Lentil vicilin allergen, Len c 1.02, has been demonstrated to have a greater than 50% identity with Ara h 1 and Soybean conglutinin subunits (60). A protein of Lupine, a beta-conglutin precursor, was shown to be significantly homologous to Ara h 1 (61). Lupine has become a significant allergen as a result of its large-scale introduction into processed foods and frequent cross-reactions with other members of the legume family (61).

Nonetheless, cross-reactivity between vicilin proteins are not a certainty: although Cashew and Peanut vicilins share 27% identity, they do not share linear epitopes, and hence do not appear to be cross-reactive in spite of other similarities such as the presence of multiple linear IgE binding epitopes, a lack of any common primary structural characteristics of the linear IgE binding epitopes, positional overlap of some of the IgE binding epitopes, and the presence of immunodominant IgE binding epitopes (62).

One known IgE-binding epitope of Ara h 1 has been shown to have an 80% homology with the corresponding area of Ses i 3, a Sesame seed protein to which 75% of the Sesame-allergic patients are sensitized to (63).

f423 rAra h 2

ImmunoCAP®: f423 rAra h 2

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Arachis hypogaea* allergen Ara h 2

Common name:	Conglutin
Biological function:	2S albumin trypsin inhibitor
Mw:	17.5 kDa

Allergen description

Ara h 2, a 2S albumin is homologous to and functions as a trypsin inhibitor, and is related to the 2S albumin superfamily of seed storage proteins (7,12,14,25,33,37,40,43-45,64-79). It is also known as Conglutin (12). Ara h 2 contributes up to 9% of the total protein content in peanut extracts (25). Ara h 2 is a 17.5 kDa protein and has a 30% homology with 2S albumins, but appears to have the closest homology with conglutin from Lupin. It does not appear to be made up of subunits like Jug r 1 or Ber e 1 (72,80). Ara h 2 has eight cysteine residues that could form up to four disulfide bonds (81).

Ara h 2 consists of two isoforms, namely Ara h 2.0101 and Ara h 2.0201. Ara h 2.0201 has similar but higher IgE binding than Ara h 2.0101 isoform (81% *vs*. 77%) and contains other IgE epitopes (73,82).

Ara h 2 is a protein that causes sensitization in >90% of patients with Peanut allergy (14,23-24,27,33-34,71). The prevalence of sensitization to a specific Peanut allergen varies between population groups (32).

Ara h 6 has homology to Ara h 2, especially in the middle part and at the C-terminal part of the protein. Almost complete inhibition of IgE-Ara h 6 interaction with Ara h 2 demonstrates that at least part of the epitopes of Ara h 6 are cross-reactive with epitopes on Ara h 2. Therefore Peanut-allergic patients recognize Ara h 6 both *in vitro* and *in vivo* to a similar extent as to that of Ara h 2 (15). However, Ara h 2 appears to be the more potent allergen, even though the two Peanut allergens share substantial cross-reactivity (7).

Ara h 2 (and the homologous Ara h 6) contains cores that are highly resistant to proteolytic digestion and to temperatures of up to 100 °C (7). This extreme immunological stability of the core structures of Ara h 2 provides an explanation for the persistence of the allergenic potency even after food processing (7).

Roasting of Peanut was shown to cause a 3.6-fold increase in trypsin inhibitory activity, i.e., resistant to trypsin digestion and are more likely to remain intact in the gastrointestinal tract, and functional and structural comparison of the purified Ara h 2 from roasted Peanut to native and reduced Ara h 2 from raw Peanut showed that the roasted Ara h 2 mimics the behavior of native Ara h 2 in a partially reduced form (12). Furthermore, thermal treatment of rAra h 2 in the presence of reactive carbohydrates and carbohydrate breakdown products has been shown to induce a strong increase of the IgE-binding activity (69).

Digestion of Ara h 2 with trypsin, chymotrypsin, or pepsin results in a number of relatively large fragments that are resistant to further enzymatic digestion. These peptide fragments contain intact IgE-binding epitopes and several potential

enzyme cut sites that are protected from the enzymes by the compact structure of the protein. Furthermore, the resistant protein fragments contain most of the immunodominant IgE-binding eptiopes (81). Furthermore, even though IgE antibody binding capacity is reduced by protease treatment, the mediator release from functional equivalent of mast cells or basophils, and the humanized RBL cell, demonstrated that the reduction in IgE antibody binding capacity did not necessarily translate into reduced allergenic potency (7).

Ara h 1 and Ara h 2 were also reported to bind higher levels of IgE and were more resistant to heat and digestion by gastrointestinal enzymes once they had undergone the Maillard reaction (58). Roasted Peanut from two different sources bound IgE from patients with Peanut allergy at approximately 90-fold higher levels than the raw Peanut from the same Peanut cultivars (58).

Greater than 75% of Peanut-allergic individuals are sensitised to Ara h 2 (14,29,33-34). In a Dutch study children with Peanut allergy recognized predominantly Ara h 2 and Ara h 6, and the pattern remained stable over a period of time, whereas in Peanut-allergic adults, IgE was mainly directed to Ara h1 and Ara h2 (31).

Sensitisation and clinical effects of maternal peanut intake may occur soon after birth in breast-fed infants. Both major Peanut allergens Ara h 1 and Ara h 2 were detected (83).

Recombinant allergens may also play a role in the evaluation of cross-reactivity between plant families. Ara h 2 has a 30% homology with 2S albumins, but appears to have the closest homology with conglutin from Lupin (80). Cross-reactivity may therefore occur between Ara a 2 and other foods containing 2S albumins, dependent on the degree of homology. However, cross-reactivity is not a certainty. For example, conformational analysis of the linear IgE-binding epitopes mapped on the molecular surface of Ara h 2 showed no structural homology with the corresponding regions of the walnut Jug r 1, the pecan nut Car i 1 or the Brazil nut Ber e 1 allergens. This suggests that the cross-reactivity observed between these three may depend on other ubiquitous seed storage protein allergens, namely the vicilins. However, the major IgE-binding epitope identified on the molecular surface of the walnut Jug r 1 allergen shared a pronounced structural homology with the corresponding region of the pecan nut Car i 1 allergen. The authors concluded that with the exception of Peanut, 2S albumins could thus account for the IgE-binding cross-reactivity observed between some other dietary nuts, e.g. Walnut and Pecan nut (75).

Ara h 2 has been shown to share common IgE-binding epitopes with Almond and Brazil nut allergens (67).

f424 rAra h 3

ImmunoCAP®: f424 rAra h 3

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Arachis hypogaea* allergen Ara h 3

Common name:	Glycinin
Biological function:	11S globulin trypsin inhibitor
Mw:	57 kDa

Allergen description

Ara h 3 is a glycinin, a member of the 11S globulin family, and may also function as a trypsin inhibitor (13,20,44,84-89). Ara h 3 was in first identified as a 14 kDa protein (90), but cloning revealed a 57 kDa protein that appears to be posttranslationally cleaved to smaller subunits (91).

Ara h 3 consists of a series of polypeptides ranging from approximately 14 to 45 kDa that can be classified as acidic and basic subunits, similar to the subunit organization of soy glycinin. Ara h 4 and Ara h 3 are considered to be the same allergen (13). Iso-allergens may be as a result of medication by post-translational cleavage (92).

A recent study also concluded, that Peanut-derived Ara h 3, in contrast to earlier reported recombinant Ara h 3, resembles, to a large extent, the molecular organization typical for proteins from the glycinin family. Posttranslational processing of Ara h 3 was shown to affect the IgE-binding properties and have impact on the allergenicity of Ara h 3 (13).

A comparison of the Peanut allergen sequences of Ara h 3/4, Ara h 3, Ara h 4 and Peanut trypsin inhibitor and the proteins Gly 1 and iso-Ara h 3 (not yet described as allergens), concluded that these proteins are isoallergens of each other, and that these isoallergens are post-translationally cleaved and held together by disulfide bonds in accordance to the 11S plant seed storage proteins signature (20).

The 11S globulins, also known as legumins, are classified into the Cupin superfamily, and are composed of 2 polypeptide chains of different molecular masses and amino acid sequences (heterodimeric form composed of a 20- to 40-kDa chain plus a 20- to 25-kDa chain), which are linked together by one disulfide bridge (93)

Between 20%-55% of Peanut-allergic individuals are sensitised to Ara h 3 (13,26,29,91). The prevalence of sensitization to a specific Peanut allergen varies between population groups (32). Ara h 3 was regarded as a minor allergen, but it was found that a group of Peanut-allergic Italian children were specifically sensitised to the basic subunit of Ara h 3. The authors stated their surprise that the dominant immunoreactivity in these patients was in a basic subunit of Ara h 3 because previous studies had indicated that Ara h 3 was only a minor Peanut allergen and that the identified allergenic epitopes occurred mainly in the acidic Ara h 3 subunit (88). It is therefore evident that sensitization to Ara h 3 depends on the population group studied and the methodology of the study, but there is a suggestion that the frequency of Ara h 3 sensitisation may indeed vary between population groups. In another study, recombinant Ara h 3 was recognized by IgE antibodies from approximately 45% of a Peanut-allergic patient population (91)

In a study that evaluated the pattern of IgE binding to specific Peanut allergens with the severity of clinical symptoms, 40 Peanut-allergic patients underwent a double-blind placebo-controlled low-dose Peanut challenge, during which the severity of the patients' Peanut allergy was scored. Seventeen IgE binding bands were identified between 5 and 100 kDa with eight bound by >50% of patients and the total number of bands per patient correlated significantly with challenge score and serum IgE. However, two protein bands, identified as subunits of Ara h 3/4, had peak intensities that correlated positively with challenge score and a third band (Ara h 1) that correlated negatively. The study concluded that promiscuity of IgE binding appears more important than the recognition of individual proteins (30).

It has been argued that in contrast to recombinant Ara h 3, the allergen isolated from its native source is extensively proteolytically processed, and that native Ara h 3 polypeptides are much more complex than the recombinant protein used for epitope mapping experiments. The authors concluded that characterization of the allergenicity of Ara h 3 should therefore also include IgE-binding studies with Peanut-derived Ara h 3, providing the high degree of variation in the Ara h 3 protein structure, as this is what Peanut-allergic individuals are confronted with (94).

Ara h 3 is an 11S globulin and shares homology, and therefore varying degrees of cross-reactivity, with other 11S globulins. Sin a 2, a major allergen from Yellow mustard seed, was shown to have a sequence identity with other allergenic 11S globulins ranging between 27% and 38%. Three peptides described as epitopes in Ara h 3 were moderately conserved in Sin a 2 (95). Similarly, IgE-binding epitopes of Ara h 3 exhibited some structural homology among Peanut and tree nut allergens (Jug r 4 of Walnut, Cor a 9 of Hazelnut, Ana o 2 of Cashew nut) to account for the IgE-binding cross-reactivity observed. IgE-binding epitopes similar to those found in 11S globulin allergens do not apparently occur in other vicilin allergens with the cupin fold from Peanut (Ara h 1) or tree nuts (Jug r 2 of Walnut, Cor a 1 of Hazel nut, Ana o 3 of Cashew nut) (96).

Cross-reactivity has also been demonstrated between homologous Ara h 3 proteins (but not related) in Lupin (Lupin conglutin gamma) and Soybean (Soybean Bg7S) (97). A sequence similarity between Ara h 3 and the glycinins in Soybean and Pea of 62% to 72% has been reported (98).

f352 rAra h 8

ImmunoCAP®: f352 rAra h 8
Recombinant non-glycosylated protein produced in an *E. coli* strain strain carrying a cloned cDNA encoding *Arachis hypogaea* allergen Ara h 8

Common name:	A Bet v 1-homologous allergen, Group 1 *Fagales*-related protein, PR-10 protein
Biological function:	Plant defence protein, a pathogenesis-related protein
Mw:	17 kDa

Allergen description

Ara h 8 (14,27) is a Bet v 1-homologous panallergen. Ara h 8 appears to have a low stability to roasting and no stability to gastric digestion (14). A study was done of 9 Swiss and 11 Dutch patients with Peanut and Birch pollen allergy, and with positive double-blind, placebo-controlled food challenges to Peanut. All patients experienced symptoms of the oral cavity on exposure to Peanut; these progressed to more-severe symptoms in 40% of patients; serum IgE to recombinant Ara h 8 was detected in 85%. The study concluded that Peanut allergy may be mediated in a subgroup of patients by means of cross-reaction of Bet v 1 induced antibodies with the homologous Peanut allergen Ara h 8 (14).

Ara h 8 belongs to the PR-10 potein family. It has also been designated a Group 1 *Fagales*-related potein. Pathogenesis-related (PR) proteins of class 10 are abundant in higher plants. Some of these proteins are induced under stress conditions as part of the plant's defence mechanism. Other homologues are developmentally regulated, and their expression varies in different plant organs. The PR-10 proteins are encoded by multigene families, have a weight of about 17 kDa and are found in the cytosol (28). They are common panallergens in *Fagales* pollens (Alder, Hornbeam, Beech, Chestnut) and may be present in a number of vegetables and fruits, e.g., Apple and Hazelnut. Pyr c 1, the major allergen from Pear (*Pyrus communis*), along with Lupine (*Lupinus albus*), is a homologous Bet v 1 allergen (29-30). Patients suffering from Birch pollen allergy can also exhibit allergic symptoms on exposure to the pollen of trees from the *Fagales* (Alder, Hazel, Hornbeam) and Oak and Chestnut, because all contain this panallergen. Recombinant marker allergens are therefore of value for more-accurate diagnoses and subsequent immunotherapy (31).

Due to cross-reactivity between Bet v 1 and Ara h 8, sensitisation to other PR-10 proteins might be evaluated to some extent using rAra h 8. For example, in a study that evaluated whether *Fagales* sensitisation occurred within a population not exposed to Birch pollen, reactivity to Bet v 1 was recorded in 84% of the Birch/Hazel/Oak co-reactivity group. Bet v 1 prevalence ranged between 48% and 21% among subgroups of patients coming from different areas (32).

Lupine is an emerging cause of food allergy, as a result of recent large-scale introduction into processed foods and frequent cross-reactions with other members of the legume family. Significant sequence homology and molecular similarity were found between the allergen Ara h 8 of Peanut and the pathogenesis-related protein PR-10 of White lupine (33).

Ara h 8 is also cross-reactive with Gly m 4 from Soya bean and Pru av 1 from Cherry. Nonetheless, although common binding epitopes do occur for this panallergen, patient-specific IgE epitope patterns also occur (29).

In a study evaluating severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in Soya bean, Gly m 4/SAM22, immediate-type allergic symptoms in patients with Birch pollen allergy after ingestion of Soy protein-containing food items were reported to occur from cross-reactivity of Bet v 1-specific IgE to homologous pathogenesis-related proteins, particularly the PR-10 protein Gly m 4/SAM22 (34). Similar cross-reactions can also be expected to Ara h 8.

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Shrimp allergen component

Penaeus aztecus

Available ImmunoCAP®:

f351 rPen a 1

Summary

Shrimp are small invertebrate marine crustaceans with 10 jointed legs (decapod) on the thorax, well-developed swimmerets on the abdominal segments, and a body that is compressed laterally. They live on the floor of oceans and lakes. There are over 2,000 different species of Shrimp worldwide.

One common commercial Shrimp is of the genus *Peneus*.

There are several other *crustacean* forms that are commonly called Shrimp although they do not belong to the same order as the true Shrimp, order *Decapoda* (phylum *Arthropoda*, subphylum *Crustacea*, class *Malacostraca*), which also includes the Lobsters and Crabs.

Shrimp may be divided into 3 basic categories: cold-water or northern; warm-water, tropical, or southern; and freshwater. However the nomenclature is complicated and the term "Shrimp" sometimes applies to all crustaceans of the *Natantia* group, regardless of size. The terms "Prawn" and "Scampi" are often used interchangeably with Shrimp.

rPen a 1 from Brown shrimp (*P. aztecus*), is representative of other shrimp tropomyosin.

Recombinant allergens, which are genetically engineered isoforms resembling allergen molecules from known allergen extracts, have immunoglobulin E (IgE) antibody binding comparable to that of natural allergens and generally show excellent reactivity in *in vitro* and *in vivo* diagnostic tests (1). To date, many different recombinant allergens of pollens, molds, mites, bee venoms, foods and latex have been cloned, sequenced, and expressed. Recombinant allergens have a wide variety of uses, from the diagnosis and management of allergic patients to the development of immunotherapy to the standardisation of allergenic test products as tools in molecular allergology.

Allergens from *Penaeus aztecus* listed by IUIS*

Pen a 1

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

Recombinant Pen a 1 and natural Pen a 1 are structurally and immunologically identical (2).

At least 13 allergens are found in extracts of cooked Brown shrimp, and the 36-kDa muscle protein tropomyosin (Pen a 1) has been identified as a major Shrimp allergen. It is detected by sera of more than 80% of all subjects allergic to Shrimp and binds up to 75% of all Shrimp-specific IgE antibodies (2-3). Amino acid sequence identities with natural allergenic and non-allergenic tropomyosins ranged from 80% to 99% and 51% to 58%, respectively (2). Since Beef, Pork and Chicken are other tropomyosin-containing foods that are not very allergenic, tropomyosins can serve in investigations of the contribution of the varying structural properties of a protein to its allergenicity (4).

Recombinant allergens are particularly useful in addressing allergies that manifest wide cross-reactivity, such as allergies to crustaceans, Cockroaches and House dust mites (5).

f351 rPen a 1

ImmunoCAP®: f351 rPen a 1
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Penaeus aztecus* allergen Pen a 1

Common name:	Tropomyosin
Biological function:	Muscle protein
Mw:	36 kDa

Allergen description

Pen a 1 (4,6-7), the major protein of Brown shrimp (*Penaeus aztecus*), is a muscle protein tropomyosin (5,7-10). The allergen is heat-stable and is found in both raw and cooked Shrimp (11).

Tropomyosin comprises a class of highly conserved proteins with multiple isoforms found in both muscle and nonmuscle cells of all species of vertebrates and invertebrates. It is an abundant and heat-stable protein that constitutes up to 20% of total protein in the edible part of the animal. It is physically associated with actin and myosin in muscIe fibres and other motile filaments. Allergenic tropomyosins are found in invertebrates such as crustaceans (Shrimp, Lobster, Crab, Crawfish), arachnids (House dust mites), insects (Cockroaches), and mollusks (e.g., Squid); but there is a distinct lack of allergenic cross-reactivity between these tropomyosins and those from vertebrates: their tropomyosins are virtually nonallergenic (11). Tropomyosins present in vertebrate sources of food, e.g., bony fish, Beef, Pork and Chicken, are rarely allergenic to human beings, as compared to Lobster, Shrimp and Cockroach. Moreover, the invertebrate tropomyosins have high IgE cross-reactivity, and have therefore been referred to as panallergens. Tropomyosin is major allergen in crustaceans. Pen a 1 is quite important: approximately 80% of individuals sensitised to the Brown shrimp were reported to show IgE antibody reactivity to Pen a 1 (6,12).

Studies suggest that IgE-binding epitopes are restricted to certain parts of the Pen a 1 molecule and that Pen a 1 may have several similar epitopes. Pen a 1 epitopes do not appear to be located in the highly homologous parts of the molecule (9).

Studies have demonstrated that tropomyosin is an important allergen in crustaceans other than Shrimp, such as Lobster - both the Spiny lobster *Panulirus stimpsoni* and the American lobster *Homarus americanus* (Pan s 1, Hom a 1) (13-14) – the Crab *Charybdis feriatus* (Cha f 1) (15), mollusks such as the Squid *Todarodes pacificus* (Tod p 1) (16), the Snail *Turbo cornutus* (Tur c 1) (17), the Oyster *Crassostrea gigas* (Cra g 1) (18), and other invertebrates such as the House dust mites *Dermatophagoides farinae* (Der f 10) and *Dermatophagoides pteronyssinus* (Der p 10) (19-20), and the Cockroach *Periplaneta americana* (Per a 7) (21-22). Immunological relationships based on tropomyosin have also been demonstrated between crustaceans, Cockroaches and House dust mites, suggesting that tropomyosin is an important cross-sensitising panallergen (5).

In further evidence, rPen a 1 has been shown to extensively and specifically compete for IgE binding to extracts of other crustacean species, House dust mites and the German cockroach (6). The tropomyosins Pan s 1 (Spiny lobster, *Panulirus stimpsoni*) and Hom a 1 (American lobster, *Homarus americanus*) have been shown to have significant homology to Shrimp tropomyosin (14).

Nonetheless, studies suggest that there may be species-specific allergens in shrimps. In a comparision of the allergens from the edible Shrimp species *Penaeus setifecus* (White shrimp) and *Penaeus aztecus* (Brown shrimp) in 31 individuals with a history of immediate hypersensitivity reactions after Shrimp ingestion, skin-specific IgE to both types of extract was observed in 77% (23/30) of the subjects; 1 individual reacted to Brown shrimp extract only. Serum-specific IgE to both extracts was demonstrated in 16/31 study participants; 1 subject reacted only to White shrimp extract, and 2 subjects to Brown shrimp extract alone. Species specificity is important because it may explain the intermittent symptoms of some study subjects (23).

Recombinant Pen a 1 has been demonstrated to have allergenic activity not only similar to that of its own native allergen (6), but also very similar to that of the Greasyback shrimp (*Metapenaeus ensis*) tropomyosin (Met e 1) (7). Four recombinant, IgE-reactive Pen a 1 peptides, isolated in a study, showed various degrees of sequence identity with tropomyosins of other arthropods such as fruitfly (*Drosophila melanogaster*), House dust mite, helminths and vertebrates (4,7,11).

Tropomyosin from the Mite *Blomia tropicalis* (Blo t 10) was demonstrated to have cross-reactivity with Der p 10 of *Dermatophagoides pteronyssinus*, and to share up to 96% amino acid identity to tropomyosin of other Mites. Although Blo t 10 and Der p 10 were shown to be significantly cross-reactive, unique IgE epitopes do exist (24).

Tropomyosin has also been described in arthropods, namely Fly (*Musca domestica*), Moth (*Ephestia spp.*) and Spider (*Tegenaria spp.*). In a study of 100 patients allergic to household arthropods, cross-reactivity due to tropomyosin was demonstrated in a large variety of extracts obtained from insects, mites, crustaceans, mollusks and parasites (25).

While tropomyosin is a major factor in food allergy to invertebrates, it plays a less prominent role in inhalation allergy to Mites and Cockroaches. Tropomyosin has been isolated from *Anisakis* but does not appear to be an important allergen in *Anisakis* sensitisation (26). Similarly, Tur c 1, the tropomyosin from the gastropod *Turbo cornutus*, has an IgE-binding epitope that is dissimilar to those proposed for Cra g 1 from the Oyster *Crassostrea gigas*, and to Pen i 1 from the Shrimp *Penaeus indicus* (17).

Clinical and serological reactivity to both Mites and Snails has been described, and the development of sensitisation and allergic symptoms to Snail and Shrimp following immunotherapy treatment with Mite extract has been reported (6). IgE antibody reactivity to Shrimp can occur in an unexposed population of individuals; a study of Orthodox Jews unexposed to Shrimp demonstrated that some subjects allergic to HDM and/or Cockroach showed substantial IgE antibody reactivity to the Shrimp tropomyosin Pen a 1. Based on inhibition with Cockroach and/or Dust mite extracts, this reactivity appeared to be due to cross-reacting tropomyosins (27).

Therefore, as IgE-mediated food allergy to crustaceans and mollusks is relatively common, and affected individuals typically react to a range of different species, tropomyosin sensitivity may be useful as a diagnostic marker for allergic sensitisation to invertebrate foods. rPen a 1 has potential use as a diagnostic reagent to determine not only sensitisation specifically to Brown shrimp, but also sensitisation to and cross-reactivity with tropomyosin allergens from other species.

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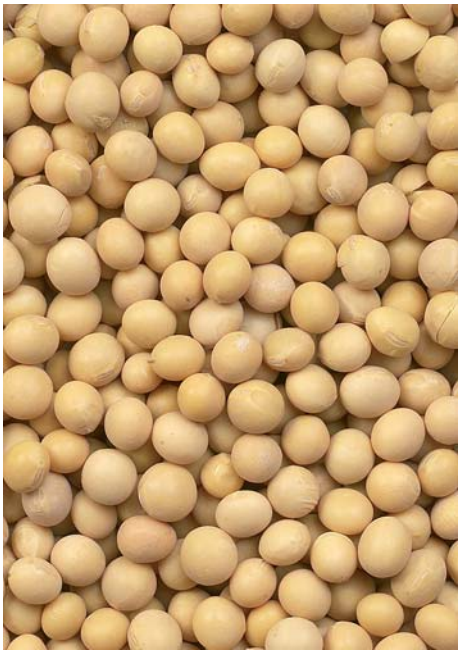
Soybean allergen components

Glycine max

Available ImmunoCAP®:
f353 rGly m 4



Summary

Soy is one of the world's most important legumes. The bean can be used fresh or processed into flour, flakes, grits, sauce, bran, or Soya milk, or pressed for oil. The list of food products presenting allergy risk is expanding. Soya protein has been found in foods that was not supposed to contain it (1).

There are more than 200 varieties of Soya bean (2-3).

Soya bean is not only a food allergen but also an occupational aero-allergen inducing asthma. Soya bean hull allergens were responsible for an outbreak of epidemic asthma in Barcelona (4). Soy may also result in Baker's Asthma (5).

Soya protein consists of more than 130 phytochemicals (6) and at least 21 allergenic proteins that have been identified (7-8). Seed proteins in Soya bean comprise 3 major fractions that account for 70% to 80% of total protein composition: 11S, 7S and 2S globulins (9-10).

The following allergens have been characterised:

Gly m 1 (11).

Gly m 2 (12).

Gly m 3 (13).

Gly m 4 (14).

Gly m 2S Albumin (8).

Gly m 39kD (13).

Gly m Bd28K (15).

Gly m Bd30K (16).

Gly m Lectin (17).

Gly m Bd 60K (18).

Gly m Oleosin (19).

Gly m Trypsin Inhibitor (20).

Allergens from *Glycine max* listed by IUIS*

rGly m 1	rGly m 2	rGly m 3
rGly m 4		

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

Some Soya bean allergens responsible for food allergy are different from those responsible for respiratory allergies. One important Soya bean food allergen is a protein termed P34, which is abundant in the seeds and other parts of the plant (3). This protein is present in significant amounts in all cultivars studied (21).

The allergens involved in occupational asthma caused by Soya bean flour are mainly high-MW proteins that are present in both Soybean hull and flour, and they are different from the allergens causing asthma outbreaks, which are mainly low-MW proteins concentrated in the hull (5). Soybean hulls contain 3 main allergens, with MW's of 8, 7.5 and 7 kDa (22). The major allergen causing the epidemics in Barcelona, Spain, was a glycopeptide less than 14 kDa in size and found in Soybean dust. This allergen occurs in all parts of the Soybean plant at all stages of growth, but the telae (hulls) and pods are by far the richest source (23,24).

Soybean allergen components

Fresh Soybeans are less allergenic than stored Soybeans, suggesting that new Soybean allergens are created by increases in temperature upon storage and transportation: During the process of harvest, transport and storage, microbial and mold contamination can raise the temperature of Soybeans to 75 °C or higher. This heat could generate 2 new allergen determinants or increases in epitope exposure as a result of conformational changes. The full significance of these new IgE and IgG4 binding proteins has yet to be determined (25).

Gly m 3, a profilin, is a major Soya bean allergen and an important panallergen (12).

Recombinant allergens, which are genetically engineered isoforms resembling allergen molecules from known allergen extracts, have immunoglobulin E (IgE) antibody binding comparable to that of natural allergens and generally show good reactivity in *in vitro* and *in vivo* diagnostic tests (26). To date, many different recombinant allergens of various pollens, molds, mites, bee venom, latex and foods have been cloned, sequenced, and expressed.

Recombinant allergens have a wide variety of uses, from the diagnosis and management of allergic patients to the development of immunotherapy to the standardisation of allergenic test products as tools in molecular allergology. Recombinant allergens are particularly useful for investigations in allergies manifesting wide cross-reactivity.

f353 rGly m 4

ImmunoCAP®: f353 rGly m 4

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Glycine max* allergen Gly m 4

Common names:	A Bet v 1-homologous allergen, Group 1 *Fagales*-related protein, PR-10 protein, SAM22
Biological function:	Plant defence protein, pathogenesis-related protein
Mw:	17 kDa

Allergen description

Gly m 4 (8) belongs to the PR-10 protein family. It has also been designated a Group 1 *Fagales*-related protein. Pathogenesis-related (PR) proteins of class 10 are abundant in higher plants. Some of these proteins are induced under stress conditions as part of the plant's defence mechanism. Other homologues are developmentally regulated, and their expression varies in different plant organs. The PR-10 proteins are encoded by multigene families, have a weight of about 17 kDa and are found in the cytosol (27). They are common panallergens in *Fagales* pollens (Alder, Hornbeam, Beech, Chestnut) and may be present in a number of vegetables and fruits, e.g., Apple and Hazelnut. Pyr c 1, the major allergen from Pear (*Pyrus communis*), along with Lupine (*Lupinus albus*), is a homologous Bet v 1 allergen (28-29). Patients suffering from Birch pollen allergy can also exhibit allergic symptoms on exposure to the pollen of trees from the *Fagales* (Alder, Hazel, Hornbeam) and Oak and Chestnut, because all contain this panallergen. Recombinant marker allergens are therefore of value for more-accurate diagnoses and subsequent immunotherapy (30).

Due to cross-reactivity between Bet v 1 and Gly m 4, sensitisation to other PR-10 proteins might be evaluated using rGly m 4. For example, in a study that evaluated whether *Fagales* sensitisation occurred within a population not exposed to Birch pollen, combined reactivity to the 3 species was recorded in 80% of this cohort. Reactivity to Bet v 1 was recorded in 84% of the Birch/Hazel/Oak co-reactivity group. Bet v 1 prevalence ranged between 48% and 21% among subgroups of patients coming from different areas (31).

Twenty-two patients, allergic to Birch pollen and with Soy allergy confirmed by means of positive double-blind, placebo-controlled food challenge results (n = 16) or a convincing history (n = 6), were investigated for IgE reactivity to Birch pollen and Soy allergens. ImmunoCAP analysis revealed Gly m 4-specific IgE in 96% (21/22) of the patients. All patients had Bet v 1-specific IgE antibodies, and 23% (5/22) had positive Bet v 2 results. In IgE immunoblotting, 25% (6/22) of the patients recognised Soya profilin (Gly m 3), and 64% (14/22) recognised other Soya proteins. IgE binding to Soya was at least 80% inhibited by Birch pollen and 60% inhibited by rGly m 4 in 9 of 11 sera tested. Seventy-one percent (67/94) of highly Bet v 1-sensitised patients with Birch pollen allergy were sensitised to Gly m 4, and 9 (9.6%) of those patients reported Soya allergy. The Gly m 4 content in Soya products ranged between 0 and 70 ppm (milligrams per kilogram). The

study concludes that Soya bean is another Birch pollen-related allergenic food. Gly m 4 is the major Soy allergen for patients allergic to Birch pollen and also Soy. The content of Gly m 4 in Soy food products strongly depends on the degree of food processing (8).

Ara h 8 is also cross-reactive with Gly m 4 from Soya bean and Pru av 1 from Cherry. Nonetheless, although common binding epitopes do occur for this panallergen, patient-specific IgE epitope patterns also occur (32).

In a study evaluating severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in Soya bean, Gly m 4/SAM22, immediate-type allergic symptoms in patients with Birch pollen allergy after ingestion of Soy protein-containing food items were reported to occur from cross-reactivity of Bet v 1-specific IgE to homologous pathogenesis-related proteins, particularly the PR-10 protein Gly m 4/ SAM22 (33).

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Timothy grass allergen components

Phleum pratense

Available ImmunoCAP®:

g205 rPhl p 1
g206 rPhl p 2
g208 nPhl p 4
g215 rPhl p 5b
g209 rPhl p 6
g210 rPhl p 7
g211 rPhl p 11
g212 rPhl p 12, profilin
g213 rPhl p 1, rPhl p 5b
g214 rPhl p 7, rPhl p 12

Summary

Allergen components from timothy grass, *Phleum pratense*, available for allergen-specific IgE antibody testing, are produced either with recombinant technique or as purified native proteins (1). The next generation of immunotherapy may be based on recombinant allergen components, possibly modified to reduce the risk of anaphylaxis. If the sensitisation profile to an allergen such as timothy is known, only those components to which the patient is actually sensitised should be relevant for therapy. This would eliminate the risk that the therapeutic reagent would induce IgE antibodies to additional components. The allergen components of timothy in IgE antibody tests may also be used for monitoring immunotherapy that is done with the naturalextract.

Studies have evaluated different combinations of recombinant allergens for diagnostic use in grass pollen allergy. The fact that only a limited number of recombinant timothy grass pollen allergens account for the detection of a high percentage of patients with grass pollen-specific IgE suggests the usefulness of recombinant allergens not only for *in vitro* diagnosis but also for patient-tailored immunotherapy (2).

For example, a study used sera from 193 European, American, and Asian subjects to evaluate the percentage of IgE directed to rPhl p 1, rPhl p 2, rPhl p 5, and rBet v 2. The study also used natural pollen extracts from *Anthoxanthum odoratum*, *Avena sativa*, *Cynodon dactylon*, *Lolium perenne*, *Phragmites australis*, *Poa pratensis*, *Secale cereale*, *Triticum sativum*, *Zea mays*, IgE antibodies directed to these 4 recombinant pollen allergens was detected in 59% of these patients (3).

Allergens from *Phleum pratense* listed by IUIS*

Phl p 1	Phl p 2	Phl p 4
Phl p 5	Phl p 6	Phl p 7
Phl p 11	Phl p 12	Phl p 13

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

A similar study, examined the *in vitro* IgE antibody-binding capacity to the 3 recombinant timothy allergens, rPhl p 1, rPhl p 2, rPhl p 5, and birch profilin in sera from 183 patients allergic to grass pollen from different populations in Europe, Japan, and Canada. More than ninety-four percent of the patients could be diagnosed with a combination of recombinant Phl p 1, Phl p 2, Phl p 5, and Birch profilin. Sera that did not react with the recombinant allergens contained low levels of timothy grass pollen-specific IgE antibodies. The study pointed out that although considerable variability in the IgE antibody recognition frequency of the recombinant allergens was observed in certain populations, a good correlation was

found between natural timothy-serum specific IgE antibodies and the combination of recombinant allergens in all 183 tested sera. The authors suggested that the addition of other recombinant allergens (e.g., recombinant Phl p 4) would only slightly improve the *in vitro* test sensitivity (4).

rPhl p 1, rPhl p 2, rPhl p 5 and Birch pollen recombinant allergens (rBet v 1, rBet v 2) were used for the measurement of allergen-specific IgE and IgG subclass antibody responses in fifty-five pollen-allergic patients, allowing allergy diagnosis in 52 of 54 of the grass pollen and in 35 of 36 of the Birch pollen-allergic patients (5).

A larger study, evaluating sensitisation to timothy grass pollen using sera from 749 patients and a timothy extract compared to 8 recombinant timothy allergens, found that 95% had detectable IgE antibodies to the timothy extract. The prevalence of IgE antibody reactivity increased from 86.8% to 93.3% as the number of combined recombinant allergens rose from 2 to 8. The prevalences for each allergen were: rPhl p 1 = 83%, rPhl p 2 = 55%, nPhl p 4 = 70%, rPhl p 5 = 50%, rPhl p 6 = 44%, rPhl p 7 = 7%, rPhl p11 = 43% and rPhl p 12 = 15%. Monosensitisation to rPhl p 1 occurred in 6% patients and was negligible for the remaining molecules (6).

A study evaluating the same group of 8 allergens, using sera of 77 patients allergic to grass pollen, found a similar frequency of sensitisation to these allergens. This study also demonstrated a good correlation, as expected, between the calcium-binding proteins of rPhl p 7 and Bet v 4, and between the profilin of rPhl p 12 and rBet v 2. Nevertheless, as with other studies, highly variable individual sensitisation patterns were seen (7).

Clearly IgE antibody reactivity profiles will vary from country to country and will depend on the prevalence of pollen allergens. In an evaluation of the IgE antibody reactivity profile to individual recombinant and native allergens in sera from 1,177 subjects sensitised to timothy and/or birch pollen and living in Finnish and Russian Karelia, the IgE antibody reactivity to pollen extracts and 8 Timothy allergens (rPhl p 1, 2, 5, 6, 7, 11, 12 and nPhl p 4) and 3 Birch pollen allergens (rBet v 1, 2 and 4) revealed that the levels of IgE antibodies to timothy and Birch pollen were higher in Finnish (median 5.2 kU_A/L) than in Russian Karelia (median 1.8 kU_A/L). There was a significantly higher prevalence of IgE reactivity to 3 timothy pollen allergens in Finnish (n=57) than in Russian Karelia (n=12): rPhl p 2, 28 vs. 0%; rPhl p 5, 60 *vs.* 0%; rPhl p 6, 47 vs. 0%. The prevalence of IgE antibody reactivity to the birch pollen allergens was similar in the 2 populations. IgE antibody reactivity to rPhl p 2, 5, 6 and 11 was associated with hayfever symptoms (8).

Because of patients being sensitised to minor timothy allergens, occasional subjects may demonstrate allergen-specific IgE antibodies to timothy extract but not to individual recombinants (9).

Assessing patients' sera for allergen-specific IgE and IgG_4 antibody reactivity to individual recombinant *P. pratense* allergens after immunotherapy has been reported to be useful in defining optimal allergen extract doses. For example, a study that found no significant rPhl p 12-specific IgG_4 antibody increase after immunotherapy, suggesting that Phl p 12 was underrepresented in the extract used. The simple detection of specific serum IgG_4 antibodies a few weeks after the start of immunotherapy was a valuable tool for estimating the presence of relevant allergens in a given immunotherapeutic allergen extract (10). Grass pollen immunotherapy elicits an array of antibody specificities and these reflect the allergen content and the potency of allergen extracts, which may contribute to defining optimal allergen extract doses (11).

g205 rPhl p 1

ImmunoCAP®: g205 rPhl p 1
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Phleum pratense* allergen Phl p 1

Common name:	Group 1 grass allergen
Biological function:	β-Expansin
Mw:	27 kDa

Allergen description

Phl p 1 (1-4,7-9,12-26) is a group 1 grass pollen allergen, a family of allergens present in all grass species (12). Group 1-grass pollen allergens are glycosylated proteins that show 60-70 % sequence identity to expansins, a family of proteins involved in cell wall loosening and extension in plants 1. IgE antibodies in almost 40% of allergic individuals, representing around 400 million allergic patients (15,19), recognize group 1 allergens. More than 95% of grass pollen-allergic patients display IgE-reactivity to group 1 grass pollen allergens of different grass species (17). A major IgE-reactive segment of Phl p 1 also exhibits a significant sequence identity of 43% with the family of immunoglobulin domain-like group 2/3 grass pollen allergens (12).

Recombinant Phl p 1 has been shown to resemble native Phl p 1, closely binding to IgE in up to 87% of patients with grass pollen allergy, indicating that rPhl p 1 shares many of the IgE epitopes with natural group 1 grass pollen allergens (17,20). rPhl p 1 produced in *Escherichia coli* (*E. coli*) is not glycosylated in difference to the native molecule. Group1 allergens have been cloned from at least 10 grass species (1).

rPhl p 1 has also been shown to inhibit IgE antibody binding to most of group 1 isoallergens from 7 to 8 grass species in studies (17,20), showing extensive cross-reactivity between species. Thus, a single recombinant group 1 allergen contains many of the IgE epitopes of group 1 isoallergens from a number of different grass species (20) and may represent a useful tool for specific diagnosis and therapy of grass pollen allergy (1).

Phl p 1 displays sequence identities of greater than 85% and homologies of greater than 90% with Lol p 1 (rye grass) and Hol I 1 (velvet grass) (27). However, despite the high degree of homology, amino acid differences occur in immunodominant positions, which may be responsible for the differing immune response also found to group 1 allergens of different grass species (3,27).

g206 rPhl p 2

ImmunoCAP®: g206 rPhl p 2
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Phleum pratense* allergen Phl p 2

Common name:	Group 2 grass allergen
Biological function:	Unknown
Mw:	13 kDa

Allergen description

Phl p 2 (1-3,7-9,18-19,22-24,28-34) is representative of the large family of cross-reacting plant allergens classified as grass allergens group 2/3. These comprise 10-12 kDa non-glycosylated proteins of 95-98 amino acid residues which exhibit 85-90 % sequence identity between grass species. Group 2 and 3 allergens share a high degree of sequence homolgy with the C-terminal part of group 1 allergens but are sufficiently different to give a more or less separate antibody recognition. Cross-reactivity between group 1 and group 2/3 allergens has not so far been shown for human IgE antibodies (1). Recombinant Phl p 2 has been demonstrated by immunological cross-reactivity studies to be immunologically equivalent to the natural protein (30).

g208 nPhl p 4

ImmunoCAP®: g208 nPhl p 4
Natural protein purified from *Phleum pratense*

Common name:	Group 4 grass allergen
Biological function:	Berberine bridge enzyme
Mw:	55 kDa

Allergen description

Phl p 4 (7-8,35) is a major allergen which reacts with IgE antibodies of approximately 75% of grass pollen-allergic patients (35-39). Phl p 4 belongs to the Group 4 grass pollen allergens, which are present in many grass species, including timothy grass and Mugwort (35,40). Group 4 allergens are highly basic glycoproteins with Mw 50-67 kDa. They carry 10-15% carbohydrates and some of the IgE antibody responses obtained are probably to the carbohydrate determinants.

This group of allergens has been located in the wall of pollens, and in timothy grass and birch pollens also in the cytoplasm. In the foods peanut, apple, celery, and carrot, only cytoplasmic areas contained this allergen. As Group 4-related allergens occur in a range of pollens of unrelated plants and in plant foods, they contribute to cross-reactivity between some pollens and foods (41).

It is therefore not surprising that Phl p 4-specific IgE antibodies will cross-react with allergens present in pollen of trees, grasses, and weeds, as well as in plant-derived food (36). Cross-reactivity has been demonstrated between the pollen allergen Dac g 4 in orchard grass (*Dactylis glomerata*) (42) and similar allergens in pollen of *Secale cereale* (cultivated rye), *Lolium perenne* (rye grass), *Festuca elatior* (meadow fescue), *Holcus lanatus* (velvet grass), *Bromus arvensis* (field brome), *Poa pratense* (Kentucky blue grass), *Hordeum sativum* (barley), and *Phleum pratense* (timothy grass) (39,42). Nevertheless, the expression of Group 4 allergens in these plants varies considerably (35).

g215 rPhl p 5b

ImmunoCAP®: g215 rPhl p 5b
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Phleum pratense* allergen Phl p 5b

Common name:	Group 5 grass allergen, Ag25
Biological function:	Not confirmed but possibly a ribonuclease
Mw:	32 kDa
Isoforms:	Phl p 5a, a 38 kDa protein; Phl p 5b, a 32 kDa protein (53).

Allergen description

Phl p 5 (1-3,7-9,18-19,22-24,26,43-52) is a major allergen from Timothy grass pollen and is one of the most reactive of the group 5 allergens, inducing allergic rhinitis and bronchial asthma in grass pollen-allergic patients. Group 5 allergens seem to be restricted to the *Pooideae* subfamily of grasses. Between 65-90% of grass pollen-allergic patients in temperate climate areas are reported to be sensitized against group 5 grass pollen allergens (1,45). Rainfall contributes to an increase in respirable particles containing group 5 allergens, which bursts the pollen grains (54).

Two isoforms exists, denoted "a" and "b," where Phl p 5b, although beeing the smaller of the two isoforms, have been demonstrated to contain at least one more IgE antibody binding epitope than Phl p 5a (1).

rPhl p 5 has been shown to be very similar to natural Phl p 5, and to have a moderately high homology to other Group 5 allergens (1,49). rPhl p5 reacts with serum IgE antibodies in up to 90% of grass pollen-allergic patients (43,51).

rPhl p 5, has been shown to be cross-reactive with similar Group 5 allergens from several grass and grain species 45, including Lol p 5 from Rye grass pollen (*Lolium perenne*) and Poa p 9 from Meadow grass (*Poa pratensis*). Nevertheless, variable IgE immunoreactivity does occur to these allergens and more diversity has been shown for goup 5 allergens than for group 1 allergens (1,50).

g209 rPhl p 6

ImmunoCAP®: g209 rPhl p 6
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Phleum pratense* allergen Phl p 6

Common name:	Group 6 grass allergen
Biological function:	Unknown
Mw:	15 kDa

Allergen description

Phl p 6 (1,7-8,18,55-58) binds IgE antibodies from 60-75% of grass pollen-sensitised subjects (1,55). Phl p 6 is one of the group 6 grass allergens and has so far only been identified in timothy and Kentucky blue grass.

Group 6 allergens are acidic non-glycosylated proteins with a Mw of about 13 kDa. They have a fairly high degree of amino-acid sequency homolgy to the C-terminal part of group 5 allergens and IgE antibodies to Phl p 6 in most cases cross-react to group 5 allergens (1).

Studies, including structural and detailed localisation (55), have resulted in the development of recombinant rPhl p 6, which has been shown to have the same reactivity with serum IgE antibodies as the native molecule.

g210 rPhl p 7

ImmunoCAP®: g210 rPhl p7
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Phleum pratense* allergen Phl p 7

Common names:	2-EF-hand, Ca^{2+}-binding protein, CBP, Polcalcin
Biological function:	Calcium-binding protein
Mw:	9 kDa

Allergen description

Phl p 7 (1,7-8,59-60) is a minor, non-glycosylated, allergen of Timothy grass pollen, recognising serum IgE antibodies in 10-15% of grass pollen-sensitized subjects. It is a 2-EF-hand, Ca^{2+}-binding protein with a high sequence identity to homologous pollen proteins found in a number of other plants (1,60-61).

Ca^{2+}-binding plant allergens can be grouped in different families according to the number of Ca^{2+}-binding domains (EF hands). 2 EF-hand Ca^{2+}-binding proteins include Phl p 7 (Timothy grass) and Aln g 4 (Alder), 3 EF-hand Ca^{2+}-binding proteins include Bet v 3 (Birch), and 4 EF-hand Ca^{2+}-binding proteins Jun o 4 (Prickly juniper). Through molecular modeling, structural similarities have been found among the allergens with 2, 3, and 4 EF-hands. In a study evaluating pollens from 16 unrelated plants, 22% of the patients with multiple pollen sensitization reacted to at least one of the Ca^{2+}-binding allergens. A hierarchy of IgE antibody cross-reactivity was noted (rPhl p7 > rAln g 4 > rJun o 4 > rBet v 3). rPhl p 7 was identified as the EF-hand allergen containing the most IgE antibody-binding epitopes in the population studied (59).

Similarly, a high degree of cross-reactivity has been demonstrated among plants containing a Ca^{2+}-binding protein, including members of the *Brassica* species, and *Alnus glutinosa*, *Olea europea*, *Betula verrucosa* (Bet v 4) and *Cynodon dactylon* (Cyn d 7) (60,62). Che a 3 from *Chenopodium album* pollen has also been reported to have a high similarity with calcium-binding protein allergens from pollens of olive, birch, alder, rapeseed, and timothy grass (63-64).

rPhl p 7 is therefore likely to cross-react with pollen proteins from most plants, in particular with other grass species, trees of the *Fagales* order such as birch tree, and olive trees and weeds (65).

g211 rPhl p 11

ImmunoCAP®: g211 rPhl p 11
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Phleum pratense* allergen Phl p 11

Common name:	Group 11 grass allergen
Biological function:	Unknown
Mw:	20 kDa

Allergen description

Phl p 11 (7-8,66) is an allergen with structural similarity to the Soybean trypsin inhibitor family of proteins, however no enzymatic activity has been found. Note that the allergen now known as Phl p 12 was initially described as Phl p 11 (67-68).

Group 11 allergens are glycoproteins where the carbohydrate moities have been found to consist mainly of MUXF3 and MMXF3 structures. These glycan determinants are frequently found in a number of plants and are commonly called CCD (Cross-reactive Carbohydrate Determinants).

Up to 70% of grass pollen sensitized individuals in temperate climates have been reported to react with group 11 allergens. It has been suggested that a part (up to 25%) of the IgE-binding to group 11 allergens might be directed to the carbohydrate epitopes (1,69).

Recombinant Phl p 11 lacks carbohydrate modification. One-third of 184 grass pollen-sensitised subjects showed allergen-specific IgE reactivity to recombinant Phl p 11 (66).

This class of grass pollen allergen was first described in *Lolium perenne* (Lol p 11) (69); significant levels of IgE antibodies binding to the purified native protein were found in 66% (n=270) of grass pollen-sensitised subjects. Phl p 11 shows 94% sequence identity to the homologous Lol p 11, and 33%-47% to described pollen proteins from a wider range of different plant species, including *Oryza sativa*, *Zea mays*, *Betula pendula*, *Olea europea* (Ole e 1), *Syringa vulgaris* (Syr v 1) and *Ligustrum vulgare* (Lig v 1).

g212 rPhl p 12

ImmunoCAP®: g212 rPhl p 12
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Phleum pratense* allergen Phl p 12

Common name:	Profilin
Biological function:	Actin-binding protein
Mw:	14 kDa

Allergen description

Phl p 12 (7-8,26,28,67-68,70-71) is a pollen profilin. It has the characteristics of a minor allergen, binding IgE antibodies from approximately 15-30% of grass pollen-allergic subjects with varying proportions in different geographical regions (1,72).

Note that the allergen now known as Phl p 12 was initially described as Phl p 11 (67-68).

Profilins are 14 kDa acidic proteins involved in cytoskeleton dynamics by binding to actin (1).

Profilin are ubiquitous proteins present in all eukaryotic organisms. Phl p 12 has >75% sequence identity with profilins of a wide range of species, from pollen as well as various plant-derived foods and latex (28,70). The sequence identity between Phl p 12 and animal profilins ranges between approximately 30% and 45%. Immunological cross-reactivity among pollen profilins and profilins of plant-derived foods is well documented. Profilins with high sequence identity have been described in *Phleum pratense*, *Olea europaea*, *Cynodon dactylon*, *Parietaria judaica*, and *Helianthus annuus* pollen (73-74). Nonetheless, it has been reported that Phl p profilin is in part responsible for the T-cell mediated immunological response in patients allergic to timothy, but that the response is very specific, since Phl p profilin-specific T-cell lines did not show cross-reactivity with a highly homologous profilin from *Parietaria judaica* (68,75).

Other profilins include Bet v 2 from birch (*Betula verrucosa*), Hev b 8 from latex (*Hevea braziliensis*), and Pho d 2 from date palm pollen (76-77). Profilin allergens also play an important role in banana and pineapple allergy, and other exotic fruits (78). Similarly, 2 rice profilin cDNAs were reported to have an 83% to 89% similarity to profilin from maize, *C. dactylon*, *H. brasiliensis* and timothy grass (79).

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Wall pellitory allergen components

Parietaria judaica

Available ImmunoCAP®:
w211 rPar j 2

Summary

Parietaria is a genus of dicotyledonous weeds of the *Urticaceae* family. Its pollen grains are among the most important allergenic triggers in the Mediterranean area and along the West coast of Europe as far north as central England. It is found in Australia and Argentina and two closely related species are found in the U.S. and one in Brazil. The genus *Parietaria* has about 10 species, which are highly crossreactive to each other (1). In some geographical areas a single species may dominate, and IgE antibodies to only one of the species can be found in sensitised individuals. Two species are commonly referred to as Wall pellitory: *Parietaria judaica* and *Parietaria officinalis*. *Parietaria* has a very long period of pollination, and often reaches peaks of more than 500 grains/m^3 of air at the beginning of June (2).

The following allergens have been characterised so far.

Par j 1, a lipid transfer protein (3).

Par j 2, a lipid transfer protein (3).

Par j 3, a profilin (4).

Par j Calcium-binding protein (5).

Parietaria pollen allergens have been reported to be quite heterogeneous and to range from high- to low-molecular mass (6). Studies of allergens of the most common species, *P. judaica* and *P. officinalis*, have shown that the allergens of the extracts are highly cross-reactive. Allergenic components are most highly concentrated in the pollen; but they are present throughout the plant, including in the leaves and, in traces, in the stems (7).

Both Par j 1 and Par j 2 are major allergens of *P. judaica* and belong to the nonspecific lipid transfer protein family. Par j 1 and Par j 2 represent major allergenic components of *Parietaria judaica* pollen, since they are able to induce an immunoglobulin E (IgE) response in 80-95% of Pj-allergic patients (1,8-9).

Allergens from *Parietaria judaica* listed by IUIS*

Par j 1	Par j 2	Par j 3
Par j 4		

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

Although *P. judaica* also contains a profilin allergen, less than 50% of patients sensitised to Birch and Grass profilin cross-react to *Parietaria* profilin; this is in contrast to a high prevalence of cross-reactivity of profilin from other pollens (4). Due to structurally similar pollen antigens in different *Parietaria* species, these are all equally useful in diagnosis, regardless of the pollen species to which the patient is sensitive or the prevalent species in the area (2).

Recombinant Par j 1 and Par j 2 allergens have been shown to possess immunological properties equivalent to those of their natural counterparts (1). As Par j 1 and Par j 2 have

similar IgE epitopes, rPar j 2 may be a useful assessment tool for the diagnosis and therapy of *Parietaria* pollen allergy (3). (Despite their structural similarities, however, Par j 1 and Par j 2 are independent allergens, as demonstrated by cross-inhibition experiments showing that they possess an independent repertoire of IgE epitopes (9)).

Recombinant allergens, which are genetically engineered isoforms resembling allergen molecules from known allergen extracts, have immunoglobulin E (IgE) antibody binding comparable to that of natural allergens and generally show good reactivity in *in vitro* and *in vivo* diagnostic tests (10). To date, many different recombinant allergens of pollens, molds, mites, bee venom, latex and foods have been cloned, sequenced, and expressed.

Recombinant allergens have a wide variety of uses, from the diagnosis and management of allergic patients to the development of immunotherapy to the standardisation of allergenic test products as tools in molecular allergology. Recombinant allergens are particularly useful for investigations in allergies manifesting wide cross-reactivity.

w211 rPar j 2

ImmunoCAP®: w211 rPar j 2

Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding *Parietaria judaica* allergen Par j 2 (3,5)

Common names:	Lipid transfer protein 2, LTP 2
Biological function:	Nonspecific lipid transfer protein
Mw:	14 kDa
Other allergens isolated:	rPar j 2.0101 (9)

Allergen description

rPar j 2 is a 14 kDa lipid transfer protein. Approximately 83% of Mediterranean weed-allergic patients, and 7% of non-Mediterranean weed-allergic patients, have been shown to be sensitised to this allergen (5). Lipid transfer proteins are represented in pollen, fruit and vegetables of a wide range of plant species.

rPar j 2 and the isoform rPar j 2.0101 are representative of Par j 2, a major allergen in *P. judaica* pollen. rPar j 2.0101 showed an allergenic activity and a capacity to bind IgE that are almost identical to those of the native allergens purified from aqueous pollen extract (11). Approximately 80% of individuals allergic to this pollen have been shown to be sensitised to these recombinant allergens (5,9). Furthermore, there is an amino acid sequence homology with rPar j 1.0101 of 45%, along with similar immunoglobulin E epitopes (3). The epitope of the major allergen Par j 1.0101 also present on Par j 2.0101, is an immuno-dominant epitope and is capable of inhibiting 30% of all specific IgE against the *P. judaica* major allergens (12).

A study evaluated the allergen profile of *P. judaica* IgE-reactive sera from 36 weed pollen-sensitised allergic individuals from the Mediterranean region with high *Parietaria* pollen exposure. They were compared with 69 weed pollen-allergic patients with little or no *Parietaria* exposure; 83% of the Mediterranean weed pollen-allergic patients mounted high IgE antibody levels (mean 20.89 kU_A/L) against recombinant rPar j 2, whereas only 7% of the non-Mediterranean weed-allergic patients showed low level IgE reactivity to rPar j 2 (mean 1.03 kU_A/L). The authors concluded that rPar j 2 might be used as a diagnostic marker allergen to identify weed pollen-allergic patients who are genuinely sensitised against *Parietaria* pollen and thus would be particularly suited for specific immunotherapy with *Parietaria* pollen extract (5).

In a study of sera of 29 *P. judaica*-allergic individuals tested against 5 recombinant peptides, at least 4 putative IgE-binding epitopes were identified. These results suggest that the recombinant rPar j 2 allergen contains IgE epitopes that are heterogeneously recognised by sensitive patients (13).

Lipid transfer proteins are panallergens that have a ubiquitous distribution in tissues of many plant species, resulting in variable degrees of cross-reactivity and particularly relevant cross-reactivity in fruits and vegetables (14-15).

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Wheat allergen components

Triticum aestivum

Available ImmunoCAP®:
f416 rTri a 19; Omega-5 Gliadin (1)

Summary

Wheat is one of the major cereal grains belonging to the grass family (*Poaceae* or *Gramineae*) and is a staple food item in most diets worldwide. The hexaploid *Triticum aestivum* is by far the most important of all wheat species, the highest yielding and the widest ranging, as well as the one most suited to bread making. All varieties of wheat contain soluble and insoluble (gluten) proteins. The softer wheat with the lowest protein content, *T. aestivum* and the varieties closest to it, is used for biscuits, cakes and pastry. Harder wheat with higher protein content is used for bread, semolina, couscous, macaroni and pasta. *T. durum* (Durum wheat) is a source of Italian pasta, Indian chappatis and Chinese noodles.

Wheat, like all other foods, contains a number of proteins. Over 300 proteins have been matched to established protein database information (2) and some have been identified as allergens.

The major proteins in wheat vary in proportion according to the type of wheat. This variability is one reason reactions to different wheat products are not consistent.

Wheat proteins can be classified into different groups:

Albumins (water-soluble; not similar to egg or milk albumin).

Globulins (salt-soluble, water-insoluble).

Glutens (the water/salt-insoluble wheat proteins)

Glutens can be further divided into:

Gliadins (28-42%; the major prolamin protein in wheat, soluble in 70-90% alcohol).

Glutenins (42-62.5%; the major glutenin proteins in wheat, soluble in dilute acid or alkali solutions).

Allergens from *Triticum aestivum* listed by IUIS*

Tri a 12	Tri a 14	Tri a 18
Tri a 19	Tri a 25	Tri a 26

*International Union of Immunological Societies (www.allergen.org) Jan. 2008.

The following allergens have been identified and characterized:

Tri a 18 (3).

Tri a 19, a gliadin, also known as fast ω-gliadin (4-18).

Tri a Chitinase (19).

Tri a LTP (20-21).

Tri a Germin, a glycoprotein expressed in many plants in response to biotic and abiotic stress (22).

Tri a aA/TI, an alpha-amylase/trypsin inhibitor, (approximately 14-15 kDa in size) (23-28) implicated as a major allergen associated with baker's asthma (24) and, less commonly, with food allergy (29). In particular, the subunits of the tetrameric alpha-amylase inhibitor, CM2, CM3 and CM16, are known to be major allergens for baker's asthma.

Wheat allergen components

In Japanese patients with atopic dermatitis, serum IgE bound only to CM3 and not to CM2 and CM16, suggesting that CM3 may be involved in both atopic dermatitis and baker's asthma (25).

Tri a Bd 36K, a peroxidase purified from Wheat albumin and an inhalant allergen (30).

Tri a Bd 17K, identified as alpha-amylase inhibitor CM16 (31-32).

Tri a Peroxidase, a 60 kDa protein (33).

Tri m Peroxidase, a 36 kDa seed-specific peroxidase, found specifically in *T. monococcum*, but also present in flour from diploid, tetraploid (pasta) and hexaploid (bread) wheats. Sensitisation occurs via inhalation. Sera from 6 out of 10 patients hypersensitive to wheat flour were shown to have specific IgE directed to this allergen (34). This allergen is one of the most reactive with some patients' sera (35).

Tri a TPIS, a triosephosphate isomerase, an allergen via inhalation in bakers (36).

f416 rTri a 19

ImmunoCAP®: f416 rTri a 19
Recombinant non-glycosylated protein produced in an *E. coli* strain carrying a cloned cDNA encoding a part of ω-5 gliadin from *Triticum aestivum*

Common names:	ω-5 gliadin, fast ω-gliadin
Biological function:	A plant proline- and glutamine-rich storage protein
Mw:	27 kDa

Allergen description

Gliadins include more than 40 monomeric water/salt-insoluble but ethanol soluble proteins with molecular weights in the range of 30-70 kDa. According to amino acid sequence and electrophoresis mobility they are classified into α-, β-, γ-, and ω-gliadins, all rich in non-polar amino acids and glutamine. ω-gliadins, consisting to about 80 % of the amino acids glutamine, proline and phenylalanine, are almost completely repetitive in sequence. The greater molecular weight and poor content of cysteines distinguish ω-gliadins from the sulphur rich α-, β- and γ-gliadins. The ω-gliadins are further classified as slowly-migrating ω-1 and ω-2, ω-3 and fast-migrating ω-4 and ω-5. Tri a 19 (ω-5 gliadin), a component of the fast ω-gliadin fraction, is a major allergen among water/salt-insoluble proteins. rTri a 19, mw ~27 kDa, is a recombinant peptide representing the immuno-dominant part of ω-5 gliadin (1).

IgE antibodies against fast ω-gliadin cross-react with g-gliadin and slow ω-gliadin (4). Further studies have reported that γ-70 and γ-35 secalins in rye and γ-3 hordein in barley cross-react with ω-5 gliadin, suggesting that rye and barley may elicit symptoms in patients sensitized to ω-5 gliadin.

In immunoblotting, anti-ω-5 gliadin antibodies bound to 70 kDa and 32 kDa proteins in rye and to a 34-kDa protein in barley, but not to any proteins in oats. The cross-reactive proteins were identified as rye γ-70 secalin, rye γ-35 secalin and barley γ-3 hordein, respectively. In ELISA studies, 21/23 (91%) patients with Wheat-Dependent Exercise-Induced Anaphylaxis (WDEIA) showed IgE antibodies to purified γ-70 secalin, 19/23 (83%) to γ-35 secalin and 21/23 (91%) to γ-3 hordein. Skin prick testing gave positive reactions to γ-70 secalin in 10/15 (67%) patients, to γ-35 secalin in 3/15 (20%) patients and to γ-3 hordein in 7/15 (47%) patients (5).

Of the wheat proteins, ω-5 gliadin has been reported as a major allergen in Wheat-Dependent Exercise-Induced Anaphylaxis (WDEIA) (1,4-16). Although the mechanism is not fully understood, a study reports that ω-5 gliadin-derived peptides are cross-linked by tissue transglutaminase (tTG), which causes a marked increase in IgE antibody binding both *in vitro* and *in vivo*. Activation of tTG in the intestinal mucosa during exercise in patients with WDEIA may lead to the formation of large allergen complexes capable of eliciting anaphylactic reactions (11).

In addition, ω-5 gliadin has been shown to be a major allergen in children with immediate allergy to ingested wheat. After oral wheat challenge 40 children with suspected wheat allergy presented atopic dermatitis and/or gastrointestinal and/or respiratory symptoms. 19 children (48%) had immediate reactions and 8 children (20%) had delayed hypersensitivity symptoms. Sixteen (84%) of those with immediate symptoms had IgE antibodies to ω-5 gliadin whereas none of the children with delayed or negative challenge test results had IgE antibodies to ω-5 gliadin. Moreover, in children with wheat induced anaphylaxis, ω-5 gliadin seems to be a major sensitizing allergen (17).

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